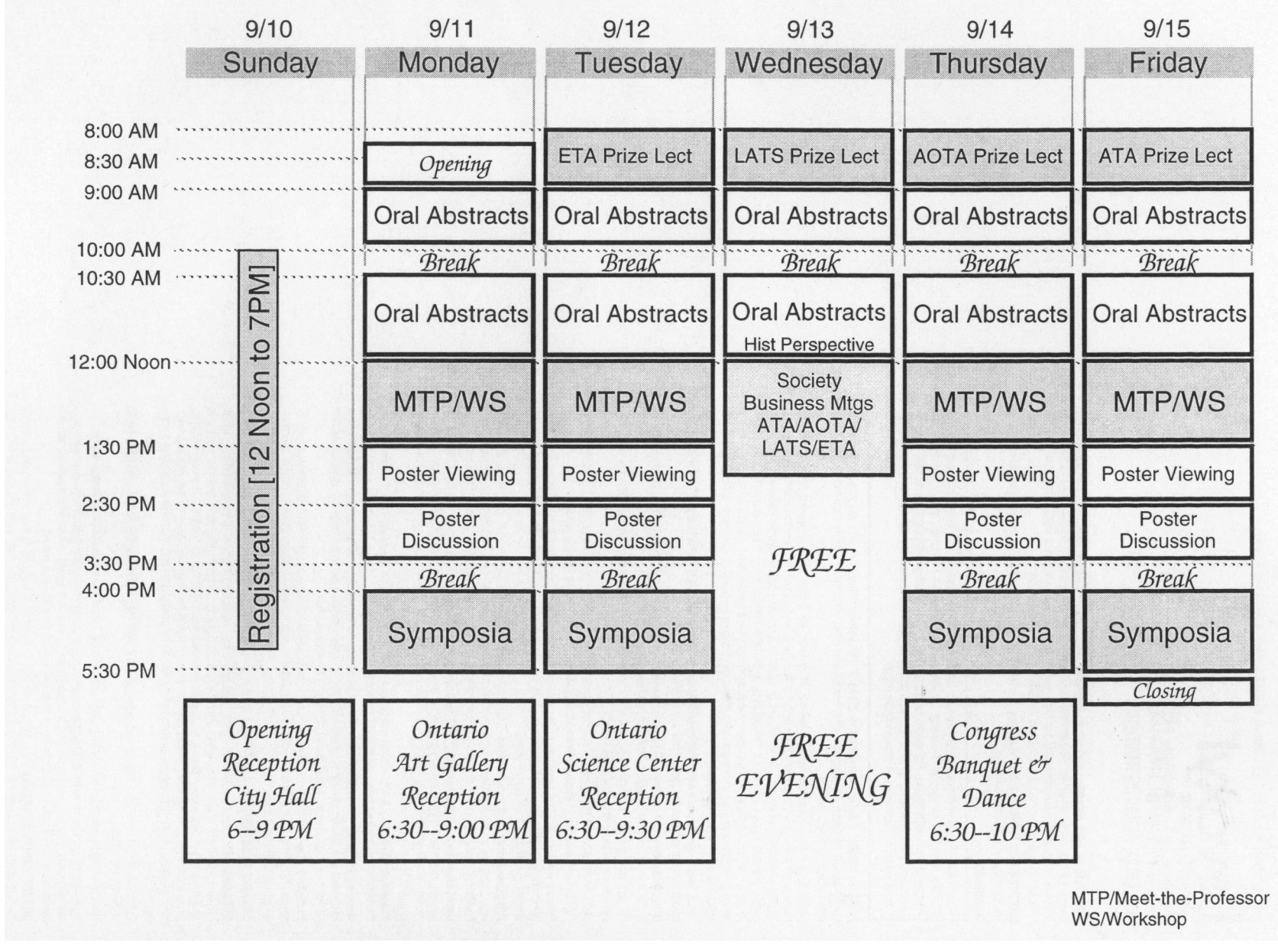
11th International Thyroid Congress

Sheraton Centre, Toronto, Canada; September 10-15, 1995



11TH INTERNATIONAL THYROID CONGRESS

The Sheraton Centre - Toronto, Canada
September 10 - 15, 1995

HOST ORGANIZATION:
AMERICAN THYROID ASSOCIATION

Martin I. Surks, M.D., Secretariat, 11th International Thyroid Congress
Professor of Medicine and Pathology
Montefiore Medical Center & The Albert Einstein College of Medicine
111 East 210th Street
Bronx, New York 10467

Diane P. Miller, Administrator
Telephone: 718 882-6047
Fax: 718 882-6085

MONTEFIORE

MONTEFIORE MEDICAL CENTER
THE UNIVERSITY HOSPITAL
FOR THE ALBERT EINSTEIN COLLEGE OF MEDICINE

CME ACCREDITATION: 39 CREDIT HOURS

THE ALBERT EINSTEIN COLLEGE OF MEDICINE IS ACCREDITED BY THE ACCREDITATION COUNCIL FOR CONTINUING MEDICAL EDUCATION TO SPONSOR CONTINUING MEDICAL EDUCATION FOR PHYSICIANS.

THE ALBERT EINSTEIN COLLEGE OF MEDICINE HAS DESIGNATED THIS CONTINUING MEDICAL EDUCATION ACTIVITY FOR 39 CREDIT HOURS IN CATEGORY 1 OF THE PHYSICIAN'S RECOGNITION AWARD OF THE AMERICAN MEDICAL ASSOCIATION.

ACKNOWLEDGMENTS

The committees, officers, members and friends of the
11th International Thyroid Congress
gratefully acknowledge the generous support of this meeting from the following:

ABBOTT LABORATORIES

CANADA LIFE ASSURANCE COMPANY

CORNING NICHOLS INSTITUTE

DIAGNOSTICS PRODUCTS CORPORATION

E. MERCK

ELSCINT CANADA LIMITED

FOREST PHARMACEUTICALS, INC.

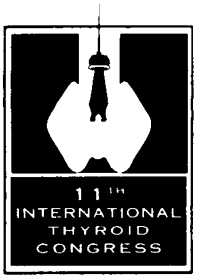
GLAXO WELLCOMBE, INCORPORATED

GENZYME CORPORATION

KNOLL PHARMACEUTICAL COMPANY
(FORMERLY BOOTS PHARMACEUTICALS)
USA AND CANADA

KRONUS

MC DONALD'S RESTAURANTS OF CANADA LIMITED



INTERNATIONAL ORGANIZING COMMITTEE

American Thyroid Association
Leonard Wartofsky, President
Martin I. Surks, Secretary

Asia & Oceania Thyroid Association
Shigenobu Nagataki, President
Junji Konishi, Secretary

European Thyroid Association
E. Dillwyn Williams, President
Albert G. Burger, Secretary
Aldo Pinchera , Recording Secretary

Latin American Thyroid Society
Joao H. Romaldini, President
Rui M.B. Maciel, Secretary

PROGRAM ORGANIZING COMMITTEE

American Thyroid Association
William W. Chin, Chairman
Jerome M. Hershman
E. Chester Ridgway

Asia & Oceania Thyroid Association
Nobuyuki Amino
Bo Youn Cho
Duncan J. Topliss

European Thyroid Association
Georg Hennemann
Bernard Rousset
Gilbert Vassart

Latin American Thyroid Society
Antonio C. Bianco
Maria Guadalupe Lopez Carrasco
Aldo H. Coleoni

LOCAL ARRANGEMENTS COMMITTEE

Paul G. Walfish, Chairman
Sandy Walfish
Robert Volpe
Ruth Volpe
John Chan
Lily Chan
Arnold Bayley
Jean Bayley
Jay Silverberg
Linda Miller-Silverberg
William Singer
Patricia Singer
Irving B. Rosen
Helen Rosen
Ali Khalil
Rhafif Khalil
Jeremy Freeman
Elayne Freeman

THE AMERICAN THYROID ASSOCIATION EXECUTIVE COUNCIL

PRESIDENT
LEONARD WARTOFSKY, M.D.
WASHINGTON D.C.

PRESIDENT-ELECT
COLUM GORMAN, M.B.
ROCHESTER, MINNESOTA

TREASURER
MANFRED BLUM, M.D.
NEW YORK, NEW YORK

SECRETARY
MARTIN I. SURKS, M.D.
AMERICAN THYROID ASSOCIATION, INC.
MONTEFIORE MEDICAL CENTER
111 EAST 210TH STREET
BRONX, NEW YORK 10467

ADMINISTRATOR
DIANE P. MILLER

TELEPHONE: 718 882-6047
FAX: 718 882-6085

DIRECTORS
P. REED LARSEN, M.D.
BOSTON, MASSACHUSETTS

ARTHUR B. SCHNEIDER, M.D., PH.D.
CHICAGO, ILLINOIS

J. ENRIQUE SILVA, M.D.
MONTREAL, QUEBEC, CANADA

BRUCE D. WEINTRAUB, M.D.
BETHESDA, MARYLAND

WILLIAM W. CHIN, M.D.
BOSTON, MASSACHUSETTS

MARGUERITE T. HAYS, M.D.
PALO ALTO, CALIFORNIA

HAROLD L. SCHWARTZ, PH.D.
MINNEAPOLIS, MINNESOTA

WOLFGANG DILLMAN, M.D.
SAN DIEGO, CALIFORNIA

MARGITA ZAKARIJA, M.D.
MIAMI, FLORIDA

ASIA AND OCEANIA THYROID ASSOCIATION EXECUTIVE COUNCIL

PRESIDENT
PROF. SHIGENOBU NAGATAKI
NAGASAKI, JAPAN

VICE-PRESIDENTS
DR. CHANG-SOON KOH
SEOUL, KOREA

DR. CHEN JIA-LUN
SHANGHAI, CHINA

DR. JAN R. STOCKIGT
MELBOURNE, AUSTRALIA

SECRETARY/TREASURER
DR. JUNJI KONISHI
DEPARTMENT OF NUCLEAR MEDICINE
KYOTO UNIVERSITY SCHOOL OF MEDICINE
KYOTO 606-01, JAPAN

TELEPHONE: 81-958-49-7260
FAX: 81-958-49-7270

COUNCIL MEMBERS
DR. NOBUYUKI AMINO
OSAKA, JAPAN

DR. MUNHO LEE
SEOUL, KOREA

DR. LELAND S. VILLADOLID
MANILA, PHILIPPINES

DR. FANG-WU CHEN
TAIPEI, CHINA

PROF. MA TAI
TIANJIN, CHINA

DR. PETER P.B. YEO
SINGAPORE

DR. CRESWELL J. EASTMAN
SYDNEY, AUSTRALIA

PROF. SUNTHORN TANDHANAND
BANGKOK, THAILAND

DR. ROSE T.T. YOUNG
HONG KONG

DR. YOICHI KONDO
MAEBASHI, JAPAN

DR. DUNCAN TOPLISS
MELBOURNE, AUSTRALIA

LATIN AMERICAN THYROID SOCIETY

PRESIDENT
JOAO H. ROMALDINI, M.D.
SAO PAULO, BRAZIL

PRESIDENT- ELECT
GUSTAVO PINEDA, M.D.

SECRETARY/TREASURER
RUI M.B. MACIEL, M.D.
ENDOCRINOLOGY/ MEDICINE
ESCOLA PAULISTA DE MEDICINA
UNIVERSIDADE FEDERAL DE SAO PAULO
CAIXA POSTAL 20266
04034-970 SAO PAULO, SP, BRAZIL

TELEPHONE: 55-11-571-9826
FAX: 55-11-575-0311

COUNCIL MEMBERS
ALDO COLEONI, PH.D.
CORDOBA, ARGENTINA

ANTONIO C. BIANCO, M.D.
SAO PAULO, BRAZIL

DORIS ROSENTHAL, M.D.
RIO DE JANEIRO, BRAZIL

GERARDO SARTORIO, PH.D.
BUENOS AIRES, ARGENTINA

GUILLERMO JUVENAL, PH.D.
BUENOS AIRES, ARGENTINA

HANS GRAF, M.D.
CURITIBA, BRAZIL

JAYME AGUAYO, M.D.
SANTIAGO, CHILE

MARIA C. DE BLANCO, M.D.
CARACAS, VENEZUELA

MARIA G. LOPEZ, PH.D.
MEXICO CITY, MEXICO

THE EUROPEAN THYROID ASSOCIATION EXECUTIVE COUNCIL

PRESIDENT
SIR DILLWYN WILLIAMS, M.D.
CAMBRIDGE, UNITED KINGDOM

PRESIDENT-ELECT
JACQUES DUMONT, M.D.
BRUSSELS, BELGIUM

SECRETARY-TREASURER
ALBERT BURGER, M.D.
THYROID UNIT - HCUG
RUE MICHELI-DU-CREST, 24
1211 GENEVA 14, SWITZERLAND

ADMINISTRATOR
CATHERINE SIEGRIST KAISER, PH.D.

TELEPHONE: +41 22 372 9195
FAX: +41 22 347 6486

COUNCIL MEMBERS
DANIEL CHRISTOPHE, PH.D.
BRUSSELS, BELGIUM

ROLAND GARTNER, M.D.
MUNCHEN, GERMANY

JOHN LAZARUS, M.D.
CARDIFF, UNITED KINGDOM

MARIAN LUDGATE, PH.D.
CARDIFF, UNITED KINGDOM

STEFANO MARIOTTI, M.D.
TIRRENIA-PISA, ITALY

HARALD MEINHOLD, PH.D.
BERLIN, GERMANY

LUCIO NITSCH, M.D.
NAPOLI, ITALY

MARIA-JESUS OBREGON, PH.D.
MADRID, SPAIN

WILMAR WIERSINGA, M.D.
AMSTERDAM, THE NETHERLANDS

SOCIAL PROGRAM - SPECIAL TOURS AND EVENTS

Saturday September 9, 1995

8:00 am to 4:00 pm	Niagara Falls and Niagara-on-the-Lake
9:00 am to 12:00 noon	History and Heritage - The St. Lawrence Market
2:00 pm to 4:30 pm	Get Acquainted Tour of Toronto

Sunday September 10, 1995

8:00 am to 5:00 pm	Niagara Falls and the Niagara Wine Region
9:00 am to 11:30 am	Get Acquainted Tour of Toronto
1:00 pm to 5:00 pm	Royal Ontario Museum, George R. Gardiner Museum of Ceramic Art and Yorkville
6:00 pm to 9:00 pm	**WELCOME RECEPTION** - City Hall Rotunda

Monday September 11, 1995

8:00 am to 12:00 pm	Breakfast at Casa Loma and Gardens Tour
9:00 am to 12:30 pm	Vaudeville and the Opulent Twenties
2:00 pm to 4:30 pm	Get Acquainted Tour of Toronto
6:30 pm to 9:00 pm	**ART GALLERY OF ONTARIO RECEPTION**

Tuesday September 12, 1995

7:30 am to 9:30 am	**THYROID ASSOCIATES PROGRAM** Sheraton Centre - Cinema Continental Breakfast & Film: GLENN GOULD
8:00 am to 5:00 pm	Mennonite Country
9:30 am to 11:30 am	Royal Doulton Presentation
1:00 pm to 4:30 pm	Vaudeville and the Opulent Twenties
6:30 pm to 9:30 pm	**ONTARIO SCIENCE CENTER RECEPTION**

Wednesday September 13, 1995

1:30 pm to 5:00 pm	McMichael Canadian Art Collection
1:30 pm to 5:00 pm	Harbour Cruise and Promenade
1:30 pm to 5:00 pm	Hockey Canada-Maple Leaf Gardens/Hockey Hall of Fame

Thursday September 14, 1995

9:00 am to 12:00 noon	The CN Tower and Spectacular SkyDome
1:00 pm to 5:00 pm	Hidden Art, Hidden Toronto and the Design Exchange
1:00 pm to 5:00 pm	Royal Ontario Museum George R. Gardiner Museum of Ceramic Art and Yorkville
2:00 pm to 3:30 pm	**THYROID ASSOCIATES PROGRAM** Sheraton Centre - City Hall Foyer "Tea With Glenn Gould"
6:30 P.M. to 10:30 P.M.	**FAREWELL RECEPTION & BANQUET**

Friday September 15, 1995

2:00 pm to 12:00 midnight	Niagara Falls with Theatrical Entertainment
9:00 am to 6:00 pm	The Stratford Festival

11th International Thyroid Congress

cordially invites you to attend a gala evening featuring

gallery visits, hors d'oeuvres and music

at

THE ART GALLERY OF ONTARIO

Monday, September 11th, 1995

From 6:30 P.M. to 9:00 P.M.

This evening is sponsored by:

knoll®

Knoll Pharmaceutical Company
(formerly Boots Pharmaceuticals, Inc.)

Admission by ticket only - Buses depart at 6:15 P.M.
from the Sheraton Centre (lower level)

EXHIBITORS

ABBOTT LABORATORIES - Abbott Park, Illinois

BERLIN-CHEMIE AG - Berlin, Germany

DANIELS PHARMACEUTICALS, INCORPORATED - St. Petersburg, Florida

DIAGNOSTICS PRODUCTS CORPORATION - Los Angeles, California

FOREST PHARMACEUTICALS, INCORPORATED - St. Louis, Missouri

HARPELL ASSOCIATES, INCORPORATED - Oakville, Ontario, Canada

KNOLL PHARMACEUTICAL COMPANY
FORMERLY BOOTS PHARMACEUTICALS - Lincolnshire, Illinois

KRONUS - San Clemente, California

LIPPINCOTT - RAVEN PUBLISHERS - Philadelphia, Pennsylvania

MARY ANN LIEBERT, INCORPORATED - Larchmont, New York

E. MERCK - Darmstadt, Germany

NICHOLS INSTITUTE - San Juan Capistrano, California

THE ENDOCRINE SOCIETY - Bethesda, Maryland

THYROID FOUNDATION OF AMERICA - Boston, Massachusetts

THYROID FOUNDATION OF CANADA - Toronto, Ontario, Canada

NATIONAL GRAVES' DISEASE FOUNDATION - Brevard, North Carolina

W.B. SAUNDERS COMPANY - Toronto, Ontario, Canada

EUROPEAN JOURNAL OF ENDOCRINOLOGY - Geneva, Switzerland

THERAMED LIMITED - Toronto, Ontario, Canada

Exhibits open at 10 A.M. Daily.
Visit the exhibitor area and demonstrate your appreciation for the vital support they provide...

11th International Thyroid Congress

cordially invites you to attend our

"Farewell Banquet"

Reception and Dinner

at

THE SHERATON CENTRE
GRAND BALLROOM

Thursday, September 14th, 1995

Reception begins at 6:30 P.M.

followed by:
Awards, Dinner and Dancing

This evening is sponsored in part by both:

Forest Pharmaceuticals, Inc.

Corning Nichols Institute

Tickets may be purchased at the ITC registration area - Concourse Level

SHERATON CENTRE - MEETING LOCATION MAPS

CONCOURSE LEVEL

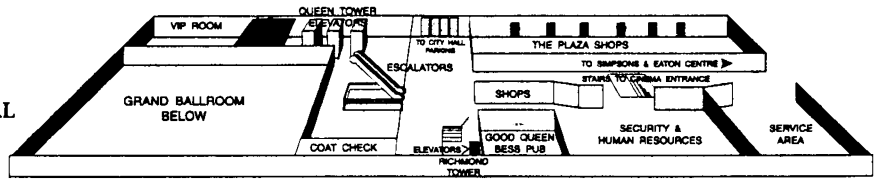
MEETING REGISTRATION AREA
CONCOURSE LEVEL FOYER

SPECIAL TOURS DESK
CO ORDINATION PLUS
CONCOURSE LEVEL FOYER

AUDIO ARCHIVES INTERNATIONAL
CONCOURSE LEVEL FOYER

ITC/ATA OFFICE
COAT CHECK ROOM

SPEAKER READY ROOM
VIP ROOM



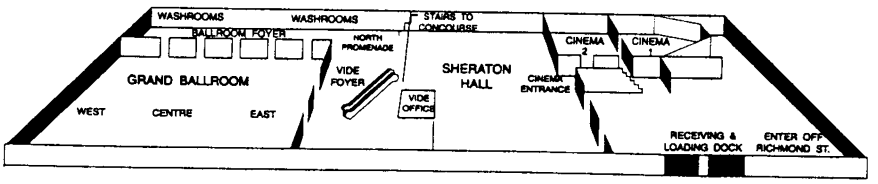
MEETING ROOMS - LOWER CONCOURSE LEVEL

GENERAL SESSION
SIMULTANEOUS SESSIONS
GRAND BALLROOM
WEST, CENTRE & EAST

POSTER PRESENTATIONS
EXHIBITION HALL
SHERATON HALL

THYROID ASSOCIATES PROGRAM
CINEMA

BANQUET
GRAND BALLROOM WEST & CENTRE



HOSPITALITY SUITE - SPIN DRIFT SPRING SONG - QUEEN TOWER - 4TH FLOOR - ROOM 440

SHERATON CENTRE - MEETING LOCATION MAPS

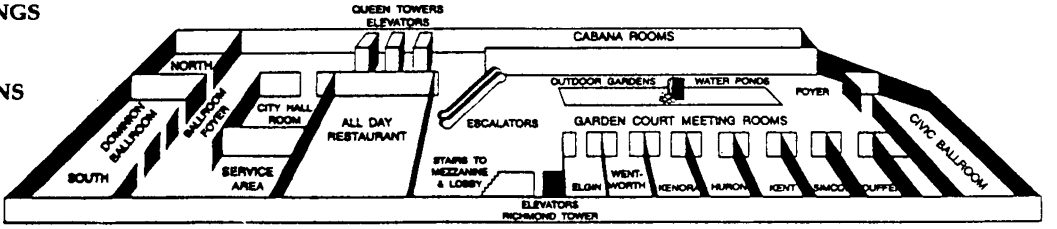
MEETING ROOMS - SECOND LEVEL

MEET THE PROFESSOR
LUNCHEONS
SIMCON/DUFFERIN & CITY HALL

WORKSHOPS
DOMINION NORTH
DOMINION SOUTH
CIVIC BALLROOM

ATA, AOTA & ETA
EXECUTIVE COUNCIL MEETINGS
KENT ROOM

POSTER DISCUSSION SESSIONS
SIMCO/DUFFEN, CITY HALL,
DOMINION NORTH
DOMINION SOUTH, CIVIC,
WINDSOR EAST AND
WINDSOR WEST



THYROID ASSOCIATES
AFTERNOON TEA
CITY HALL FOYER

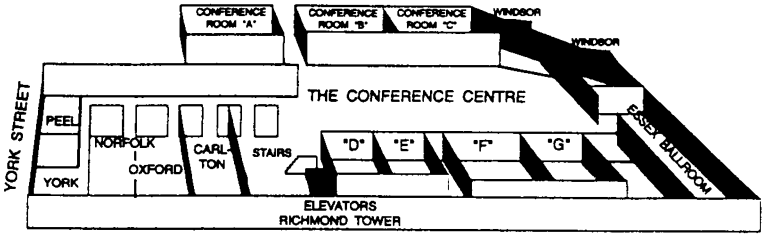
ATA - BUSINESS MEETING
DOMINION NORTH
AOTA - GENERAL ASSEMBLY
CITY HALL ROOM
LATS - GENERAL ASSEMBLY
DOMINION SOUTH
ETA - GENERAL ASSEMBLY
SIMCO/DUFFIN ROOM

MEETING ROOMS - MEZZANINE RICHMOND TOWER

MEET THE PROFESSOR LUNCHEONS
ESSEX BALLROOM

ATA COMMITTEE
BREAKFAST MEETINGS
ESSEX ROOM

ATA "BLUE RIBBON PANEL"
NORFOLK ROOM



CERTIFICATION OF ATTENDANCE

THIS IS TO CERTIFY THAT

_____

ATTENDED THE
11th International Thyroid Congress
Hosted by:
THE AMERICAN THYROID ASSOCIATION, INC.

HELD AT THE SHERATON CENTRE HOTEL

TORONTO, ONTARIO, CANADA

SEPTEMBER 10TH TO SEPTEMBER 15TH, 1995

Martin I. Surks, MD

MARTIN I. SURKS, SECRETARY
THE AMERICAN THYROID ASSOCIATION, INC.

11th International Thyroid Congress
Sunday, September 10, 1995

THE SHERATON CENTRE - TORONTO, CANADA

CONCOURSE LEVEL FOYER

12:00 P.M. TO 7:00 P.M. **REGISTRATION OPENS**
11th International Thyroid Congress

Daily hours - Monday - Friday 8 A.M. to 5 P.M.

REGISTRATION
SPECIAL TOURS AND EVENTS
Co Ordination Plus

AUDIO ARCHIVES INTERNATIONAL, INC.
Desk - Tapes of Congress Proceedings may be purchased here

CONCOURSE LEVEL - VIP ROOM

SPEAKER READY ROOM

SHERATON HALL

2:00 P.M. **EXHIBITORS SET-UP**
Exhibits open - Monday 10 AM
Daily hours 10 AM - 4 PM
Special hours Wednesday (only) - 10 AM to 12 Noon

6:00 P.M. to 9:00 P.M. **WELCOME TO CANADA RECEPTION**
CITY HALL ROTUNDA
(Directly across from The Sheraton Centre)

8:00 P.M. MEETING - KENT ROOM
AMERICAN THYROID ASSOCIATION
EXECUTIVE COUNCIL

11th International Thyroid Congress
Monday, September 11, 1995

MORNING SESSION

WEST & CENTRE BALLROOM
THE SHERATON CENTRE - TORONTO, CANADA

8:15 A.M. **OPENING CEREMONIES**

9:00 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

AUTOIMMUNITY - West & Centre Ballroom
CHAIRPERSONS DRS. CHO AND REES-SMITH

9:00
1. CLONING OF A PROTEIN, SOX-4, INVOLVED IN METHIMAZOLE (MMI), INSULIN, AND THYROTROPIN (TSH) REGULATION OF THE MAJOR HISTOCOMPATIBILITY (MHC) CLASS I AND THYROGLOBULIN (TG) GENES.
K. Suzuki, H. Shimura, G. Napolitano, V. Montani, C. Giuliani, Y. Shimura, M. Ohmori, M. Ohta, D.S. Singer and L D. Kohn
NIH, Bethesda, MD, USA; Chieti University Medical School, Chieti, Italy; and Yamanashi School of Medicine, Yamanashi, Japan

9:15
2. IODIDE-INDUCED B CELL AUTOIMMUNE REACTION IN INTERLEUKIN 9 (IL9) TRANSGENIC MICE.
M.-C. Many, J.-C. Renauld and J.-F. Denef
Histology Unit and Experimental Medicine Unit, Ludwig Institute for Cancer Research, Catholic University of Louvain, Brussels, Belgium

9:30
3. CYTOKINES AND THYROID EPITHELIAL INTEGRITY: INTERLEUKIN-1 ALPHA INDUCES PARACELLULAR LEAKAGE AND ZO-1 REDISTRIBUTION IN FILTER-CULTURED HUMAN THYROCYTES.
L.E. Ericson, J. Husmark, B. Nilsson and L.-E. Tisell
Institute of Anatomy & Cell Biology, Göteborg University, and Department of Surgery, Sahlgrenska Hospital, Göteborg, Sweden

9:45
4. REGULATION OF INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1) EXPRESSION IN HUMAN THYROID TRANSPLANTS IN THE NUDE MOUSE IN VIVO.
R. Hoermann, S. Poertl, K. Mann and P.M. Schumm-Draeger
Division of Endocrinology, Department of Medicine, University of Essen, Essen; and Department of Endocrinology, Center of Internal Medicine, University of Frankfurt, Frankurt, Germany

CANCER - East Ballroom
CHAIRPERSONS DRS. GERBER AND WALFISH

9:00
5. ALTERED REGULATION OF THE STIMULATORY G PROTEIN(Gs) -ADENYLATE CYCLASE CASCADE IN DIFFERENTIATED THYROID CARCINOMAS: EVIDENCE FOR UPREGULATION OF INHIBITORY G PROTEIN BY Gs PROTEIN.
M. Derwahl, C. Hamacher, G. Papageorgiou and H. Schatz
Medizinische Universitätsklinik Bergmansheil, Bochum, Germany; and Evangelismos Hospital, Athens, Greece

9:15
6. NEW MECHANISMS OF ESCAPE FROM IMMUNOSURVEILLANCE OF THYROID CARCINOMA CELLS: INVOLVEMENT OF PKC PATHWAY.
V. Bassi, S. De Riu, M. Vitale, E. Carbone, M. Maio, G. Rossi and G.F. Fenzi
Dipartimento di Endocrinologia ed Oncologia Clinica e Molecolare, Dipartimento di Biologia e Patologia Cellulare e Molecolare,Universita di Medicina e Chirurgia, "Federico II", Napoli; Immunotherapeutics Advanced Unit, CRO, Aviano, Italy

MORNING SESSION - Monday, September 11, 1995

9:30 7. EVIDENCE FOR PARTIAL GROWTH INHIBITION BY EXCESSIVE AUTOCRINE SECRETION OF TRANSFORMING GROWTH FACTORα (TGFα) AND PLATELET-DERIVED GROWTH FACTOR B (PDGF-B) IN HTC-TSHr THYROID CARCINOMA CELLS.
M. Bröcker, J. Hammer and M. Derwahl
Medizinische Universitätsklinik, Bochum, Germany

9:45 8. GLUT1 GLUCOSE TRANSPORTER EXPRESSION IN BENIGN AND MALIGNANT THYROID CELLS: AN IMMUNOHISTOCHEMICAL STUDY.
K.R. Weiser, D.E. Burstein, I. Reder, A. Pritsker and R.S. Haber
Mount Sinai School of Medicine, New York, NY USA

10:00 A.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

10:30 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

AUTOIMMUNITY- West & Centre Ballroom
CHAIRPERSONS DRS. BROWN AND NIEPOMNISZCZE

10:30 9. DETERMINANT SPREADING SHOWN BY MONOCLONAL THYROID STIMULATING ANTIBODY PRODUCED AGAINST A TSH RECEPTOR PEPTIDE.
H. Sugawa, Y. Ueda, M. Zhang, S. Kosugi, M. Ueda and T. Mori
Kyoto University, Kyoto, Japan

10:45 10. EXPERIMENTAL THYROTROPIN RECEPTOR (TSHr) ANTIBODIES: INVESTIGATION OF MECHANISMS BY WHICH THEY CAN INHIBIT TSH-MEDIATED cAMP PRODUCTION BY THYROID CELLS.
J.S. Dallas and S.J. Cunningham
Department of Pediatrics, The University of Texas Medical Branch, Galveston, TX USA

11:00 11. HETEROGENEITY IN EPITOPE(S) FOR THYROID STIMULATING ANTIBODIES (TSAb) FROM GRAVES' SERA: A POSSIBLE LINK TO DIFFERENCES IN RESPONSES TO ANTITHYROID DRUG TREATMENT.
Won Bae Kim, Bo Youn Cho, Hae Young Park, Hong Kyu Lee and Chang-Soon Koh
Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea

11:15 12. IDENTIFICATION OF BINDING SITE OF TSH AND ANTI-TSH RECEPTOR ANTIBODIES IN AUTOIMMUNE THYROID DISEASES : SIGNIFICANCE OF SUGAR CHAINS AND DISULFIDE BONDS OF THE RECEPTOR ON SIGNAL TRANSDUCTION.
T. Yoshida, H. Kameda, K. Arikawa, I. Saito, S. Nagano, Y. Ichikawa, K. Ito and Y. Ikeda
Department of Internal Medicine, School of Medicine, Keio University, Tokyo, St. Marianna University, Kawasaki and Ito Thyroid Clinics and Hospital, Tokyo, Japan

11:30 13. HUMAN IgG AUTOANTIBODIES FROM EPSTEIN BARR VIRUS TRANSFORMED B LYMPHOCYTES OF GRAVES' DISEASE PATIENTS: SPECIFIC ANTIBODY TO THYROTROPIN RECEPTOR AS DETERMINED BY IMMUNOPRECIPITATION ANALYSIS AND BIOLOGICAL ACTIVITY.
W.A. Scherbaum, N.G. Morgenthaler, M.R. Kim, W. Richter, J. Tremble, G.C. Huang and M. Gupta
Medizinische Klinik III, Universitat Leipzig, Leipzig, Germany and Department of Medicine, King's College School of Medicine, London, United Kingdom; The Department of Clinical Pathology, The Cleveland Clinic Foundation, Cleveland, Ohio, USA

11:45 14. PRIMARY HORMONOGENIC SITES AS CONSERVED AUTOEPITOPES IN MURINE AUTOIMMUNE THYROIDITIS: ROLE OF MHC CLASS II.
Q. Wan, R.W. Motte, B.E. Fuller, A.A. Giraldo, D.J. McCormick, C.S. David and Y.M. Kong
Wayne State University School of Medicine and St. John Hospital, Detroit, MI and Mayo Clinic, Rochester, MN USA

MORNING SESSION - Monday, September 11, 1995

CANCER - East Ballroom
CHAIRPERSONS DRS. SCHULMBERGER AND TAKANO

10:30 15. STUDIES ON THE PREVALENCE OF TSH-R MUTATIONS IN A LARGE SERIES OF CONSECUTIVE HYPERFUNCTIONING THYROID ADENOMAS.
A. Porcellini, V. Tassi, I. Ciullo, S. Pannain, G. Amabile, C. Cisternino, A. DiCerbo, E. Papini, V.E. Avvedimento and G.F. Fenzi
Catt. Endocrinologia & Dip. Biol. Pat. Cell. Mol. University Napoli; Division Endocrinologia IRCCS Casa Sollievo Sofferenza, S. Giovanni Rotondo; Division of Endocrinologia Osp. Regina Apostolorum, Albano Laziale, Italy

10:45 16. CYS-618-ARG MUTATION IN EXON 10 OF THE RET PROTO-ONCOGENE IN PATIENTS WITH MEN 2A PHENOTYPE AND HIRSCHSPRUNG'S DISEASE.
Ph. Caron, T. Attié, D. David, J. Amiel, F.L. Brousset, P. Roger and S. Lyonnet
Service d'Endocrinologie, CHU Rangueil, Toulouse et Hôpital Haut-Leveque, Bordeaux, Service de Génétique Médicale, INSERM U-393, Hôpital des Enfants-Malades, Paris, France

11:00 17. INCIDENCE OF RET PROTO-ONCOGENE MUTATIONS IN SPORADIC MTC.
N. Wohllk, G.J. Cote, M.M.J. Bugalho, D. Evans, H. Goepfert, S. Khorana, P. Schultz, N. Ordonez and R.F. Gagel
IEMA and Hosp del Salvador (U de Chile) Santiago, Chile and Sect of Endocrinol, Depts. of Surgery & Pathology, UT M.D. Anderson Cancer Center, Houston, TX USA

11:15 18. LOCALISATION OF RET PEPTIDE IN THYROID TUMORS.
G.A. Thomas, G.H. Williams, H.G. Davies, N. Hooton and E.D. Williams
Department of Histopathology, University of Cambridge, Cambridge, United Kingdom

11:30 19. RESTORATION OF P53 FUNCTION MODULATES PROLIFERATION AND DIFFERENTIATION OF A HUMAN ANAPLASTIC CARCINOMA CELL LINE.
F. Moretti, A. Farsetti, S. Soddu, S. Misiti, M. Andreoli, A. Sacchi and A. Pontecorvi
Molecular Oncogenesis Laboratory, 1st Regina Elena, 2nd Chair of Endocrinology, University Rome "La Sapienza," Institute Experimental Medicine, CNR, Institute of Medical Pathology, Catholic University, Rome, Italy

11:45 20. p53 GENE MUTATIONS IN RADIATION-INDUCED THYROID CANCER.
L. Fogelfeld and T.K. Bauer
Division of Endocrinology, Michael Reese Hospital, University of Illinois, Chicago, IL USA

MONDAY, SEPTEMBER 11TH, 1995 - ADMISSION BY TICKET ONLY
12:00 P.M. TO 1:30 P.M.

MEET THE PROFESSORS LUNCHEONS

Simco/Duffen Room - Thermogenic effects of thyroid hormone
Dr. Antonio Bianco

City Hall Room - Surgical therapy of thyroid cancer
Dr. Orlo Clark & Dr. Shiro Noguchi

Essex Room - Treatment of hot thyroid nodules with ethanol injection
Dr. Enio Martino

WORKSHOPS

Dominion North Ballroom - Spectrum of IDD in 1995
Drs. Boyages, Madeiros-Neto & Pinchera - Moderator, Dr. Dunn

Dominion South Ballroom - Immunologic effects of antithyroid drugs
Drs. Volpe & Weetman

Civic Ballroom - Thyroid hormone and the brain
Drs. Escobar & Nunez - Moderator, Dr. Oppenheimer

11th International Thyroid Congress
Monday, September 11, 1995

AFTERNOON SESSION

SHERATON HALL
THE SHERATON CENTRE - TORONTO, CANADA

1:30 P.M. **POSTER VIEWING**
POSTERS IN PLACE AT 9 AM - REMOVED AT 8 PM

Autoimmunity -1

21. P1 SOLUBLE INTERLEUKIN -1 RECEPTOR INHIBITS INTERLEUKIN-1 INDUCED GLYCOSAMINOGLYCAN PRODUCTION IN CULTURED HUMAN ORBITAL FIBROBLASTS.
G.H. Tan, C.M. Dutton and R.S. Bahn
Division of Endocrinology/Metabolism and Department of Internal Medicine, Mayo Clinic/Foundation, Rochester, MN USA

22. P2 IMMUNODETECTION AND LOCALIZATION OF MANGANESE SUPEROXIDE DISMUTASE (MnSOD) IN RETROOCULAR FIBROBLASTS (ROF) FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY (GO).
H.B. Burch, S.G. Barnes, O. Arseven, D. Sellitti and S. Lahiri
Walter Reed Army Medical Center, Washington, D.C. USA

23. P3 STUDIES OF RETROORBITAL TISSUE XENOGRAFTS FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE: A NEW ANIMAL MODEL.
S. Mori, N. Yoshikawa, T. Tokoro and S. Ikehara
Second Dept. of Internal Medicine, Dept. of Pathology, Kansai Medical University, Moriguchi, Osaka, Japan

24. P4 LEUKOREGULIN INDUCED CHANGES IN CELL GENE EXPRESSION SPECIFIC FOR ORBITAL AND PRETIBIAL FIBROBLASTS DEFINE PHENOTYPIC DIFFERENCES AND NOVEL PROTEIN INDUCTION OF POTENTIAL RELEVANCE TO GRAVES' OPHTHALMOPATHY.
D.A. Young, C.H. Evans and T.J. Smith
University of Rochester School of Medicine, Rochester, N.Y. and Albany Medical College and Veterans' Affairs Medical Center, Albany, NY USA

25. P5 ANTIBODIES AGAINST STRIATED MUSCLE, CONNECTIVE TISSUE AND NUCLEAR ANTIGENS IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY: IS GRAVES' DISEASE A COLLAGEN DISORDER?
J. Kiljanski, A. Barsouk, K. Peele, I. Stachura, J. Pickeral, V. Nebes, J. Kennerdell and J.R. Wall
Thyroid-Eye Disease Center, Allegheny-Singer Research Institute, Pittsburgh, PA USA

26. P6 EFFECTS OF BRADYKININ AND TSH ON PHOSPHOLIPASE C AND A_2 SYSTEMS IN RETROOCULAR FIBROBLASTS OBTAINED FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY.
M.A. Atwa, R.C. Smallridge, I.D. Gist, E. M. Abo-Hashem, M.H. El-Kannishy and K.D. Burman
Washington Hospital Center and Walter Reed Army Institute of Research, Washington, D.C. USA

27. P7 PREVALENCES OF ANTIBODIES REACTIVE WITH PIG EYE MUSCLE MEMBRANE ANTIGENS IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY.
S. Wengrowicz, M. Puig-Domingo, J. Soldevila and A. DeLeiva
Endocrine Research Laboratory, Hospital de la Santa Cruz y San Pablo, Barcelona's Autonomous University, Barcelona, Spain

28. P8 ANTIBODIES CROSS-REACTING WITH THYROGLOBULIN AND ACETYLCHOLINESTERASE IN GRAVES' DISEASE ARE ASSOCIATED WITH OPHTHALMOPATHY.
G. Philippou, D.G. Mappouras, A. Souvatzoglou and P. Lymberi
1st Endocrine Section, "Alexandra" General Hospital and Department of Immunology Hellenic Pasteur Institute, Athens, Greece

POSTER SESSION - Monday, September 11, 1995

29. P9 RETROBULBAR AUTOANTIGENS AND CELLULAR IMMUNITY IN GRAVES' OPHTHALMOPATHY.
G. Kahaly, K. Ochs, C. Hansen, J. Beyer and E. Otto
Third Medical Department, University Hospital, Mainz, Germany

30. P10 INCREASED SUPEROXIDE DISMUTASE ACTIVITY IN LACRIMAL SECRETIONS (TEARS) FROM PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY.
A. Barsouk, B. Fraiture, K.A. Peele, C. Stolarski, M. Hayes, J.S. Kennerdell and J.R. Wall
Thyroid-Eye Disease Center, Allegheny-Singer Research Institute, Pittsburgh, PA USA

31. P11 DEVELOPMENT OF A MOUSE MODEL OF THYROID ASSOCIATED OPHTHALMOPATHY.
V.L. Nebes and S.M. Longinotti
Allegheny-Singer Research Institute, Pittsburgh, PA USA

32. P12 OCCURRENCE AND FEATURES OF OCULAR INVOLVEMENT IN GRAVES' PATIENTS WITH ASSOCIATED MYASTHENIA GRAVIS.
M. Marinò, R. Ricciardi, G. Barbesino, L. Manetti, P. Caturegli, L. Petrone, B. Rossi, A. Muratorio and S. Mariotti
Institute of Endocrinology and Institute of Neurology, University of Pisa and Endocrinology, University of Cagliari, Cagliari, Italy.

33. P13 IMMUNOMODULATORY EFFECT OF NICOTINAMIDE ON CULTURED HUMAN ORBITAL FIBROBLASTS FROM PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY.
Y. Hiromatsu, G. Gen, M. Migita and J. Kamachi
Department of Medicine, Kurume University School of Medicine, Kurume, Japan

Autoimmunity - 2

34. P14 ANTIBODY TO gp39, THE LIGAND FOR CD40 SIGNIFICANTLY INHIBITS HUMORAL RESPONSE FROM GRAVES' THYROID TISSUES XENOGRAFTED INTO SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE.
E. Resetkova, K. Kawai, T. Enamoto, T.M. Foy, R.J. Noel and R. Volpé
Endocrinology Research Laboratory, The Wellesley Hospital, University of Toronto, Toronto, Ontario, Canada; Department of Microbiology, Dartmouth Medical School, Lebanon, NH USA

35. P15 A PATHOGENIC LYMPHOCYTE LINE FROM SPONTANEOUSLY-OCCURRING THYROGLOBULIN-REACTIVE BB/WOR RAT T CELLS IS CYTOTOXIC.
E.M. Allen and J. N. Thupari
University of Maryland Medical Center and Baltimore Veteran's Administration Medical Center, Baltimore, MD USA

36. P16 THYROID TRANSCRIPTION FACTOR-1 (TTF-1) REGULATES MAJOR HISTOCOMPATIBILITY (MHC) CLASS I GENE EXPRESSION IN THYROID CELLS AND IS THYROTROPIN (TSH) CONTROLLED.
M-H. Shong, M. Ohta, S.-I. Taniguchi, Y. Shimura and H. Shimura
NIH, Bethesda, MD USA and Yamanashi School of Medicine, Yamanashi, Japan

37. P17 TSEP-1, A Y-BOX PROTEIN, IS A NEGATIVE REGULATOR OF MAJOR HISTOCOMPATIBILITY (MHC) CLASS I, TSH RECEPTOR (TSHR) AND MHC CLASS II GENES: IT IS A TARGET OF METHIMAZOLE (MMI) ACTION.
M. Ohmori, M.H. Shong, G. Napolitano, D.S. Singer, and L.D. Kohn
NIH, Bethesda, MD USA

38. P18 IODIDE AND TPA CAN DECREASE MAJOR HISTOCOMPATIBILITY (MHC) CLASS I GENE EXPRESSION IN THYROID CELLS VIA NFκB, BUT THEY USE DIFFERENT PHOSPHOLIPASE C (PLC)- ACTIVATED SIGNAL PATHWAYS.
S-I. Taniguchi, V. Montani, C. Giuliani, and M. Saji
NIH, Bethesda, MD and Dept. of Surgery, Johns Hopkins University, Baltimore, MD USA

39. P19 IMMUNOSUPPRESSION OF THYROIDITIS.
V. Guimaraes, J. Quintans, M-E. Fisfalen, and L.J. DeGroot
Thyroid Study Unit and Department of Pathology, The University of Chicago, Chicago, IL USA

POSTER SESSION - Monday, September 11, 1995

40. P20 ANTIBODY-DEPENDENT CELL MEDIATED-CYTOTOXICITY IN AUTOIMMUNE THYROID DISEASE; RELATIONSHIP TO THYROPEROXIDASE ANTIBODIES.
P. Rodien, A.M. Madec, H. Bornet, A. Stefanutti, J. Ruf and J. Orgiazzi
INSERM U197, Faculté de Médecine A Carrel, Lyon Cédex, France and INSERM U38, Faculté de Médecine La Timone, Marseille, France

41. P21 CLONAL T-CELL RESPONSE TO hTSHR-PEPTIDE REVEALED BY RADIO-LABELLED PCR.
Munetoshi Nakashima and Andreas Martin
Department of Medicine, Mount Sinai School of Medicine, New York, NY USA

42. P22 CONGENITAL HYPOTHYROIDISM SCREENING PROGRAMS PROVIDE A SENSITIVE METHOD FOR THE IDENTIFICATION OF POPULATIONS AT RISK FOR ENDEMIC GOITER.
G. Costante, O. Ludovico, M. Nocera, G. Parlato, E. Schifino, M.F. Marasco, U. Crocetti, C. Capula and S. Filetti
Dipartimento di Medicina Sperimentale e Clinica and Istituto di Chimics Clinica - University of Reggio Calabria, Catanzaro, Italy

43. P23 STUDIES OF RE-XENOGRAFTED THYROID TISSUE OF GRAVES' DISEASE (GD) AFTER SOJOURN IN NUDE MICE INTO SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE: THE EFFECT OF ADDING AUTOLOGOUS PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) WITH A SURFEIT OF CD8+ T CELLS.
T. Mukuta, G. Arreaza, E. Resetkova, K. Kawai, T. Enomoto and R. Volpé
Endocrinology Research Laboratory, Wellesley Hospital, University of Toronto, Toronto, Ontario, Canada

44. P24 ACTIVATED AND IFN-γ PRODUCING PERIPHERAL AND THYROID DERIVED T CELLS ARE DETECTED IN GRAVES' DISEASE, THYROID AUTONOMY AS WELL AS IN NON-TOXIC MULTINODULAR GOITER
G. Aust, I. Lehmann, S. Laue and H.-J. Heberling
Department of Internal Medicine III and Institute of Immunology and Transfusion Medicine, University of Leipzig, City Hospital Leipzig, Germany

45. P25 IMPAIRED AUTOLOGOUS MIXED LYMPHOCYTES REACTION IN PATIENTS WITH HYPOTHYROIDISM UNDERGOING HEMODIALYSIS TREATMENT.
T. Tokoro and Y. Ogawa
The 2nd Dept. of Internal Medicine, Kansai Medical University, Moriguchi, Osaka, Japan

46. P26 T-LYMPHOCYTES RESPONSE TO *Yersinia enterocolitica 0:9* IN PATIENTS WITH AUTOIMMUNE THYROID DISEASES (AITD).
I. Zubaschev, E. Gaspar, A. Peters and B.E. Wenzel
Haynal University, Budapest, Cell & Immunobiol. Lab., Dept. Internal Medicine, Medical University Lübeck, Germany

47. P27 CLASS II EXPRESSION AND FUNCTION OF DENDRITIC CELLS FROM THYROID GLAND DRAINING LYMPH NODES OF THE BB RAT.
G.A. Delemarre, P.J. Simons and H.A. Drexhage
Dept. of Immunology, Erasmus University, Rotterdam, The Netherlands

48. P28 EXPRESSION OF HLA-DR, THYROID PEROXIDASE AND THYROGLOBULIN ON CULTURED HUMAN THYROID CELLS.
Å.K. Rasmussen, M.-L. Hartoft-Nielsen, P. Carayon, U. Feldt-Rasmussen and K. Buschard
University Depart. of Endocrinology, Rigshospitalet, Bartholin Institute, Copenhagen, Denmark; U38 INSERM Marseille Medical School, France

49. P29 INTRATHYROIDAL INFILTRATING LYMPHOCYTES (ITL) FROM PATIENTS WITH GRAVES' DISEASE (GD) INDUCE FUNCTIONAL AND HISTOLOGICAL CHANGES IN HUMAN THYROID TRANSPLANTS IN VIVO.
P.M. Schumm-Draeger, K. Rippegather, Z. Abasi, C. Müller, H.J.C. Wenisch, G. Herrmann and K.H. Usadel
Department of Internal Medicine I, Centres of Animal Research Surgery and Pathology, University Clinics Frankfurt/Main, Germany

POSTER SESSION - Monday, September 11, 1995

Cancer - 3

50. P30 EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND P53 GENES IN THYROID CARCINOMA.
Chen Lin, Huai-Yin Shi and Li Rong
General Hospital of PLA, Beijing, China

51. P31 THYROID CARCINOMA IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS - REPORT OF TWO CASES AND REVIEW OF THE LITERATURE.
Kaoru Kobayashi, Hiroyuki Maeta, Yoshimasa Suzuki, Yoshiyuki Tanaka, Tohru Mori and Chiaki Shigemasa
Second Department of Surgery and First Department of Internal Medicine, Tottori University Faculty of Medicine, Yonago, Tottori, Japan

52. P32 COMPARISON OF P53 MUTATION BETWEEN CHINESE AND JAPANESE PATIENTS WITH POORLY DIFFERENTIATED THYROID CARCINOMA.
R.X. Chen, T. Masuda, M. Saito, H. Naganuma, M. Watanabel, J.S. Cui, J.H. Dong and S. Mori
Tohoku University School of Medicine, Sendai, Japan; Norman Bethune University of Medical Sciences, Changchun, China; Akita University School of Medicine, Akita, Japan; Sendai City Hospital, Sendai, Japan

53. P33 THYROGLOBULIN, bcl-2 AND p53 IMMUNOREACTIVTY IN POORLY DIFFERENTIATED CARCINOMA VARIANTS OF THE THYROID GLANDS.
P. Collini, S. Rao, C. Lavarino and F. Rilke
Istituto Nazionale Tumori, Milan, Italy

54. P34 UTILIZATION OF 'COLD'-SSCP SCREENING FOR CLINICAL DIAGNOSTIC LABORATORY MUTATION DETECTION IN THE HUMAN THYROID RECEPTOR-β AND THE p53 TUMOR SUPRESSOR GENES.
M.B. Grace, A.M. Hruszkewycz, R.M. Delgado, W.P. Bennett and G.S. Buzard
MCEB/NIDDK, Clin Path. Dept.Clin. Cntr., DCE/NCI, PRI/DynCorp NCI-FCRDC; National Institutes of Health, Bethesda and Frederick, MD USA

55. P35 CHARACTERIZATION OF THE *ret*/PTC CHIMERIC INTRONS IN VARIOUS HUMAN PAPILLARY THYROID CARCINOMAS.
P.A. Smanik, T.L. Furminger, E.L. Mazzaferri, and S.M. Jhiang
The Ohio State University, Columbus, OH USA

56. P36 THYROID CANCER IN THE UKRAINE POST CHERNOBYL.
T. Bogdanova, M. Bragamik, N.D. Tronko, H.R. Harach, G.A. Thomas and E.D. Williams
Institute of Endocrinology, Kiev, Ukraine and Department of Histopathology, University of Cambridge, Cambridge, United Kingdom

57. P37 THYROID TUMOURS IN PERSONS FROM TWO REGIONS OF RUSSIA AFFECTED BY CHERNOBYL ACCIDENT RADIATION.
M. Bronstein, V. Bogdanov and I. Panteleev
Department of Pathomorphology and Department of Surgery, Endocrine and Science Center, Moscow, Russia

58. P38 EXPERIENCE WITH ORAL IODIZED OIL IN THE REGION OF RUSSIA WITH MILD IODINE DEFICIENCY.
N. Sviridenko, N. Mayorova, B. Mishenko and M. Arbusova
Russian Endocrinology Research Centre, Moscow, Russia

59. P39 MANAGEMENT OF CHILDHOOD THYROID CANCER FOLLOWING THE CHERNOBYL ACCIDENT.
T. Delbot, L. Leenhardt, A. Moutet, D. Leguillouzic, B. Gameiro, M.L. Simonet and A. Aurengo
Department of Nuclear Medicine, Pitié Hôpital, Les Enfants de Tchernobyl, Paris, France

60. P40 THYROID CANCER IN CHILDREN AFTER CHERNOBYL NUCLEAR DISASTER.
Lidio Baschieri, Alessandro Antonelli, Marco Ferdeghini, Baldassare Alberti, Marco Puccini, Giuseppe Boni and Paolo Miccoli
Departments of Internal Medicine, Surgery and Nuclear Medicine, University of Pisa, Pisa, Italy

POSTER SESSION - Monday, September 11, 1995

Cell Biology - 4

61. P41 THE PROLIFERATIVE RESPONSE OF PORCINE THYROID FOLLICULAR CELLS TO EPIDERMAL GROWTH FACTOR REFLECTS THE AUTOCRINE INVOLVEMENT OF TRANSFORMING GROWTH FACTOR -β1 AND INSULIN-LIKE GROWTH FACTOR-I.
S. Bidey, J. Soden, H. Beere and A. Cowin
Endocrine Sciences Research Group and School of Biological Sciences, University of Manchester, Manchester, United Kingdom

62. P42 TSH AND cAMP POSITIVELY CONTROL GI/S AND NEGATIVELY G2/MITOSIS TRANSITIONS OF THE CELL CYCLE IN DOG THYROID PRIMARY CULTURES. CORRELATION WITH THE EXPRESSION AND CELLULAR LOCALIZATION OF CYCLIN A AND CYCLIN-DEPENDENT KINASES (CDKs).
M. Baptist, F . Depoortere, R. Burikhanov and P.P. Roger
I.R.I.B.H.N., Free University of Brussels, Campus Erasme, Brussels, Belgium

63. P43 EFFECT OF EPIDERMAL GROWTH FACTOR ON POLARIZED PROTEIN SECRETION IN FILTER-CULTURED PIG THYROID EPITHELIAL CELLS.
J. Husmark and M. Nilsson
Institute of Anatomy & Cell Biology, Göteborg University, Göteborg, Sweden

64. P44 ANGIOGENESIS IN THE HYPERPLASTIC RAT THYROID: CONTROL BY FIBROBLAST GROWTH FACTOR (FGF)- 2 AND THROMBOSPONDIN (TSP)?
V.A. Patel, D.J. Hill, M.C. Eggo, G.P. Becks, M.C. Sheppard and A. Logan
Departments of Clinical Chemistry and Medicine, University of Birmingham and Department of Medicine, Lawson Research Centre, London, Ontario, Canada

65. P45 SELENIUM DEFICIENCY, DECREASED THYROID EPITHELIAL CELL PROLIFERATION AND THYROID-FIBROSIS. ROLE OF THE INFLAMMATORY CELLS AND CYTOKINES. POSSIBLE INVOLVEMENT OF AN EXCESS OF TGF-β.
B. Contempre, O. Le Moine, J.E. Dumont and J.-F. Denef
IRIBHN and Service of Gastroenterology, Free Univ. of Brussels; and Laboratory of Histology, Catholic University of Louvain, Brussels, Belgium

66. P46 EFFECTS OF GROWTH FACTORS ON RAF-1, MEK AND MAP KINASE ACTIVITY IN PORCINE THYROID CELLS IN CULTURE.
T. Tsushima, H. Murakami, O. Isozaki, H. Demura, K. Shizume and Y. Nozoe
Department of Medicine, Tokyo Women's Medical College, and Institute of Growth Schience (K.H., Y.N.), Tokyo, Japan

67. P47 TRANSFORMING GROWTH FACTOR-β1 (TGF-β1) INHIBITS Na^+/K^+ -ATPase EXPRESSION IN FRTL-5 RAT THYROID CELLS.
A.E. Pekary, S.R. Levin, D. Johnson, L. Berg and J.M. Hershman
Endocrinology and Metabolism Section, West Los Angeles VA Medical Center and UCLA Department of Medicine, Los Angeles, CA USA

68. P48 EGF BUT NOT IODIDE ENHANCES TGFβ PRECURSOR, TGFβ-1mRNA AND TISSUE PLASMINOGEN ACTIVATOR IN PORCINE THYROID FOLLICLES EX VIVO.
G. Bechtner, A. Erdmann, D. Schopohl, M. Rafferzeder, and R. Gärtner
Medizinische Klinik Innenstadt, Universität München, Germany

69. P49 TRANSFORMING GROWTH FACTOR-β_1 (TGF-β_1) IN OVINE THYROID: AUTOCRINE PRESENCE AND EFFECTS ON IODINE METABOLISM AND RELEASE OF INSULIN-LIKE GROWTH FACTOR (IGF) BINDING PROTEINS (BPs).
G.P. Becks, J.-F. Wang and D.J. Hill
The Lawson Research Institute, St. Joseph's Health Centre and University of Western Ontario, London, Canada

POSTER SESSION - Monday, September 11, 1995

Clinical - 5

70. P50 PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM IN PATIENTS WITH DOWN'S SYNDROME AND RELATION TO PSYCHOMOTOR DEVELOPMENT.
F. Baptista, M. Palha, A. Costa, P. Oom, L. Sampaio, C. Freitas, H. Proenca, T. Condeco, L. Cotrim and S. Jorge
Endocrine Unit, Child Development Center , and Clinical Chemistry Lab, Sta. Maria Hospital University of Lisbon, Lisbon, Portugal

71. P51 INCREASED THYROID VOLUME IN CHILDREN LIVING IN AN AREA SUBMITTED TO A RECENT IODINE PROPHYLAXIS: A CLUE TO PAST EXPOSURE TO IODINE DEFICIENCY.
F. Aghini-Lombardi, L. Antonangeli, T. Rago, A.M. Bartolomei and P. Vitti
Istituto di Endocrinologia, University of Pisa, Pisa, Italy and Divisione di Pediatria, Ospedale di San Sepolcro, Italy

72. P52 ULTRASOUND MASS SCREENING OF THE THYROID GLAND IN AN ENDEMIC AREA OF CHINA.
K. Fujimori, F. Takaya, H. Ohtomo, T. Takahashi, Y. Taguchi, D. H. Zhang and X. L. Sun
Tohoku University School of Medicine, Sendai, Japan; The 3rd Teaching Hospital of Norman Bethune University, Changchun, China; The 2nd Jilin Provincial Institute for Endemic Disease Control and Research, Jilin, China.

73. P53 INCIDENCE OF CONGENITAL HYPOTHYROIDISM (CH): AN INDICATOR OF IODINE DEFICIENT AREAS IN ITALY.
M. Sorcini, A. Olivieri, C. Fazzini, E. Medda, M.E.Grandolfo, F. Aghini Lomhardi, P. Balestrazzi, G. Giovannelli and S. Carta
Istituto Superiore di Sanita, Roma, Istituto di Endocrinologia, Universita Pisa, Clinica Pediatrica, Universita, Parma, Italy

74. P54 LONGITUDINAL STUDY OF IODINE DEFICIENCY IN NEWBORNS OF THE REUNIFIED PARTS OF THE CITY OF BERLIN.
A. Grüters, K.P. Liesenkötter, B. Stach, H. Willgerodt and H. Helge
University Childrens Hospital KAVH, Free University Berlin and University Childrens Hospital, Leipzig, Germany

75. P55 EARLIEST PREVENTION OF ENDEMIC GOITER BY IODINE SUPPLEMENTATION DURING PREGNANCY.
K.P. Liesenkötter, H. Helge, W. Göpel, B. Stach and A Grüters
University Childrens Hospital KAVH, Free University Berlin and University Childrens Hospital, Leipzig, Germany

76. P56 THE EFFECT OF IODINE DEFICIENCY ON NEONATAL - MATERNAL THYROID RELATIONSHIP.
R. Rajatanavin, L. Chailurkit, K. Suthivechvorakul, P. Winichakoon, P. Tananchai and S. Srinawat
Faculty of Medicine, Ramathibodi Hospital, Mahidol University and The Ministry of Public Health, Bangkok, Thailand

77. P57 THE PATHOGENESIS OF ENDEMIC GOITRE IN AN AFRICAN POPULATION.
D.F. Smith, C. Darke, A.B. Parkes, A.M. Hetherton and J.H. Lazarus
University College, Dublin, Ireland; University of Wales, College of Medicine, Cardiff and NBTS, Cardiff, United Kingdom

78. P58 THE PREVALENCE OF THYROID DYSFUNCTION IN DIFFERENT GERIATRIC SUBPOPULATIONS FROM A MODERATELY IODINE DEFICIENT REGION. COMPARATIVE CLINICAL AND HORMONAL SCREENING.
O. Dohán, I. Szabolcs, Zs. Kovács, J. Gönczi, T. Kákosky, M. Góth, L. Kovács, G. Szilágyi and F.A. Horster
Haynal Imre University of Health Sciences; Istvan Hospital, Budapest, Hungary; University of Düsseldorf, Germany

79. P59 LONG TERM SEQUELAE OF HEARING IMPAIRMENT IN CONGENITAL HYPOTHROIDISM.
W. Walker and J. Rovet
The Hospital for Sick Children, Toronto, Ontario, Canada

80. P60 BREAD IODIZATION FOR MICRONUTRIENT SUPPLEMENTATION IN IODINE DEFICIENT REGION OF RUSSIA.
G. Gerasimov, A. Nazarov, N. Mayorova, A. Schischkina, M. Arbusova, B. Mischenko and I. Dedov
Russian Endocrinology Research Centre, Moscow, Russia

POSTER SESSION - Monday, September 11, 1995

81. P61 A VARIANT OF ADENOMATOUS GOITER WITH CHARACTERISTIC HISTOLOGY AND POSSIBLY THYROGLOBULIN ABNORMALITY.
S. Yoshida, S. Sakane, N. Kobe and N. Ohsawa
Kuma Hospital, Kobe, and First Department of Internal Medicine, Osaka Medical College, Takatsuki, Japan

82. P62 LOWERED LEARNING POTENTIAL AND ACHIEVEMENT MOTIVATION DUE TO PROLONGED IODINE DEFICIENCY.
B.D. Tiwari, L.B. Tripathi and M.G. Karmarkar
Department of Psychology, Kashi Vidyapith, Varanasi, India; Gorakhpur University, Gorakhpur, India; Mehrauli Institutional Area, New Delhi, India

83. P63 HYPOECHOICITY OF THYROID SONOGRAPHY IN CHILD IN IODINE DEFICIENCY AREA CANNOT BE CONSIDERED AS AN INDEX FOR THYROID AUTOIMMUNITY.
Mihaela Simescu, M. Varciu, I. Podoba, Gabriela Voiculescu, Emilia Nicolaescu and Mariana Sava
Institute of Endocrinology, Bucharest, District Hospital, Brasov, Romania and Cathedra of Internal Medicine and Endocrinology, Bratislava, Romania

84. P64 EFFECT OF SMALL DOSES OF IODINE ON THYROID FUNCTION IN PATIENTS WITH HASHIMOTO'S THYROIDITIS RESIDING IN AN IODINE DEFICENT AREA.
W. Reinhardt, M. Luster, K.H. Rudorff, Ch. Heckmann, R. Haase, K. Cissewski and D. Reinwein
Medical Clinic, Division of Endocrinology, University of Essen, Essen; Endocrine Outpatient Clinic, Wuppertal, Germany

85. P65 THYROXINE DETERMINES INSULIN-LIKE GROWTH FACTORS AND IGF-BINDING PROTEIN-3 LEVELS IN PREPUBERTAL BUT NOT PUBERTAL MALNOURISHED CHILDREN IN IODINE-DEFICIENT AREAS.
W.M. Wan Nazaimoon, A. Osman, M. Norhazwati and B.A.K. Khalid
Division of Endocrinology, Institute for Medical Research, Kuala Lumpur, Malaysia

Clinical - 6

86. P66 BONE MINERAL DENSITY (BMD) AND PROLONGED THYROID SUPPRESSIVE THERAPY IN PREMENOPAUSAL WOMEN.
F. Matteucci, D. Bacciardi, R. Bonini, G. Ciranna, A. De Biase, M. Grosso, A. Lanzillotta, G. Manca, M. Ravani and R. Bianchi
Nuclear Medicine Center of the University of Pisa, Pisa, Italy

87. P67 NO ASSOCIATION EXISTS BETWEEN EVIDENCE OF THYROID FAILURE AND DEVELOPMENT OF ISCHAEMIC HEART DISEASE (IHD) IN A TWENTY-YEAR FOLLOW-UP STUDY OF A COMMUNITY.
W.M.G. Tunbridge, M.P.J. Vanderpump, J.M. French, D. Appleton, F. Clark, J. Grimley Evans and E.T.Young
Department of Medicine, Newcastle General Hospital, Newcastle upon Tyne, United Kingdom

88. P68 SCINTIGRAPHIC EVALUATION OF HEART FUNCTION IN REST IN UNTREATED HYPOTHYROIDISM AND HYPERTHYROIDISM.
E. Tielens, M. Pillay, C. Storm and A. Berghout
Depts. of Internal Medicine and Cardiology, Zuiderziekenhuis, and Dept. of Nuclear Medicine, Dr. Daniel Den Hoed Kliniek, Rotterdam, The Netherlands

89. P69 PREVENTION OF THYROXINE - INDUCED BONE LOSS IN POSTMENOPAUSAL WOMEN - THE EFFECT OF CALCIUM SUPPLEMENTATION AND INTRANASAL CALCITONIN.
Annie W.C. Kung and S.C. Yeung
Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong

90. P70 L-THYROXINE TREATMENT AND BONE DENSITY: RESULTS OF A LONGITUDINAL STUDY.
C. Marcocci, F. Golia, E. Vignali, G. Bruno-Bossio, A. Picone, S. Puccinelli and R. Bonacci
Istituto di Endocrinologia, Università de Pisa, Pisa, Italy

91. P71 URINARY COLLAGEN CROSS-LINKS EXCRETION CORRELATES BETTER WTH FREE T3 THAN FREE T4 IN PATIENTS WTH UNTREATED HYPERTHYROIDISM.
E.C.J. Amarante, T.S. Kasamatsu, J.G.H. Vieira and R.M.B. Maciel
Division of Endocrinology, Department of Medicine, Escola Paulista de Medicina, Universidade Federal de Sao Paula, Sao Paulo, SP Brazil

POSTER SESSION - Monday, September 11, 1995

92. P72 MECHANISMS OF THE IMPAIRED EFFORT TOLERANCE IN HYPERTHYROIDISM AND EFFICACY OF THERAPY.
G: Mercuro, M. G. Panzuto, A. Poddighe, M. Leo, R. Cabula, L. Petrini and A. Cherchi
Institutes of Cardiology and Endocrinology, University of Cagliari, Sardinia, Italy

93. P73 CARDIAC PERFORMANCE AND EXERCISE TOLERANCE ARE IMPAIRED IN PATIENTS ON TSH SUPPRESSIVE THERAPY WITH LEVOTHYROXINE. BENEFICIAL EFFECT OF ADRENERGIC BETA-BLOCKADE.
L. Saccà, B. Biondi, S. Fazio, A. Cuocolo, D. Sabatini, M. Salvatore and G. Lombardi
Internal Medicine, Endocrinology, and Nuclear Medicine, Federico II University, Naples, Italy

94. P74 EVIDENCE FOR A LONG-STANDING CALCIUM DEFICIENCY AFTER TREATMENT FOR HYPERTHYROIDISM.
E. Nyström, K. Stenlöf, L. Sjöström, L.-E. Tisell, P.-A. Lundberg, A. Michanek, G. Berg and G. Lindstedt
The Thyroid Unit, Depts. of Endocrinology, Medicine, Surgery, Oncology and Clinical Chemistry, Sahlgren's University Hospital, Götborg, Sweden

95. P75 IMPROVEMENT OF SKELETAL MUSCLE FUNCTION AND KINANTHROPOMETRIC PROFILE AFTER TREATMENT OF HYPERTHYROIDISM.
M. Vaisman, A.C.L. Nobrega, C.G.S. Araujo, J.D.M. Nunes, F.D. Oliverira, and M.O. Annarumma
Division of Endocrinology and Cardiology, HUCFF, University Fed. Rio de Janeiro, Brazil

96. P76 EFFECTS OF SHORT-TERM THYROXINE TREATMENT ON BONE METABOLISM IN HEALTHY VOLUNTEERS.
W.S. Huang and W.L. Chen
Department of Nuclear Medicine, Tri-Service General Hospital, Taipei, Taiwan and Thyroid Research Laboratory, VA-UC Irvine Medical Center, Long Beach, CA USA

97. P77 BONE DENSITY IN TREATED THYROTOXIC AND HYPOTHYROID WOMEN RECEIVING THYROXINE THERAPY.
W. Evans, F. Ammari, R. Pettit, D. Coleman, S. Sandeman and R. John
Departments of Medicine, Medical Physics, Medical Biochemistry, University of Wales College of Medicine, Cardiff, United Kingdom

98. P78 LEFT VENTRICULAR CONTRACTILITY MONITORING IN HYPERTHYROIDISM DURING THE TREATMENT.
G. Kotova and F. Burumkulova
Russian Endocrinology Research Center, Moscow, Russia

99. P79 EVIDENCE FOR AN EFFECT OF PRIOR HYPERTHYROIDISM ON THE PATTERN OF BONE MINERAL DENSITY AS ASSESSED BY INDICES OF TRABECULAR AND CORTICAL BONE.
J. C. Krakauer and M. Kochan
Henry Ford Hospital Medical Center, West Bloomfield, MI and Osteoporosis Testing Centers, Inc., Southfield, MI USA

100. P80 BONE TURNOVER IN PATIENTS WITH THYROID DYSFUNCTION.
Birgitta Norstedt-Wikner, Per Bjellerup, Anders Kallner and O. Tørring
Department of Clinical Chemistry and Molecular Medicine, Karolinska Hospital, Stockholm, Sweden

Thyroid Hormone Action - 7

101. P81 TRANSGENIC MICE BEARING HUMAN MUTANT THYROID HORMONE β1 RECEPTOR (TRβ1): A MODEL OF RESISTANCE TO THYROID HORMONE (RTH) ASSOCIATED WITH FAT LOSS AND HYPERACTIVITY.
R. Wong, D.I. Kutler, V.V. Vasilyev, Y.T. Ting, M. Willingham, S.Y. Cheng and B.D. Weintraub
National Institutes of Health, Bethesda, MD and Medical University of South Carolina, SC USA

102. P82 HISTONE ACETYLATION INFLUENCES THYROID HORMONE AND RETINOIC ACID-MEDIATED GENE EXPRESSION.
P. Garcia-Villalba, A. Jimenez-Lara, A.I. Castillo and A. Aranda
Instituto de Investigaciones Biomedicas, CSIC, Madrid, Spain

103. P83 T3 ACTIONS AND T3 RECEPTORS IN THE T3-SENSITIVE MURINE OB 17 PREADIPOCYTE CELL LINE : INTERFERENCES EXERTED BY RETINOIDS AND 1,25-DIHYDROXYVITAMIN D3.
A. Dace, C. Lenoir, C. Martin, J. Bonne, M. Teboul, R. Planells and J. Torresani
INSERM Unité 38, Biochimie Médicale, Fac. Médecine, Marseille Cédex, France

POSTER SESSION - Monday, September 11, 1995

104. P84 THE INHIBITION OF RETINOID X RECEPTOR RESPONSIVENESS BY THYROID HORMONE RECEPTORS REQUIRES HETERODIMERIZATION AND IS ISOFORM-SPECIFIC.
A. O'Donnell
Department of Medicine, State University of New York at Buffalo and VAMC, Buffalo, NY USA

105. P85 PLASMINOGEN ACTIVATOR INHIBITOR TYPE - 1 IS REGULATED BY THYROID HORMONES IN HUMANS.
A. Bennet, F. Brousset, S. Dumoulin, P. Sié and P.J. Caron
Service d'Endocrinologie, CHU Purpan et Rangueil, Service d'Hémostase, CHU Purpan, Toulouse, France

106. P86 RETINOIC ACID MODULATES T_3-RESPONSIVENESS BY ALTERING THE LEVEL OF MAXIMUM STIMULATION IN HepG2 CELLS.
T.C. Crowe, N.M. Loidl, N.L. Cowen and J.W. Barlow
Ewen Downie Metabolic Unit, Alfred Hospital, Melbourne, Australia

107. P87 INTERACTION BETWEEN NUCLEAR PROTEINS AND LIGAND-BINDING POSITIVE MUTANT TRβ IS DIFFERENT FROM THAT OF WILD-TYPE TRβ.
A. Sakurai, H. Kobayashi, M. Katai, Y. Itakura, K. Ichikawa and K. Hashizume
Department of Geriatrics, Endocrinology and Metabolism, Shinshu
University School of Medicine, Matsumoto, Japan

108. P88 EVIDENCE OF TRIIODOTHYRONINE (T3) BINDING TO PURIFIED MICROTUBULE COMPONENTS IN CHICK EMBYRO BRAIN.
A. Giguère, C. Beaudry and D. Bellabarba
Laboratoire d'endocrinologie, Faculté de Médecine, Université de Sherbrooke, Québec, Canada

109. P89 IN VITRO BINDING OF IODOTHYRONINES AND ANALOGS TO THE ANGIOTENSIN RECEPTOR OF BOVINE ADRENAL CORTICAL MICROSOMES.
C. Horst, C. Wolff, T. Chen and H. Rokos
Henning Berlin GmbH, Research, Berlin, Germany

110. P90 NORMALIZATION OF CARDIAC CONTRACTILITY AND MYOSIN HEAVY CHAIN GENE EXPRESSION WITH TRIIODOTHYRONINE DURING CHRONIC ENERGY RESTRICTION.
H.L. Katzeff, S. Powell and K. Ojamaa
North Shore University Hospital, Comell University Medical College, Manhasset, NY USA

111. P91 PROTEIN KINASE C ACTIVATION IS ESSENTIAL FOR POTENTIATION BY THYROXINE OF INTERFERON-γ-INDUCED HLA EXPRESSION.
F.B. Davis, H.R. Thacore, H.-Y. Lin, and P.J. Davis
Division of Molecular and Cellular Medicine, Department of Medicine, Albany Medical College and Stratton Veterans Affairs Medical Center, Albany, NY, and Department of Microbiology, State University of New York School of Medicine and Biomedical Sciences, Buffalo, NY USA

112. P92 METHOXATIN (COENZYME PQQ) IN MITOCHONDRIAL COMPLEX I: A TARGET FOR THYROID HORMONE ACTION.
P.M. Gallop, J. Mah, M.A. Paz, R. Flückiger, P. Martin and P.J. Davis
Children's Hospital, Harvard Medical and Dental Schools, Boston, MA, Ciba-Geigy, Basel, Switzerland and Albany Medical College, Albany, NY USA

113. P93 DISTINCT RESPONSES OF SERUM THYROTROPIN CONCENTRATION TO ADMINISTRATION OF TRIIODOTHYRONINE AND TRIIODOTHYROACETIC ACID IN RESISTANCE TO THYROID HORMONE.
S. Ueda and M. Ito
First Department of Medicine, Osaka Medical College, Takatsuki, Japan

TRH-TSH - 8

114. P94 IMPORTANCE OF AMINO ACID RESIDUES 33-44 OF THE ALPHA SUBUNIT IN THE SECRETION, RECEPTOR BINDING AND BIOACTIVITY OF HUMAN TSH.
M. Grosmann, J.E. Tropea, J.A. Dias, H. Xia, D. Puett, N. Teh and M.W. Szkudlinski
MCEB, NIDDK, National Institutes of Health, Bethesda, MD; Wadsworth Center, Albany, NY and Dept. of Biochemistry, University of Georgia, Athens, GA USA

POSTER SESSION - Monday, September 11, 1995

115. P95 UPTAKE OF TETRAIODOTHYROACETIC ACID (TETRAC) AND ITS EFFECT ON TSH SECRETION IN CULTURED RAT ANTERIOR PITUITARY CELLS.
M.E. Everts, R. Docter, H. van Toor and G. Hennemann
Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands

116. P96 EFFECT OF THYROID HORMONES ON RAT PITUITARY CONTENT OF NEUROMEDIN B (NB) CORRELATED WITH THYROTROPIN (TSH) SECRETION.
T.M. Ortiga-Carvalho and C.C. Pazos-Moura
Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil

117. P97 EFFECT OF GASTRIN-RELEASING PEPTIDE (GRP) AND GRP ANTAGONISTS ON TSH SECRETION FROM RAT ISOLATED PITUITARIES.
C.V. Santos and E.G. Moura
Department of Physiological Sciences, Biology Institute, State University of Rio de Janeiro, R.J. Brazil

118. P98 EFFECTS OF TRH AND DOPAMINERGIC RECEPTOR BLOCKADE ON CIRCULATING TSH BIOACTIVITY IN NORMAL SUBJECTS.
L. Persani, E. Giammona and G. Faglia
Institute of Endocrine Sciences, Ospedale Maggiore IRCCS and Centro Auxologico Italiano IRCCS, University of Milan, Milan, Italy

119. P99 EFFECTS OF METOCLOPRAMIDE ON FASTING-INDUCED TSH SUPPRESSION.
M.H. Samuels, P. Kramer and D. Wilson
Oregon Health Science Univ. Portland, OR and Univ. of Texas HSC at San Antonio, TX USA

120. P100 EFFECTS OF HUMAN RECOMBINANT INTERLEUKIN-1 BETA (IL-1β) ON TSH RELEASE BY CULTURED RAT ANTERIOR PITUITARY CELLS.
F.W.J.S. Wassen, E.P.C.M. Moerings and M.E. Everts
Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands

121. P101 EFFECT OF FEMALE SEX STEROIDS ON RAT THYROTROPIN (TSH) SECRETION IN VITRO.
R.M. Moreira, P.C. Lisboa, F.H. Curty and C.C. Pazos-Moura
Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil

122. P102 ISOSMOLAR ETHANOL OR UREA STIMULATE HYPOPHYSIOTROPIC TRH SECRETION FROM BOTH THE HYPOTHALAMIC PARAVENTRICULAR NUCLEI (PVN) AND MEDIAN EMINENCE (ME).
M. Nikodemova, V. Strbak, S.E. Greer and M.A. Greer
Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia, and Oregon Health Sciences University, Portland, OR USA.

123. P103 POTENTIAL THYROID HORMONE RESPONSE ELEMENTS (TREs) WITHIN THE DNA SEQUENCES OF GLYCOSYLTRANSFERASES: IMPLICATIONS FOR THE HORMONAL MODULATION OF TSH GLYCOSYLATION.
J.A. Magner and J.B. Menke
Section of Endocrinology, Department of Medicine, East Carolina University School of Medicine, Greenville, NC USA

124. P104 TIME-DEPENDENT RECOVERY OF TSH SECRETION AFTER WITHDRAWAL OF LEVOTHYROXINE SUPPRESSIVE THERAPY: ASSESSMENT BY 3RD GENERATION TSH-ASSAY.
L. Duntas, D.K. Nelson, B.M. Grab and K. Hatzimichail
Department of Internal Medicine I, University Clinic of Ulm, Ulm, Germany. The Genesee Hospital, University of Rochester, NY USA; Hellenic Laboratory of Complex Systems, Drama, Greece

125. P105 HEAT STRESS UNCOUPLES CYTOSOLIC CALCIUM AND THYROTROPIN RELEASING HORMONE EFFECTS ON PROLACTIN SYNTHESIS AND SECRETION IN GH_3 CELLS.
R.J. Galloway, J.M. Carrick, I.D. Gist, C.U. Fisher and R.C. Smallridge
Walter Reed Army Institute of Research, Washington D.C. USA

126 P106 A NATURALLY OCCURRING INHIBITOR OF THYROID HORMONE UPTAKE INTO CELL NUCLEI.
M. Lakshmanan and S. Benvenga
Dept. of Medicine, CWRU, Cleveland, OH USA and Dept of Endocrinology, University of Messina, Messina, Italy

AFTERNOON SESSION - Monday, September 11, 1995

2:30 P.M. **POSTER DISCUSSIONS**

Simco/Duffen Room
AUTOIMMUNITY - 1
DISCUSSION LEADER - DR. WEETMAN

City Hall Room
AUTOIMMUNITY - 2
DISCUSSION LEADER - DR. BAKER

Civic Room
CANCER - 3
DISCUSSION LEADER - DR. YAMASHITA

Dominion Ballroom South
CELL BIOLOGY - 4
DISCUSSION LEADER - DR. ENDO

Essex Room
CLINICAL - 5
DISCUSSION LEADER - DR. GAITAN

Dominion Ballroom North
CLINICAL - 6
DISCUSSION LEADER - DR. CULLEN

Windsor East Room
THYROID HORMONE ACTION - 7
DISCUSSION LEADER - DR. ARANDA

Windsor West Room
TRH/TSH - 8
DISCUSSION LEADER - DR. WILBER

3:30 P.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

4:00 P.M. **SYMPOSIA - SIMULTANEOUS SESSIONS**

GRAND BALLROOM WEST & CENTER

"The thyrocyte: cell-cell and cell-matrix interactions"

Dr. Yvonne Munari-Silem
A Carrel Faculté Médecine, Lyon Cédex , France
Dr. Lucio Nitsch
University Napoli, Italy
Dr. Mikael Nilsson
University of Göteborg, Sweden

Chairpersons - Drs. Roger & Rousset

AFTERNOON SESSION - Monday, September 11, 1995

4:00 P.M. GRAND BALLROOM EAST

"Retinoid X receptors in thyroid hormone action"

Dr. Ronald J. Koenig
University of Michigan Medical Center, Ann Arbor, MI USA
Dr. Tomoaki Mitsuhashi
Faculty of Medicine - University of Tokyo, Tokyo , Japan
Dr. Paul M. Yen
Brigham & Women's Hospital, Boston, MA USA

Chairpersons - Drs. S.Y. Cheng & Chin

6:30 P.M to 9:00 P.M. **ONTARIO ART GALLERY RECEPTION**

Admission by ticket only

Buses depart at 6:15 P.M. from the lower level of the Sheraton Centre

11th International Thyroid Congress
Tuesday, September 12, 1995

MORNING SESSION

WEST & CENTRE BALLROOM
THE SHERATON CENTRE - TORONTO, CANADA

7:00 A.M. Kent Room - **AOTA Executive Council Meeting**

Essex Room - **ATA Committee Meetings & Breakfast**

8:00 A.M. **European Thyroid Association Prize Lecture**

9:00 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

CLINICAL - West & Centre Ballroom
CHAIRPERSONS DRS. BURGI AND HASHIZUME

9:00
127 TREATMENT OF EUTHYROID SICK SYNDROME IN PATIENTS UNDERGOING CARDIAC SURGERY.
J.D. Klemperer, M. Gomez, K. Ojamaa, K. Krieger and I. Klein
Department of Cardiothoracic Surgery, New York Hospital-Cornell University Medical College, New York, NY USA

9:15
128. HEART RATE, HEART RATE VARIABILITY, AND ARRHYTHMIAS IN PATIENTS RENDERED SUBCLINICALLY HYPERTHYROID.
F.S. Keck, S. Wieshammer, G. Grossmann, A.Ch. Schauffelen and Ch.F. Wolf
University of Ulm, Germany

9:30
129. DETERMINATION OF SERUM IL-6 CONCENTRATION IS USEFUL TO DIRECT THE CHOICE OF TREATMENT OF AMIODARONE-INDUCED THYROTOXICOSIS (AIT).
E. Martino, S. Brogioni, L. Grasso, L. Manetti, G. Scarcello, A. Burelli, F. Bogazzi and L. Bartalena
Ist. di Endocrinologia, University of Pisa, Pisa, Italy

9:45
130. ORGAN SPECIFIC EFFECTS OF TIRATRICOL (TRIAC).
S.I. Sherman, M.J. Smith, W. Zoghbi, M.D. Ringel, and P.W. Ladenson
U.T.M.D. Anderson Cancer Center, Baylor College of Medicine, and Johns Hopkins University School of Medicine, Houston, TX and Baltimore, MD USA

CELL BIOLOGY - East Ballroom
CHAIRPERSONS DRS. MURATA AND SANTISTEBAN

9:00
131. GENETICALLY TRANSMITTED GOITROUS HYPOTHYROIDISM DUE TO A DEFECT IN THYROGLOBULIN FOLDING.
P. Kim, 0. Kwon and P. Arvan
Beth Israel Hospital, Boston, MA USA

9:15
132. MULTIMERIZATION OF THYROGLOBULIN (TG) BY COVALENT CROSS-LINKING: A UBIQUITOUS PHENOMENON AND A PREREQUISITE FOR THE STORAGE OF TG IN OSMOTICALLY INERT FORM.
U. Berndorfer, Y. Saber and V. Herzog
Institut für Zellbiologie der Universität Bonn, Bonn, Germany.

9:30
133. ANALYSIS OF THYROID PEROXIDASE-CATALYZED IODINATION AND COUPLING MECHANISMS.
Alvin Taurog and Martha L. Dorris
University of Texas Southwestern Medical Center, Dallas, TX USA

MORNING SESSION - Tuesday, September 12, 1995

9:45 CHARACTERIZATION OF THE SODIUM/IODIDE SYMPORTER OF THE THYROID GLAND.
134. Orlie Levy, Ge Dai and Nancy Carrasco
Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY USA

10:00 A.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

10:30 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

CLINICAL - West & Centre Ballroom
CHAIRPERSONS DRS. KIM AND SINGER

10:30 INCIDENCE AND MECHANISMS OF HEARING LOSS IN PATIENTS WITH RESISTANCE TO THYROID
135. HORMONE (RTH+).
F. Brucker-Davis, A.Pikus, D. Ishizawar and M. Skarulis
National Institutes of Health, Bethesda, MD USA

10:45 FAMILIAL RESISTANCE TO THYROID HORMONE (RTH) NOT LINKED TO DEFECTS IN THE THYROID
136. HORMONE RECEPTORS (TR) α OR β GENES.
R.E. Weiss, Y. Hayashi, T. Nagaya, K.J. Petty, Y. Murata, H. Seo, and S. Refetoff
Departments of Medicine and Pediatrics, University of Chicago, Chicago, IL; Institute of Environmental Medicine, Nagoya University, Nagoya, Japan; and Department of Medicine, Southwestern Medical School, Dallas, TX USA

11:00 A STUDY OF SUSCEPTIBILITY GENES OF HLA CLASS II ANTIGEN IN GRAVES' PATIENTS WITH
137. METHIMAZOLE-INDUCED AGRANULOCYTOSIS.
H. Tamai, T. Sudo, S. Matsubayashi, A. Kimura and T. Sasazuki
Department of Psychosomatic Medicine, Faculty of Medicine, and Department of Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

11:15 OCTREOSCAN IN THE EVALUATION OF ACTIVE GRAVES' OPHTHALMOPATHY.
138. R. Moncayo, I Baldissera, R. Metzler, C. Decristoforo, and G. Riccabona
Department of Nuclear Medicine and Ophthalmology, University of Innsbruck, Austria

11:30 FURTHER STUDIES ON THE COURSE OF GRAVES' OPHTHALMOPATHY (GO)
139. FOLLOWING RADIOACTIVE IODINE (RAI) ADMINISTRATION.
L. Bartalena, C. Marcocci, F. Bogazzi, G. Bruno-Bossio, M.L. Tanda, G. Vanni, E. Dell'Unto, E. Martino and A. Pinchera
Istituto di Endocrinologia, University of Pisa, Pisa, Italy

11:45 RECURRENCE OF GRAVES' DISEASE IS INDEPENDENT OF THYROXINE ADMINISTRATION AFTER
140. MEDICAL THERAPY.
A. Doufas, G. Mastorakos, J. Mantzos and D.A. Koutras
Endocrine Unit, Department of Clinical Therapeutics, Athens University, Evgenidion Hospital, Athens, Greece

THYROID AND THE BRAIN - East Ballroom
CHAIRPERSONS DRS. JACKSON AND MORI

10:30 THYROID HORMONE REGULATES THE EXPRESSION OF LAMININ IN THE DEVELOPING RAT
141. CEREBELLUM.
A.P. Farwell and A. Duhord
Molecular Endocrinology Lab, University of Massachusetts Medical School, Worcester, MA USA

10:45 IDENTIFICATION OF RC3/NEUROGRANIN-EXPRESSING, THYROID HORMONE-SENSITIVE NEURONS
142. IN THE RAT FOREBRAIN.
M.A. Iñiguez, B. Morte and J. Bernal
Instituto de Investigaciones Biomedicas, CSIC, Madrid, Spain

11:00 EVIDENCE FOR CIRCADIAN VARIATIONS OF THYROID HORMONE CONCENTRATIONS AND TYPE 11
143. 5'- IODOTHYRONINE DEIODINASE ACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE RAT.
A. Campos-Barros, A. Musa, A. Flechner, C. Hessenius, U. Gaio, M. Eravci, H. Meinhold and A. Baumgartner
Dept. of Nuclear Medicine and Psychiatric Clinic, Klinikum Benjamin Franklin, Free University of Berlin, Germany

MORNING SESSION - Tuesday, September 12, 1995

11:15 144. SUPERAGONISTS OF RECOMBINANT HUMAN TSH PROVIDE A MODEL OF RATIONAL DESIGN OF GLYCOPROTEIN HORMONE ANALOGS: SITE-SPECIFIC BOVINIZATION OF THE ALPHA SUBUNIT INCREASES IN VITRO AND IN VIVO BIOACTIVITY.
M.W. Szkudlinski, N.G. Teh, M. Grossmann, J.E. Tropea, J. East-Palmer, C. DaCosta, N.R. Thotakura and B.D. Weintraub
MCEB, NIDDK, NIH, Bethesda, MD USA

11:30 145. ASPARAGINE OF THE HIGHLY CONSERVED $NP(X)_{2-3}$ Y SEQUENCE IN THE SEVENTH TRANSMEMBRANE DOMAIN OF G PROTEIN COUPLED RECEPTORS IS IMPORTANT FOR BINDING AND AGONIST-INDUCED INTERNALIZATION OF THE THYROTROPIN-RELEASING HORMONE (TRH) RECEPTOR.
J.H. Perlman, E. Geras-Raaka, W. Wang and M.C. Gershengorn
Cornell University Medical College, New York Hospital, New York, NY USA

11:45 146. TRH GENE EXPRESSION IN THE ANTERIOR PITUITARY: EVIDENCE FROM TRANSPLANTATION STUDIES FOR IN VIVO HYPOTHALAMIC INHIBITION.
I.M.D. Jackson, B. Monfils, J. Kile and R.B. Todd
Division of Endocrinology, Brown University, Rhode Island Hospital, Providence, RI USA

TUESDAY, SEPTEMBER 12TH, 1995 - ADMISSION BY TICKET ONLY
12:00 P.M. TO 1:30 P.M.

MEET THE PROFESSORS LUNCHEONS

Simco/Duffen Room - Thyroid hormones and the heart
Dr. Paul Ladenson

City Hall Room - Postpartum thyroid disease
Dr. Nobuyuki Amino

Essex Room - Thyroid hormone transport
Dr. Georg Hennemann

WORKSHOPS

Dominion North Ballroom- Clinical aspects of thyroid hormone resistance
Drs. Beck-Peccoz & Refetoff - Moderator, Dr. Chatterjee

Dominion South Ballroom - Techniques in molecular thyroidology
Drs. Brent & Petty - Moderator, Dr. Jameson

Civic Ballroom - Radiation and the thyroid: Chernobyl update
Drs. Nagataki & Williams - Moderator, Dr. Becker

11th International Thyroid Congress
Tuesday, September 12, 1995

AFTERNOON SESSION

SHERATON HALL
THE SHERATON CENTRE - TORONTO, CANADA

1:30 P.M. **POSTER VIEWING**
POSTERS IN PLACE AT 9 AM - REMOVED AT 8 PM

Autoimmunity - 1

147. P1 CLINICAL SIGNIFICANCE OF MEASUREMENTS OF ANTITHYROGLOBULIN AND ANTIPEROXIDASE ANTIBODIES IN THE DIAGNOSIS OF HASHIMOTO'S THYROIDITIS; COMPARISON WITH HISTOLOGICAL FINDINGS.
K. Kasagi, T. Kousaka, K. Higuchi, S. Sasayama, S. Miyamoto, T. Misaki and J. Konishi
Department of Nuclear Medicine and Clinical Pathology, Kyoto University, Kyoto, Japan

148. P2 CRYSTALLISATION OF THE HUMAN AUTOANTIGEN, THYROID PEROXIDASE FOR STRUCTURAL ANALYSIS.
A. Gardas, M.K. Sohi, B.J. Sutton and A.M. McGregor
Medical Centre Postgraduate Education, Warsaw, Poland, Department of Medicine and The Randall Institute, King's College, London, United Kingdom

149. P3 ESTIMATION OF INDUCTION TO HUMAN ANTI-HUMAN THYROGLOBULIN AUTOANTIBODY PRODUCTION LED BY ANALYSIS OF B CELL EPITOPE.
M. Horimoto, Y. Nakajima, T. Suzuki and S. Mori
Section of Internal Medicine, Kobe City General Hospital, Kobe, and The Second Department of Internal Medicine, Kansai Medical University, Osaka, Japan

150. P4 EFFECTS OF THE TREATMENT OF HYPERTHYROIDISM ON THE LEVELS OF EYE MUSCLE AUTOANTIBODIES IN GRAVES PATIENTS.
A. Boucher, G. Emond, M. Richard, N. Griffiths, H. Beauregard and R. Comtois
Louis-Charles Simard Research Center, Montreal, Canada

151. P5 THYROID PEROXIDASE IS AN AUTOANTIGEN IN NON-OBESE DIABETIC MICE WITH THYROIDITIS.
A.S. Motani,P. Arscott, M.R. Shen, L. Hennessey, M. McInery, and J.R. Baker, Jr.
University of Michigan Medical School, Ann Arbor, MI and University of Toledo, Toledo, OH USA

152. P6 DETECTION OF ANTI-THYROGLOBULIN AUTOANTIBODIES WITH A HIGHLY SENSITIVE IRMA IN HASHIMOTO'S THYROIDITIS AND NON TOXIC NODULAR GOITER.
G. Barbesino, M. Marino, L. Petrone, G. Bendinelli, T. Giacomelli, L. Grasso and L. Chiovato
Institute of Endocrinology, University of Pisa, Pisa, Italy

153. P7 PROBING THE EPITOPE RECOGNITION OF THYROID PEROXIDASE AUTOANTIBODIES IN SERA OF PATIENTS WITH AUTOIMMUNE THYROID DISEASE.
B. Czarnocka, D. Pastuszko, U. Malczewska and A. Gardas
Dept. of Clinical Biochemistry, Med. Ctr. Postgraduate Education, Warsaw, Poland

154. P8 INTERACTIONS OF THYROCYTES ISOLATED FROM TOXIC AND EUTHYROID GOITERS WITH ANTITHYROID AUTOANTIBODIES.
V.I. Kandror, S.I. Krajnova, N. Yu. Sviridenko and E.N. Bazarova
Endocrinology Research Center, Academy of Medical Sciences, Moscow, Russia

155. P9 THYROID ANTIBODY PREVALENCE IN SCHOOLCHILDREN: GEOGRAPHICAL DISTRIBUTION AND RELATIONSHIP TO ENDEMIC GOITER IN THE MEDITERRANEAN ISLAND SARDINIA.
A. Loviselli, M.A. Cambosu, F. Velluzzi, P. Mele, A. Balestrieri and S. Mariotti
Endocrinology, University of Cagliari, Cagliari, Italy

POSTER SESSION - Tuesday, September 12, 1995

156. P10 CLINICAL FEATURES AND THYROID FUNCTION OF AUTOIMMUNE THYROIDITIS WITH ISOLATED ANTIBODIES AGAINST THYROGLOBULIN OR THYROID PEROXIDASE.
N. Kobe, J. Takamatsu, S. Yoshida, Y. Yamano, A. Fukao and K. Hirai
First Department of Medicine, Osaka Medical College, Takatsuki, and The Kuma Hospital, Kobe, Japan

157. P11 THYROID PEROXIDASE (TPO.Ab) AND THYROID ENLARGEMENT IN BREAST CANCER.
P.P.A. Smyth, D.F. Smith, A. Zbar, M.J. Murray, E.W.M. McDermott and N.J. O'Higgins
Endocrine Laboratory, Depts of Medicine & Surgery, University College Dublin; St. Vincent's Hospital, Dublin, Ireland

Thyroid and the Brain - 2

158. P12 DIFFERENTIAL REGULATION OF GENE EXPRESSION IN THE SAME CELL BY THE INTERACTION OF THYROID HORMONES WITH THE THYROID RECEPTOR β1 (TRβ1).
J. Puymirat and J.H. Dussault
Department of Medicine and Molecular Genetics, CHU Laval Research Center, Ste.-Foy, Quebec, Canada

159. P13 ASSESSMENT OF DYSLEXIA IN SUBJECTS WITH RESISTANCE TO THYROID HORMONE.
P. Hauser, F. Brucker-Davis and E. Wiggs
Baltimore VA Medical Center and University of Maryland School of Medicine, Baltimore, MD and National Institutes of Health, Bethesda, MD USA

160. P14 DETECTION OF WIDESPREAD DISTRIBUTION OF β2 THYROID HORMONE RECEPTOR mRNA IN RAT BRAIN USING cRNA in SITU HYBRIDISATION HISTOCHEMISTRY.
M. Li and S.C. Boyages
Department of Endocrinology, Westmead Hospital, Westmead, Sydney, Australia

161. P15 MATERNAL-NEONATAL IODINE DEFICIENCY DECREASES MYELIN BASIC PROTEIN (MBP) IMMUNOREACTIVE STRUCTURES IN THE STRIATUM (STR) WHICH MIGHT AFFECT CORTICAL-SUBCORTICAL PROJECTIONS.
J.R. Martinez-Galan, P. Pedraza, M. Santacana-Altimiras, F. Escobar del Rey, G. Morreale de Escobar and A. Ruiz-Marcus
Cajal Institute: Instituto de Investigaciones Biomédicas, CSIC & UAM, Madrid, Spain

162. P16 EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR m-RNA AND PROTEIN IN HUMAN THYROID NEOPLASIA.
E.Y. Soh, Q.Y. Duh, M.C. Wong, D.M. Young, H.D. Epstein, R.F. Grossman, S.A. Sobhi, A.E. Siperstein and O.H. Clark
Surgery and Pathology Service, UCSF/ Mount Zion Medical Center; Veterans Affairs Medical Center and Department of Surgery and Pathology, University of California, San Francisco, CA USA

163. P17 mRNA EXPRESSION OF RBI 1 - AN IODINE DEFICIENT RAT BRAIN SUBTRACTED CLONE.
R. Kher, N. Chattopadhyay, A. Mandal, J. Virmani, P. Jaiswal, A. Mithal and M.M. Godbole
Sanjay Gandhi PG Institute of Medical Sciences, Lucknow, India; National Botanical Research Institute, Lucknow, India

164. P18 EVIDENCE THAT TYPE III DEIODINASE IN RAT ASTROCYTES IS A SELENOPROTEIN.
F. Courtin, M. Ramaugé, S. Pallud, J.-M. Gavaret, A.-M. Lennon and M. Pierre
INSERM U-96, Le Kremlin-Bicêtre Cedex, France

165. P19 3,3',5 - TRIIODOTHYRONINE EFFECTS ON ASTROCYTE DIFFERENTIATION DURING BRAIN DEVELOPMENT.
F. Lima, A. Trentin, D. Rosenthal, C. Chagas and V. Moura-Neto
Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

166. P20 BATCH TESTING OF NEWBORNS IN EIGHT ASIAN CAPITAL CITIES REVEALS A MARKED SHIFT IN THYROTROPIN LEVELS TO HIGHER LEVELS.
G.F. Maberly, W. May, R. Houston, F. van de Haar, and K. Sullivan.
Rollins School of Public Health, Emory University, Atlanta, Georgia USA

167. P21 PSYCHOEDUCATIONAL OUTCOME IN CHILDREN WITH EARLY-TREATED CONGENITAL HYPOTHYROIDISM.
J. Rovet, R. Ehrlich and D. Altmann
The Hospital for Sick Children, Toronto, Ontario, Canada

POSTER SESSION - Tuesday, September 12, 1995

168. P22 HYPERPROLACTINAEMIA AND PITUITARY LESIONS IN PRIMARY HYPOTHYROIDISM.
T. Tchernova, V. Kandror and N. Goncharov
National Endocrinology Research Centre, Moscow, Russia

169. P23 IODISED OIL IMPROVES BRAIN FUNCTION IN EUTHYROID IODINE DEFICIENT CHILDREN.
P. Mirmiran, A. Sarshar, M. Nafarabadi and M. Kimiagar
Endocrine Research Center, Shaheed Behesthi University of Medical Sciences, Tehran, I.R. Iran

170. P24 INHIBITION OF TYPE II-DEIODINASE BY OXYDATIVE STRESS IN RAT ASTROCYTES.
S. Pallud, M. Ramaugé, A.-M. Lennon, F. Courtin, M. Pierre and J.-M. Gavaret
INSERM U-96, Le Kremlin-Bicêtre Cedex, France

171. P25 CLINICAL NEUROPHYSIOLOGICAL, CT AND MR FINDINGS IN ENDEMIC CRETINISM.
A. Antonelli, S.C. Boyages, J.P. Halpern, F. Falaschi, F. Sartucci, M. Ferdeghini, M. Mascalchi, G. Tognoni, S. Venturi, M. Sturniolo, C. Kotopulos, P. Fallahi, L. Murrio and L. Baschieri
Departments of Internal and Nuclear Medicine, Radiology and Neurology, University of Pisa, Italy; Departments of Endocrinology and Neurology, Westmead Hospital, Westmead, Australia

172. P26 EFFECTS OF ACTIVELY INDUCED THYROGLOUBLIN IMMUNITY IN THE VASCULAR DEPOSTION OF AMYLOID PROTEINS IN THE BRAIN OF RABBITS.
H.T. Blumenthal, B.N. Premachandra, R.G. Naidu and I.K. Williams
VA Medical Center and Washington University, St. Louis, MO USA

173. P27 ENDOGENOUS TRIIODOTHYRONINE (T3) LOCALIZATION IN FIBER TRACTS AND NEURONAL CELL GROUPS OF RAT FOREBRAIN IS REDUCED BY ACUTE ADMINISTRATION OF A SPECIFIC LOCUS COERULEUS (LC) LESIONING AGENT.
J.T. Gordon, C.B. Rozanov and M.B. Dratman
Medical College of Penn. and VA Medical Center, Philadelphia, PA USA

Cancer - 3

174. P28 CHARACTERIZATION OF THYROID HORMONE RECEPTOR (TR) IN HUMAN HEPATOMA CELL LINES AT DIFFERENT STAGES OF DIFFERENTIATION.
K.-H. Lin
Chang Gung College of Medicine and Technology, Kwei-San, Taoyuan, Taiwan, R.O.C.

175. P29 DETECTION OF CD44 VARIANT USING A NEW METHOD TO EXTRACT RNA FROM FINE NEEDLE ASPIRATION BIOPSIES OF THYROID TUMORS.
T. Takano, H. Sumizaki, S. Umena and N. Amino
Department of Laboratory Medicine, Osaka University Medical School, Suita, Osaka, Japan

176. P30 TSH SUPPRESSION IN THE COURSE OF DIFFERENTIATED THYROID CANCER.
C. Jaffiol, J.P. Daurès, P. Pujol, N. Nsakala, J. Guerenova, L. Baldet, D. Vannereau and J. Bringer
Department of Endocrinology and Epidemiology, University Hospital of Montpellier, France

177. P31 GROWTH CHARACTERISTICS OF HUMAN ANAPLASTIC THYROID CARCINOMA CELLS TRANSFECTED WITH A HUMAN THYROTROPIN RECEPTOR cDNA.
N.-E. Heldin, B.Gustavsson, A. Hermansson, A.C. Andersson, L. Grimelius, J. Bergh and B. Westennark
Dept. of Pathology and Oncology, University Hospital, Uppsala, Sweden

178. P32 VISUALIZATION OF NON-MEDULLARY THYROID CANCER BY (In-111-DTPA-D-Phe 1) OCTREOTIDE (OCT) SCINTIGRAPHY (OCTREOSCAN).
P.T.E. Postema, W.W. de Herder, J.C. Reubi, H.Y. Oei, H.J. Bruining and E.P. Krenning
University Hospital Rotterdam, The Netherlands and University of Berne, Switzerland

179. P33 DIPEPTIDYL PEPTIDASE IV (DPP IV / CD26) ACTIVITY STAINING AS SPECIFIC DIAGNOSTIC ASSAY FOR DIFFERENTIATED THYROID CARCINOMA.
T. Tanaka, K. Umeki, I. Yamamoto and S. Ohtaki
Department of Obstetrics & Gynecology, Osaka City University Medical School, Abeno-ku, Osaka, Japan.
Department of Laboratory Medicine, Miyazaki Medical College, Kiyotake, Miyazaki, Japan

POSTER SESSION - Tuesday, September 12, 1995

180. P34 A NOVEL MONOCLONAL ANTIBODY JT-95 SPECIFICALLY REACTS WITH SIALYL FIBRONECTIN OF THYROID CARCINOMA.
H. Takeyama, N. Kimura, T. Hosoya, N. Shinozaki, K. Uchida, A. Yamashita, S. Nagahara, T. Fukushima, N. Shioya, S. Nakano, H. Kurihara, N. Akiba and K. Itsubo
Jikei University School of Medicine and Faculty of Engineering, Soka University, Tokyo, Japan

181. P35 DETERMINATION OF MALIGNANCY IN THYROID NODULES BY FACTOR ANALYSIS OF SPECTRAL AND DYNAMIC STRUCTURES WITH SIMULTANEOUS DUAL-ISOTOPE: THALLIUM-201 AND IODINE -123.
B.O. Helal, F. Frouin, D. Leguillouzic, E. Cosson, M.E. Pueyo, F. Archambaud and R. Di Paola
Nuclear Medicine Bicetre, U66 INSERM Villejuif, France

182. P36 VALUE OF TECHNETIUM (Tc)-99m-HYDROXYMETHYLENE DIPHOSPHONATE (HMDP) BONE SCAN SUPPLEMENTED WITH THALLIUM (T1)-201 SCAN IN DETECTING BONE METASTASES FROM DIFFERENTIATED THYROID CARCINOMA.
M.S. Alam, R. Takeuchi, T. Misaki, S. Miyamoto, Y. Iida, K. Kasagi and J. Konishi
Department of Nuclear Medicine, Kyoto University, Kyoto, Japan

183. P37 THYROID CANCER AND CONCURRENT HYPERTHYROIDISM.
M. Auersperg, M. Hočevar, M. Us-Krašovec and T. Movrin
Institute of Oncology, Ljubljana, Slovenia

184. P38 MIBI-Tc FOR DETECTION OF RECURRENCES IN A PATIENT WITH HURTLE CELL CARCINOMA.
J.L. Wémeau, D. Huglo, S. Coequyt, S. Petit, J.F. Cussac, M. Leconte-Houcke, B. Maes and Ch. Proye
Centre Hospitalier Régional et Universitaire, Lille, France

185. P39 THE USEFULNESS OF THE CONCANAVALIN A-THYROGLOBULIN AFFINITY TEST FOR THE DIAGNOSIS OF THYROID CANCER.
M. Maruyama, R. Kato, A. Sugenoya, S. Kobayashi and Y. Kasuga
Department of 2nd Surgery, Shinshu University School of Medicine, Matsumoto, Japan

Cancer - 4

186. P40 PROGNOSTIC FACTORS IN MEDULLARY THYROID CARCINOMA (MTC): A CLINICOPATHOLOGICAL STUDY ON 53 PATIENTS.
M.E. Dottorini, A. Assi, M. Sironi and G. Sangalli
Nuclear Medicine Department and Pathology Department, General Hospital Busto Arsizio, Italy; Pathology Department General Hospital Legnano, Italy.

187. P41 SUBNORMAL TSH LEVELS MODULATE SERUM THYROGLOBULIN (Tg) IN PATIENTS WITH DIFFERENTIATED THYROID CANCERS (DTC).
C.A. Spencer, C. Wang, R.B. Guttler, S. Fatemi and J.S. LoPresti
Department of Medicine, USC, Los Angeles, CA USA

188. P42 SOMATIC MUTATION OF THE RET PROTO-ONCOGENE IN SPORADIC MEDULLARY THYROID CARCINOMA ARE NOT RESTRICTED TO EXON 16 AND ARE ASSOCIATED WITH TUMOR RECURRENCE.
C. Romei, R. Elisei, I. Ceccherini, E. Molinaro, F. Mancusi, G. Romeo and F. Pacini
Istituto de Endocrinologia, University of Pisa, Pisa, Italy and Genetica Molecolare, Istituto G. Gaslini, Genova, Italy

189. P43 CHARACTERIZATION OF RET ONCOGENIC ACTIVATION IN MeN2 INHERITED CANCER SYNDROMES.
S. Xing, P.A. Smanik, E.L. Mazzaferri and S.M. Jhiang
The Ohio State University, Columbus, OH USA

190. P44 MEDULLARY THYROID CANCER - PROGNOSTIC FACTORS AND THE ROLE OF EXTERNAL BEAM RADIATION THERAPY.
J. Brierley, R. Tsang, W. Simpson, M. Gospodarowicz, S. Sutcliffe and T. Panzarella
Princess Margaret Hospital, Toronto, Ontario, Canada

191. P45 MEDULLARY THYROID CARCINOMA - SPORADIC OR FAMILIAL?
B.G. Robinson, D.J. Marsh, D.L. Learoyd and S. Andrew
Departments of Molecular Genetics and Endocrinology, Royal North Shore Hospital and University of Sydney, Australia

POSTER SESSION - Tuesday, September 12, 1995

192. P46 ^{99m}Tc SESTAMIBI SCANNING IN RECURRENT MEDULLARY THYROID CARCINOMA.
D.L. Learoyd, P. Roach, G. Briggs, L. Delbridge, A. Poole, E. Wilmshurst and B.G. Robinson
Molecular Genetics Unit, Kolling Institute of Medical Research, Departments of Nuclear Medicine, Radiology, Surgery, and Endocrinology, Royal North Shore Hospital and University of Sydney, Australia

193. P47 A NEW SCHEDULE IN THE TREATMENT OF ADVANCED MEDULLARY THYROID CARCINOMA.
G. Lupoli, E. Cascone, F. Arlotta, G. Vitale, V. Nuzzo, E. Rampone and G. Lombardi
Department of Molecular and Clinical Endocrinology and Oncology, "Frederico II" University, Naples, Italy

194. P48 MEDULLARY CARCINOMA OF THE THYROID ASSOCIATED WITH MULTIPLE NON-ENDOCRINE TUMOURS: A MOLECULAR STUDY.
M. Alevizaki, L. Sarika,K. Karaiskos, E. Anastasiou and A. Souvatzoglou
1st Endocrine Section, Alexandra Hospital Department of Medical Therapeutics Athens University, Athens, Greece

195. P49 DISTANT METASTASES IN DIFFERENTIATED THYROID CARCINOMA. MULTIVARIATE ANALYSIS OF PROGNOSTIC VARIABLES.
C. Schvartz, S. Theobald, M.J. Delisle and the Thyroid Cancer Group of Champagne-Ardenne.
Institut Jean Godinot, Reims, France

196. P50 DNA, RNA AND PROTEIN ANALYSES OF MUC1 MUCIN IN BENIGN ADENOMA AND PAPILLARY CARCINOMA OF THE THYROID.
I. Bièche, E. Ruffet, A. Zweibaum, R. Lidereau and B. Franc
Department of Oncogenetic, René Huguenin Center, Saint-Cloud, Department of Pathology, Ambroise Paré Hospital, Boulogne-Billancourt, INSERM U178, Paul Brousse Hospital, Villejuif, France

197. P51 K-RAS AND P53 ONCOGENE MUTATIONS IN THYROID NEOPLASMS.
S. Suzuki, M. Onda, S. Ohki, H. Ami, H. Sakuma, K. Kiman, A. Tsuchiya and R. Abe
The Department of Surgery II, Fukushima Medical College, Fukushima, Japan

Cell Biology - 5

198. P52 IODIDE AND CHLORIDE CHANNEL OF THE THYROID.
A. Yoshida and C. Shigemasa
First Department of Internal Medicine, Tottori University, Yonago, Japan

199. P53 IDENTIFICATION OF ANION EXCHANGER AE2 IN THYROID AND ITS ROLE IN IODIDE LOSS.
A. Fanelli, D. Tombaccini, S.Q. Doi, M.C. Baggio and E.F. Grollman
NIDDK, National Institutes of Health, Bethesda, MD USA

200. P54 AMMONIUM PERSULFATE: AN ALTERNATIVE OXIDIZING REAGENT FOR MEASURING URINARY IODINE.
S. Pino, S.-L. Fang and L.E. Braverman
Division of Endocrinology, University of Massachusetts Medical School, Worcester, MA USA

201. P55 RESORCINOL EFFECT ON IODIDE UPTAKE IN FRTL-5 THYROID CELLS.
E. Gaitan, R.C. Cooksey and J. Legan
Division of Endocrinology, University of Mississippi Medical School and VA Medical Center, Jackson, MS, USA

202. P56 THYROID HORMONOGENESIS IS DIRECTLY MODULATED BY N-GLYCANS AT THE N-TERMINAL DOMAIN OF HUMAN THYROGLOBULIN.
P.J. Lejeune, B. Mallet, N. Baudry, P. Niccoli and P. Carayon
Unité 38 INSERM, Faculté de Médecine, Marseille Cédex, France

203. P57 THYROGLOBULIN ENDOCYTOSIS IS SELECTIVE. BUT DOES IT INVOLVE A SPECIFIC RECEPTOR?
A. Giraud, J. Lanet and J.L. Franc
INSERM U38, Faculté de Médecine, Marseille Cédex, France

204. P58 NEW SPECIFIC INHIBITORS OF Na^+/I^- SYMPORTER, TETRAHYDROPYRIDINE DERIVATIVES, IN CULTURED RAT THYROID (FRTL-5) CELLS.
T. Saito
Third Department of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi, Japan

POSTER SESSION - Tuesday, September 12, 1995

205. P59 ROLE OF GUANYLATE CYCLASE-cGMP IN THE REGULATION OF IODIDE UPTAKE BY CALF THYROID PRIMARY CULTURES.
L.V. Bocanera, L. Krawiec, D. Silberschmidt, O. Pignataro, G.J. Juvenal, L.B. Pregliasco and M.A. Pisarev
Div. Bioquim Nuclear, CNEA; IBYME and CONICET, Buenos Aires, Argentina

206. P60 DISSOCIATION AND REASSOCIATION OF NON-COVALENTLY LINKED DIMERS OF HUMAN 19 S THYROGLOBULIN TREATED WITH UREA.
F. Gentile, B.M. Veneziani and C. Sellitto
Centro di Endocrinologia e Oncologia Sperimentale del Consiglio Nazionale delle Ricerche, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Universita "Federico II," Naples, Italy

207. P61 mRNA'S OF SEVERAL LENGTHS ENCODE THYROGLOBULIN'S C TERMINUS.
M.E. Mason, B. Struyk and J.T. Dunn
University of Virginia, Charlottesville, VA USA

208. P62 THE TRANSIENT EXPRESSION OF LARGE HUMAN THYROGLOBULIN FRAGMENTS IN MAMMALIAN CELLS.
S.A.R. van de Graaf, Erwin Pauws, C. Ris-Stalpers and J. J.M. de Vijlder
Academic Medical Center, Experimental Pediatric Endocrinology,
University of Amsterdam, The Netherlands

209. P63 URSODEOXYCHOLIC ACID (UDCA) INHIBITS IODINE METABOLISM IN PORCINE THYROID CELLS IN PRIMARY CULTURE: IS UDCA A BENEFICIAL ANTITHYROID DRUG?
Y. Takiyama, R. Kanri and I. Makino
The Second Department of Internal Medicine, Asahikawa Medical College, Asahikawa, Japan

210. P64 IODOAMINO ACID DETERMINATION IN THYROGLOBULIN: A RAPID AND SENSITIVE MICROMETHOD.
N. Baudry, R. Koukiekolo, L. Vinet, P.J. Lejeune, J.L. Franc and B. Mallet
Unité 38 INSERM, Faculté de Médecine, Marseille Cédex, France

Clinical - 6

211. P65 MATERNAL "COMPOUND W," A POSSIBLE INDICATOR OF FETAL THYROID FUNCTION DURING MATERNAL HYPERTHYROIDISM ON PTU THERAPY.
L. VanMiddlesworth, S.M. Guerra, B.M. Mercer, S.R. Rose, S. Burstein , and S.Y. Wu
Dept. of Medicine, Dept. Obstetrics & Gynecology, Dept. of Pediatrics, University of Tenn., Memphis, TN; Depts. of Nuclear Medicine and Medicine, VA-University of California, Irvine Medical Center, Long Beach, CA USA

212. P66 GRAVES' DISEASE - HIGH DOSED METHIMAZOLE THERAPY AND LEVOTHYROXINE ADMINISTRATION DO NOT LEAD TO BETTER LONG TIME RESULTS.
W. Meng, Th. Niemeyer, G. Kirsch, S. Krabbe and A. Schindler
Medizinische Universitaetsklinik, Greifswald, Germany

213. P67 ROLE OF THYROID PEROXIDASE IN MINOCYCLINE-INDUCED BLACK PIGMENTATION OF THE THYROID: ANTITHYROID EFFECTS OF MINOCYCLINE.
Martha L. Dorris and Alvin Taurog
University of Texas Southwestern Medical Center, Dallas, TX USA

214. P68 EFFECT OF THE PRESENCE OF ANTITHYROID PEROXIDASE (TPO) ANTIBODY ON THE DEVELOPMENT OF POSTPARTUM THYROID DISEASE (PPT) IN A SUBSEQUENT PREGNANCY.
F. Ammari and C.J. Richards
Department of Medicine, University of Wales College of Medicine, Cardiff; and Department of Obstetrics, Caerphilly Miners District Hospital, Cardiff, United Kingdom

215. P69 METHIMAZOLE TREATMENT OF MATERNAL HYPERTHYROIDISM DURING LACTATION.
F. Azizi
Endocrine Research Center, Shaheed Beheshti University of Medical Sciences, Eveen, Tehran, I.R. Iran

216. P70 ANTITHYROID TREATMENT OF MATERNAL HYPERTHYROIDISM DURING LACTATION.
Y. Abe, H. Sato, H. Sakai, and N. Ooyama
Department of Internal Medicine, School of Medicine, Tokai University, Isehara; Department of Pediatrics, School of Medicine, Kitasato University, Sagamihara, Japan

POSTER SESSION - Tuesday, September 12, 1995

217. P71 EXCESSIVE THYROIDAL STIMULATION IN GEMELLAR PREGNANCY.
J.P. Grün, S. Meuris, P. De Nayer and D. Glinoer
University Hospital St.-Pierre (ULB) and UCL, Brussels, Belgium

218. P72 A RANDOMIZED TRIAL OF ANTITHYROID DRUG TREATMENT IN GRAVES' DISEASE: FOLLOW-UP REPORT FOR UP TO 7 YEARS.
D. Reinwein, A. Rawert, G. Benker and The European Multicenter Study Group
University of Essen, Essen, Germany

219. P73 DETERMINANTS OF THE RESPONSE TO METHIMAZOLE (MMI) IN GRAVES' DISEASE. A STUDY IN 509 PATIENTS.
G. Benker, D. Reinwein, H. Hirche and The European Multicenter Study Group
University of Essen, Essen, Germany

220. P74 ANTITHYROID DRUG TREATMENT OF HYPERTHYROIDISM FOR 10 TO 28 YEARS.
D.W. Slingerland and B.A. Burrows
DVA Medical Center, Boston, MA USA

221. P75 RESPONSE TO METHIMAZOLE IN THYROTOXIC PATIENTS WITH LOW AND OPTIMUM IODINE INTAKE.
F. Azizi
Endocrine Research Center, Shaheed Beheshti University of Medical Sciences, Eveen, Tehran, I.R. Iran

222. P76 POST-ABORTION THYROID DYSFUNCTION: RESULTS OF A PROSPECTIVE STUDY.
A. Stagnaro-Green and A.G. Thomas
Mount Sinai School of Medicine, New York, NY USA

223. P77 FOLLOW-UP OF PATIENTS WITH GRAVES' DISEASE AFTER METHIMAZOLE TREATMENT.
T. Rago, C. Mammoli, S. Pallini, E. Fiore, R. Rocchi, F. Latrofa, P. Agretti and P. Vitti
Istituto di Endocrinologia, University of Pisa, Pisa, Italy

224. P78 PERI-PARTUM THYROID DEFICIENCY.
E.A. Laryea and J.H. Dusseault
McGill University and Universite de Laval, Montreal, Quebec, Canada

Clinical - 7

225. P79 PAINFUL GRAVES' DISEASE: CLINICAL PICTURES AND ITS UNIQUE OUTCOME.
S. Fukata, K. Kuma and M. Sugawara
Kuma Hospital, Kobe, Japan; West Los Angeles, VA Medical Center and UCLA School of Medicine, Los Angeles, CA USA

226. P80 FLUCTUATION OF PANCREATIC BETA-CELL FUNCTION AND PERIODIC PARALYSIS IN PATIENTS WITH GRAVES' DISEASE.
K. Notsu, M. Imaoka, Y. Ito, J. Saito, S. Ohguni and Y. Kato
Departments of Medicine and Neurology, Shimane Prefectural Central Hospital and First Division Department of Medicine, Shimane Medical University, Izumo, Japan

227. P81 PHOSPHOLIPASE C AND CA^{2+} RESPONSES INDUCED BY UNTREATED GRAVES' BUT NOT REMISSION GRAVES' IGGS AND THEIR POTENTIATION BY AN ADENOSINE DERIVATIVE.
A. Kuwabara, F. Okajima, K. Sho, I. Kobayashi and Y. Kondo
Institute for Mol. Cell. Regulation and Dept. Laboratory Medicine, Gunma University, Maebashi, Japan

228. P82 CLINICAL APPLICATION OF COLOR DOPPLER IMAGING TO THE DIFFERENTIAL DIAGNOSIS OF THYROTOXICOSIS.
M. Kitaoka and H. Ishido
Division of Endocrinology and Metabolism, Showa General Hospital, Kodaira, Tokyo, Japan

229. P83 SUCCESSFUL THERAPY RESTORES TO NORMAL THE INCREASED LEVELS OF ENDOTHELIN-1 IN ADULT HYPERTHYROID PATIENTS.
C. Letizia, M. Centanni, R. Cesareo, A. De Ciocchis, S. Cerci, A. Di Gregorio and D. Scavo
I Patologia Medica & Endocrine Section, Dept. of Experimental Medicine, University "La Sapienza," Roma, Italy

POSTER SESSION - Tuesday, September 12, 1995

230. P84 SERUM THYROGLOBULIN CONCENTRATION AS AN INDICATOR OF REMISSION IN ANTI-THYROID DRUG TREATMENT FOR GRAVES' DISEASE.
Y. Murakami, S. Mortia and T. Yokozawa
First Department of Internal Medicine, Osaka Medical College, Takasuki, The Kuma Hospital, Kobe, Japan

231. P85 PHENOTYPE OF FAMILIAL NON AUTOIMMUNE DIFFUSE THYROTOXICOSIS.
J.L. Leclère, C. Schvartz, J. Parma, J. Van Sande, M. Decoulx and J. Orgiazzi
Clinique d'Endocrinologie, Hopital de Brabois, CHU de Nancy, France; Service de Médecine Nucléaire, Institut J. Godinot, CHU de Reims, France; Institut de Recherche Interdisciplinaire, Université de Bruxelles, Belgium, Clinique Médicale, CHU de Lille, France; Service de Médecine Interne, CHU de Lyon Sud, Lyon, France

232. P86 GRAVES' DISEASE AND AUTOIMMUNE FACTOR VIII DEFICIENCY.
R. Sievert and M.I. Surks
Department of Medicine, Division of Endocrinology, Montefiore Medical Center and The Albert Einstein College of Medicine, Bronx, NY USA

233. P87 THE CLINICAL DILEMMAS PRESENTED BY PATIENTS WITH DOWN'S SYNDROME (DS) AND GRAVES' DISEASE (GD)
J.M. Hughes
Mary Imogene Bassett Hospital, Cooperstown, NY USA

234. P88 RELATIONSHIPS BETWEEN PROGNOSIS OF GRAVES' DISEASE AND THYROID RELATED ANTIBODIES.
T. Morita, S. Kubota, S. Murakami, I. Hayaki, K. Tamagawa and A. Oshima
Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan

235. P89 A CASE REPORT OF GRAVES' DISEASE TREATED BY ULTRASOUND GUIDED PERCUTANEOUS ETHANOL INJECTION.
A. Kawauchi, Y. Ban, M. Taniyama, M. Kaname, H. Nagakura, M. Kaihara, K. Kushima, Y. Hashimoto, T. Sawada and M. Kusano
Depts. of Surgery, 3rd Internal Medicine and Clinical Pathology, Showa University School of Medicine, Tokyo, Japan

236. P90 CHRONIC STRESS ASSOCIATED WITH PANIC DISORDER DOES NOT PRECIPITATE GRAVES' HYPERTHYROIDISM.
E. Fiore, G. Perugi, P. Lapi, L. Montanelli, G. B. Cassano and C. Mammoli
Institute of Endocrinology and Institute of Psychiatry, University of Pisa, Pisa, Italy

237. P91 ALTERATIONS IN CORTICOSTEROIDS WITH LOW ALDOSTERONE LEVELS IN GRAVES' DISEASE.
B.A.K. Khalid, M.L. Ng, M.S.L. Lo and H. Kamarulzaman
Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

238. P92 LONG PROGNOSIS OF THYROTOXICOSIS TREATED WITH GLUCOCORTICOID WITH OR WITHOUT THIONAMIDE IN GRAVES' DISEASE.
T. Yagura, K. Sugiyama, T. Yamamoto, T. Yamada and H. Ishii
Tenri Hospital, Tenri, Nara, Japan

Thyroid Hormone Metabolism - 8

239. P93 THE TYPE III 5-DEIODINASE GENE IN *RANA CATESBEIANA* (RC) RESPONDS TO THYROID HORMONE (TH) IN BOTH THE TADPOLE AND THE ADULT FROG.
Kathryn B. Becker and Valerie Anne Galton
Darmouth Medical School, Lebanon, NH USA

240. P94 EFFECT OF CERAMIDE AND PROTEIN KINASE C ON THE REGULATION OF TYPE I 5'- DEIODINASE.
K. Mori, S. Stone, and W.J. DeVito
Division of Endocrinology, University of Massachusetts Medical School, Worcester, MA USA

241. P95 CHARACTERIZATION OF IODOTHYRONINE SULFOTRANSFERASE ACTIVITY IN RAT LIVER.
T.J. Visser and E. Kaptein
Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands

POSTER SESSION - Tuesday, September 12, 1995

242. P96 SELECTIVE MACROPHAGE DEPLETION IN THE LIVER DOES NOT PREVENT THE DEVELOPMENT OF THE LOW T_3 SYNDROME IN THE MOUSE.
A. Boelen, M.C. Platvoet-ter Schiphorst and W.M. Wiersinga
Dept. of Endocrinology, Academic Medical Center, University of Amsterdam, The Netherlands

243. P97 EFFECT OF T_3 ADMINISTRATION ON THE EXPRESSION OF TYPE I 5'-DEIODINASE MESSENGER RNA OF THE LIVER IN STREPTOZOTOSIN INDUCED DIABETIC RATS.
S. Tabata, T. Yonemoto, and M. Horimoto
2nd Department of Internal Medicine, Kansai Medical University, Moriguchi, Osaka, Japan

244. P98 INHIBITION OF IODIDE TRANSPORT AND ORGANIFICATION BY SODIUM NITROPRUSSIDE IN CULTURED BOVINE THYROID CELLS
M.E. Costamagna, A.M. Masini-Repiso, A.M. Cabanillas, C.G. Pellizas, M. Di Fulvio and A.H. Coleoni
Department of Clinical Biochemistry, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina

245. P99 ALTERED THYROID HORMONE METABOLISM IN EUTHYROID PATIENTS WITH COMPLEX VENTRICULAR ARRHYTHMIAS AND ITS NORMALIZATION AFTER CHRONIC AMIODARONE TREATMENT.
G. Iervasi, A. Clerico, A. Pilo, S. Berti, R. Bonini, S. Turchi, C. Manfredi, A. Biagini, R. Bianchi, and L. Donato
CNR Institute of Clinical Physiology, Pisa, Italy

246. P100 2-IODOHEXADECANAL, THE PUTATIVE XI, DERIVES FROM PLASMALOGENS, IS PRESENT IN THE HUMAN THYROID AND BINDS COVALENTLY TO THYROID PROTEINS.
V. Panneels, H. Van Den Bergen, P. Niccoli, J. Van Sande, J.C. Braekman and J.M. Boeynaems
Institute of Interdisciplinary Research, School of Medicine, Université Libre de Bruxelles, Brussels, Belgium

247. P101 ONTOGENY OF THE DIFFERENCES IN THYROID HORMONE LEVELS AMONG OUTBRED RAT STOCKS AND BETWEEN GENDERS.
A.G. Amador, D. Pittman and A. Caruso
Division of Research, Department of Obstetrics & Gynecology, SIU School of Medicine, Springfield, IL USA

248. P102 ^{125} 1-FLAVONOID CROSSES THE PLACENTA AND ACCUMULATES IN FETAL COMPARTMENT IN THE RAT.
J.P. Schroder-van der Elst, D. van der Heide, P.M. Versloot, T. Chen and H. Rokos
Human and Animal Physiology, Agricultural University, Wageningen; Endocrinology, University Hospital; Leiden, The Netherlands, Henning Berlin GmbH, Germany

249. P103 GENDER-SPECIFIC CHANGES IN HEPATIC THYROID HORMONE METABOLISM DURING SHORT-TERM FASTING AND LONG-TERM FOOD RESTRICTION IN RATS.
G.A.C. van Haasteren, E. Linkels, E. Kaptein, W.J. de Greef and T.J. Visser
Departments of Endocrinology and Reproduction, and Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands

250. P104 T_2 AND TRIAC ARE SULFATED IN HEPG2 CELLS, WHEREAS T_3 SULFATION IS ABSENT.
M. de Jong, E.J. Rolleman and H.F. Bernard
Departments of Internal Medicine III and Nuclear Medicine, Erasmus University Hospital, Rotterdam, The Netherlands

251. P105 THYROXINE BINDING GLOBULIN (TBG) INHIBITS TRANSTHYRETIN (TTR) BINDING BY MONOCYTES.
G.C. Schussler and M.G. Yap
State University of New York Health Science Center, Brooklyn, NY USA

252. P106 SERUM IODTHYRONINE CONCENTRATIONS IN INTESTINALLY DECONTAMINATED RATS TREATED WITH TYPE I T4 5'-DEIODINASE INHIBITOR.
I.E. Veronikis, S. Alex, S.L. Fang, G.E. Wright, and C.H. Emerson
University of Massachusetts Medical School, Worcester, MA USA

253. P107 RELATIONSHIP BETWEEN SERUM T_3 AND SERUM INTERLEUKIN-8, INTERLEUKIN-10 OR INTERFERONγ IN PATIENTS WITH NONTHYROIDAL ILLNESS.
M.C. Platvoet-ter Schiphorst and A. Boelen
Department of Endocrinology, Academic Medical Center, University of Amsterdam, The Netherlands

AFTERNOON SESSION - Tuesday, September 12, 1995

2:30 P.M. **POSTER DISCUSSIONS**

Simco/Duffen Room
AUTOIMMUNITY - 1
DISCUSSION LEADER - DR. BURMAN

City Hall Room
THYROID AND THE BRAIN - 2
DISCUSSION LEADER - DR. BERNAL

Civic Room
CANCER - 3
DISCUSSION LEADER - DR. HAY

Dominion Ballroom South
CANCER - 4
DISCUSSION LEADER - DR. SCHNEIDER

Essex Room
CELL BIOLOGY - 5
DISCUSSION LEADER - DR. ARVAN

Dominion Ballroom North
CLINICAL - 6
DISCUSSION LEADER - DR. TOPLISS

Windsor East Room
CLINICAL - 7
DISCUSSION LEADER - DR. COOPER

Windsor West Room
THYROID HORMONE METABOLISM - 8
DISCUSSION LEADER - DR. LARSEN

3:30 P.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

4:00 P.M. **SYMPOSIA - SIMULTANEOUS SESSIONS**

GRAND BALLROOM WEST & CENTER

"Thyroid cell proliferation and oncogenesis"

Dr. Jacques E. Dumont
IRIBHN/Univ. Libre de Bruxelles, Bruxelles, Belgium
Dr. James A. Fagin
University of Cincinnati School of Medicine, Cincinnati, Ohio
Dr A. Fusco
Dipartimento Di Biologia & Patologia, Napoli, Italy

Chairpersons - Drs. Tsushima & Vecchio

AFTERNOON SESSION - Tuesday, September 12, 1995

4:00 P.M. GRAND BALLROOM EAST

"Is thyroid hormone therapy a risk for osteoporosis?"

Dr. Lewis E. Braverman
University of Massachusetts Medical Center, Worcester, MA USA
Dr. Jayne A. Franklyn
University of Birmingham, Birmingham, United Kingdom
Dr. Douglas S. Ross
Massachusetts General Hospital, Boston, MA USA

Chairperson - Dr. Tunbridge

6:30 P.M. to 9:30 P.M. **ONTARIO SCIENCE CENTER RECEPTION**
Admission by ticket only
Buses depart at 6:15 P.M. from the lower level of The Sheraton Centre

11th International Thyroid Congress
Wednesday, September 13, 1995

MORNING SESSION

WEST & CENTRE BALLROOM
THE SHERATON CENTRE - TORONTO, CANADA

7:00 A.M. **American Thyroid Association - Executive Council Meeting**

8:00 A.M. **Latin American Thyroid Society Prize Lecture**

9:00 A.M. **ORAL PRESENTATIONS - PLENARY SESSION**

West & Centre Ballroom
CHAIRPERSONS DRS. BURGER AND EASTMAN

Cancer

9:00 254. TSH RECEPTOR MUTATIONS IN THYROID DIFFERENTIATED CARCINOMAS WITH CONSTITUTIVE ACTIVATION OF ADENYLATE CYCLASE.
D. Russo, F. Arturi, M. Schlumberger, J.A. DuVillard, B. Caillou, R. Monier, H.G. Suarez and S. Filetti
Cattedra di Endocrinologie, Dipartimento di Medicina Sperimentale e Clinica; Cattedra di Farmacologia, Facoltà di Farmacia, Universita di Reggio Calabria, Catanzaro, Italy; Institut de Recherches Scientifiques sur le Cancer, CNRS, Villejuif, and Institut Gustave-Roussy, Villejuif, France

Cell Biology

9:15 255. INTERFERON-γ (IFNγ) SUPPRESSES THYROTROPIN RECEPTOR (TSHR) PROMOTER ACTIVITY BY REDUCING THE DNA BINDING OF THYROID TRANSCRIPTION FACTOR-1 (TTF-1) TO ITS RECOGNITION SITE.
K. Ohe, S. Ikuyama and H. Nawata
Third Department of Internal Medicine, Kyushu University Faculty of Medicine, Fukuoka, Japan

9:30 256. CLONING OF THE cDNA FOR THE THYROID TRANSCRIPTION FACTOR-2.
M. Zannini, K. Sato, M.I. Arnone and R. DiLauro
Stazione Zoologica "Anton Dohm," Naples, Italy

Autoimmunity

9:45 257. ISOLATION OF LYMPHOCYTES PRODUCING HUMAN IgG CLASS MONOCLONAL TSAb AND ANALYSIS OF VARIABLE REGIONS OF THEIR Ig GENES.
T. Akamizu, H. Li, J. Okuda, H. Sugawa and F. Matsuda
Department of Laboratory Medicine, Faculty of Medicine, and The Center for Molecular Biology and Genetics, Kyoto University, Kyoto, Japan

10:00 A.M. **INTERMISSION - COFFEE & EXHIBITS - SHERATON HALL**

EXHIBIT HOURS - WEDNESDAY, SEPTEMBER 13TH
10 AM TO 12 NOON

MORNING SESSION - Wednesday, September 13, 1995

10:30 A.M. **ORAL PRESENTATIONS - PLENARY SESSION**

West & Centre Ballroom
CHAIRPERSONS DRS. ROMALDINI AND SURKS

Clinical

10:30 258. A RANDOMISED OPEN TRIAL OF CARBIMAZOLE ALONE VERSUS CARBIMAZOLE AND THYROXINE IN THE TREATMENT OF GRAVES' DISEASE.
B. McIver, P.H. Rae, G. Beckett, A.E. Gold and A.D. Toft
University Department of Medicine and University Department of Clinical Biochemistry, Royal Infirmary, Edinburgh, United Kingdom

Thyroid Hormone Metabolism

10:45 259. IDENTIFICATION OF A T3 RESPONSIVE ELEMENT IN THE UPSTREAM REGULATORY REGION OF THE HUMAN TYPE I 5'-DEIODINASE GENE.
T. Jakobs, C. Schmutzler and J. Köhrle
Klinische Forschergruppe, Medizinische Poliklinik, University of Würzburg, Würzburg, Germany

Thyroid and the Brain

11:00 260. EXPRESSION OF THE MYELIN BASIC PROTEIN GENE IN DIFFERENTIATING PRIMARY OLIGODENDROCYTES: THE T3RE SEQUENCE (-186 TO -163) IS NECESSARY FOR BOTH T3-DEPENDENT AND T3-INDEPENDENT REGULATION.
K.A. Strait and J.H. Oppenheimer
Dept. of Medicine and Dept. of Cell Biology, University of Minnesota, Minneapolis, MN USA

Thyroid Hormone Action

11:15 261. CROSS-TALK BY MUTANT T_3 β_1 RECEPTORS INTERFERES WITH DUAL HORMONAL SYNERGY: A NEW MOLECULAR MECHANISM OF HORMONE RESISTANCE?
P.G. Walfish, Y.-F. Yang, L.A. Chang, T. Yoganathan, E. Pisano and T.R. Butt
Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, Ontario, Canada; SmithKline Beecham, King of Prussia, PA USA

TRH-TSH

11:30 262. THYROID HORMONE RECEPTOR AND CREB ASSOCIATE BY PROTEIN-PROTEIN INTERACTION.
A. Lin, B. Stevenin and S.L. Lee
Division of Endocrinology, Metabolism, and Molecular Medicine, New England Medical Center Hospital, Tufts University School of Medicine, Boston, MA USA

11:45 **"Historical orgin of anti-thyroid drugs for hyperthyroidism"**
Dr. Clark T. Sawin, Chief, Endocrine-Diabetes Section
V.A. Medical Center, Boston, Massachusetts

12:00 P.M. **ANNUAL BUSINESS MEETINGS**

City Hall Room
AOTA - General Assembly

Dominion North
ATA - Business Meeting

Dominion South
LATS - General Assembly

Kent Room
ETA - Executive Committee

1:00 P.M. Simco/Duffin
ETA - General Assembly

FREE AFTERNOON AND EVENING

11th International Thyroid Congress
Thursday, September 14, 1995

MORNING SESSION

WEST & CENTRE BALLROOM
THE SHERATON CENTRE - TORONTO, CANADA

7:00 A.M. Kent Room - **AOTA Executive Council Meeting**

7:00 A.M. Norfolk Room - **ATA - Blue Ribbon Panel**

8:00 A.M. **Asia Oceania Thyroid Association Prize Lecture**

9:00 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

CLINICAL/CANCER - West & Centre Ballroom
CHAIRPERSONS DRS. PINEDA AND ROBBINS

9:00 263. PAPILLARY THYROID CANCER IN CHILDREN EXPOSED TO CHERNOBYL NUCLEAR ACCIDENT: MOLECULAR ANALYSIS OF TUMOR SPECIMENS.
L. Fugazzola, I. Bongarzone, S. Pilotti, T.V. Vorontsova, P. Collini, L. DeGregorio, S. Rao, L. Astakhova, E.P.Demidchik, M. Prat, F. Pacini, A. Pinchera and M.A. Pierotti
Istituto Nazionale Tumori, Milano; Dipartimento di Scienze Biomediche e Oncologia, Torino; Istituto di Endocrinologia, Pisa, Italy; Institute of Endocrinology, Minsk, Byelorussia

9:15 264. PROGNOSTIC VALUE OF c-MET EXPRESSION IN PAPILLARY THYROID CARCINOMAS.
A. Belfiore, P. Gangemi, M.G. Santonocito, G. L. La Rosa, A. Costantino, A. Fiumara and R. Vigneri
Cattedra di Endocrinologia, University of Catania and Servizio di Anatomia Patologica, V. Emanuele Hospital, Catania, Italy

9:30 265. RELATIONSHIP OF SPECIFIC MUTATIONS OF THE RET PROTO-ONCOGENE TO DISEASE PHENOTYPE IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A.
H.M. Heshmati, H. Gharib, H. Abu-Lebdeh, S. Khosla, N.M. Lindor and S.N. Thibodeau
Divisions of Endocrinology/Metabolism, Medical Genetics, and Laboratory Genetics, Mayo Clinic and Mayo Foundation, Rochester, MN USA

9:45 266. MEDULLARY CARCINOMA OF THE THYROID. A STUDY ON PROGNOSTIC FACTORS IN 117 CONSECUTIVE PATIENTS.
L. Scopsi, G. Sampietro, P. Boracchi, M. Gullo, F. Cusumano and S. Pilotti
Endocrinology Unit, Division of Pathological Anatomy and Division of Surgical Oncology C, Istituto Nazionale Tumori, Milan, Italy; Istituto di Statistica Medica e Biometria, University of Milan, Milan, Italy

THYROID HORMONE ACTION - East Ballroom
CHAIRPERSONS DRS. CHIN AND COLEONI

9:00 267. THE TRANSCRIPTION FACTOR GHF-1, BUT NOT THE SPLICE VARIANT GHF-2, COOPERATES WITH THYROID HORMONE AND RETINOIC ACID RECEPTORS IN RAT GROWTH HORMONE GENE EXPRESSION.
P. Peña, A. Sanchez-Pacheco, T. Palomino and A. Aranda
Instituto de Investigaciones Biomedicas. CSIC, Madrid, Spain

9:15 268. DIRECT PROTEIN INTERACTIONS BETWEEN THYROID HORMONE RECEPTOR ISOFORMS AND GENERAL TRANSCRIPTION FACTORS.
Kevin J. Petty
University of Texas Southwestern Medical Center, Dallas, Texas USA

THE MORNING SESSION - Thursday, September 14, 1995

9:30 269. INTERACTION OF NATURAL AND ARTIFICIAL THYROID HORMONE RECEPTOR MUTANTS WITH A POTENTIAL TRANSCRIPTIONAL CO- ACTIVATOR.
T.N. Collingwood, V. Cavaillès, O. Rajanayagam, M. Adams, C. Matthews, M.G. Parker and V.K.K. Chatterjee
Department of Medicine, University of Cambridge, Adenbrooke's Hospital, Hills Rd, Cambridge, United Kingdom; Molecular Endocrinology Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, England

9:45 270. STUDIES OF THE FUNCTIONS OF DIFFERENT MUTANT T3 RECEPTORS WITH TRUNCATION OF THE EXTREME C-TERMINAL ACTIVATION DOMAIN.
H. Nakamura, Y. Miyoshi, K. Nishiyama, K. Komatsu and K. Nakao
Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; Kyoto University School of Medicine, Sakyoku, Kyoto, Japan

10:00 A.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

10:30 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

CLINICAL - West & Centre Ballroom
CHAIRPERSONS DRS. CAVALIERI AND ROSENTHAL

10:30 271. A LONGITUDINAL STUDY OF THYROID DISORDERS IN THE COMMUNITY.
M.P.J. Vanderpump, W.M.G. Tunbridge, J.M. French, D. Appleton, F. Clark, J. Grimley Evans and E.T. Young
Department of Medicine, Newcastle General Hospital, Newcastle upon Tyne, United Kingdom

10:45 272. IODINE SUPPLEMENTATION MUST BE MONITORED AT THE POPULATION LEVEL IN IODINE DEFICIENT AREAS.
A.M. Ermans, D. Gullo, S.G. Mugisho, M. Tshibangu, R. Tonglet, P. Iurato, M.W. Mukalay, E. Mahangaiko and P. Bourdoux
ULB-Cemubac Brussels, University of Catania, Kirotshe Zaire, UCL Woluwe, Belgium

11:00 273. HIGH FREQUENCY OF GOITRE AND SUBCLINICAL HYPERTHYROIDISM IN A LOW IODINE INTAKE AREA VERSUS HIGH FREQUENCY OF SUBCLINICAL HYPOTHYROIDISM IN A HIGH IODINE INTAKE AREA. A COMPARATIVE EPIDEMIOLOGICAL STUDY IN ELDERLY SUBJECTS.
P. Laurberg, A.B. Hreidarsson, K.M. Pedersen, N. Sigfusson and E. Iversen
Department of Endocrinology, Aalborg, Denmark; Department of Medicine Landspitalinn and The Heart Preventive Clinic, Reykjavik, Iceland

11:15 274. RARITY OF ONCONGENIC MUTATIONS IN THE TSH RECEPTOR OF AUTONOMOUSLY FUNCTIONING THYROID NODULES IN JAPAN.
Y. Nagayama, A. Takeshita, N. Ishikawa, T. Yamashita and T.Obara
Nagasaki University School of Medicine, Nagasaki Ito Hospital, Tokyo; Tokyo Women's Medical College, Tokyo, Japan

11:30 275. MULTIPLE CHANGES IN THYROID STATUS IN PATIENTS TREATED WITH RECOMBINANT INTERFERON-α (rIFN-α).
R. Minelli, T. Giuberti, C. Schianchi, S. Marchelli, E. Gardini, M. Salvi, L. Braverman and E. Roti
Thyroid and Infectious Disease Units , University of Parma, Parma, Italy and the Endocrine Division, University of Massachusetts Medical School, Worcester, Massachusetts USA

11:45 276. THYROXINE ADMINISTRATION TO INFANTS OF LESS THAN 30 WEEKS GESTATIONAL AGE DECREASES PLASMA TRIIODOTHYRONINE CONCENTRATIONS; RESULTS OF A RANDOMIZED TRIAL.
AG van Wassenaer, J.H. Kok and T. Vulsma
Academic Medical Center, Amsterdam, The Netherlands

THYROID HORMONE ACTION - East Ballroom
CHAIRPERSONS DRS. MARIASH AND TORRESANI

10:30 277. HETERODIMERIZATION OF THYROID HORMONE RECEPTOR α1 AND ITS VARIANT ERBα2 WITH RETINOID X RECEPTOR.
Y.-Z. Yang and R.J. Koenig
Division of Endocrinology, University of Michigan Medical Center, Ann Arbor, Michigan USA

THE MORNING SESSION - Thursday, September 14, 1995

10:45 278. AMINO ACID CHARGE OF THE NINTH HEPTAD REPEAT OF THE THYROID HORMONE RECEPTOR DICTATES HOMO AND HETERODIMERIZATION ON A NUMBER OF THYROID HORMONE RESPONSE ELEMENTS.
T. Monden, J.D. Safer, A.N. Hollenberg, and F.E. Wondisford
Thyroid Unit, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston MA USA

11:00 279. THE FUNCTION OF RETINOID X RECEPTOR (RXR) ON A NEGATIVE T3 RESPONSE ELEMENT (nTRE).
T. Takeda and T.Nagasawa
Thyroid Study Unit, Department of Medicine, The University of Chicago, Chicago, IL USA

11:15 280. ROLE OF RXRα ON THE STIMULATION OF THE RAT UNCOUPLING PROTEIN GENE BY T_3.
R. Rabelo, C. Reyes, A. Schifman and J.E. Silva
Division of Endocrinology, Jewish General Hospital, Lady Davis Institute, McGill University, Montreal, Ouebec, Canada

11:30 281. MOLECULAR BASIS OF DIFFERENTIAL INTERACTIONS OF HUMAN THYROID HORMONE RECEPTOR ISOFORMS WITH HORMONE RESPONSE ELEMENTS.
X.-G. Zhu, P. McPhie and S.Y. Cheng
Laboratory of Molecular Biology, NCI and Laboratory of Biochemical Pharmacology, NIDDK, National Institutes of Health, Bethesda, Maryland USA

11:45 282. VITAMIN D RECEPTORS (VDRs) HAVE DOMINANT NEGATIVE ACTIVITY ON T3- RECEPTOR (TR) TRANSCRIPTIONAL ACTIVITY.
P.M. Yen, Y. Liu and W.W. Chin
Division of Genetics, Department of Medicine, Brigham & Women's Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts USA

THURSDAY, SEPTEMBER 14TH, 1995 - ADMISSION BY TICKET ONLY
12:00 P.M. TO 1:30 P.M.

MEET THE PROFESSORS LUNCHEONS

Simco/Duffen Room - Thyroid hormones and lipids
Dr. Michael Sheppard

City Hall Room - Thyroid growth factors
Dr. Rui Maciel

Essex Room - TRAbs - when to measure
Dr. Toru Mori & Dr. Jacques Orgiazzi

WORKSHOPS

Dominion North Ballroom - Pregnancy and the thyroid
Drs. Glinoer & Yoshimura - Moderator, Dr. Hershman

Dominion South Ballroom - Treatment of malignant Graves' ophthalmopathy
Drs. Wiersinga & Yokoyama - Moderator, Dr. Gorman

Civic Ballroom - Non-nuclear actions of thyroid hormone
Drs. Davis & Klein - Moderator, Dr. DeGroot

11th International Thyroid Congress
Thursday , September 14, 1995

AFTERNOON SESSION

SHERATON HALL
THE SHERATON CENTRE - TORONTO, CANADA

1:30 P.M. **POSTER VIEWING**
POSTERS IN PLACE AT 9 AM - REMOVED AT 8 PM

Autoimmunity - 1

283. P1 ALPHA-INTERFERON (IFN) THERAPY AND AUTOIMMUNE THYROID DISEASE.
K. Tsuboi, H. Oshima, R. Yuasa, T. Nagayama, F. Ihara, S. Otsuka and Y. Miyachi
Toho University School of Medicine, Tokyo, Japan

284. P2 GENETICS OF GRAVES' DISEASE: LINKAGE TO HLA AND IMMUNOGLOBULIN (Gm and Km) ALLOTYPES IN PATIENTS AND THEIR FAMILIES.
S. Ratanachaiyavong, D.C. Shields and A.M. McGregor
Department of Medicine, Songkla University, Songkla, Thailand; Department of Genetics, Trinity College, Dublin, Ireland; Department of Medicine, King's College School of Medicine, London, United Kingdom

285. P3 EFFECTS OF EXOGENOUS HUMAN INTERFERON (IFN) - γ OR IFN-NEUTRALIZING MONOCLONAL ANTIBODY ON XENOGRAFTED HUMAN GRAVES' THYROID TISSUE IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE.
N. Yoshikawa, S. Ikehara, H. Kumazawa, T. Yamashita and M. Inada
The Second Department of Internal Medicine, Department of Pathology and Otorhinolaryngology, Kansai Medical University, Moriguchi, Osaka, Japan

286. P4 THYROID HORMONE AUTOANTIBODIES AFTER FINE NEEDLE BIOPSY OF THE THYROID.
S. Benvenga, L. Bartolone, S. Squadrito, S. Battiato and F. Trimarchi
Cattedra di Endocrinologia, University of Messina, Messina, Italy

287. P5 LACK OF B7-1/BB1 AND B7-2/B70 EXPRESSION ON THYROCYTES FROM PATIENTS WITH GRAVES' DISEASE: DELIVERY OF THE COSTIMULATORY SIGNALS FROM BYSTANDER PROFESSIONAL ANTIGEN PRESENTING CELLS.
N. Matsuoka, A. Kawakami, M. Tsuboi, H. Kimura, M. Kita and K. Eguchi
The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki City, Nagasaki, Japan

288. P6 REGULATION OF FAS ANTIGEN-MEDIATED APOPTOSIS ON HUMAN THYROCYTES BY THYROID - STIMULATING HORMONE(TSH), INTERLEUKIN-1 (IL-1), AND INTERFERON-γ [IFN-γ).
A. Kawakami, K. Eguchi, N. Matsuoka, M. Tsuboi, M. Kita, H. Kimura and S. Nagataki
The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan

289. P7 ASSOCIATION OF HLA-A ALLELES WITH GRAVES' DISEASE AND HASHIMOTO'S THYROIDITIS: IMPLICATIONS OF NATURALLY PROCESSED MHC-BOUND PEPTIDES.
T. Sudo, H. Tamai, CJ. Savoie, T. Morita and T. Sasazuki
Department of Psychosomatic Medicine (T.S., H.T.,T.M.), Faculty of Medicine, and Department of Genetics, Medical Institute of Bioregulation (T.S., CJ.S.), Kyushu University, Fukuoka, Japan

290. P8 INDUCTION OF THYROID AUTOANTIBODIES IN NATIVE MICE BY IDIOTYPIC MANIPULATION.
Y. Tomer, B. Gilburd, J. Sack, A. Meshorer, C.L. Burek, N.R. Rose and Y. Shoenfeld
Division of Endocrinology, Mount Sinai School of Medicine, New York, NY; Sheba Medical Center, Weizmann Institute of Science, Israel; Department of Immunology, The Johns Hopkins University, Baltimore, Maryland USA

291. P9 UNIQUE HLA CLASS II ASSOCIATION IN JUVENILE GRAVES.
L. Lavard, H. Perrild, B.B. Jacobsen, H.O. Madsen and A. Svejgaard.
Departments of Pediatrics, Internal Medicine and Clinical Immunology, Glostrup, Bispebjerg, Odense University Hospitals and Rigshospitalet, Denmark

POSTER SESSION - Thursday , September 14, 1995

292. P10 IMMUNOGENETIC MARKERS IN MEXICAN PATIENTS WITH GRAVES DISEASE.
V. Gomez, O. González and J. Granados
Hospital Adolfo López Mateos, ISSSTE, Instituto Nacional de la Nutrición Salvador Zubirán, México, D.F. México

293. P11 IMMUNE FUNCTION IN THYROID DISORDERS.
P.D. Kallio and E.D. Murphy
Evanston Hospital, Evanston, Illinois USA

294. P12 HLA CLASS II GENOTYPE ANALYSIS IN THYROPEROXIDASE (TPO) ANTIBODY POSITIVE PATIENTS EXAMINED THE IMMUNE-DOMINANT LESIONS OF TPO BY RECOMBINANT ANTIGEN-BINDING FRAGMENTS [F(ab)].
T. Nishikawa, Y. Shirahige, T. Tominaga, K. Ashizawa, T. Kiriyama and N. Yokoyama
The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagaski, Japan

295. P13 IMMUNOHISTOCHEMICAL DETECTION OF APOPTOSIS IN AUTOIMMUNE THYROID DISORDER.
M. Koga, M. Sato, M. Migita and K. Nonaka
Department of Medicine, Kurume University School of Medicine, Kurume, Japan

296. P14 CD31, CD 62P AND CD62E IDENTIFY A SPECIFIC PATTERN OF ENDOTHELIAL ACTIVATION IN GRAVES' DISEASE (GD).
V.D. Aubert, M.C. Bene, J. Leclère, G.C. Faure.
Clinique Medicale & Endocrinologique and Laboratoire d'Immunologie, Faculté de Médecine & CHU Nancy, France

Autoimmunity - 2

297. P15 NO DOSE RELATED EFFECT OF METHIMAZOLE ON THE INTENSITY OF THE INTRATHYROIDAL AUTOIMMUNE PROCESS IN RELAPSING GRAVES' DISEASE.
R. Paschke and M. Vogg
Service de Génétique Médicale, Université Libre de Bruxelles, Belgium

298. P16 DOES THYROXINE ADMINISTRATION POSPARTUM (PP) REDUCE ANTI TPO ANTIBODY ASSOCIATED DEPRESSION?
J.H. Lazarus, B. Harris, A.B. Parkes, C.J. Richards and R.G. Newcombe
Departments of Medicine, Psychological Medicine, Obstetrics, Medical Computing and Statistics, University of Wales College of Medicine, Cardiff, Wales, United Kingdom

299. P17 THYROID HYPOFUNCTION WITH Ab-TPO MAY BE A FURTHER RISK FACTOR OF PROGRESSION IN HIV INFECTION.
A. Olivieri, E. Medda, M. Sorcini, P. Narciso, M. D'Archivio, S. Baccarini, M.E. Grandolfo and S. Carta
Istituto Superiore di Sanità, Roma and Ospedale L. Spallanzani, Roma, Italy

300. P18 AUTOIMMUNE PRONE BB RATS SHOW A HIGH INTRINSIC THYROCYTE METABOLISM AND ARE CAPABLE TO COPE WITH MILD IODINE DEFICIENCY.
P.J. Simons, P. Mooij and H.A. Drexhage
Department of Immunology, Erasmus University, Rotterdam, The Netherlands

301. P19 THYROID AUTOIMMUNITY ASSOCIATED WITH LIPOPROTEIN(a) INCREASE.
Helga Lotz and Giovanni B. Salabè
Istituto di Medicina Sperimentale, Consiglio Nazionale delle Ricerche, Viale Marx, Roma, Italy

302. P20 THYROID-STIMULATING HORMONE-LIKE ACTIVITY OF THE ANTI-α GALACTOSYL ANTIBODY (ANTI-GAL) IN ENDEMIC GOITER PATIENTS.
G. Medeiros-Neto, ES Umezawa, MJF Martins, MLC Correa, VCC Guimarães, M. Knobel and D. Gianella-Neto
Inst Tropical Med and Thyroid Laboratory, University of São Paulo Medical School, São Paulo, Brazil

303. P21 AMIODARONE INDUCES A DIFFERENT PATTERN OF ULTRASTRUCTURAL CHANGES TO IODINE EXCESS ALONE IN BB/W RAT THYROID.
Vicki Pitsiavas, Mu Li, Peter Smerdely and Steven C. Boyages
Departments of Clinical and Laboratory Endocrinology, Westmead Hospital, Westmead and Department of Aged Care, St. George Hospital, Kogarah, Australia

POSTER SESSION - Thursday , September 14, 1995

304. P22 TISSUE EOSINOPHILIA AND EOSINOPHIL DEGRANULATiON IN RIEDEL'S INVASIVE FIBROUS THYROIDITIS.
A.E. Heufelder, J.R. Goellner, G.J. Gleich and I.D. Hay
Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität, München, Germany, and Departments of Pathology, Immunology, and Internal Medicine, Mayo Clinic/Foundation, Rochester, MN USA

305. P23 GLUCOSE METABOLISM IN PERIPHERAL LYMPHOCYTES IN GRAVES' DISEASE: RELATIONSHIP WITH SERUM THYROID AUTOANTIBODIES.
Marisa C. Werner, João H. Romaldini, Luis F. B. Costa Rosa and Rui Curi
Department Endocrinology HSPE-IAMSPE, The Institute of Biomedical Sciences, University of São Paulo, Brazil

306. P24 FREE RADICALS AND ANTIOXIDANT DEFENSES IN GRAVE'S DISEASE.
M. Abalovich, D. Bequelman, C. Reides, M. Repetto, S. Llesuy, S. Gutierrez and A. Guitelman
Endocrinology Division, Durand Hospital; General and Inorganic Chemistry Division, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

307. P25 EFFECT OF METHIMAZOLE (MMI) ON T CELL RECOGNITION OF HUMAN THYROGLOBULIN (hTg).
H. Sarui, S. Sakata, H. Takuno, I. Matsui and K. Yasuda
The Third Department of Internal Medicine, Gifu University School of Medicine, Gifu, Japan

308. P26 HODGKIN'S DISEASE IS ASSOCIATED WITH INCREASED ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY AGAINST HUMAN ORBITAL MUSCLE CELLS (ADCC).
M.D. Ringel, T. Taylor, C.E. Freter, L. Diehl, R. Howard and K.D. Burman
The Washington Hospital Center, Georgetown University Medical Center, and Walter Reed Army Medical Center, Washington D.C. USA

Cancer - 3

309. P27 IMMUNOHISTOCHEMICAL STUDY OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THYROID DISORDER.
A. Inagaki, K. Iwase, T. Tsujimura, S. Jimbo and K. Miura
Division of Endocrine Surgery, Fujita Health University School of Medicine, Toyoake, Aichi, Japan

310. P28 IN SITU HYBRIDISATION AND IMMUNOHISTOCHEMICAL STUDY OF THYROPEROXIDASE EXPRESSION IN THYROID TUMORS.
C. de Micco, M. Grino, F. Kopp and S. Garcia
Pathology Institute, Faculty of Medicine, INSERM U38, INSERM U297, Marseille, France

311. P29 GROWTH ACTIVITY IN HYPERPLASTIC AND NEOPLASTIC HUMAN THYROIDS AS ESTIMATED BY IMMUNOHISTOCHEMISTY USING ANTIBODY MIB-1.
R. Katoh, C.E. Bray, K. Suzuki, A. Komiyama, A. Hemmi and A. Kawaoi
Department of Pathology, Yamanashi Medical University, Tamaho, Japan

312. P30 THE NATURAL PROTEIN KINASE Cα MUTANT IS PRESENT IN HUMAN THYROID NEOPLASMS.
Prévostel, V. Alvaro, F. de Boisvilliers, A. Martin, C. Jaffiol and D. Joubert
INSERM U401 et Hôpital Lapeyronie, Montpellier Cedex, France

313. P31 IMMUNOHISTOCHEMICAL STUDY OF THROMBOSPONDIN AND ITS RECEPTORS IN NORMAL THYROID AND IN BENIGN AND MALIGNANT THYROID TUMORS.
M. Patey, B. Delemer, D. Claisse, G. Bellon, M. Pluot and B. Haye
Department of Pathology. C.H.U. Robert Debré; Department of Biochemisty. Université Reims Champagne Ardenne; Department of Endocrinology. C.H.U. Maison Blanche, Reims, France

314. P32 ALTERED METABOLISM OF SUPEROXIDE RADICALS IN THYROID CANCER.
DJ Conway, K. Sheahan, J. Jacob, NJ O'Higgins and PPA Smyth
Endocrine Laboratory, Departments of Medicine & Surgery, University College Dublin; St Vincent's Hospital, Dublin, Ireland

315. P33 TYROSINE PHOSPHORYLATION PLAYS AN IMPORTANT ROLE IN IFNγ INDUCED MHC CLASS I AND CLASS II EXPRESSION IN THYROID CANCER.
Y. Suzuki, Y. Tanaka, H. Nakamura, K. Kobayashi and T. Mori
2nd Department of Surgery, Tottori University, Yonago, Japan

POSTER SESSION - Thursday , September 14, 1995

316. P34 SEQUENTIAL CHANGES IN SERUM TPO FOLLOWING RADIOIODINE THERAPY OF PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA.
M. Ozata, H. Bayhan, N. Bingöl, S. Dündar, S. Ilgin, I. Kurt, Z. Beyhan, A. Çorakci and M.A. Gündogan
Departments of Endocrinology and Metabolism and Nuclear Medicine, Gülhane School of Medicine, Etlik- Ankara and Bayindir Medical Center, Ankara, Turkey

317. P35 IMMUNOHISTOCHEMICAL STUDY OF SMALL HEAT SHOCK PROTEIN, HSP28 IN VARIOUS THYROID DISORDERS.
K. Iwase, K. Kato, T. Tsujimura, A. Inagaki, S. Jimbo and K. Miura
Division of Endocrine Surgery, Fujita Health University School of Medicine, Toyoake, Aichi, Japan. Institute for Developmental Research, Aichi Prefecture Colony, Aichi, Japan

318. P36 IODINE THERAPY REDUCES INDUCED CARCINOGENESIS IN MAMMARY GLANDS OF NORMAL AND IODINE DEFICIENT RATS.
B.A. Eskin, C.E. Grotkowski and C. P. Connolly
Medical College of Pennsylvania and Hahnemann University, Philadelphia, Pennsylvania USA

319. P37 EFFICACY OF VITAMIN D3 AND ITS ANALOG, 22-OXA-1,25-DIHYDROXYVITAMIN D3(OCT) ON TSH DEPENDENCY, AND INHIBITION OF PROMOTION IN THE THYROID ANAPALASTIC CARCINOMA CELL LINE,TTA-I.
H. Furukawa, S. Suzuki, K. Kiman, A. Tsuchiya and R. Abe
The Department of Surgery II, Fukushima Medical College, Fukushima, Japan

Cell Biology - 4

320. P38 BOTH THYROID-SPECIFIC AND UBIQUITOUS FACTORS ARE NECESSARY TO INDUCE HORMONAL REGULATION OF RAT THYROPEROXIDASE GENE PROMOTER.
L. Ortiz, P. Aza-Blanc and P. Santisteban
Instituto de Investigaciones Biomédicas (CSIC) Arturo Duperier, Madrid, Spain

321. P39 RELATIONSHIP BETWEEN THE EXPRESSION OF THYROID-SPECIFIC GENES AND THEIR TRANSCRIPTION FACTORS IN HUMAN THYROID TUMOR CELL LINES.
P. Ros, D. Rossi, C. Dominguez and P. Santisteban
Institute de Investigaciones Biomedicas. CSIC. Servicie de Endocrinologia Pediatrica, Hospital Ramon y Cajal, Madrid, Spain

322. P40 THE REASON WHY THE THYROID SECRETES MAINLY THYROXINE
J.J.M. de Vijlder and M.T. den Hartog
Academic Medical Center, University of Amsterdam, Pediatric Endocrinology, Emma Children's Hospital AMC, The Netherlands

323. P41 MENADIONE (VITAMIN K3) IS THE MOST POTENT STIMULATOR OF SUPEROXIDE AND HYDROGEN PEROXIDE IN THE THYROID CELL.
M. Sugawara and K. Wen
West Los Angeles VA Medical Center and University of California School of Medicine, Los Angeles, California USA

324. P42 THYROTROPIN MODULATES THE NADPH OXIDASE ACTIVITY IN PRIMARY CULTURE OF PORCINE THYROID CELLS.
D.P. Carvalho, B. Haye, C. Dupuy, J. Pommier and A. Virion
Unité de Recherche sur la Glande Thyroïde,INSERM U-96, Bicêtre and Université de Reims Champagne Ardenne, UFR Sciences, ERS-CNRS, Reims, France

325. P43 NADPH OXIDASE FROM THYROID PLASMA MEMBRANE: IRREVERSIBLE ATP ACTIVATION AND ATP DEPENDENT Ca^{2+} DESENSITIZATION.
Y. Gorin, C. Dupuy, J. Pommier and A. Virion
Unité de Recherche sur la Glande Thyroïde, INSERM U-96, Bicetre, France

326. P44 ENZYMATIC ACTIVITY OF HUMAN THYROID PEROXIDASE PRODUCED IN INSECT CELLS IN THE PRESENCE OF HEMATIN.
Ji-Lao Fan and G.S. Seetharamaiah
Department of Microbiology/Immunology, University of Texas Medical Branch, Galveston, Texas USA

POSTER SESSION - Thursday , September 14, 1995

327. P45 CONSTITUTIVE cAMP STIMULATION DECREASES MYC/MAX AND AP-I SITE BINDING PROTEIN EXPRESSION IN FRTL-5 THYROID CELLS.
M.A. Zeiger, P. Catureguli, M.A. Levine and M. Saji
Departments of Surgery, Pathology and Medicine, Johns Hopkins University, Baltimore MD USA

328. P46 THYROID PEROXIDASE ACTIVITY IS INHIBITED BY SOME AMINO ACIDS.
D. Rosenthal, D.P. Carvalho, S.M. Coelho and M.A.S. Camacho
Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

329. P47 MUC1 MUCIN EXPRESSION IN PAPILLARY THYROID CARCINOMA: COMPARISON OF MICRO CARCINOMA, MACR0CARCINOMA AND CASES AFTER CHERNOBYL ACCIDENT.
C. Julie, T. Delbot, L. Leenhardt, A.M. Bergemer, B. Helal and B. Franc
Department of Pathology, Ambroise Paré Hospital, Boulogne-Billancourt, Department of Nuclear Medicine, Pitié Hospital, Paris, Department of Nuclear Medicine, Kremlin-Bicêtre Hospital, Le Kremlin-Bicêtre, France

330. P48 EXPRESSION PATTERNS OF ECM/BM COMPONENTS IN NATIVE AND CULTURED HUMAN THYROID TISSUE.
U. Bürgi, M.E. Bürgi, F. Simon, M. Paulsson, A. Sidiropoulos, D. Aeschlimann, C. Glaser, H. Wagner, Ch. Ruchti, H. Gerber and H.J. Peter
Departments of Endocrinology, Visceral Surgery, Biomechanics, Pathology, University Hospital, Bern, Switzerland

331. P49 REGULATION AND METABOLIC ROLE OF PHOSPHOLIPASE D ACTIVITY IN HUMAN THYROID AND CULTURED DOG THYROCYTES.
C. Lejeune, J. Mockel and M. Taton
Institute of Interdisciplinary Research (IRIBHN) and Dept. of Endocrinology, Hospital Erasme, Université Libre de Bruxelles, Brussels, Belgium

Cell Biology - 5

332. P50 SIMILAR CHANGES OF THE THYROCYTE ULTRASTRUCTURE FOLLOWING SMALL INCREASES OF TSH OR IODINE DEFICIENCY: A MODEL FOR GOITROGENESIS.
C. Hoang-Vu, G. Brabant, W. Sierralta, H. Leitolf, A. von zur Mühlen and H. Dralle
Klinik für Allgemeinchirurgie, MLU Halle, Depts Clinical Endocrinology, Medical Hochschule Hannover and Electron Microscopy Laboratory, Max-Planck Institute, F.R. Germany

333. P51 REGULATION OF THE THYROID JUNCTIONAL COMPLEX BY PROTEIN KINASES. DIFFERENT EFFECTS OF PKC-INHIBITORS ON THE FUNCTION AND DISTRIBUTION OF E-CADHERIN.
H. Fagman, L. Rödjer and L.E. Ericson
Institute of Anatomy & Cell Biology, Göteborg University, Göteborg, Sweden

334. P52 THYROID FOLLICLE RECONSTRUCTION: CALCIUM, HEXAMETHYLENE BISACETAMIDE, COMPONENTS OF THE NCTC 109 MEDIUM AND COCULTURES WITH THE ENTEROBACTERIA ESCHERICHIA COLI. NEW DATA IN FAVOUR OF A cAMP-INDEPENDENT MECHANISM.
G. Fayet, S. Hovsépian, T. Fazekas and A. Aouani
Laboratoire de Biochimie Médicale, Unité INSERM 38, Marseille, France

335. P53 TSH-REGULATED EXPRESSION, GLYCOSYLATION AND SECRETION OF THE ALZHEIMER PRECURSORPROTEIN: POSSIBLE IMPLICATION FOR THE PROLIFERATION OF THYROCYTES.
G.M. Popp,. K.S. Graebert, S. Rosentreter, P. Lemansky and V. Herzog
Institut für Zellbiologie der Universität Bonn, Bonn, Germany

336. P54 FOLLICLE FORMING CAT THYROID CELL LINES SYNTHESIZING EXTRACELLULAR MATRIX AND BASAL MEMBRANE COMPONENTS: A NEW TOOL FOR THE STUDY OF THYROIDAL MORPHOGENESIS.
C.Tognella, H.J. Peter, H. Wagner, J. Kaempf, F. Simon, C. Glaser, D.C. Ferguson, M.E. Peterson, H.J. Häuselmann, M.Paulsson. Ch. Ruchti, M.E- Bürgi-Saville, U. Bürgi and H. Gerber
Depts. of Clin. Chemistry, Visceral Surgery, Internal Medicine, Pathology and M.E. Müller-Institute, University Hospital, Bern, Clinic of Surgery, District Hospital, Thun; Dept of Physiol and Pharm., Univ. of Georgia College of Vet. Medicine, Athens, GA, USA; Dept. of Medicine, Animal Medical Center and Center for Res. Animal Resources, Comell University, New York, NY USA

POSTER SESSION - Thursday , September 14, 1995

337. P55 COLLAGEN AND FIBRONECTIN ARE GROWTH PROMOTING FACTORS IN NORMAL AND TUMOR THYROID CELLS.
M. Vitale, M. Illario, A. Casamassima, V. Bassi, C. Sandomenico, V. Molese, GF. Fenzi and G. Rossi
Dipartimento di Biologia e Patologia Cellulare e Molecolare, Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Universita de Napoli, Italy C.E.O.S., C.N.R.

338. P56 SPONTANEOUS EARLY AGING OF THE RAT THYROID CELL LINE FRTL-5.
T. Zimmermann-Belsing, A.K. Rasmussen and U.Feldt-Rasmussen
University Department of Endocrinology, Rigshospitalet, Copenhagen, Denmark

339. P57 THE EFFECT OF INSULIN LIKE GROWTH FACTOR-1 (IGF-1) ON LIVER TRIIODOTHYRONINE (T_3) DEPENDENT ENZYME ACTIVITIES AND NUCLEAR T_3 RECEPTOR NUMBER IN RATS.
C. G. Pellizas, A.H. Coleoni, M.E. Costamagna, M. DiFulvio, A.M. Masini-Repiso.
Department of Clinical Biochemistry, Faculty of Chemical Sciences, National University of Cordoba, Cordoba, Argentina

340. P58 ELECTRON MICROSCOPY FINDINGS ARE SUGGESTIVE OF THYROCYTE APOPTOSIS DURING TSH- SUPPRESSION.
E.T. Kimura and P.A. Abrahamshon
Department of Histology & Embryology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

341. P59 INDUCTION AND MODULATION OF NITRIC OXIDE GENERATION BY CYTOKINES IN CULTURED HUMAN THYROCYTES.
S-I. Shimoda, Y. Hattori, N. Banba, S. Motohashi and K. Kasai
Department of Endocrinology, Internal Medicine, Dokkyo University School of Medicine, Mibu, Tochigi, Japan

342. P60 BASIC FIBROBLAST GROWTH FACTOR (bFGF) EXPRESSION IS MEDIATED BY PROTEIN KINASE C FOLLOWING ACTIVATION BY IONIZING RADIATION IN HUMAN THYROIDS.
T. Hara, H. Namba and S. Yamashita
Nagasaki University School of Medicine, Nagasaki, Japan

343. P61 SERUM THYROID GROWTH-BLOCKING ACTIVITY IN PATIENTS WITH CHRONIC RENAL FAILURE ON HEMODIALYSIS.
M. Nishikawa, A. Shouzu and M.Inada
Second Department of Internal Medicine, Kansai Medical University, Moriguchi, Osaka, Japan

344. P62 EFFECTS OF EPIDERMAL GROWTH FACTOR (EGF) ON THYMIDINE KINASE ACTIVITY IN THE TISSUE OF RAT THYROID LOBES INCUBATED *IN VITRO*.
M. Karbownik, J. Brzeziński, A. Lewiński, H. Modrzejewska and J. Greger
Department of Thyroidology and Department of Endocrine Surgery, Institute of Endocrinology and Department of Biochemistry, Institute of Physiology and Biochemistry, The University School of Medicine at Lódź, Poland

345. P63 HORMONAL REGULATION OF THE LEVEL OF DNA POLYMERASE β mRNA IN HUMAN THYROID OF GRAVES' DISEASE AND THYROID TUMORS.
M. Kotake, Y. Sawai, A. Nakai, R. Masunaga, T. Mano, K. Shimazaki, R. Kato, S. Kato, H. Nakagawa, M. Hukushima, M. Itoh and A. Nagasaka
Dept. of Internal Med. Fujita Health Univ. School of Medicine, Toyoake, Aichi, Japan

Clinical - 6

346. P64 PREVALENCE AND SIGNIFICANCE OF THYROID AUTOANTIBODIES IN PATIENTS WITH CHRONIC HEPATITIS C.
MJ Huang, BY Huang and YF Liaw
Division of Endocrinology and Liver Research Unit, Chang Gung Memorial Hospital and Medical College, Taipei, Taiwan, Republic of China

347. P65 A NEW TYPE OF THYROXINE-BINDING GLOBULIN DEFICIENCY.
Y. Ueta, Y. Mitani, S. Taniguchi, T. Nawada, I. Manabe, A. Ohtahara, Y. Yamamoto and Y. Tanaka
First Department of Internal Medicine, Tottori University, Yonago, Japan

POSTER SESSION - Thursday , September 14, 1995

348. P66 TSH MEASUREMENT IN SCREENING SUBCLINICAL HYPOTHYROIDISM AMONG HEALTHY PERSONS SEEKING ROUTINE CHECKUPS.
F. Akasu, J. Komatsu, M. Oritsu, H. Enomoto, T. Hiyoshi and M. Yoshitsugu
Division of Endocrinology, Japanese Red Cross Medical Center, Tokyo, Japan

349. P67 ADDITION OF PHENYTOIN TO HUMAN SERUM DISPLACES L-THYROXINE (T4) FROM SERUM BINDING PROTEINS.
M.I. Surks and C.R. DeFesi
Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY USA

350. P68 RELATIONSHIPS OF SERUM TSH TO FREE T_4 IN HEALTHY INFANTS AND CHILDREN, HEALTHY PREGNANT WOMEN, AND PATIENTS WITH UNTREATED THYROID DISEASE (PRIMARY AND SECONDARY) COMPARED TO HEALTHY NONPREGNANT ADULTS.
JC Nelson, SJ Clark, RM Nelson, El Carlton, DL Borut, DA Fisher and RB Wilcox
Loma Linda University School of Medicine, Loma Linda CA, and Corning Nichols Institute; San Juan Capistrano, CA USA

351. P69 SUPRESSION OF SERUM TSH CONCENTRATIONS FOLLOWING ORAL SALSALATE (DISALCID) ADMINISTRATION.
Rong Wong, R. Bruce Wilcox, and Jerald C. Nelson
Loma Linda University, School of Medicine, Loma Linda, CA and Corning Nichols Institute, San Juan Capistrano, CA USA

352. P70 CLINICAL COURSE OF YOUNG EUTHYROID STUDENTS WITH THYROID ANTIBODIES.
Y. Maeda, M. Nomaguchi, H. Tanaka and S. Tojo
Health Service Center; The First Department of Internal Medicine; Tojo Hospital, Kagoshima, Japan

353. P71 IODIZATION OF WATER FOR CORRECTING IODINE DEFICIENCY: A SIMPLE AND EFFICIENT TECHNIQUE.
P. Bourdoux, D. Yazipo, L. Fio-Ngaindiro, L. Namboua, J. Ndoyo, L. Barrière-Constantin and E. Pichard
ULB-Cemubac Brussels, Ministry of Health Bangui, Bangui, University of Bamako

354. P72 CLINICAL EVALUATION OF A ONE-STEP, RAPID TSH ASSAY.
J. Ehrenkranz, F. Schletter, J. Stock and K. Usiskin
Endocrine Section, Morristown Memorial Hospital, Morristown, New Jersey, USA

355. P73 ACQUIRED INAPPROPRIATE THYROTROPIN (TSH) ELEVATION IN THYROXINE-REPLACED HYPOTHYROID PATIENTS
J. Takamatsu, S. Ueda, N.R. Farid, A. Kobayashi and F. Matsuzuka
First Department of Medicine, Osaka Medical College, Takatsuki; and the Kuma Hospital, Kobe, Japan

356. P74 ELEVATED SERUM LEVELS OF ANTIBODIES AGAINST THYROGLOBULIN (TgAB) AND THYROID PEROXIDASE (TPOAb) IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION: IS IT A RISK FACTOR?
M.M. Biancalana, J.H.Romaldini, C.S. Farah, D.I. Figueiredo, I. Rassi, D. Armaganijan, M. Shibata, M. Sampaio, L.A. Mattos and M.M. Santos
Endocrinology Department, HSPE-IAMSPE and Cardiology Institute, Dante Pazzanezze, São Paulo, Brazil

357. P75 CHRONIC LYMPHOCYTIC LEUKEMIA WITH THYROID INVOLVEMENT.
W. J. Georgitis, N.H. Alex, M.M. Lieberman, and M.J. Johnson
Fitzsimons Army Medical Center, Aurora, Colorado USA

358. P76 GENE ANALYSIS OF COMPLETE THYROXINE-BINDING GLOBULIN DEFICIENCY (TBG-CD) IN A KOREAN MALE.
Youn-Bok Chang, Chang-Ho Back and Se-Chan Wee
Chang's Clinic, Seoul, Korea and Department of Genetic Engineering, Hallym University, Chuncheon, Korea

359. P77 ACUTE PRIMARY HYPERPARATHYROIDISM AND THYROTOXICOSIS: WHEN "STORM" CLOUDS MEET.
G. Tolbert, G. Walker, M. Kenecker, J. Leveque, J.W. Grosse and B. Warner
University of South Alabama College of Medicine, Mobile, Alabama, USA

360. P78 ASSOCIATION BETWEEN THYROID AUTOANTIBODIES AND ANTI-HUMAN T-LYMPHOTROPIC VIRUS TYPE-I (HTLV-I) ANTIBODY IN NAGASAKI, JAPAN.
M. Tsuruta, N. Yokoyama, Y. Shibata, M. Akahoshi, T. Matsuo, M. Tomonaga and M. Izumi
Radiation Effects Research Foundation; The First Department of Internal Medicine; Department of Hematology, Atomic Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan

POSTER SESSION - Thursday , September 14, 1995

Clinical - 7

361. P79 SOMATOSTATIN (S) TREATMENT ASSOCIATED WITH CORTICOTHERAPY (C) AND/OR RADIOTHERAPY (R) IN GRAVES OPHTHALMOPATHY (GO).
M. Simescu, I. Lancranjan, E. Nicolaescu and M. Sava
Institute of Endocrinology, Bucharest, Romania and Dept. of Neuroendocrinology Sandoz Pharm LTD.

362. P80 GRAVES' OPHTHALMOPATHY IN AN INCIDENCE COHORT.
G.B. Bartley, V. Fatourechi, E.F. Kadrmas, S.J. Jacobsen, D.M. Ilstrup, J.A. Garrity and C.A. Gorman
Mayo Clinic, Rochester, Minnesota USA

363. P81 IDENTIFICATION OF PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY WITH EARLY OPTIC NERVE INVOLVEMENT BY THE STUDY OF VISUAL EVOKED POTENTIALS.
M. Salvi, F. Neri, E. Spaggiari, C. Macaluso, E. Gardini, R. Minelli, J.R. Wall and E. Roti
Centro Tireopatie e Istituto di Oftalmologia, Università di Parma, Parma, Italy

364. P82 (In-111-DTPA-D-Phe-1) OCTREOTIDE (OCT) SCINTIGRAPHY (OCTREOSCAN) IN THYROIDAL AND ORBITAL GRAVES' DISEASE: FOLLOW-UP DURING TREATMENT.
EP Krenning, PTE Postema, R Wijngaarde, WA Vandenbosch, PPM Kooij, RML Poublon and HY Oei
University and Eye Hospitals, Rotterdam, The Netherlands

365. P83 IMAGING TECHNIQUES IN THE EVALUATION OF ENDOCRINE OPHTHALMOPATHY PATIENTS PRESENTING WITH DIPLOPIA.
E.V. Nagy, J. Toth, I. Kaldi, J. Damjanovich and A. Leovey
Dept I of Medicine, Radiology and Ophthalmology, University Medical School of Debrecen and Opthalmolgy Unit, County Hospital Debrecen, Hungary

366. P84 RECOVERY COURSE OF CHEMICAL TYPE AND IMMUNOLOGICAL TYPE REVERSIBLE HYPOTHYROIDISM.
K. Sato, K. Okamura, H. Ikenoue, T. Kuroda, T. Mizokami and M. Fujishima
Second Department of Internal Medicine, Kyushu University, Fukuoka, Japan

367. P85 CLINICAL SIGNIFICANCE OF ANTI-THYROTROPIN RECEPTOR ANTIBODIES IN THYROID-ASSOCIATED OPHTHALMOPATHY.
I. Miyake, M. Koga, N. Nagasawa, Y. Hiromatsu, K.Kojima, Y. Inoue and K. Nonaka
Department of Medicine and Radiology, Kurume University School of Medicine, Kurume, and Eye Division of Olympia Clinic, Tokyo, Japan

368. P86 NEW HPLC METHOD TO ANALYSE THE ABERRANT DISTRIBUTION OF GLYCOSAMINOGLYCAN IN GRAVES' OPTHALMOPATHY.
Ch. Hansen, B. Fraiture, R. Rouhi, K. Kuhlemann, E. Otto, J. Beyer and G. Kahaly
Dept. of Medicine III, University Hospital, Mainz, Germany

369. P87 HIGH PREVALENCE OF ACHLORHYDRIC ATROPHIC GASTRITIS IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE.
M. Centanni, M. Marignani, A. Casini, S. Terracina, G.F. Delle Fave and B. Annibale
Endocrinology Section, Dept. of Experimental Medicine and Gastroenterology Unit, University "La Sapienza" Roma, Italy

370. P88 FOLLOW-UP STUDY AND MRI FINDINGS IN PATIENTS WITH HASHIMOTO'S THYROIDITIS AND WITH HYPERPROLACTINEMIA.
C. Takagi, K. Notsu, M. Imaoka and Y. Kato
Department of Medicine, Shimane Prefectural Central Hospital and First Division, Department of Medicine, Shimane Medical University, Izumo, Japan

371. P89 UNUSUAL DEVELOPMENT OF ACTIVE GRAVES' OPHTHALMOPATHY IN AN IMMUNOSUPPRESSED-PATIENT.
G. Höfle, G. Finkenstedt and R. Moncayo
Department of Internal Medicine and Nuclear Medicine, University of Innsbruck, Austria

372. P90 SERUM CONCENTRATIONS OF NEOPTERIN, BETA-2 MICROGLOBULIN AND SOLUBLE INTERLEUKIN-2 RECEPTOR IN PATIENTS WITH GRAVES' OPHTHALMOPATHY.
M. Herold, I. Baldissera and W. Mayer
University of Innsbruck, Department of Internal Medicine,
Department of Ophthalmology, Innsbruck, Austria

POSTER SESSION - Thursday , September 14, 1995

373. P91 THYROID HORMONE AUTOANTIBODIES (THAA) IN A CASE OF CHRONIC THYROIDITIS WITH LARGE GOITER: EFFECTS OF VARIOUS TREATMENTS ON THAA TITERS.
S. Fujii, M. Oomori, U. Miwa, T. Seta and T. Ohka
Ishikawa Prefectural Central Hospital, Kanazawa, Japan

374. P92 THE EFFECT OF SOMATOSTATIN VERSUS CORTICOSTEROID IN THE TREATMENT OF DYSTHYROID EYE DISEASE.
S.C. Yeung, Annie W. C. Kung, John Michon, S.H. Cheung, K.S. Tai and F.L. Chan
Departments of Medicine and Ophthalmology, University of Hong Kong and Department of Diagnostic Radiology , Queen Mary Hospital, Hong Kong

375. P93 THE EFFECT OF A COMBINDED THERAPY OF TOTAL THYROIDECTOMY AND 131I ABLATION ON MALIGNANT EXOPHTHALMOS - A LONG TERM FOLLOW-UP STUDY.
Y. Matsumoto, S. Kubota, S. Fukata, A. Kobayashi and K. Kuma
Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Kuma Hospital, Kobe, Japan

376. P94 FACTORS ALLEVIATING THE HYPERTHYROIDISM IN GRAVES' PATIENTS WITH EXTRAOCULAR MUSCLE ENLARGEMENT.
N. Hamada, J.Y. Noh, Y. Okamoto, M. Ohno, and K. Ito
Sumire Hospital, Osaka Social Welfare Fndn.,Osaka City General Hospital, Osaka Ekisaikai Hospital, Osaka, and Ito Hospital, Tokyo, Japan

Thyroid Hormone Action - 8

377. P95 HORMONE-DEPENDENT INTERACTIONS OF THYROID HORMONE RECEPTORS WITH THEIR ANTIBODIES.
M.K. Bhat, P. McPhie and S.Y. Cheng
Laboratory of Molecular Biology NCI and Laboratory of Biochemical Pharmacology, NIDDK, NIH, Bethesda, Maryland USA

378. P96 TRANSTHYRETIN (TTR) LIGAND COMPLEXES: CRYSTAL STRUCTURE OF RAT TTR-EMD21388 COMPEX.
Vivian Cody, Andrzej Wojtczak, Joesph R. Luft and Walter Pangborn
Hauptman-Woodward Medical Research Institute, Buffalo, NY USA and N. Copernicus University, Torun, Poland

379. P97 ANTISENSE-MEDIATED BLOCKADE OF "SPOT 14" EXPRESSION SPECIFICALLY INHIBITS INDUCTION OF LIPOGENIC ENZYMES BY TRIIODOTHYRONINE (T3) AND GLUCOSE.
William B. Kinlaw and Jori L. Church
Department of Medicine, Division of Endocrinology and Metabolism, Dartmouth Medical School, Lebanon, NH USA

380. P98 ABNORMAL EXPRESSION OF T3 RECEPTOR ISOFORMS IN A NON-T3 RESPONSIVE THYROTROPIC TUMOR.
V.D. Sarapura, T.M. Bright, W.M. Wood, B.R. Haugen, D.F. Gordon and E.C. Ridgway
University of Colorado Health Sciences Center, Denver, CO USA

381. P99 EFFECT OF AMIODARONE ON RAT HEART mRNA CODING FOR Ca^{2+} ATPase OF THE SARCOPLASMIC RETICULUM.
Lea Maria Zanini Maciel, Nassim Iazigi
School of Medicine of Ribeirao Preto, Ribeirao Preto-SP, Brazil

382. P100 RELATIONSHIP BETWEEN THE THYROID HORMONE TRANSPORT SYSTEM AND THE Na^{+}/H^{+} EXCHANGER IN CULTURED RAT BRAIN ASTROCYTES.
A. Beslin, F. Chantoux, J.P. Blondeau and J. Francon
Unité Thyröide, INSERM U96, Kremlin-Bicêtre, Paris, France

383. P101 TREATMENT WITH PHYSIOLOGICAL DOSES OF L-THYROXINE PREVENTS THE OVARIECTOMY-INDUCED BONE LOSS IN RATS.
C.H.A. Gouveia and A.C. Bianco
Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paula, SP, Brazil

POSTER SESSION - Thursday , September 14, 1995

384. P102 REGULATION OF HIGH-AFFINITY THYROID-HORMONE BINDING IN HUMAN KIDNEY AND LIVER CYTOSOL BY THE REDOX STATE OF NADP. CYTOSOLIC-NUCLEAR INTERRELATIONSHIP.
M.P. Vié, M. Samson, A. Beslin, J. Francon and J.P. Blondeau
Unité de Recherches sur la Glande Thyroïde, INSERM U96, Kremlin-Bicêtre, Paris, France

385. P103 HYPOTHYROIDISM REDUCES GLUT 1 EXPRESSION DURING RAT TESTICULAR DEVELOPMENT.
MM Godbole, J. Virmani, AM Srivastava, A. Mandal, M. Shukla and A. Mithal
Sanjay Gandhi PG Institute of Medical Sciences, Lucknow, Avadh University, Faizabad, India

386. P104 GENERATION OF NONESTERIFIED FATTY ACIDS WITHOUT HEPARIN IN SERA FROM CRITICALLY ILL AND NORMAL SUBJECTS: IMPLICATIONS FOR FREE THYROXINE MEASUREMENTS.
C.-F. Lim, D. Dutta, D.J. Topliss and J.R. Stockigt
Ewen Downie Metabolic Unit, Alfred Hospital, Melbourne, Australia

387. P105 EFFECT OF DIIODOTHYRONINES ADMINISTRATION ON RESTING METABOLISM AND TISSUE OXIDATIVE CAPACITY OF HYPOTHYROID RAT.
Fernando Goglia, Maria Moreno, Assunta Lombardi and Antonia Lanni
Departmento di Fisiologia Generale ed Arnbientale, Napoli, Italy

388. P106 CHANGES IN CATECHOLAMINE AND ITS METABOLITE CONCENTRATIONS IN CEREBRAL CORTEX, CARDIAC MUSCLE, ADRENAL GLAND AND SERUM FROM HYPER- OR HYPOTHYROID RATS.
T. Mano, H. Sakamoto, T. Mokuno, Y. Itoh, R. Kato, M. Hamada, Y. Nishida, M. Hukushima, K. Asano, T. Miyamoto, T. Kawabe, K. Fujita and H. Kuzuya
Dept. of Intern. Med., and Division of Molecular Biology, Inst. for Comprehen. Med. Sci., Fujita Health Univ., School of Medicine, Toyoake, Aichi, Japan

389. P107 SUBCELLULAR ALTERATIONS OF CARDIAC FIBERS IN RATS SUBJECTED TO HYPOTHYROIDISM AND AFTER RESTORING EUTHYROIDISM.
F.J.C. Stamato, M.J.Simoes, O.A.Moura, A.C.Lopes and R.P. Furlanetto
Disciplines of Endocrinology, Histology and Medicine Urgency, Universidade Federal de São Paulo, São Paulo, Brazil

2:30 P.M. POSTER DISCUSSIONS

Simco/Duffen Room
AUTOIMMUNITY - 1
DISCUSSION LEADER - DR. FENZI

City Hall Room
AUTOIMMUNITY - 2
DISCUSSION LEADER - DR. WALL

Civic Room
CANCER - 3
DISCUSSION LEADER -DR. FELDT-RASMUSSEN

Dominion Ballroom South
CELL BIOLOGY - 4
DISCUSSION LEADER - DR. SEO

Essex Room
CELL BIOLOGY - 5
DISCUSSION LEADER -DR. KONDO

Dominion Ballroom North
CLINICAL - 6
DISCUSSION LEADER - DR. WARTOFSKY

AFTERNOON SESSION - Thursday , September 14, 1995

Windsor East Room
CLINICAL - 7
DISCUSSION LEADER DR. FABER

Windsor West Room
THYROID HORMONE ACTION - 8
DISCUSSION LEADER DR. LAZAR

3:30 P.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

4:00 P.M. **SYMPOSIA - SIMULTANEOUS SESSIONS**

GRAND BALLROOM WEST & CENTRE

"Thyroid- and pituitary-specific transcription factors"

Dr. Roberto DiLauro
Stazion Zoologica - Anton DOHRN, Napoli, Italy
Dr. Bryan R. Haugen
University Of Colorado Health Sciences, Denver, CO USA
Dr. Keita Tatsumi
Osaka University Medical School, Osaka, Japan

Chairpersons - Drs. Leedman & Wondisford

GRAND BALLROOM EAST

"Medical management of thyroid cancer"

Dr. Junji Konishi
Faculty Medicine Kyoto University Sakyo-ku, Kyoto, Japan
Dr. Harry R. Maxon
University Of Cincinnati Medical Center, Cincinnati, OH USA
Dr. Ernest L. Mazzaferri
The Ohio State University, Columbus, OH USA
Dr. Furio Pacini
Instituto di Endocrinologia, University of Pisa, Italy

Chairpersons - Drs. deMicco & Van Herle

6:30 P.M. to 10:30 P.M. **Reception and Banquet**
Grand Ballroom - Sheraton Centre
Admission by ticket only

11th International Thyroid Congress
Friday, September 15, 1995

MORNING SESSION

WEST & CENTRE BALLROOM
THE SHERATON CENTRE - TORONTO, CANADA

8:00 A.M. **American Thyroid Association - Van Meter Prize Lecture**

9:00 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

AUTOIMMUNITY - West & Centre Ballroom
CHAIRPERSONS DRS. AKAMIZU AND LE CLERE

9:00 390. THYROID-DERIVED FIBROBLASTS SUPPORT SURVIVAL, DIFFERENTIATION AND IMMUNOGLOBULIN SECRETION OF CULTURED GRAVES' THYROID-DERIVED B-LYMPHOCYTES.
A. M. Bichlmair, B. E. Wenzel, P. C. Scriba and A. E. Heufelder.
Molecular Thyroid Research Unit, Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians- Universität, München, and Cell and Immunobiology Lab, Med. Universität zu Lübeck, Germany

9:15 391. ANATOMIC SITE-SELECTIVE INDUCTION OF CYCLOOXYGENASE-2 AND PGE_2 IN HUMAN ORBITAL FIBROBLASTS.
H.S. Wang, V.D. Winn, C.H. Evans, D.A. Young and T.J. Smith
Albany Medical College and VAMC, Albany, New York; NCI, Bethesda, Maryland and University of Rochester School of Medicine, Rochester, NY USA

9:30 392. T-CELL-RECEPTOR V GENE SELECTION IN HUMAN THYROID ORGANOIDS.
Andreas Martin, Jin Zhang, Pamela Unger and Leonard D. Shultz
Departments of Medicine and Pathology, Mount Sinai School of Medicine, New York, NY and The Jackson Laboratory, Bar Harbor, Maine USA

9:45 393. THYROID TISSUE FROM PATIENTS WITH GRAVES' DISEASE CONTAINS CLONALLY EXPANDED T CELLS WITHIN A RESTRICTED BUT HETEROGENOUS T CELL POPULATION–EVIDENCE FOR A DUAL MECHANISM OF IN-SITU T CELL EXPANSION.
T.F. Davies and M. Nakashima
Department of Medicine, Mount Sinai School of Medicine, New York, NY USA

CELL BIOLOGY - East Ballroom
CHAIRPERSONS DRS. KIMURA AND SPAULDING

9:00 394. CLONING AND ANALYSIS OF RAT THYROID TRANSCRIPTION FACTOR-1 (TTF-1) GENE.
M. Ohno, M. Nakazato, T. Kogai, T. Endo and T. Onaya
Third Department of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi, Japan

9:15 395. THYROID HORMONES REGULATE MESSENGER RNA LEVELS OF THE THYROID-SPECIFIC TRANSCRIPTION FACTORS :TTF-1 AND PAX-8.
S. Selmi-Ruby, C. Watrin and B. Rousset
INSERM U369, Faculté de Médecine Alexis Carrel, Lyon, France

9:30 396. DYNAMIC CHANGES OF TIGHT JUNCTION PROTEIN ZO-1: POSSIBLE ROLE IN TGFβ1-INDUCED JUNCTIONAL DISASSEMBLY-REASSEMBLY RELATED TO EMIGRATION OF THYROID EPITHELIAL CELLS.
M. Nilsson
Institute of Anatomy & Cell Biology, Göteborg University, Göteborg, Sweden

MORNING SESSION - Friday, September 15, 1995

9:45 397. SERUM DEPRIVATION ACTIVATES APOPTOSIS IN Ki-RAS BUT NOT IN POLYOMA TRANSFORMED OR IN DIFFERENTIATED THYROID CELLS.
B. Di Jeso, L. Racioppi, A. Feliciello, F. Pacifico, P. Giuliano, L. Ulianich, E. Consiglio, S. Formisano, and E.V. Avvedimento
Centro di Endocrinologia ed Oncologia Sperimentale and Dip. di Biologia e Patologia Cellulare e Molecolare, Napoli, Italy

10:00 A.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

10:30 A.M. **ORAL PRESENTATIONS**
SIMULTANEOUS SESSIONS

THYROID HORMONE METABOLISM - West & Centre Ballroom
CHAIRPERSONS DRS. BLONDEAU AND CHOPRA

10:30 398. CLONING AND EXPRESSION OF THE HUMAN SELENOPROTEIN, TYPE 3 IODOTHYRONINE DEIODINASE.
D. Salvatore, S.C. Low, A-L. Maia, J.W. Harney, W. Croteau, D. St. Germain and P.R. Larsen
Thyroid Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA and Departments of Medicine and Physiology, Dartmouth Medical School, Hanover, NH USA

10:45 399. REGULATION OF TYPE 1 5'DEIODINASE BY INFLAMMATORY CYTOKINES IN RAT LIVER CELLS.
PH Davies, MC Sheppard and JA Franklyn
Department of Medicine, University of Birmingham, Edgbaston, Birmingham, United Kingdom

11:00 400. PRESENCE OF GROWTH FACTORS-INDUCED 5 DEIODINASE ACTIVITY IN CULTURED BROWN ADIPOCYTES.
A. Hernández and M.-J. Obregón
Inst. Investigaciones Biomédicas (CSIC), Madrid, Spain

11:15 401. REGULATORY MECHANISM OF IODOTHYRONINE 5' DEIODINASE ACTIVITY AND mRNA OF CULTURED RAT MYOCARDIAL CELLS.
T. Yonemoto, A. Gondou, Y. Mori, H. Matsubara and N. Nishikawa
The Second Dept. of Internal Medicine, Kansai Medical University, Moriguchi, Osaka, Japan

11:30 402. EXPRESSION OF THE TRANSMEMBRANE THYROID HORMONE TRANSPORT PROTEIN FROM RAT LIVER IN *XENOPUS LAEVIS* OOCYTES.
R. Docter, E.C.H. Friesema, P.G.J. van Stralen and G. Hennemann
Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands

11:45 403. SERUM "COMPOUND W" CONCENTRATIONS IN FETUSES AND THEIR MOTHERS AT VARIOUS GESTATIONAL STAGES.
P. Beck-Peccoz, D. Cortelazzi, A.M. Marconi, A.M. Baggiani, M. Buscaglia, D.H. Polk, D.A. Fisher and S.Y. WU
Institutes of Endocrine Sciences, Ospedale Maggiore IRCCS; Obstetric & Gynecology, S. Paolo Hospital, University of Milan, Milan, Italy; Perinatal Lab, Harbor-UCLA Medical Center, Torrance, CA; and Thyroid Research Lab, VAUC Irvine Medical Center, Long Beach, CA USA

CELL BIOLOGY - East Ballroom
CHAIRPERSONS DRS. BERRY AND KINLAW

10:30 404. IMPORTANCE OF ASPARTATE - 474 IN THE FIRST EXOPLASMIC LOOP OF THE THYROTROPIN RECEPTOR (TSHR) IN RECEPTOR ACTIVATION.
S. Kosugi and T. Mori
Department of Laboratory Medicine, Kyoto University, Japan

10:45 405. ADDITIVE EFFECT OF ACTIVATING POINT MUTATIONS OF THE TSH RECEPTOR GENE.
M. Tonacchera, J. Parma, F. Cetani, S. Costagliola, R. Paschke and G. Vassart
IRIBHN, Service Genetique Medicale, Université Libre de Bruxelles, Brussels, Belgium

MORNING SESSION - Friday, September 15, 1995

11:00 406. MUTUAL ANTAGONISTIC INTERACTIONS BETWEEN THE PROTEIN KINASE A AND PROTEIN KINASE C/TYROSINE KINASE PATHWAYS IN HUMAN THYROID *c-jun* AND *c-fos* PROTO-ONCOGENE EXPRESSION, CELL PROLIFERATION AND DIFFERENTIATION.
Z. Kraiem, G. Gorenstein, E. Sobel, O. Sadeh and R. Heinrich
Endocrine Research Unit, Carmel Medical Center, Haifa, Israel

11:15 407. EXPRESSION OF FUNCTIONAL TSH RECEPTOR (TSHR) IN RAT EPIDIDYMAL FAT CELLS AND 3T3L1 CELLS.
K. Haraguchi, T. Endo and T. Onaya
Third Dept. of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi, Japan

11:30 408. INCREASED cAMP STIMULATION BY THE HUMAN TSH RECEPTOR VARIANT WITH THE PRO52THR SUBSTITUTION IN THE EXTRACELLULAR DOMAIN.
S. Hagner, C.J. van Koppen, U.R.M. Bohr, K.H. Jakobs, and U. Loos
Dept. of Internal Medicine I, University of Ulm and Institute of Pharmacology, University GH Essen, Germany

11:45 409. TRANSFORMING GROWTH FACTOR β SPECIFICALLY INHIBITS THE TSH/cAMP-DEPENDENT PROLIFERATION OF DOG THYROCYTES IN PRIMARY CULTURE: ITS EFFECTS ON CELL CYCLE REGULATORY PROTEINS.
F. Depoortere, R. Burikhanov, M. Baptist and P.P. Roger
I.R.I.B.H.N., Free University of Brussels, Campus Erasme, Brussels, Belgium

FRIDAY, SEPTEMBER 15TH, 1995 - ADMISSION BY TICKET ONLY
12:00 P.M. TO 1:30 P.M.

MEET THE PROFESSORS LUNCHEONS

Simco/Duffen Room - Transgenic animals in thyroid research
Dr. Marc Parmentier

City Hall Room - Uses of recombinant TSH
Dr. Bruce Weintraub

Essex Room - Immunogenetics of autoimmune thyroid disease
Dr. Terry Davies

WORKSHOPS

Dominion North Ballroom- Free thyroid hormone levels: useful test?
Drs. Laurberg & Stockigt - Moderator, Dr. Spencer

Dominion South Ballroom - Thyroid gland autoregulation
Drs. Boyenaems & Pisarev - Moderator, Dr. Braverman

Civic Ballroom - Inherited hypothyroidism
Drs. DeVijlder & Miyai - Moderator, Dr. Dussault

11th International Thyroid Congress
Friday , September 15, 1995

AFTERNOON SESSION

SHERATON HALL
THE SHERATON CENTRE - TORONTO, CANADA

1:30 P.M. **POSTER VIEWING**
POSTERS IN PLACE AT 9 AM - REMOVED AT 8 PM

Autoimmunity - 1

410. P1 A THYROTROPIN RECEPTOR CODON 52 POLYMORPHISM AND HLA-DR3 ARE INDEPENDENTLY ASSOCIATED WITH INCREASED RISK FOR THE DEVELOPEMENT OF AUTOIMMUNE THYROID DISEASE IN CAUCASIAN FEMALES, WHILE HLA-DRB3 AND DQA_1 *0501 ARE NOT.
R.M. Cuddihy, D.J. Schaid, and R.S. Bahn
Division of Endocrinology and Section of Biostatistics, Mayo Clinic, Rochester MN USA

411. P2 HUMAN THYROTROPIN RECEPTOR TRANSLATED *IN VITRO* AS A NASCENT PROTEIN BINDS AUTOANTIBODIES FROM GRAVES' DISEASE PATIENTS.
N.G. Morgenthaler, J. Tremble, G.C. Huang, W.A. Scherbaum and J.P. Banga
Department of Medicine, King's College School of Medicine, London, United Kingdom; Medizinische Klinik III, Universitat Leipzig, Leipzig, Germany

412. P3 POLYCLONAL ANTISERA TO RECOMBINANT THYROTROPIN RECEPTOR; BIOLOGICAL PROPERTIES AND BINDING TO NATIVE RECEPTOR PROTEIN.
J.P. Banga, M.R. Kim, A. Gardas and M. Gupta
Department of Medicine, King's College School of Medicine, London, United Kingdom; The Department of Clinical Pathology, The Cleveland Clinic Foundation, Cleveland, Ohio USA

413. P4 KINETICS OF ANTIBODY RESPONSES TO THYROTROPIN RECEPTOR IN MICE THAT ARE SUSCEPTIBLE (BALB/cJ) AND RESISTANT (SJL/J) TO THE INDUCTION OF HYPERTHYROXINEMIA.
Sai A. Patibandla and Ji-Lao Fan
Department of Microbiology/Immunology, University of Texas Medical Branch, Galveston, Texas USA

414. P5 DEFINING THE MAJOR ANTIBODY EPITOPES ON THE HUMAN THYROTROPIN RECEPTOR.
H. Vlase, P.N. Graves, Y. Tomer, J. Morris and T.F. Davies
Department of Medicine, Mount Sinai School of Medicine, New York, NY; Department of Medicine, Mayo Clinic, Rochester MN USA

415. P6 TRANSFER OF THYROIDITIS WITH SPLEEN CELLS FROM MICE IMMUNISED WITH RECOMBINANT HUMAN THYROTROPIN RECEPTOR (TSHR).
S. Costagliola, M-C.Many and M. Ludgate
IRIBHN, ULB and Lab. Histology, UCL, Brussels, Belgium

416. P7 VARIABLE REGIONS OF IMUNNOGLOBULIN GENES ENCODING "blocking type" ANTITHYROTROPIN RECEPTOR ANTIBODIES OF PATIENTS WITH PRIMARY MYXEDEMA.
J. Okuda, T. Akamizu, H. Li and F. Matsuda
Department of Laboratory Medicine, Faculty of Medicine and Center for Molecular Biology and Genetics, Kyoto University, Kyoto, Japan

417. P8 DISCRETE CHARACTERISTICS OF ANTIBODIES RAISED AGAINST TSH RECEPTOR-RELATED PEPTIDES WHOSE SEQUENCES ARE NOT CONSERVED IN THE LH/CG RECEPTOR.
M. Murakami, K. Miyashita, Y. Hosoi, T. Negishi, T. Michimata, M. Yamada, T. Iriuchijima and M. Mori.
First Department of Internal Medicine, Gunma University, School of Medicine, Maebashi, Japan

POSTER SESSION - Friday, September 15, 1995

418. P9 SPECIFIC EFFECTS OF RADIOIODINE (RAI) ON THYROID STIMULATING (TSAb) AND BLOCKING (TBAb) ANTIBODIES IN GRAVES' DISEASE.
V.P. Michelangeli, C. Poon, D.J. Topliss and P.G. Colman
Department of Diabetes and Endocrinology, Royal Melbourne Hospital and Ewen Downie Metabolic Unit, Alfred Hospital, Victoria, Australia

419. P10 IN VITRO SPONTANEOUS PRODUCTION OF THYROTROPIN RECEPTOR ANTIBODIES (TRAb).
J. Aguayo, R. Arellano and G. Pineda
Endocrine Section, Dept. of Medicine, Univ. of Chile and I.E.M.A. Endocrine Lab. Santiago, Chile

420. P11 DEMONSTRATION OF THE THYROACTIVE SMALLER COMPONENTS RELEASED FROM TSAb-IgG BY PROTEASE DIGESTION OR REDUCTION.
Y. Ochi, T. Inui, W. Chen, T. Kouki, M. Ogura, T. Hachiya and Y. Kajita.
Dept. Clin. Lab. Med. , Shiga University of Medical Science, Shiga; 2nd Dept. Int. Med. , Kobe Univ., Hyogo; 2nd Dept. Int. Med. and Kyoto Pref. Univ. of Med. , Kyoto, Japan

421. P12 INFLUENCE OF ADJUVANTS ON DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNITY TO THE THYROTROPIN RECEPTOR.
G.S. Seetharamaiah and B.S. Prabhakar
Department of Microbiology/Immunology, University of Texas Medical Branch, Galveston, Texas USA

422. P13 FUNCTIONAL RELEVANCE OF DIFFERENCES IN FINE SPECIFICITY OF THYROTROPIN RECEPTOR SPECIFIC ANTIBODIES IN MICE WITH AND WITHOUT EXPERIMENTAL HYPERTHYROXINEMIA.
Bellur S. Prabhakar, Sai A. Patibandla, John S. Dallas, John C. Morris and Neelam M. Wagle
Departments of Microbiology/Immunology, and Pediatrics, University of Texas Medical Branch, Galveston, Texas USA and Division of Endocrinology, Mayo Clinic, Rochester, MN

423. P14 EXPRESSION OF SECRETED FORM OF RECOMBINANT HUMAN THYROTROPIN RECEPTOR.
Y. Okamoto, N. Hamada, T. Sato, S. Tanaka, Y. Nishizawa, S. Fujii and H. Morii
Department of Internal Medicine, Osaka City General Hospital, Sumire Hospital, and Second Department of Internal Medicine, Osaka City University, Osaka, Japan

Cancer - 2

424. P15 EFFECTS OF ANTI-CANCER DRUGS ON TWO HUMAN THYROID CELL LINES WITH DIFFERENT STAGES OF DIFFERENTIATION.
A. Hishinuma, T. Yamanaka, K. Kasai, T. Ieiri and S-I. Shimoda
Departments of Clinical Pathology and Endocrinology, Dokkyo University School of Medicine, Mibu, Tochigi, Japan

425. P16 THE CLINICAL FEATURES OF ADVANCED DIFFERENTIATED THYROID CARCINOMA UNDERGOING RADIOIODINE THERAPY.
H. Koshiishi, T. Mimura, O.Ozaki, K. Sugino, W. Kitagawa, K. Ito, Jr. and K. Ito
Ito Hospital, Tokyo, Japan

426. P17 CLINICAL OUTCOME OF HIGH-RISK PATIENTS WITH DIFFERENTIATED THYROID CANCER.
R. Vassilopoulou-Sellin, E.S. Delpassand and T.P. Haynie
M.D.Anderson Cancer Center, Houston, Texas USA

427. P18 THE CORRELATION BETWEEN PAPILLARY THYROID CARCINOMA AND LYMPHOCYTIC INFILTRATION IN THE THYROID GLAND.
S. Matsubayashi, Y. Matsumoto and F. Matsuzuka
Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University (S.M., Y.M.), Fukuoka, Kuma Hospital (F.M.), Kobe, Japan

428. P19 PREFERENTIAL EXPRESSION OF THE CELL ADHESION MOLECULE CD44 IN PAPILLARY THYROID CARCINOMA.
J. Figge, G. Gerasimov, I. Dedov, M. Bronstein. K. Troshina and G. Alexandrova
Albany Medical College, Albany, New York USA; and The Russian Endocrinology Research Center, Moscow, Russia

POSTER SESSION - Friday, September 15, 1995

429. P20 EXCESSIVE EXPRESSION OF CYTOPLASMIC C-MYC PROTEIN IN THYROID PAPILLARY CARCINOMAS.
Y. Nakamura, H. Kamma, T. Yazawa, K. Kakudo and T. Ogata
Department of Pathology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba; and Wakayama Medical College, Wakayama, Japan

430. P21 THE ROLE OF RADIATION THERAPY IN DIFFERENTIATED THYROID CANCER.
R.W. Tsang, J.D. Brierley, W.J. Simpson, T. Panzarella, M.K. Gospodarowicz and S.B. Sutcliffe
University of Toronto, Princess Margaret Hospital, Toronto, Canada

431. P22 INFLUENCE OF 3,5,3' - TRIIODOTHYROACETIC ACID TREATMENT ON TSH SUPPRESSION AND SURVIVAL OF PATIENTS WITH DIFFERENTIATED THYROID CANCER.
P. Pujol, J.P. Daurès, N. Nsakala, R. Martinel and J. Bringer
Department of Endocrinology and Epidemiology, University Hospital of Montpellier, France

432. P23 TOTAL THYROIDECTOMY AND PRESERVATION OF PARATHYROID GLAND FUNCTION IN OPERATION OF CHILDHOOD THYROID PAPILLARY CARCINOMA.
H. Funahashi, T. Imai, Y. Tanaka, M. Wada and T. Morita
Department of Surgery II, Nagoya University School of Medicine, Nagoya, Japan

433. P24 SOME MORE INFORMATION ON THE VALUE OF OUTPATIENT ABLATION OF THYROID TISSUE WITH <30 mCi OF I-131 IN PATIENTS WITH DIFFERENTIATED THYROID CANCER.
I.R. McDougall
Stanford Health Services, Stanford, California USA

434. P25 TREATMENT OF MICRONODULAR PULMONARY METASTASES OF PAPILLARY THYROID CARCINOMA WITH 131-I.
J.C. Sisson, S. Zempel and S. Spaulding
University of Michigan, Ann Arbor, Michigan USA

435. P26 IS MORE AGGRESSIVE TREATMENT NEEDED FOR FOLLICULAR THYROID CANCER?
L.J. DeGroot, E.L. Kaplan, and F. Straus
Thyroid Study Unit and Departments of Pathology and Surgery, The University of Chicago, Chicago, IL USA

Cell Biology - 3

436. P27 STRUCTURAL DETERMINANTS OF THYROTROPIN RECEPTOR INTERNALIZATION.
Yufei Shi, Minjing Zou and Nadir R. Farid
Molecular Endocrinology Laboratory, Kinq Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

437. P28 COMPARISON OF THE CONSTITUTIVE ACTIVITY OF THE TSH AND LH RECEPTORS USING DIFFERENT ASSAY CONDITIONS.
F. Cetani, M. Tonacchera and G. Vassart
IRIBHN, Service de Genetique Medicale, Universitè Libre de Bruxelles, Brussels, Belgium

438. P29 PROTEOLYTIC CLEAVAGE OF THE HUMAN TSH RECEPTOR: ANALYSIS USING EXTRACELLULAR REGION CHIMERIC TSH-LH RECEPTORS.
G.D. Chazenbalk, Y. Nagayama S.M. McLachlan and Basil Rapoport
Thyroid Molecular Biology Unit, V.A. Medical Center and the University of California, San Francisco, CA USA

439. P30 TSH-RECEPTOR EXPRESSION AND HUMAN THYROID DISEASE: RELATION TO THYROIDAL TRANSCRIPTION FACTORS.
S. Deiters, F. Schuppert, G.F.W. Scheumann, H. Dralle and A. von zur Mühlen
Department of Clinical Endocrinology, Department of Abdominal and Transplant Surgery, Hannover Medical School, Hannover, FRG; Center for Surgery I, Halle-Wittenberg University, Halle/Saale, Germany

440. P31 TSH REGULATION OF THYROID HORMONE SYNTHESIS AND PROTEIN KINASE C EXPRESSION.
M.C. Eggo, E.G. Black, H. Chahal and J.M. Lord
Departments of Medicine and Immunology, University of Birmingham, Edgbaston, Birmingham, United Kingdom

POSTER SESSION - Friday, September 15, 1995

441. P32 SPECIFICITY OF SYNTHETIC TSH ANTAGONISTS.
A.A.K. Hassoun, E.R.Bergert and J.C.Morris
Mayo Clinic, Rochester, MN USA

442. P33 ACTIVATION OF PHOSPHOLIPASE D BY THYROTROPIN IN FRTL-5 THYROID CELLS.
J. Ginsberg, S. Gupta, A. Gomez-Munoz, W.C. Matowe and D. Brindley
Signal Transduction Laboratories, University of Alberta, Edmonton, Canada

443. P34 THE AMINO TERMINUS OF THE HUMAN THYROTROPIN RECEPTOR IS A POTENT LINEAR EPITOPE.
P.N. Graves, V. Nguyen and H. Vlase
Department of Medicine, Mount Sinai School of Medicine, New York, NY USA

444. P35 PRODUCTION OF SOLUBLE PROKARYOTIC HUMAN TSH RECEPTOR EXTRACELLULAR DOMAIN BY DISULFIDE BOND FORMATION IN THE CYTOPLASM OF E. COLI.
Y. Bobovnikova and V. Nguyen
Department of Medicine, Mount Sinai School of Medicine, New York, NY USA

445. P36 DECREASED SENSITIVITY OF THYROID STIMULATING ANTIBODY ASSAY WITH CHINESE HAMSTER OVARY CELLS TRANSFECTED WITH CLONED HUMAN TSH RECEPTOR USING HYPOTONIC INCUBATION MEDIA WHICH IS DUE TO DECREASED VIABILITY OF CELLS DURING INCUBATION.
Bo Youn Cho, Won Bae Kim, Jae Hoon Chung, Hong Kyu Lee and Chang-Soon Koh
Department of Internal Medicine, Seoul National University College of Medicine; Department of Medicine, Samsung Medical Center, Seoul, Korea

446. P37 ANALYSIS OF THE BIOACTIVITY OF RECOMBINANT HUMAN THYROID STIMULATING HORMONE IN TT_4 SUPRESSED MICE.
R.J. Mattaliano, B.G. Whittaker, N.M.Losapio, L. Cheney, E.M. Cole and B.M. Pratt.
Genzyme Corporation, Therapeutic Protein Development, Framingham, Massachusetts USA

Clinical - 4

447. P38 LONG-TERM FOLLOW-UP OF CONTRALATERAL LOBE IN HEMITHYROIDECTOMIZED WOMEN DUE TO SOLITARY FOLLICULAR ADENOMA.
H. Niepomniszcze, A. Castellanos, E. Faure, A. Garcia. M.C. Zalazar. G. Bur and B. Elsner
Services of Endocrinology and Pathology, Complejo Médico "Churruca-Visca", and Divisions of Endocrinology and Pathology, Hospital de Clínicas "Jose de San Martín", School of Medicine, University of Buenos Aires, Buenos Aires, Argentina.

448. P39 RECURRENCE OF SUBACUTE THYROIDITIS LONG TERM AFTER THE FIRST ATTACK.
H. Fukazawa, K. Yoshida, K. Abe, M. Yamamoto, S. Saito and H. Kurihara
Narugo National Hospital, Narugo-Machi: The Second Dept. of Internal Medicine, Tohoku University School of Medicine and JR Hospital, Sendai: Kurihara Thyroid Clinic, Morioka, Japan

449. P40 THE CHARACTERIZATION AND RESPONSE TO TREATMENT OF THYROID NODULES IN PATIENTS WITH GRAVES' DISEASE.
N.E. Carnell and W.A. Valente
Division of Endocrinology, University of Maryland, Baltimore, Maryland USA

450. P41 CHILDHOOD THYROID DISEASES AROUND CHERNOBYL.
K. Ashizawa, S. Yamashita, M. Ito, T. Nishikawa, K. Hara, H. Namba, M. Izumi, Y. Shibata, M. Hoshi and S. Nagataki
The First Department of Internal Medicine, Atomic Disease Institute, Nagasaki University School of Medicine; Radiation Effects Research Foundation, Nagasaki; Hiroshima University, Hiroshima, Japan

451. P42 TREATMENT OF TOXIC THYROID NODULE BY PERCUTANEOUS ETHANOL INJECTION DURING PREGNANCY.
D. Cortelazzi, D. Castagnone, B. Tassis, E. Venegoni, R. Rivolta and P. Beck-Peccoz
Institute of Endocrine Sciences and Department of Radiology, Ospedale Maggiore IRCCS; and Obstetrics and Gynecology Clinic I, University of Milan, Milan, Italy

452. P43 RECURRENCE OF SUBACUTE THYRODITIS AFTER A LONG LATENT PERIOD.
Makoto Iitaka, Naoko Momotani, Jun Ishii and Kunihiko Ito
Department of Medicine 4, Saitama Medical School, Saitama, Ito Hospital, Tokyo, Japan

POSTER SESSION - Friday, September 15, 1995

453. P44 COMPARISON OF CONVENTIONAL VERSUS SONOGRAPHY-GUIDED FINE NEEDLE ASPIRATION OF THYROID NODULES.
D. Danese, S. Sciacchitano, R. Bocale, D. Pesaresi, M. Andreoli and A. Pontecorvi
Forensic Medical Institute of Rome, Italian Air Force; Department of Experimental Medicine, "La Sapienza" University of Rome;Institute of Medical Pathology, Catholic University, Italy

454. P45 CALCITONIN HORMONE RESERVE IN AUTOIMMUNE THYROIDITIS.
L.A. Verbruggen, B. Velkeniers, J.J. Body, E. Finné and L. Vanhaelst
Department of Rheumatology and Endocrinology, AZ-VUB and Service de Médecine et Laboratoire d'Investigation Clinique, Institut Bordet, ULB, Universities of Brussels (VUB-ULB), Brussels, Belgium

455. P46 ACUTE SPONTANEOUS HEMORRHAGIC DEGENERATION OF THE THYROID NODULE WITH SUBACUTE THYROIDITIS-LIKE SYNDROME.
T. Mizokami, K. Okamura, T. Hirata, K. Yamasaki, K. Sato, H. Ikenoue, and M. Fujishima
Second Department of Internal Medicine, Faculty of Medicine, and Division of Diagnostic Ultrasound, Department of Radiology, Kyushu University, Fukuoka, Japan

456. P47 FINE NEEDLE ASPIRATION BIOPSY ON NODULAR THYROID DISEASE. FOUR YEARS OF EXPERIENCE IN A GENERAL TEACHING HOSPITAL.
JJ Castro, F. Baptista, L. Lopes, R. Madureira, E. Oliveria, A.C. Fernandes and A. Galvão-Teles
Endocrine Unit and Pathology Department of Sta. Maria Hospital, University of Lisbon, Portugal

457. P48 DEVELOPMENT OF A SOFT TISSUE ABSCESS IN THE NECK: A COMPLICATION OF FINE NEEDLE ASPIRATION OF THE THYROID IN A BACTEREMIC PATIENT.
D.H. Sarne
University of Illinois at Chicago Medical Center, Chicago, Illinois USA

458. P49 SUBCLINICAL AUTOIMMUNE THYROIDITIS AS A DETERMINANT FOR RAPID CYCLING OF BIPOLAR PSYCHOSIS.
H.A. Drexhage, H.A.P.C. Oomen and A.J.M. Schipperijn
Department of Immunology, Erasmus University, Rotterdam; Langeveld Center for Psychiatry, Noordwijk, The Netherlands

459. P50 THE VALUE OF ASPIRATION NEEDLE BIOPSY IN PREOPERATIVE EVALUATION OF THYROID NODULES.
A. Carpi, E. Ferrari and G. Di Coscio
Departments of Internal Medicine and Pathology, University of Pisa, Pisa, Italy

Clinical - 5

460. P51 ALTERATIONS IN THYROID FUNCTION DURING AND AFTER CARDIOPULMONARY BYPASS IN PEDIATRIC PATIENTS: CLINICAL IMPLICATIONS.
B. Murzi, G. Iervasi, R. Moschetti, V. Vanini, S. Berti, A. Clerico, L. Salutini, S. Masini, and A. Biagini
CNR Institute of Clinical Physiology, Pisa, Italy

461. P52 THE EVOLUTION AND SIGNIFICANCE OF VENTRICULAR LATE POTENTIALS PARAMETERS DURING THYROID REPLACEMENT TREATMENT IN HYPOTHYROIDISM.
S.A Kontoyannis, D.A. Kontoyannis, G.D. Piperingos, A. Kalabalikis, P. Konaxi, and D.A. Koutras
Athens University, Department of Clinical Therapeutics, Alexandra General Hospital, Athens, Greece

462. P53 A NOVEL 4-BASE PAIR DELETION MUTATION OF GSα GENE IN A JAPANESE PATIENT WITH PSEUDOHYPOPARATHYROIDISM.
M. Yokoyama, K. Takeda, K. Iyota, M. Sasaki and K. Hashimoto
2nd Department of Internal Medicine, and Clinical Laboratory Medicine, Kochi Medical School, Nankoku, Japan

463. P54 DIRECT DETERMINATION OF FREE TRIIODOTHYRONINE (T_3) IN UNDILUTED SERUM BY EQUILIBRIUM DIALYSIS/RADIOIMMUNOASSAY (RIA).
Philip Taing, Lance Mikus and Inder J. Chopra
Department of Medicine, UCLA Center for Health Sciences, Los Angeles, CA USA

464. P55 THYROID HORMONES DO NOT ONLY REGULATE CHOLESTEROL, BUT ALSO TRIGLYCERIDE METABOLISM.
H. Engler and W.F. Riesen
Institute for Clinical Chemistry and Hematology, Kantonsspital, St. Gallen, Switzerland

POSTER SESSION - Friday, September 15, 1995

465. P56 THYROID DYSFUNCTIONS AFTER ALLOGENIC BONE MARROW TRANSPLANTATION WITHOUT THE USE OF PREPARATIVE TOTAL BODY IRRADIATION.
M.E. Toubert, G. Socié, S. Aractingi, A. Devergie, P. Ribaud, H. Bourdeau, N. Parquet, E. Gluckman and P. Vexiau
Services de Médecine Nucléaire, de Greffe de Moëlle, de Dermatologie, d 'Endocrinologie Hôpital Saint-Louis, Claude Vellefaux, Paris, France

466. P57 LEAN BODY MASS AS A DETERMINANT OF THYROID VOLUME IN HEALTHY ADULTS IN A NON-IODINE DEFICIENT AREA.
MFT Wesche and NJ Smits
Academic Medical Center, Depts. of Endocrinology and Radiodiagnostics, University of Amsterdam, Amsterdam, The Netherlands

467. P58 ULTRASONOGRAPHIC VERSUS SCINTIGRAPHIC MEASUREMENT OF THYROID VOLUME IN THYROID PATIENTS.
MMC Tiel-van Buul, MFT Wesche, NJ Smits and WM Wiersinga
Academic Medical Center, Depts. of Nuclear Medicine, Endocrinology and Radiodiagnostics, University of Amsterdam, The Netherlands

468. P59 CHANGES OF THYROID FUNCTION BY PROPHYLACTIC APPLICATION OF THYROSTATIC DRUGS IN PATIENTS WITH AUTONOMY DURING IODINE CONTAMINATION.
M. Hüfner, R. Müller, W. Nolte and D. Emrich
Department of Internal Medicine and Nuclear Medicine, University of Göttingen, Germany

469. P60 HYPROTHYROIDISM WITH LONG-STANDING GOITER AND RESISTANCE TO REPLACEMENT THERAPY.
M.-F. Langlois and D. Bellabarba
Service d'endocrinologie, Centre Hospitalier Universitaire, Sherbrooke, Québec, Canada

470. P61 COMPUTER SYSTEM FOR SEMIAUTOMATIC EVALUATION OF RELATIVE VOLUMES OF HISTOLOGICAL COMPARTMENTS IN THE THYROID GLAND.
M. Klencki, D. Slowińska-Klencka and A. Lewiński
Department of Thyroidology and Department of Experimental Endocrinology and Hormone Diagnostics, Institute of Endocrinology, The University School of Medicine at Łódź, Poland

471. P62 OBSTACLES TO REDUCED LENGTH-OF-STAY FOLLOWING THRYOIDECTOMY.
F.D. Moore, Jr.
Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts USA

472. P63 SURGERY FOR ALL: IS IT WISE TO DO MAJOR SURGERY IN REMOTE AFRICA?
T.S. Harrison, D. Downing, J.F. Seaton, J. Teeter, M. Teeter, A.O. Longombe, P. Bourdoux, D.G. Koivunen and G.W. Geelhoed
Pennsylvania State University, Hershey Medical Center, Hershey, Pennsylvania, USA and University of Brussels, Belgium

473. P64 A CASE OF THYROID HISTIOCYTOSIS X WITH SEVERE IMMUNODEFICIENCY.
S. Kitahama, M. Iitaka, J. Ishii, S. Murakami and Y. Simizu
Saitama Medical School, Saitama, Japan

474. P65 SERUM CONCENTRATIONS OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) DETERMINED BY A HIGHLY-SENSITIVE CHEMILUMINESCENT ASSAY IN THYROID DISEASES.
S. Sakane, Y. Murakami, Y. Yamano and N. Ohsawa
First Department of Medicine, Osaka Medical College, Takatsuki; The St. Maria Hospital, Ibaraki; The Kurna Hospital, Kobe, Japan

475. P66 THE SUSPENSORY LIGAMENT OF BERRY: ITS RELATIONSHIP TO THE RECURRENT LARYNGEAL NERVE.
S. Sasou and S. Nakamura
Division of Pathology, Iwate Medical University, Morioka, Iwate, Japan

POSTER SESSION - Friday, September 15, 1995

Clinical - 6

476. P67 TSH-RECEPTOR EXPRESSION AND HUMAN THYROID DISEASE: RELATION TO CLINICAL AND ENDOCRINE PARAMETERS.
F. Schuppert, S. Deiters, G.F.W. Scheumann, H. Dralle and A. von zur Mühlen
Department of Clinical Endocrinology, Department of Abdominal and Transplant Surgery, Hannover Medical School, Hannover,Germany; Center for Surgery I, Halle-Wittenberg University, Halle/Saale, Germany

477. P68 RADIOIODINE TREATMENT OF THYROID CARCINOMA IN PATIENTS ON DIALYSIS FOR CHRONIC RENAL FAILURE.
Ch. Daumerie, S. Vynckier, J. Caussin, M. Jadoul, J.P. Squifflet and A. Wambersie
Cliniques Universitaires St Luc, Brussels, Belgium

478. P69 ASSESSMENT OF REMISSION IN GRAVES' DISEASE BY MEASUREMENT OF THYROID ARTERY BLOOD FLOW USING ULTRASOUND PULSE DOPPLER
M. Taniyama, H. Nagakura, A. Kawauchi, M. Kaname and Y. Ban
Depts. of 3rd Internal Medicine, Surgery, and Clinical Pathology, Showa University, Tokyo, Japan

479. P70 LACK OF STIMULATION BY THYROTROPIN RECEPTOR ANTIBODY OF NORMAL THYROID GLANDS IN MOST FETUSES AT TERM.
J.Y. Noh, A. Nagata, N. Momotani, and K. Ito
Ito Hospital, Shibuya-ku, Tokyo; Yamasa Shoyu Co., Choshi, Chiba, Japan

480. P71 GRAVES' HYPERTHYROIDISM, WHICH WAS AGGRAVATED AFTER DELIVERY, SUBSEQUENTLY AMELIORATES TO ITS ORIGINAL STATUS.
Y. Gomi, N. Momotani and N. Ishikawa
Ito Hospital, Tokyo, Japan

481. P72 IS COMPENSATED RADIOIODINE THERAPY OF HYPERTHYROIDISM WORTHWHILE?
A.E. Jarløv, L. Hegedüs, L.Ø. Kristensen, B. Nygaard and J.M. Hansen
Department of Internal Medicine and Endocrinology and Ultrasound, Herlev Hospital, Herlev; Department of Internal Medicine and Endocrinology, Odense University Hospital, Odense, Denmark

482. P73 THE DETECTION OF REVERSE TRANSCRIPTASE IN THYROID GLAND OF GRAVES' DISEASE.
A. Nakai, A. Nagasaka, N. Oda, Y. Sawai, N. Hayakawa, M. Kotake, M. Itoh and S. Yoshida
Dept. of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Aichi, Japan; Res. Inst. Dis. Mech. Cont., Nagoya University School of Medicine, Showa-ku, Nagoya, Japan

483. P74 GROWTH HORMONE (GH) RESPONSE TO GH-RELEASING PEPTIDE-6 (GHRP-6) IN HYPERTHYROIDISM.
J.C.Ramos-Dias, E.T. Kimura, F. Pimentel Fo, A.F. Reis, L.J. Machado and A.M.J.Lengyel
Division of Endocrinology, Universidade Federal de São Paulo UNIFESP/EPM, São Paulo, Brazil

484. P75 THE EFFECT OF PSYCHOLOGICAL FACTOR ON THE PROGNOSIS OF ANTITHYROID DRUG-TREATED GRAVES' DISEASE PATIENTS.
A. Fukao, M. Ito, S. Hayashi and H. Sato
First Department of Medicine, Osaka Medical College, Takatsuki, and Department of Mental Health, School of Hygienic Sciences, Kitasato University, Sagamihara, Japan

485. P76 HYPERTHYROIDISM SECONDARY TO PITUITARY TUMOR CO-SECRETING THYROTROPIN AND PROLACTIN--REPORT OF A CASE.
Y.M. Song and W.H. Lin
Division of Endocrinology/Metabolism, Taichung Veterans General Hospital, Taiwan, Republic of China

486. P77 LATE CHANGE OF THYROID FUNCTION AND IMMUNOLOGICAL PARAMETERS AFTER SUBTOTAL THYROIDECTOMY FOR GRAVES' DISEASE.
Y. Kasuga, S. Sugenoya, S. Kobayashi and M. Maruyama
Department of Surgery, Shinshu University School of Medicine, Matsumoto, Japan

487. P78 CONVERSION OF FUNCTION OF TSH-RECEPTOR-ANTIBODIES IN A PATIENT WITH MYASTHENIA GRAVIS.
M. Weissel
Third Medical University Clinic, General Hospital Vienna, Vienna, Austria

POSTER SESSION - Friday, September 15, 1995

488. P79 DIFFERENTIAL DIAGNOSIS FOR A THYROTOXIC RELAPSE IN THE POSTPARTUM PERIOD IN GRAVES' DISEASE USING ANTITHYROID ANTIBODY MEASUREMENT BY RADIOIMMUNOASSAY.
T. Shimizu, Y. Daimon, M. Kitano, Y. Yamazaki, Y. Umezu, Y. Arakawa and Y. Shishiba
Toranomon Hospital, Tokyo, Japan

489. P80 SIGNIFICANT CHANGES IN THYROID FUNCTION BEFORE AND AFTER A FOUR WEEK VACATION PERIOD.
W. Reinhardt, E. Freygang, G. Kummer, M. Gosselink, F. Jockenhövel, D. Reinwein and K. Mann
Medical Clinic, Division of Endocrinology, University of Essen,
Essen and Nordseeklinik Borkum, Borkum, Germany

Thyroid Hormone Metabolism - 7

490. P81 THERMOGENIC EFFECTS OF TRIAC IN CULTURED BROWN ADIPOCYTES.
M.-J. Obregón and A. Hernández
Inst. Investigaciones Biomédicas (CSIC), Madrid, Spain

491. P82 PLASMA T3 AND T4 ARE MORE HIGHLY CORRELATED WITH TISSUE T3 CONCENTRATIONS THAN PLASMA TSH LEVELS, IN THYROID HORMONE-INFUSED THRYOIDECTOMIZED RATS.
H.F. Escobar-Morreale, F. Escobar del Rey and G. Morreale de Escobar
Institute de Investigaciones Biomédicas, CSIC & UAM, Madrid, Spain

492. P83 3,5,3'-TRIIODOTHYRONINE SULFATE (T3S) CONCENTRATIONS IN PREMATURE AND FULL-TERM NEWBORNS.
F. Santini, P. Lapi, P. Ghirri, E. Fiore, G. Bendinelli, D. Taddei, M. Ciampi, R. Centoni, F. Lippi, I.J. Chopra and L. Chiovato
Institute of Endocrinology and Department of Neonatology, University of Pisa, Italy; UCLA,
Los Angeles, CA USA

493. P84 1.4-DIHYDROPYRIDINES AS T3-ANTAGONISTS - FURTHER EVIDENCE FOR "MOLECULAR MIMICS"?
G.H. Scholz, S. Vieweg, M. Uhlig, S. Kistner, H.J. Hofmann, S. Goldmann, D.K. Chalmers, S.L.A. Munro, D.J. Topliss, and J.R. Stockigt
Department of Internal Medicine III, Department of Biosciences, University Leipzig, Bayer AG Leverkusen, Germany; Vic. College Pharmacy (Monash University) and Downie Metabolic Unit, Alfred Hospital, Melbourne, Australia

494. P85 REGULATION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR BY THYROID HORMONE AND AMIODARONE IN HEPG2 CELLS.
F. Hudig and O. Bakker
Dept. of Endocrinology, Academic Medical Centre, University of Amsterdam, The Netherlands

495. P86 DEMONSTRATION OF THYROMIMETIC EFFECTS OF 3,5,3'- TRIIODOTHYRONINE SULFATE (T_3S) IN EUTHYROID RATS.
Inder J. Chopra and Davis Nguyen
Department of Medicine, UCLA School of Medicine, Los Angeles, CA USA

496. P87 SOURCE OF TRIIODOTHYRONINE SULFATE (T_3S) IN MAN.
J.S. LoPresti, S.J. Eng and J.T. Nicoloff
Department of Medicine, USC School of Medicine, Los Angeles, California USA

497. P88 DIFFERENTIAL EFFECTS OF HORMONES OF THE ADRENAL AXIS ON THYROID FUNCTION IN EMBRYONIC CHICKENS.
V.M. Darras, S. Kotanen, K.L. Geris and E.R. Kühn
Zoological Institute, Catholic University of Leuven, Belgium

498. P89 EFFECTS OF CYCLIC NUCLEOTIDES AND PHOSPHODIESTERASE INHIBITOR ON TYPE II IODOTHYRONINE 5'- DEIODINASE ACTIVITY IN CULTURED RAT BRAIN MIXED GLIAL CELLS.
A. Gondou, Y. Ogawa and M. Yoshimura
Second Department of Internal Medicine, Kansai Medical University, Moriguchi, Osaka, Japan

POSTER SESSION - Friday, September 15, 1995

499. P90 CHANGES IN PROTEIN LEVEL AND ENZYME ACTIVITY OF PROTEIN DISULFIDE ISOMERASE/MEMBRANE-ASSOCIATED THYROID HORMONE BINDING PROTEIN.
T. Mori, R. Gemma, H. Natsume, R. Horiuchi and T. Yoshimi
Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; Gumma University School of Medicine, Maebashi, Gumma, Japan

500. P91 THYROID HORMONE CONCENTRATIONS AND SERUM T_4 BINDING CHARACTERISTICS IN THE KOALA (PHASCOLARCTOS CINEREUS).
Veronica Lawson, Andrea J. Curtis, Frank Carrick and John W. Barlow
Department of Zoology, University of Queensland, St. Lucia, Qld. Australia; Ewen Downie Metabolic Unit and Monash University Department of Medicine, Alfred Hospital, Melbourne, Vic. Australia

501. P92 EARLY EFFECTS OF NEONATAL DIABETES ON THYROID HORMONE DEPENDENT PARAMETERS IN THE RAT.
D. van der Heide, J.P. Schroder-van der Elst, P.M. Versloot, T.C. Viets, M.E. van Bakel, G.J.E.J. Hooiveld and I.H.C. Vos
Human and Animal Physiology, Agricultural University, Wageningen; Endocrinology, University Hospital, Leiden, The Netherlands

502. P93 UPTAKE OF TRIIODOTHYRONINE(T_3) IN THE RAT KIDNEY IS INHIBITED BY MALEIC ACID.
E.J. Rolleman, H.F. Bernard and M. de Jong
Depts. Internal Medicine III and Nuclear Medicine, Erasmus University Medical School, Rotterdam, The Netherlands

503. P94 SIGNIFICANCE OF LIVER VISUALISATION ON 131 I TOTAL BODY SCANS IN FOLLOW-UP OF THYROID CARCINOMA.
M.L. Maayan, G.C. Schussler, S.D. Sarkar, E.M. Lopez, and M.S. Axelrod
Veterans Affairs Medical Center and State University of New York Health Science Center, Brooklyn, NY USA

504. P95 LACK OF EFFICACY OF REVERSE T3 FOR DIFFERENTIATING BETWEEN HYPOTHYROIDISM AND EUTHYROIDISM IN ILLNESS.
L.A. Burmeister
University of Pittsburgh School of Medicine, Pittsburgh, PA USA

505. P96 RECOMBINANT CHICKEN PROLACTIN DECREASES PLASMA CONCENTRATIONS OF THYROXINE AND INCREASES HEPATIC INNER RING DEIONATING TYPE III ACTIVITY IN THE CHICK EMBRYO.
E.R. Kühn, K. Shimada, L.M. Vleurick, L.R Berghman and V.M. Darras
Zoological Institute, Catholic University Leuven, Belgium and Faculty of Agriculture, Nagoya University, Japan

Thyroid Hormone Action - 8

506. P97 MARKERS OF BONE METABOLISM IN PATIENTS WITH GRAVES' HYPERTHYROIDISM AND THE EFFECTS OF ANTI-THYROID DRUG THERAPY.
S-W Kuo, W-K Liao and S-L Su
Division of Endocrinology and Metabolism, Tri-Service General Hospital, Taipei, Taiwan

507. P98 EFFECT OF THYROID HORMONE ON CARBONIC ANHYDRASE I CONCENTRATION IN HUMAN ERYTHROLEUKENIC CELLS AND ERYTHROID BURST FORMING UNIT DERIVED CELLS.
Y. Kiso, K. Yoshida, N.Sayama, H. Fukazawa, K. Kikuchi, Y. Aizawa, H. Hori and K. Abe
The Second Department of Internal Medicine and Department of Cinical Biology and Hormonal Regulation, Tohoku University School of Medicine, Sendai, Japan

508. P99 FUNCTIONAL PROPERTIES OF MUTANT T 3 RECEPTOR (R338W) IDENTIFIED IN PATIENTS WITH PITUITARY RESISTANCE TO THYROID HORMONE.
H. Nakamura, S. Sasaki, S. Andoh, T. Tagami, K. Nishiyama, R. Kitahara and T. Yoshimi
Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; Kyoto University School of Medicine, Kyoto, Japan

509. P100 TISSUE DISTRIBUTION OF HUMAN T_3 INDUCIBLE GENE (I-4) PRODUCTS AND THE EFFECT OF THYROID AND GLUCOCORTICOID HORMONES ON ITS EXPRESSION.
T. Miyazaki,Y. Murata, S. Refetoff, and H. Seo
Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan;
The University of Chicago, Chicago, Illinois USA

POSTER SESSION - Friday, September 15, 1995

510. P101 POST-TRANSCRIPTIONAL REGULATION OF EGF mRNA: AU-BINDING PROTEINS AND OTHER TRANSACTING FACTORS INTERACT WITH THE 3' UTR OF EGF TRANSCRIPTS.
Lowell G. Sheflin, Elizabeth M. Brooks and Stephen W. Spaulding
Buffalo VAMC and SUNY at Buffalo, Buffalo NY USA

511. P102 A CARBOXY TERMINAL PEPTIDE FROM THE THYROID HORMONE RECEPTOR β1 BLOCKS T3 BINDING TO THYROID HORMONE RECEPTORS AND INDUCES CONFORMATIONAL AND DNA BINDING CHANGES SIMILAR TO T3.
L.R. Goldberg, M Abboud and L.D. Madison
Northwestern University Medical School, Chicago, IL USA

512. P103 EXPRESSION OF THYROID HORMONE RECEPTOR SUBTYPES α1, α2 AND β1 IN THE LIVER OF THE FASTING RAT.
O. Bakker, H. Razaki and C. Ris-Stalpers.
Departments of Endocrinology and Pediatric Endocrinology, Academic Medical Centre, Amsterdam, The Netherlands

513. P104 CHANGES IN PORCINE HEPATIC THYROID RECEPTOR RNA DURING EXTENDED EXPOSURE TO THERMAL EXTREMES.
H.L. Reed, J. Anderson, Y.Y Djuh and F. Carr
Departments of Clinical Investigation and Medicine, Walter Reed Army Medical Center, Washington, D.C. USA

514. P105 HUMAN GRANULOSA CELLS AND OOCYTES EXPRESS MULTIPLE THYROID HORMONE RECEPTOR ISOFORM mRNAs.
S.S. Zhang, A.J. Carrillo and D.S. Darling
Dept. of Biological and Biophysical Science, and Dept. of OB/GYN, University of Louisville, and Alliant Health System, Louisville, KY USA

515. P106 DEMONSTRATION OF INHIBITION OF THYROXINE BINDING TO BOTH THYROXINE BINDING GLOBULIN AND FDH-ALBUMIN IN A PATIENT WITH FAMILIAL DYSALBUMINEMIC HYPERTHYROXINEMIA (FDH).
B.N. Premachandra, J. Wortsman and I.K. Williams
VA Medical Center and Washington University, St. Louis, Missouri and SIU School of Medicine, Springfield, Illinois USA

516. P107 CHANGES IN GLUT2 IN THE LIVER OF HYPERTHYROID RAT (STUDY ON GLUCOSE TRANSPORTER IN SECONDARY DIABETES).
T. Mokuno, K. Shimazaki, Y. Itoh, N. Oda, Y. Nishida, K. Asano, N. Hayakawa, M. Hamada, R. Masunaga, S. Kato and T. Kawabe
Dept. of Internal Med. Fujita Health Univ. School of Med., Toyoake, Aichi, Japan

AFTERNOON SESSION - Friday, September 15, 1995

2:30 P.M. **POSTER DISCUSSIONS**

Simco/Duffen Room
AUTOIMMUNITY - 1
DISCUSSION LEADER - DR. KASAGI

City Hall Room
CANCER - 2
DISCUSSION LEADER - DR. GOSLINGS

Civic Room
CELL BIOLOGY - 3
DISCUSSION LEADER - DR. KOHN

Dominion Ballroom South
CLINICAL - 4
DISCUSSION LEADER - DR. RIDGWAY

Essex Room
CLINICAL - 5
DISCUSSION LEADER - DR. LOPEZ-CARRASCO

Dominion Ballroom North
CLINICAL - 6
DISCUSSION LEADER - DR. TAKAMATSU

Windsor East Room
THYROID HORMONE METABOLISM - 7
DISCUSSION LEADER - DR. SILVA

Windsor West Room
THYROID HORMONE ACTION - 8
DISCUSSION LEADER - DR. NAKAMURA

3:30 P.M. **INTERMISSION - COFFEE, POSTERS, EXHIBITS - SHERATON HALL**

4:00 P.M. **SYMPOSIA - SIMULTANEOUS SESSIONS**

GRAND BALLROOM WEST & CENTER

"Advances in thyroid hormone metabolism"

Dr. Josef Kohrle
Med Poliklinik Wurzburg, Germany
Dr. Jack Leonard
University of Massachusetts Medical Center, Worcester, MA USA
Dr. Donald St. Germain
Dartmouth Medical School, Lebanon, NH USA
Dr. Theo J. Visser
Erasmus University Medical School, Rotterdam The Netherlands

Chairpersons - Drs. Nicoloff & Nishikawa

AFTERNOON SESSION - Friday, September 15, 1995

4:00 P.M. **SYMPOSIA - SIMULTANEOUS SESSIONS**

GRAND BALLROOM EAST

"TSH receptor and autoimmunity"

Dr Marian E. Ludgate
Universite Libre de Bruxelles, Belgium
Dr. Toshimasa Onaya
University of Yamanashi Medical School, Yamanashi-ken, Japan
Dr. Basil Rapoport
University of California, San Francisco, CA USA

Chairpersons - Drs. Heufelder & Shishiba

5:30 P.M. **CLOSING CEREMONIES**

MONDAY MORNING ORAL PRESENTATIONS

9:00 A.M. 1. **CLONING OF A PROTEIN, SOX-4, INVOLVED IN METHIMAZOLE (MMI), INSULIN, AND THYROTROPIN (TSH) REGULATION OF THE MAJOR HISTOCOMPATIBILITY (MHC) CLASS I AND THYROGLOBULIN (TG) GENES.** K. Suzuki, H. Shimura, G. Napolitano, V. Montani, C. Giuliani, Y. Shimura, M. Ohmori, M. Ohta, D.S. Singer and L.D. Kohn. NIH, Bethesda, MD, USA; Chieti University Medical School, Chieti, Italy; and Yamanashi School of Medicine, Yamanashi, Japan.

The TG insulin-response site (IRE) interacts with more than one protein. One, thyroid transcription factor (TTF)-2, is thyroid-specific. The other is ubiquitously expressed and regulates an upstream silencer/enhancer complex of the MHC class I promoter, important for the determination of class I levels in different tissues; in the thyroid, it is important for insulin-dependent TSH- and MMI-induced decreases in MHC class I levels. We have cloned the latter protein and confirm, by Northernanalysis, it is present in many tissues. The protein, termed Sox-4, is a high mobility group protein which regulates DNA structure. Like TTF-2, it binds to the IRE of the TG and thyroid peroxidase (TPO) promoters, footprints the IRE of the TG promoter, and does not bind to oligonucleotides with the structure of the TTF-1 or Pax-8 sites of the TG, TPO, or TSHR promoters. As with TTF-2, complex formation with the TG IRE is increased by insulin; unlike TTF-2, complex formation is decreased by TSH in the presence of insulin. Insulin increases complex formation with the upstream silencer of the class I promoter; insulin plus TSH decreases complex formation. The basis for both effects appears to be the ability of insulin to increase Sox-4 mRNA levels, whereas TSH plus insulin decreases them. Antisera to a peptide of Sox-4 indicate that Sox-4 interacts with both the silencer and enhancer. The silencer complex with which Sox 4 interacts is an adduct with the p65 subunit of NF-κB; the enhancer complex involves Sox-4 and c-jun. MMI decreases complex formation with the former. We suggest this protein is important for the regulation of class I levels in different tissues. In the thyroid, its activity is normally regulated by TSH and is important for self-tolerance during TSH-induced changes in growth and function which increase the expression of many proteins, i.e. TG or TPO. Thus, insulin/TSH-induced increases in TG or TPO, mediated by TTF-2 and TTF-1/Pax-8, would be accompanied by an insulin/TSH-induced decrease in class I involving Sox-4. In autoimmune disease, it is one of the targets of MMI action to decrease MHC class I expression and reverse the immune response. MMI/TSH regulation of Sox-4 functions coordinately with MMI/TSH regulation of TSEP-1 which acts on a downstream 48 bp silencer in the class I promoter; we have cloned TSEP-1 and characterize its action separately. We propose that TSH/MMI action to decrease Sox-4 acts like a clutch, disengaging the upstream silencer and engaging the TSEP-1-regulated downstream silencer; this action reflects the ability of Sox-4 to alter DNA structure.

9:15 A.M. 2. IODIDE-INDUCED B CELL AUTOIMMUNE REACTION IN INTERLEUKIN 9 (IL9) TRANSGENIC MICE. M-C. Many, J-C. Renauld*, J-F. Denef. Histology Unit and *Experimental Medicine Unit, Ludwig Institute for Cancer Research, Catholic University of Louvain, Brussels, Belgium.

The administration of a high dose of iodide to goitrous mice initiates an immune reaction with thyroidal infiltration of numerous Ia+ antigen presenting cells and T cells. In most strains of mice (Balb/c, C3H, CBA), this reaction is transient, whereas in autoimmune prone Non Obese Diabetic (NOD) mice, it persists, and a destructive Hashimoto's thyroiditis is obtained after 64-96 days. Ia+ macrophages, CD4+ T helper (T_H) cells and CD8+ T cytotoxic (T_C) cells are predominant, while B cells are found later. In this study, we analyzed the effects of iodide in IL9 transgenic (IL9-TG) mice. IL9 is produced by T_H cells of TH2 type, also producing IL4, IL5, and regulating the B cell response. IL9 is a T and mast cells growth factor, and potentiates the IL4-induced Ig production by murine peripheral B cells.

Two-month-old female control (FVB) and IL9-TG mice, expressing high levels of biologically active IL9 without tissue specificity, were divided into four groups (n = 5). The untreated group was fed a standard diet (1 µg I/day). The goitrous group received a low iodine diet (LID: 0.1 µg I/day) plus 0.25% PTU for 10 days and LID alone for 2 days. In the third and fourth groups, goitrous mice received a high iodide dose (10 µg I/day) for 4 and 64 days respectively. Thyroid glands, thyroid and mesenteric lymph nodes were processed for light microscopy and for immunohistochemical typing of inflammatory cells on frozen sections. Blood samples were collected to determine antithyroid autoantibodies (ATAb).

Untreated IL9-TG mice had a normal thyroid morphology as in controls, and a typical hyperplastic goiter was obtained after the goitrogenic treatment. In both IL9-TG and control mice, the administration of the high iodide dose, after withdrawal of the goitrogenic treatment, early induced thyroid cells death, and infiltration of immune cells. These were numerous Ia+ macrophages and T cells, CD4+ T_H cells, among which numerous were activated expressing the receptor for IL2, and CD8+ T_C cells. However, in IL9-TG mice, but not in controls, numerous polymorphonuclear eosinophils and B220+ B cells infiltrated the thyroid interstitium, as soon as after 4 days of iodide treatment. At this time, thyroid lymph nodes of IL9-TG mice showed evident signs of humoral stimulation with enlargement of the cortical zone and formation of germinative centers. ATAb were detected in the serum of IL9-TG mice treated with iodide. They were absent in untreated and goitrous IL9-TG mice as well as in all FVB control mice. After 64 days of iodide treatment, the inflammatory infiltrate was reduced. However, in thyroids of IL9-TG mice, B cells were still more numerous than in controls. Signs of humoral stimulation persisted in the thyroid lymph nodes, and ATAb were still present.

Taken together, our results indicate that overexpression of a TH2 cytokine, namely IL9, provokes a persistent iodide-induced anti-thyroid B cell response, but without development of tissular damages, as observed in autoimmune prone NOD mice. These data raise the hypothesis that the consequences of an anti-thyroid immune response could be dependent on the balance between secretion of TH1 and TH2 cytokines.

9:30 A.M. 3. CYTOKINES AND THYROID EPITHELIAL INTEGRITY: INTERLEUKIN-1 ALPHA INDUCES PARACELLULAR LEAKAGE AND ZO-1 REDISTRIBUTION IN FILTER-CULTURED HUMAN THYROCYTES. L.E. Ericson, J. Husmark, B. Nilsson*, L-E. Tisell*. Institute of Anatomy & Cell Biology/Göteborg University, and *Department of Surgery/Sahlgrenska Hospital, Göteborg, Sweden.

Effects of inflammatory cytokines produced by intrathyroidal lymphocytes and macrophages or the follicular epithelium itself may be pathophysiologic in autoimmune thyroid diseases. Previous studies have mainly considered cytokine effects on the expression of thyroid-specific functions and thyroid growth. Here we have examined the influence of tumor necrosis factor-alpha (TNFa;10ng/ml), interleukin-1 alpha (IL-1a; 10-1000U/ml), IL-6 (100U/ml), interferon-gamma (INFg;100U/ml), and transforming growth factor-beta (TGFb;10ng/ml) on the thyroid epithelial barrier, the maintenance of which is essential to create high concentrations of the components (iodide, thyroglobulin and H_2O_2) used for thyroid hormone synthesis in the follicular lumen. For this purpose, the bicameral chamber culture technique was adopted to human thyrocytes isolated from normal tissue (obtained at surgery of follicular adenomas) and toxic, diffus goitre (Graves' disease).

Culture. Isolated, open thyroid follicles were seeded on collagen-coated permeable filter of Transwells and grown to confluence in EMEM, 5H or 6H media with 5% FCS. The monolayer cultures developed a transepithelial resistance (500-2000 $\Omega \times cm^2$) which was promoted by 6H. Immunoreactivities of ZO-1 and E-cadherin, components of tight and adherens junctions, respectively, were highly concentrated at the cell borders. Synthesis and apical secretion of thyroglobulin were up-regulated by 6H. Exposure to cytokines (48 h). All cytokines but IL-6 had specific effects on the pattern of apical and basal protein secretion in SDS-PAGE/[^{35}S]methionine autoradiography, and INFg induced HLA-DR expression as well. Only IL-1a abolished the resistance and increased dose-dependently the paracellular permeability to [^{3}H]inulin. IL-1a altered the ZO-1 immunofluorescence from a linear and continuous to a zigzag and partly disrupted appearance. IL-1a decreased thyroglobulin secretion but stimulated the release of several other high and low molecular weight proteins.

In conclusion, the epithelial barrier function of filter-cultured human thyrocyte monolayers is compromised by IL-1a, but not other cytokines, without signs of general cytotoxicity. The tight junction protein ZO-1 may be negatively controlled by IL-1a. The IL-1a effect suggests mechanisms by which transepithelial migration of macrophages into the follicular lumen and leakage of lumenal content may occur in autoimmune thyroid disease.

9:45 A.M. 4. REGULATION OF INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1) EXPRESSION IN HUMAN THYROID TRANSPLANTS IN THE NUDE MOUSE IN VIVO. R. Hoermann, S. Poertl, K. Mann and P. M. Schumm-Draeger*, Division of Endocrinology, Department of Medicine, University of Essen, Essen and *Department of Endocrinology, Center of Internal Medicine, University of Frankfurt, Frankfurt a. Main, Germany.

Adhesion molecules are intimately involved in the interaction of antigen-presenting cells and autoreactive T-cell clones. They may propagate the homing of lymphocytes in the thyroid gland and thereby participate in the induction of autoimmune thyroid disease. We have therefore studied the expression of ICAM-1 in human thyroid tissue transplanted to nude mice. The animals were injected intravenously 8 weeks after the transplantation of normal human thyroid tissue on two consecutive days with single doses of vehicle (saline), bTSH (1 - 10 mIU/animal), Protein A Sepharose purified IgG's from patients with active untreated Graves'disease (0.05 - 1 mg/animal), or a combination of the stimulators and asialoagalacto-hCG (0.1 - 1 mg/animal), an in vivo active human TSH receptor blocker. Experiments were performed in the presence and absence of γ-interferon (200 IU/day), which was continously supplied by an i.p. minipump (Alzet). ICAM-1 expression was evaluated by immunoperoxidase staining of frozen transplant sections with monoclonal ICAM-1 antibody (Dianova). Results are expressed as the percentage of ICAM-1 positive thyroid follicular cells. bTSH had little effect by itself ($5 \pm 2.1\%$, mean ± SD, n = 6; controls < 3%), but did enhance γ-interferon-induced ICAM-1 expression from $35 \pm 4.9\%$ to $51 \pm 4.2\%$. Graves' IgG's (0.05 - 1 mg/animal), on the other hand, were able to increase, in the absence of γ-interferon, the expression of ICAM-1 by thyroid follicular cells in the human transplants in a dose dependent manner from < 3% up to $50 \pm 5.9\%$. Treatment of the animals with asialoagalacto-hCG (1 mg/animal) nearly completely abolished the stimulatory effect of Graves' IgG's on ICAM-1 expression, significantly reduced the combined bTSH/γ-interferon effect and did not affect the action of γ-interferon alone. In conclusion, Graves' immunoglobulins strongly stimulate the expression of ICAM-1 by human thyroid follicular cells in the nude mouse model, as does γ-interferon. The stimulatory action of the immunoglobulins is apparently related to their specific interaction with hTSH receptor. Thus, expression of ICAM-1 by normal thyrocytes, which facilitate their interaction with T-cells, may be brought about by two distinct pathways, either T-cell activation and cytokine release or Graves' immunoglobulins action and TSH receptor activation.

9:00 A.M. 5. ALTERED REGULATION OF THE STIMULATORY G PROTEIN(Gs) -ADENYLATE CYCLASE CASCADE IN DIFFERENTIATED THYROID CARCINOMAS: EVIDENCE FOR UPREGULATION OF INHIBITORY G PROTEIN BY Gs PROTEIN. M. Derwahl, C. Hamacher, G. Papageorgiou* and H. Schatz, Medizinische Universitätsklinik Bergmansheil, 44789 Bochum, Germany and Evangelismos Hospital Athens*, Greece.

We have recently reported on the overexpression of functional Gsα protein in nonfunctioning thyroid adenomas (1) and differentiated thyroid carcinomas. Unexpectedly, we had found that high levels of ADP-ribosylated Gsα protein were not correlated neither to the basal nor to the TSH-stimulated adenylate cyclase activities. To investigate this discrepancies we extended our studies to analyses of the expression of ß-gamma complex and functional inhibitory G proteins ($Gi\alpha_1$ and $_2$). We isolated membranes from tissue of 15 different thyroid carcinomas (13 papillary and 2 follicular carcinomas) and studied pertussis toxin-catalyzed ADP-ribosylation of Giα proteins and basal and TSH-stimulated adenylate cyclase activities. To analyze regulatory aspects of Gsα expression we additionally performed studies with specific Gsα and Giα antisense oligodeoxynucleotides in HTC-TSHr thyroid carcinoma cells. We found high expression of Giα in all tumors with high Gsα expression, but unchanged expression of ß-gamma complex. There was a significant correlation between expression of functional Gsα and functional Giα in carcinoma tissues. As in thyroid cancer tissue we also found both high expression of cholera toxin-catalyzed ADP-ribosylated Gsα protein and of pertussis toxin-catalyzed ADP-ribosylated Giα protein in HTC-TSHr thyroid carcinoma cells. Treatment of cells with Gsα antisense oligodeoxynucleotides decreased expression of both Gsα and Giα protein and TSH-stimulated adenylate cyclase activities. In contrast, treatment with Giα antisense oligodeoxynucleotides only decreased Giα, but not Gsα expression and increased TSH-stimulated adenylate cyclase activities. We conclude that in thyroid cancers Giα protein, at least in part, antagonizes the effect of high Gsα protein levels on adenylate cyclase activities that are normal or only moderately increased. Our data aadditionally indicate that high levels of functional Gsα protein upregulate expression of functional Giα protein in differentiated thyroid cancers.

Hamacher C, Studer H, Zbaeren J, Schatz H, Derwahl M J Clin Endocrinol Metab (in press 5-95)

Supported in part by a grant of Dr. Mildred-Schell-Stiftung for Cancer Research.

9:15 A.M. 6. NEW MECHANISMS OF ESCAPE FROM IMMUNOSURVEILLANCE OF THYROID CARCINOMA CELLS: INVOLVEMENT OF PKC PATHWAY.

V. Bassi*, S. De Riu*, M. Vitale°, E. Carbone°, M. Maio^, G. Rossi°, GF. Fenzi. Dipartimento di Endocrinologia ed Oncologia Clinica e Molecolare*, Dipartimento di Biologia e Patologia Cellulare e Molecolare°, Università di Medicina e Chirurgia, "Federico II", Napoli; Immunotherapeutics Advanced Unit, CRO, Aviano^.

Protein kinase-C (PKC) pathway, able to increase proliferation and dedifferentiation in thyroid cells in vitro, seems to be involved in thyroid tumor growth and progression. The aim of this study was to investigate new possible mechanisms involved in the escape of thyroid tumors from immunosurveillance. Human anaplastic carcinoma cell line (ARO) and human papillary carcinoma cell lines (NPA and TPC-1) were incubated in RPMI 1640/10% FCS/2 mM L-glutamine with or without TPA, a well known PKC activator. An overnight stimulation with 100 nM TPA was able to increase cluster aggregation between tumor cells and lymphokine-activated killer (LAK) cells, as demonstrated with flow cytometry techniques. A parallel increase in intercellular adhesion molecule-1 (ICAM-1) levels, usually involved in lymphocyte/target cell adhesion, was also observed using flow cytometry. Surprisingly, in TPA-treated tumor cells no increase in LAK-mediated cytotoxicity with a classic ^{51}Cr test was found, but, on the contrary, a marked reduction in the percent of lysis in TPA-treated cells as compared to control cells. Therefore, these data suggest that TPA could impair in tumor cells the triggering mechanisms involved in LAK-induced cell lysis, although an increase of cell adhesion was observed. Moreover, spent media of tumor cells treated with 20 nM TPA for different time periods (12, 24 and 48 h) showed significantly higher levels of soluble form of ICAM-1 (measured with an ELISA sICAM-1 kit, Genzyme), usually considered as an active molecule able to induce immunosuppressive effects. In conclusion, these data suggest that PKC pathway may be involved in thyroid tumor growth and/or progression, also by inhibiting in tumor cells the intrinsic ability to be specifically lysed by immune mechanisms. These results may give a possible functional role to the finding of an increased PKC activity in human thyroid tumors in vivo (Hatada et al. Cancer. 1992. 70, 12: 2918).

9:30 A.M. 7. **EVIDENCE FOR PARTIAL GROWTH INHIBITION BY EXCESSIVE AUTOCRINE SECRETION OF TRANSFORMING GROWTH FACTOR α (TGFα) AND PLATELET-DERIVED GROWTH FACTOR B (PDGF-B) IN HTC-TSHr THYROID CARCINOMA CELLS.** M. Bröcker, J. Hammer and M. Derwahl, Medizinische Universitätsklinik, 44789 Bochum, Germany.

Autocrine stimulation of cell growth by growth factors is a hallmark of many carcinoma cells, including thyroid carcinoma cells. We have recently demonstrated that HTC-TSHr thyroid carcinoma cells express EGF and PDGFα and ß receptors. While the former are synthesized also by normal thyroid cells, expression of PDGF receptors is normally restricted to cells of mesenchymal or glial origin. In order to analyse potential autocrine growth stimulation of the cells, we investigated expression of growth factors and their secretion into the culture medium. We additionally treated the cells with growth factor-neutralizing antibodies, tyrosine kinase inhibitors and with EGF, PDGF-AA and -BB. We found that HTC-TSHr cells express TGFα and PDGF-A and -B and secrete these factors into the culture medium suggesting an autocrine stimulation of their corresponding receptors. Interestingly, treatment of the cells with exogenous EGF or PDGF-B resulted in growth inhibition. In contrast, incubation with lavendustin A or tyrphostin 25, two specific tyrosine kinase inhibitors, decreased growth rate of the cells. A more complex picture emerged, when cells were treated with neutralizing antibodies: Low concentrations of anti-TGFα antibodies increased growth rate, while high concentrations of the antibodies inhibited growth. No effect on growth was observed with low concentrations of anti-PDGF-B antibodies, while high concentrations of the antibodies also inhibited growth. Independent of the concentrations anti-PDGF-A antibodies did not affect cellular growth. We conclude that growth of HTC-TSHr thyroid carcinoma cells is partially inhibited by excessive endogenous production of TGFα and probably also of PDGF-B. The failure of low anti-PDGF-B antibody concentrations to stimulate growth, is most likely due to the fact that binding of PDGF to its receptor is, at least in part, an intracellular process that is unaffected by neutralizing antibodies.

9:45 A.M. 8. GLUT1 GLUCOSE TRANSPORTER EXPRESSION IN BENIGN AND MALIGNANT THYROID CELLS: AN IMMUNOHISTOCHEMICAL STUDY. K.R. Weiser, D.E. Burstein, I. Reder, A. Pritsker, and R.S. Haber, Mount Sinai School of Medicine, New York, NY, USA.

Malignant cells exhibit increased rates of glycolysis and glucose uptake, and several types of cancer have been reported to overexpress the GLUT1 glucose transporter. We hypothesized that overexpression of GLUT1 might distinguish malignant from benign thyroid tissue. Formalin-fixed paraffin-embedded archival thyroid tissue obtained at surgery was immunostained for GLUT1 protein using antiserum against the C-terminus peptide of GLUT1 and avidin-biotin-peroxidase immunohistochemistry. To confirm specificity parallel tissue sections were stained using antiserum that had been pre-incubated with the immunizing peptide. There were 31 benign cases (19 follicular adenoma, 1 Hurthle cell adenoma, 6 nodular goiter, 3 lymphocytic thyroiditis, 2 Graves' disease) and 23 cases of thyroid cancer (9 papillary, 4 follicular variant of papillary, 5 follicular, 1 Hurthle cell, 2 anaplastic, 2 medullary). Normal thyroid tissue adjacent to nodules showed no thyrocyte staining in any case. As expected, there was strong specific GLUT1 staining in erythrocyte membranes and in perineurium. No GLUT1 staining was seen in thyrocytes in benign nodular tissue, except for a single case of thyroiditis in which some foci of Hurthle cells showed weak staining. Among the thyroid cancers, 9/23 (39%) showed GLUT1 staining in tumor cells. This included 6/13 cases of papillary carcinoma and its follicular variant, 1/5 cases of follicular carcinoma and 2/2 cases of anaplastic carcinoma. Tumor cell GLUT1 staining was seen in two patterns: focal circumferential plasma membrane staining at the center of tumor cell nests, or asymmetric staining of the basilar aspect of tumor cells adjacent to stroma in some cases of papillary carcinoma . We conclude that GLUT1 protein is frequently overexpressed in thyroid cancer. GLUT1 immunostaining may be potentially useful in the cytologic diagnosis of thyroid nodules.

10:30 A.M.
9. **DETERMINANT SPREADING SHOWN BY MONOCLONAL THYROID STIMULATING ANTIBODY PRODUCED AGAINST A TSH RECEPTOR PEPTIDE.** **H. Sugawa, Y. Ueda, M. Zhang, S. Kosugi, M.Ueda and T. Mori. Kyoto University, Kyoto, Japan**

Since the cloning and sequencing of the TSH receptor (TSHR) cDNA, epitope analyses of TSHR antibody, especially of thyroid stimulating antibody (TSAb), have been studied widely. Site-directed mutagenesis and synthetic TSHR peptide application are the major methods used and there has been a great discrepancy on the understanding of the TSHR region corresponding to amino acid residues 354-367. When a deleted mutant cDNA of this region was transfected into the non-thyroidal cells, TSAb exerted cAMP increase in the cell. On the other hand, most of Graves' IgGs bind this region and further incubation of a peptide 354-367 with Graves' IgGs shows significant inhibition of TSAb activity.

To elucidate the significance of the 354-367 region which contains the "immunogenic peptide (352-366)" of the receptor, we produced monoclonal antibody with TSAb activity and analyzed its characters. One of monoclonal TSAbs (G4) was characterized for its antigenic epitopes on a TSH-R molecule using binding assay to various synthetic peptides. Among 27 kinds of synthetic peptides covering most of extracellular domain of TSH-R, four peptides (P91-129, P172-189, P261-280 and P287-304) in addition to P354-367 showed significant binding to G4. Three kinds of peptides within the residue 354-367 were similarly analyzed, and G4 bound to P358-367 but not to P354-363 and P358-363.

TSAb activity of proteinG-purified G4 IgG was eliminated by absorption with Sepharose column fixed with P354-367, but TSAb activity was recovered in the unbound fraction from a column fixed with glycine or reverse-ordered peptide of residue 355-367. G4 IgG enhanced cAMP production in COS-7 cell transfected with both wild type TSH-R gene and mutated receptor cDNA with deletion of residues 339-367 .

Thus, various anti-TSH-R antibodies were induced by immunizing mice with a synthetic peptide in immunogenic region of TSH-R. One of thyroid stimulatory antibody (G4) recognized several discontinuous regions on a TSH-R molecule including the immunogenic peptide. These results suggest that (1) immunogenic region of TSH-R can induce several kinds of TSH-R antibodies, (2) combination of epitopes recognized by TSH-R antibody decides the final biological activity of the receptor antibody, (3) immunogen of small linear peptide can induce antibodies recognizing conformational structure, which agrees with the concept "Determinant Spreading" (Steinman,Cell 80:7, Kaufman, Nature 355:69, Tisch, Nature 366:72).

10:45 A.M.
10. **EXPERIMENTAL THYROTROPIN RECEPTOR (TSHr) ANTIBODIES: INVESTIGATION OF MECHANISMS BY WHICH THEY CAN INHIBIT TSH-MEDIATED cAMP PRODUCTION BY THYROID CELLS.** J.S. Dallas and S.J. Cunningham. Department of Pediatrics, The University of Texas Medical Branch, Galveston, TX. 77550-0363.

Autoantibodies that inhibit TSH-mediated activation of thyroid cells (TSBAb) have been detected in sera from patients with various thyroid disorders. When tested by radioreceptor assay (RRA), sera which contain TSBAb (TSBAb+) either block (TBII+) or have no effect (TBII-) on TSH binding to the TSHr. In the present study, we used rabbit antibodies with TSBAb activity to further investigate potential mechanisms by which TSHrAb can inhibit thyroid cell function. The antibodies were produced against 2 synthetic peptides corresponding to AA 357-372 (p357) and 367-386 (p367) of the human ETSHr. By ELISA, both antisera (α357 and α367) had high titers (>1:100,000) of IgG against their respective peptides and recombinant ETSHr protein; α357 had a low IgG titer to p367 (<1:800), and α367 had a low IgG titer to p357 (<1:400). Based on competitive inhibition studies, α357 and α367 displayed similar relative binding affinities for their respective peptides and for recombinant ETSHr. When tested by commercial RRA, α357 did not block (TBI index -3.7%), whereas α367 blocked TSH binding to TSHr (TBI index 53.9%). The blocking effect of α367 could be reversed by preincubating the antiserum with p367 before assay. IgG from both α357 and α367 were able to inhibit ($p<0.001$) TSH-mediated cAMP production by FRTL-5 cells (bTSH 2.5×10^{-10}M; cAMP in pmol/ml, **Normal rabbit IgG (NRI)** 62.5±6.1; **α357** 12.2±2.4; **α367** 36.2±3.5). α357 continued to inhibit ($p<0.05$) cAMP production by FRTL-5 cells in 10^{-8}M bTSH, whereas α367 no longer inhibited cAMP production at concentrations $>5\times10^{-10}$M. When compared to NRI, both α357 and α367 were also able to inhibit ($p<0.001$) Graves' IgG-mediated cAMP production by FRTL-5 cells. When IgG were tested on FRTL-5 cells in the presence of 10^{-7}M forskolin, only α357 inhibited ($p<0.001$) cAMP production (**NRI** 75.1±4.8; **α357** 52.3±4.5; **α367** 77.2±1.4). To determine if the inhibitory effect of α357 on forskolin stimulation was thyroid cell-dependent, IgG were tested on CHO cells transfected with cDNA of the hTSHr. Again, α357 inhibited ($p<0.005$) cAMP production by forskolin (at 10^{-7}M; **NRI** 68.7±4.4; **α357** 36.8±5.7; **α367** 64.6±8.6). α357 did not inhibit forskolin-mediated cAMP production in non-transfected CHO cells, indicating that the inhibitory effect of α357 on forskolin stimulation was TSHr-dependent. Neither α357 nor α367 inhibited cholera toxin-mediated cAMP production in FRTL-5 cells. In all relevant bioassays, the inhibitory effects of α357 and α367 could be reversed by preincubating the IgG with respective peptides before assay. We conclude: (1) α367 (TSBAb+/TBII+) binds to the ETSHr and blocks TSH-mediated cAMP production by competitively inhibiting TSH from binding to its receptor; (2) α357 (TSBAb+/TBII-) binds to the TSHr, and without blocking TSH binding, appears to inhibit TSH-mediated cAMP production through a postreceptor mechanism(s) that affects adenylate cyclase activity; and (3) forskolin-mediated cAMP production by thyroid cells can be inhibited by IgG which bind directly to the TSHr.

11:00 A.M. 11. **HETEROGENEITY IN EPITOPE(S) FOR THYROID STIMULATING ANTIBODIES (TSAb) FROM GRAVES' SERA : A POSSIBLE LINK TO DIFFERENCES IN RESPONSES TO ANTITHYROID DRUG TREATMENT.** **Won Bae Kim, Bo Youn Cho, Hae Young Park, Hong Kyu Lee and Chang-Soon Koh. Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.**

To evaluate the clinical significance of heterogeneity in epitope(s) for TSAb in Graves' disease, we measured TSAb in 66 untreated Graves' patients using Chinese hamster ovary (CHO) cells transfected with wild-type human TSH receptor (WT) or human TSH receptor/rat LH-CG receptor chimeras. Substitution of amino acid residue 8-165 of the TSH receptor with the corresponding segment of LH-CG receptor (Mc 1+2) or of residue 90-165 (Mc 2) did not alter the degree of cyclic AMP responses to bTSH as compared with those of WT. But, when measured with Mc 1+2 and Mc 2, IgG's from 86.4% (57/66) and 77.3% (51/66) of Graves' disease patients lost their TSAb activities (positive TSAb with WT), respectively. According to the 'TSAb positivity' with Mc 1+2 or with Mc 2, the Graves' disease patients were divided into two groups, one with 'Mc 1+2 TSAb' - negative and 'Mc 2 TSAb' - negative (Group A, n=45) and the other with 'Mc 1+2 TSAb' - positive or 'Mc 2 TSAb' - positive (Group B, n=21). The patients in group A and those in group B were not different in age, sex distribution, goiter size, prevalence in ophthalmopathy, initial thyroid function parameters, prevalence or titer of thyroid autoantibodies including TBII activities. Those in group B had higher initial TSAb activities than those in group A in both assay with CHO cells (WT), (933.6±705.2 and 533.3±470.7%, respectively, $p<0.05$) and with FRTL-5 cells (336.7±184.7 and 230.5±145.5%, respectively, $p<0.05$). Of those patients treated with antithyroid drug (n=55), excluding patients treated with radioactive iodine in initial period, 11 of 37 (29.7%) in group A failed to respond to antithyroid drugs requiring radioactive iodine therapy in their course but, only one of 18 (5.6%) in group B did (Odd ratio=0.139, $p<0.05$). And the total cumulative dose to render the serum free T4 level normal in patients treated with antithyroid drug only (n=45) was higher in group A than in group B (20.8±9.9 vs. 16.2±3.2 gram, $p<0.05$), even with lower initial (pretreatment) thyroid hormone level and lower TSAb activities in patients in group A. Our results suggest that the region of amino acid residue 90-165 may be more important or that non-continuous region encompassing both N-terminal portion and portion distal to residue 165 is important for receptor stimulation by TSAb in most cases. More importantly, there is heterogeneity in epitope(s) for TSAb and such heterogeneity may be responsible for the differences in natural course or in responses to drug therapy in patients with Graves' diseases.

11:15 A.M. 12. IDENTIFICATION OF BINDING SITE OF TSH AND ANTI-TSH RECEPTOR ANTIBODIES IN AUTOIMMUNE THYROID DISEASES : SIGNIFICANCE OF SUGAR CHAINS AND DISULFIDE BONDS OF THE RECEPTOR ON SIGNAL TRANSDUCTION. T. Yoshida, H. Kameda, K. Arikawa, I. Saito, S. Nagano, Y. Ichikawa*, K. Ito** and Y. Ikeda, Department of Internal Medicine, School of Medicine, Keio University, Tokyo, *St. Marianna University, Kawasaki and **Ito Thyroid Clinics and Hospital, Tokyo, Japan

Anti-TSH receptor antibodies are considered to be involved in the pathogenesis of autoimmune thyroid diseases. To elucidate the structure of the human TSH receptor and binding sites of TSH and the antibodies and the significance of sugar chains and disulfide bonds of the receptor on signal transduction, the receptors were expressed in eukaryotic cells ($CHO\text{-}K_1$), using the pECE vector. Recombinant TSH receptor demonstrated bioactivities as the TSH receptors and activated the adenylate cyclase specifically in response to TSH stimulation and anti-TSH receptor antibodies ($Ka=0.9\times10^{10}/M$, Mr. 108 kDa). In order to ascribe specific bioactivities to the particular region of the receptor and define structure-function relationships, several mutants have been prepared, using deletion with Exonuclease III and site-directed mutagenesis and mutated receptors were also stably expressed. The analysis of the data of the deleted mutants revealed that both stimulatory and inhibitory antibodies bound to the amino-terminal 80 amino acid residues of the receptor and that the bindings were not inhibited by excess TSH. We investigated the role of asparagine (N)-linked glycosylation and disulfide bridges. The receptor mutated at Asn^{113} to Gln displayed lower affinity for TSH ($Ka=0.1\times10^{10}/M$, Mr. 102 kDa) when compared with wild type. In the receptor mutated at Asn^{99}, activities of anti-TSH receptor antibodies to inhibit TSH binding and stimulation decreased. Replacement of Cys with Ser in the extracellular and the second extracellular loop also affected affinity for TSH ($Ka=0.4\times10^{10}/M$, Mr. 108 kDa) and ability to mediated an increase in intracellular cAMP levels. Substitution of amino acid residues at Asn^{113} corresponding to N-glycosylation and disulfide bridge resulted in a significant reduction in the ability to respond with TSH and stimulatory anti-TSH receptor antibodies, but the reduction of the binding activity of antibodies to the mutated receptor of disulfide bridge was less pronounced.

These data suggest that the mechanism of action of anti-TSH receptor antibodies on the receptor might be partially different from that of TSH and that the extracellular region of the receptor and its sugar chains and disulfide bonds might play an important role in signal transduction.

11:30 A.M.
13. HUMAN IgG AUTOANTIBODIES FROM EPSTEIN BARR VIRUS TRANSFORMED B LYMPHOCYTES OF GRAVES' DISEASE PATIENTS: SPECIFIC ANTIBODY TO THYROTROPIN RECEPTOR AS DETERMINED BY IMMUNOPRECIPITATION ANALYSIS AND BIOLOGICAL ACTIVITY. W. A. Scherbaum, N. G. Morgenthaler, M. R. Kim, W. Richter, J. Tremble, G.C. Huang and M Gupta. Medizinische Klinik III, Universitat Leipzig, Leipzig, Germany. Department of Medicine, King's College School of Medicine, London, UK. and The Department of Clinical Pathology, The Cleveland Clinic Foundation, Cleveland, Ohio, USA.

It is well recognised that antibodies to the thyrotropin receptor (TSH-R) in Graves' disease patients are heterogeneous in nature and have multiple effects on thyroid function. The heterogeneity of different antibody specificities together with the low serum concentration of these potent antibodies has made it difficult to analyse the epitopes recognised on the TSH-R. Indeed, it has also proved difficult to reproduce these antibody specificities in xenogeneic immunisations with purified TSH-R preparations. Human monoclonal antibodies (mab) would provide the ideal tools to study the pathogenetic epitopes on TSH-R but so far, the human mabs to TSH-R that have been described are frequently IgM in nature or without biological activity. Furthermore, the biochemical reactivity of these human mabs to TSH-R has never been ascertained. Using Epstein Barr Virus (EBV) transformed human B lymphocytes coupled with magnetic selection of IgG producing immortalised B cells, we have obtained stable B cell lines (n=11) and clones (n=2) secreting IgG autoantibody to the TSH-R from peripheral blood of four Graves' disease patients. The human mabs immunoprecipitate ^{35}S-methionine labelled receptor in insect cells and also react with nascent TSH-R polypeptide generated in *in vitro* translation experiments. Culture supernatants from seven lines have blocking activity (TBAb activity) where inhibition of TSH mediated stimulation of cAMP in FRTL-5 cells is demonstrable. Six supernatants showed mild thyroid stimulating activity (TSAb) (200-300% increase in cAMP in relation to media control) and one showed significant TSAb (>300%) increase. Interestingly, none of culture supernatants from lines and clones showed any significant inhibition of ^{125}I-TSH binding in a commercial radioreceptor assay (TBII activity). This is likely to be related to the low senstivity of the assay. Our data demonstrates that stable EBV transformed B cell lines producing IgG antibody to the TSH-R can be established from patients. The IgG antibodies react with the receptor protein in two different immunoprecipitation experiments, have biological activity and can, after repeated cloning, be used to study further the epitopes recognised on the TSH receptor.

11:45 A.M.
14. PRIMARY HORMONOGENIC SITES AS CONSERVED AUTOEPITOPES IN MURINE AUTOIMMUNE THYROIDITIS: ROLE OF MHC CLASS II. Q. Wan, R.W. Motte*, B.E. Fuller, A.A. Giraldo*, D.J. McCormick°, C.S. David° and Y.M. Kong, Wayne State Univ. School of Medicine and *St. John Hospital, Detroit, MI, and °Mayo Clinic, Rochester, MN.

A few synthetic peptides corresponding to a.a. sequences on human thyroglobulin (hTg) have been reported to induce moderate thyroiditis or activate mouse Tg (mTg)-primed T cells to transfer thyroiditis in mice susceptible to experimental autoimmune thyroiditis (EAT). One conserved, 12mer peptide containing tyrosine at the hTg hormonogenic site at position 2553 was not thyroiditogenic unless the tyrosine had been substituted with thyroxine (T4) (Hutchings et al., J. Exp. Med. 175:869, 1992). However, since noniodinated, thyronine (T0)-containing peptide was not compared in parallel, we derivatized three pairs of 12mer peptides (1-12, 2549-2560, 2559-2570), with T4 or T0 at hormonogenic site 5, 2553 or 2567 respectively, using N^{α}-FMOC protection chemistry on Rink polystyrene resin. We reported subsequently that iodination was not a requisite for T4(2553) to be immunogenic; hT0(2553) also activated mTg-primed T cells to transfer thyroiditis and was immunogenic for mice, producing antibodies and primed T cells which can be expanded with peptide in vitro to transfer EAT. We report here that hT4(5), but not hT4(2567), can also stimulate mTg-primed cells to transfer EAT. To determine the relative importance of MHC class II and T cell repertoire in the response to conserved epitopes, we compared two EAT-susceptible k & s (CBA & A.SW) haplotypes to their respective MHC-identical strain (C57BR & SJL) with 50% genomic deletion of TCR Vβ genes. We find that: 1) Whereas k and s strains are susceptible to mTg-induced EAT, vigorous immunizing regimens with T4 or T0 at either 5 or 2553 lead to mild (10-20%) thyroiditis only in some mice of either k strain, but not in the s strains; 2) regardless of TCR Vβ gene differences, T cell responses to all peptide pairs are similar in CBA and C57BR mice. Moreover, cross-stimulation studies show that both hT0(2553) and hT4(2553) reciprocally prime and stimulate T cell responses. By comparing T4- and T0-containing peptides in different susceptible haplotypes, we show further that antigenicity of conserved hormonogenic sites is intrinsic, dependent more on a.a. sequence and binding to appropriate class II molecules, and less on iodine residues. Iodination may enhance in vitro stimulation, possibly to T cells, but is not required for a hormonogenic site to be an autoepitope. (Supported by NIH DK 45960 and St. John Hospital)

10:30 A.M. 15. **STUDIES ON THE PREVALENCE OF TSH-R MUTATIONS IN A LARGE SERIES OF CONSECUTIVE HYPERFUNCTIONING THYROID ADENOMAS**

Porcellini A.^; Tassi V°; Ciullo I.; Pannain S.; Amabile G.; Cisternino C.°; Di Cerbo A.°; Papini E.*; Avvedimento V.E.^; Fenzi G.F. Catt. Endocrinologia & ^Dip. Biol. Pat. Cell. Mol. Università Napoli; ° Div. Endocrinologia IRCCS Casa Sollievo Sofferenza, S.Giovanni Rotondo; * Div. Endocrinologia Osp. Regina Apostolorum, Albano Laziale -ITALY.

In the last two years several somatic mutations have been reported in the TSH receptor gene (TSH-R) in hyperfunctioning thyroid adenomas. These mutations constitutively activate TSH-R and result in clonal growth and stimulation of cAMP pathway, ultimately responsible of the hyperthyroidism. These TSH-R mutations were located in a 25 aminoacid region spanning between the third cytoplasmic loop (J. Parma et al. *Nature* 1993; **365**:649-651) and the sixth transmembrane domain of the receptor (A. Porcellini et al. JCE&M 1994; **79**:657:661). Moreover, most of these mutations lead to gain or loss of a site of a restriction enzyme. Thus *TaqI, Eco RI, Hph I* and *Asp 718* enzymes can be used as tools for the rapid identification of the mutations by restriction analysis on the amplified cDNA obtained from fine needle aspiration biopsy samples (FNAB). To evaluate the frequency of these mutations we analysed the TSH-R cDNA from fine needle aspiration biopsy in 40 patients (15 males and 25 females, aged between 25 and 70 years) with hyperfunctioning thyroid adenomas. All patients presented a single "hot" nodule and complete suppression of the remaining thyroid tissue by ^{131}I scan, undetectable serum TSH and normal or elevated total and free serum thyroid hormones levels. The restriction analysis of the PCR products revealed 11 mutations (see table below).

ENZYME	CODON	MUTATION	# OF CASES
Asp 718	**619**	**D->G**	**1**
Hph I	**631***	**F->C***	**4**
Taq I	**632**	**T->I**	**4**
Eco RI	**633**	**D->E**	**2**

*All possible nucleotides substitutions at codons 631 and 632 lead to loss of the Hph I site; these mutations must be confirmed by DNA sequencing.

Our preliminary results indicate that at least 25% of thyroid hyperfunctioning adenomas are caused by TSH-R activating mutations screened by restriction analysis on amplified cDNA. Since this analysis is not able to identify all possible mutations of TSH receptor gene involved in thyroid hyperfunctioning adenomas, this frequency represents a minimum estimate.We are currently performing DNA sequence analysis to evaluate the actual frequency of the TSH-R mutations.

10:45 A.M. 16. CYS-618-ARG MUTATION IN EXON 10 OF THE RET PROTO-ONCOGENE IN PATIENTS WITH MEN 2A PHENOTYPE AND HIRSCHSPRUNG'S DISEASE. Ph Caron, T Attié, D David, J Amiel, Fl Brousset, P Roger, S Lyonnet. Service d'Endocrinologie, CHU Rangueil, Toulouse et Hôpital Haut-Leveque, Bordeaux, Service de Génétique Médicale, INSERM U-393, Hôpital des Enfants-Malades, Paris, France.

Point mutations in exons 10 and 11 of the RET proto-oncogene are present in greater than 95 % of patients with MEN 2A phenotype characterized by medullary thyroid carcinoma (MTC), phaeochromocytoma (phaeo) and hyperparathyroidism (HPT). On the other hand, variable mutations of the RET proto-oncogene have been recently identified in patients with familial Hirschsprung's disease (HSCR) attributed to the absence of autonomic ganglion cells in the terminal hindgut. It has been speculated that MEN 2A may be due to gain of functional mutation of RET exon 10 whereas loss of function mutation in the same domain resulted in HSCR. The association of MEN 2A with HSCR in patients with mutation of the RET proto-oncogene is infrequent. A 30 year-old man was referred for screening of MTC. In his past history, surgery for HSCR was performed in the first years of life. Basal (59 pg/ml) and pentagastrin-stimulated calcitonin levels (979 pg/ml) were abnormal. Twenty-four hour urinary catecholamine and metanephrine levels were normal. A total thyroidectomy with prophylactic central node dissection was performed. Histological examination of the thyroid confirmed bilateral MTC. His mother has been treated for a MTC and screening for phaeo was negative. In second-degree relatives, a 50 year-old woman died with metastatic MTC ; her daughter had unilateral phaeo and MTC ; one grand-daughter with HSCR presented a histologically confirmed MTC while her sister had an abnormal pentagastrin stimulated calcitonin test. All other members of the family were free of HSCR, MTC as well as phaeo. Screening for HPT was negative in all members of the family. A Cys-618-Arg mutation was identified in the proband and his mother as well as in second-degree relatives with MEN 2A phenotype. In conclusion, we report patients with MEN 2A phenotype and HSCR associated with a point mutation (Cys-618-Arg) at one of five cysteine codons in the extracellular domain of RET proto-oncogene. Based on the data obtained in this novel family, we hypothesized that Cys-Arg mutation in codon 618 may not only lead to MEN 2A tumor formation, but could also inactivate the RET product resulting in abnormal enteric neurogenesis and HSCR with low penetrance.

11:00 A.M. 17. INCIDENCE OF RET PROTO-ONCOGENE MUTATIONS IN SPORADIC MTC. N Wohllk, GJ Cote, MMJ Bugalho, D Evans, H Goepfert, S Khorana, P Schultz, N Ordonez, RF Gagel. IEMA and Hosp del Salvador (U de Chile) Santiago, Chile and Sect of Endocrinol, Depts of Surgery & Pathology, UT M.D. Anderson Cancer Center, Houston, TX.

RET proto-oncogene mutations affecting codons 609, 611, 618, 620, 634, 768 and 918 have been identified in multiple endocrine neoplasia (MEN) type 2. To determine the frequency of MEN 2-like mutations in sporadic medullary thyroid carcinoma (MTC), we analyzed exons 10, 11, 13 and 16 of the RET gene by direct PCR-based DNA sequencing on 84 DNA samples from patients with apparent sporadic MTC. We found 5 (6%) had mutations in their peripheral blood DNA which were more consistant with hereditary disease. Two of these individuals were found to have codon 609 (cys to arg or tyr) mutations; one had a codon 611 (cys to tyr); the other two had codon 634 (cys to arg) mutations. The individual with the codon 609 cys to tyr mutation was the proband of a previously unrecognized kindred. The two patients with codon 634 mutations were found to be examples of *de novo* mutations with no other affected family member. It has not yet been possible to determine the mutation origin of the remaining two individuals because the unavailability of DNA samples. To determine the frequency of somatic RET proto-oncogene mutations in sporadic MTC, DNA from 34 tumors was included in our analysis (excluding the 4 patients with peripheral blood mutations). We found a codon 918 mutation in seven (20%) of these samples. No significant correlation between the existence of a codon 918 mutation and patient survival was observed for this patient population. The frequency of MEN 2 mutations in apparent sporadic MTC and the identification of a previously unrecognized kindred indicates that mutational analysis should performed on all patients with apparent sporadic MTC to define the risk for other family members.

11:15 A.M. 18. LOCALISATION OF RET PEPTIDE IN THYROID TUMOURS. GA Thomas, GH Williams, HG Davies, N Hooton, ED Williams. Department of Histopathology, University of Cambridge, Cambridge, UK. CB2 2QQ

The ret oncogene has been implicated in the genesis of two types of thyroid carcinoma. Germ line mutations of ret have been shown to give rise to the MEN2 syndrome, and somatic mutations have been recorded in a proportion of sporadic medullary carcinoma s (MCT). Translocations of ret have been implicated in a proportion of papillary carcinomas (PTC). We have used immunocytochemistry (ICC) with two different rabbit polyclonal antibodies to study the localisation of ret peptide in paraffin embedded material from a variety of thyroid tumours, 10 MTCs, 12 follicular carcinomas (FC), 9 follicular adenomas (FA), 33 PTCs and 6 oxyphil tumours. Thyroglobulin or calcitonin antibodies were used as positive controls and an inappropriate rabbit polyclonal antibody as one of the negative controls. Neonatal mouse brain was used as a positive tissue control. Western blots showed that each of the two ret antibodies, both recognising the tyrosine kinase domain, detected two bands of 150 and 170 kDa respectively, corresponding to the two glycosylation states of the ret protein. Both antibodies gave similar results on ICC.

70% of MTCs showed some degree of positivity for ret located to the cell membrane. In half of the cases there were occasional strongly positive cells, in a background of weak cytoplasmic positivity, in 20% of the cases there was generalised strong membrane positivity.. 85% of PTCs were positive for ret, the majority (70%) exhibiting widespread focal positivity at the base of the cell. A minor degree of apical and basal membrane staining was also seen in these tumours. In contrast, the majority of the FCs (82%) and FAs (66%) were completely negative for ret expression. The solitary FC and 2 of the FAs which showed moderate positivity for ret expression were both of trabecular/solid architecture. Two follicular FAs, showed a small number of positive cells., 5 were completely negative. Two of the 6 oxyphil tumours were positive for ret, both showing apical membrane staining and contained some papillary architecture. Interestingly 3 of the 5 PTCs which were negative for ret expression, showed strong epithelial positivity for IGF1 receptor and a widespread lymphoplasmacytic infiltrate which was positive for IGF1 mRNA expression. Normal follicular cells where present on the sections studied were negative.

The antibodies do not distinguish between activation of normal ret, point mutation or translocation. The altered cellular distribution observed in the PTCs may however reflect translocation - further molecular biological studies are underway to confirm this. Application of RT PCR for ret to 10 cases showed a good correlation with the ICC results. These results show that in thyroid tumours the ret gene product is predominantly observed in MTC and PTC, and that different morphological subtypes within these two groups show different ret expression. They suggest that overexpression of ret, whether mutated or not is important in a larger proportion and in a wider range of thyroid tumours than previously identified.

11:30 A.M. 19. RESTORATION OF P53 FUNCTION MODULATES PROLIFERATION AND DIFFERENTIATION OF A HUMAN ANAPLASTIC CARCINOMA CELL LINE

F.Moretti*°, A. Farsetti*°, S. Soddu*, S.Misiti*^, M. Andreoli^, A. Sacchi* and A. Pontecorvi*#.
*Molecular Oncogenesis Laboratory, Ist. Regina Elena, ^2nd Chair of Endocrinology, University of Rome "La Sapienza", °Inst. Experimental Medicine, CNR, #Inst. of Medical Pathology, Catholic University, Rome, Italy.

Alterations of the tumor suppressor gene p53 are detected with high frequency in undifferentiated thyroid carcinomas while are very rare in differentiated thyroid neoplasms. Impairment of p53 function may be responsible for loss of differentiation and acquisition of an aggressive biological behaviour by thyroid tumors. Aim of the study was to evaluate the effects of restoration of wild type p53 activity on cell growth and differentiation of an anaplastic thyroid carcinoma cell line (ARO), characterized by the presence of a mutated p53. ARO cell clones were generated by stable transfection with a temperature-sensitive mutant p53 which exhibited wild type-like properties at 32°C, but was inactive at 37°C. When grown at 32°C, cell clones expressing exogenous p53, as assessed by indirect immünofluorescence, showed a significant reduction of cell proliferation rate (≅40%), as compared to controls transfected with vector alone. Inhibition of cell growth was characterized by an increase of cells accumulating in the G1 phase of the cell cycle. Expression of thyroid-specific genes was evaluated by semi-quantitative RT-PCR measurement of TSH-receptor (TSH-r), Thyroglobulin (Tg) and Thyroid peroxidase (TPO) mRNA levels on cell clones grown at 32°C or 37°C. In the presence of TSH (10mU/ml) an increased expression of all thyroid-specific differentiation markers was observed when cell clones were cultured at 32°C, but not at 37°C, as compared to controls. Our results suggest that restoration of wild type p53 activity is able to partly inhibit ARO cell proliferation by accumulating cells in the G1 phase of the cell cycle. In addition, the reappearance of thyroid-specific gene expression suggests that p53-mediated changes promote cell differentiation. In conclusion, the likelihood of re-inducing follicular differentiation properties may lead to a more efficient approach to thyroid cancer treatment.
(The work has been supported by a grant from AIRC).

11:45 A.M. 20. **p53 GENE MUTATIONS IN RADIATION-INDUCED THYROID CANCER.** L. Fogelfeld and T.K. Bauer. Division of Endocrinology, Michael Reese Hospital, University of Illinois, Chicago IL 60616.

Little information is available about the pathogenetic role of specific oncogenes and tumor suppressor genes in radiation-induced thyroid cancer (RITC). The p53 tumor suppressor gene is the most commonly mutated gene in cancer. In thyroid cancer, p53 gene mutations have been linked to the more aggressive anaplastic forms, but mutations have also been found in well differentiated cancers. We have studied the occurrence of point mutations in p53 tumor suppressor gene in 22 patients exposed in childhood to radiation for benign conditions of head and neck who developed well differentiated, mixed papillary and follicular thyroid cancers (RITC). After histological identification, DNA was extracted from paraffin embedded specimens of the RITC patients. Polymerase chain reaction (PCR) amplification of exons 5-8, containing most of the reported point mutations, and screening for mutations by single strand conformation polymorphism (SSCP) analysis was performed. For confirmation and identification of each mutation, DNA extracted from the shifted SSCP bands and from the paraffin specimens was PCR amplified and subjected to direct cycle sequencing. PCR-amplified DNA from patients without shifted SSCP bands was also subjected to direct cycle sequencing. Five out of 22 RITC patients (22%) showed missense point mutations by SSCP and sequencing analysis in the coding regions of p53. The missense mutations involved codon 178, codon 217 and codon 208 in 3 patients. The mutations were transitions from G to A and C to T. In addition, one patient had neutral mutation and two had intronic mutations. Three of the 5 patients with p53 missense mutations had invasion of the cancer beyond the thyroid capsule compared to 2 of the 17 remaining RITC patients. None of the patients with p53 mutations had distant metastases or recurrence of the tumor. These results suggest that p53 gene point mutations may play a pathogenetic role in some radiation-induced, well differentiated thyroid cancers and in their local spread.

21. P1 SOLUBLE INTERLEUKIN-1 RECEPTOR INHIBITS INTERLEUKIN-1 INDUCED GLYCOSAMINOGLYCAN PRODUCTION IN CULTURED HUMAN ORBITAL FIBROBLASTS.

G.H. Tan, C.M. Dutton, R.S. Bahn. Division of Endocrinology/Metabolism and Department of Internal Medicine. Mayo Clinic/Foundation, Rochester,Minnesota, 55905

An accumulation of cytokine-stimulated glycosaminoglycan (GAG) production with attendant edema within the perimysial and retroocular connective tissues is largely responsible for the clinical manifestations of Graves' ophthalmopathy (GO). IL-1 has been shown to be a potent stimulator of GAG production by cultured human orbital fibroblasts. We studied in vitro the effects of treatment with the IL-1 antagonist, recombinant soluble IL-1 receptor (sIL-1R), on IL-1 mediated GAG synthesis in these cells.

MATERIALS AND METHODS:
Retroocular connective tissues were obtained from patients with GO undergoing surgical orbital decompression and from normal individuals during eye surgeries for trauma, tumors or to correct strabismus. Orbital fibroblasts were isolated and grown to confluency. Monolayers were incubated in fresh medium containing [^{3}H] glucosamine and bovine serum albumin, IL-1 alone (10U/ml), sIL-1R alone (1 ng/ml), or IL-1 (10 U/ml) in combination with sIL-1R (0.25-10 ng/ml). [^{3}H] GAG production was quantitated following 48-hour incubation. Experiments were performed in triplicate using fibroblasts from normal individuals (n=2) and patients with GO (n=2).

RESULTS:
Treatment of cultures with IL-1 alone stimulated GAG synthesis by 19%-63% (mean=39% $p=<0.05$). A 0%-45% inhibition (mean=29%) of IL-1 induced GAG production followed treatment of cultures with 0.25 ng/ml sIL-1R, a 62%-96% inhibition (mean=79%, $p=<0.05$) with 0.5 ng/ml of sIL-1R, and 66%-100% inhibition (mean=89%, $p=<0.05$) with 1.0 ng/ml sIL-1R. No differences in response between normal and patient cells were observed.

CONCLUSION:
Soluble IL-1 receptor is a potent inhibitor of IL-1 induced GAG production by cultured human orbital fibroblasts. Complete inhibition was achieved with a low concentration of the soluble receptor (1 ng/ml). These results suggest that this compound, known to be relatively safe when administered to humans, may be useful in the treatment or prevention of GO.

22. P2 **IMMUNODETECTION AND LOCALIZATION OF MANGANESE SUPEROXIDE DISMUTASE (MnSOD) IN RETROOCULAR FIBROBLASTS (ROF) FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY (GO).**

H.B. Burch, S.G. Barnes, O. Arseven, D. Sellitti, S. Lahiri. Walter Reed Army Medical Center, Washington, D.C.

Proximate events in the pathogenesis of GO are being actively investigated. Factors linking the autoimmune response against thyroid antigens to ocular disease are of particular interest. We have recently identified MnSOD as a 23 kDa ROF protein using microsequencing techniques (Presented at the 77th Annual Endocrine Society Meeting, June, 1995, Washington, D.C.). This protein was detected using polyclonal rabbit antiserum against an immunogenic portion of the human TSH-receptor (hTSH-R), and has limited homology with the hTSH-R immunizing peptide (AA 352-367). The current study examines intracellular localization of MnSOD using immunofluorescent staining techniques. In addition, simultaneous immunoblotting studies were performed using specific MnSOD antiserum and sera from patients with Graves' disease (GD) to examine MnSOD immunoreactivity in these patients. Cultured human ROF obtained from patients with severe GO (generously provided by Dr. Rebecca Bahn, Mayo Clinic) were grown on glass cover slips and fixed in cold methanol for indirect immunofluorescent studies. After washing with PBS, and incubating with a 1:100 dilution of MnSOD antibody (kindly provided by Dr Lawrence Oberly, U. Iowa), cells were washed again and incubated with fluorescein-isothiocyanate labeled goat anti-rabbit IgG. Moderate staining in the form of discrete cytoplasmic granules was observed using MnSOD antiserum, compared to an absence of significant staining using control serum. Immunoblotting studies using GD patient sera showed a high prevalence of immunity against a protein band coinciding with that recognized by MnSOD antisera. Two patients with GD acquired an enhanced immunoreactivity against this protein following 131-I ablation therapy. These studies demonstrate: 1) a high level of MnSOD protein expression in cultured ROF; 2) immune recognition of this protein in patients with GD; 3) a potential role for oxidative damage in the pathogenesis of GO; and 4) a mechanism through which cellular defenses against oxidative injury may be impaired in this disorder. The latter hypotheses are currently under investigation.

23. P3 **STUDIES OF RETROORBITAL TISSUE XENOGRAFTS FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE : A NEW ANIMAL MODEL.**

S. Mori, N. Yoshikawa, T. Tokoro, S. Ikehara*. The Second Dept. of Internal Medicine, Dept. of Pathology*, Kansai Medical University, Moriguchi, Osaka 570, Japan.

Retroorbital fat tissues and eye muscle tissues from patients with Graves' ophthalmopathy (GO) were separately xenografted to severe combined immunodeficient (SCID) mice. Autologous peripheral blood mononuclear cells (PBMC) were engrafted into separate SCID mice. The production of human IgG, thyroglobulin antibody (Tg-Ab) and thyroid peroxidase antibody (TPO-Ab) in the SCID recipients were monitored for up to 8 weeks. Retroorbital tissues were histologically analyzed before and after transplantation. The immunohistochemical analysis of human lymphocytes subset in the tissue was performed using monoclonal antibody to human T and B lymphocytes. Retroorbital tissues from 2 of 9 GO patients produced substantial IgG (>100mg/dl) peaking at 6-8 weeks after xenografting, although their values were lower than those seen for mice engrafted autologous PBMC. Engraftment of eye muscle tissues from 6 GO patients was not followed by the appearance of human IgG. The titer of Tg-Ab and TPO-Ab, in contrast, were comparable in the mice with retroorbital tissues and PBMC, suggesting the existence of larger number of B cell clones which produce thyroid autoantibody in mice with retroorbital tissue than those with PBMC. On the other hand, retroorbital tissues from control subjects did not produce any detectable levels of human IgG, TPO-Ab, or Tg-Ab. Immunohistochemical findings showed T cell dominant lymphocytic infiltration in GO retroorbital graft. In conclusion, we have successfully reconstituted the SCID mice with human lymphocytes of retroorbital tissues from patients with GO, and this animal model may help to elucidate the pathogenesis of GO.

24. P4 **Leukoregulin Induced Changes in Cell Gene Expression Specific for Orbital and Pretibial Fibroblasts Define Phenotypic Differences and Novel Protein Inductions of Potential Relevance to Graves Ophthalmopathy.** Donald A. Young, MD*, CH Evans and Terry J. Smith, MD#. *University of Rochester School of Medicine, Rochester, N.Y. 14620, and # Albany Medical College and Veterans' Affairs Medical Center, Albany, N.Y 12208.

We have studied changes in proteins in cultured cells with the aim of discovering differences of relevance to the hypertrophic and inflammatory changes in orbital tissues in Graves Ophthalmopathy. The comparisons were among dermal fibroblasts from abdominal (ABD) and pretibial (PT) skin and orbital fibroblasts (GO) from the same patient. Protein analyses by giant-2-D-gel electrophoresis reveal distinct phenotypes of fibroblasts from the different sites. GO were much more like PT than ABD. An unanticipated finding was a remarkable differences in their responsiveness to leukoregulin (LR), a factor recently isolated from killer-T cells. A few proteins (most notably a p35) were induced similarly in all cells by treatment with LR However, both GO and PT shared additional inductions and repressions totally absent in other fibroblasts. Among these were inductions in 2 p40 proteins, and dramatic, seemingly *de novo* inductions in a group of 5 p45-65 proteins. Moreover, the GO responses were further distinguished by large inductions in the inflammatory prostaglandin GH synthase-2 (O'Banion et al. PNAS, 89 4888-92 1992) suggesting it as the likely cause of the inflammation in Graves ophthalmopathy (see also another abstract by HS Wang, VD Winn, et al.). No inductions were produced by addition of PGE2 to the medium and all were inhibited by the glucocorticoid dexamethasone.

These studies demonstrate a number of new protein responses to LR. They are also the first to describe a protein phenotype for Graves orbital fibroblasts, and distinctive responses to cytokines that distinguish orbital fibroblasts from others. Moreover, similarities in some of the protein inductions in orbital and pretibial fibroblasts suggests an involvement of these proteins in Graves ophthalmopathy and dermopathy. Studies aimed at the molecular cloning and characterization of these proteins are in progress. Interim progress will be described. (Supported by NIH R01s, DK 16177 and EY 08976, and a VA merit award).

25. P5 ANTIBODIES AGAINST STRIATED MUSCLE, CONNECTIVE TISSUE AND NUCLEAR ANTIGENS IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY: IS GRAVES' DISEASE A COLLAGEN DISORDER? J. Kiljanski, A. Barsouk, K. Peele, I. Stachura, J. Pickeral, V. Nebes, J. Kennerdell and J. R. Wall. Thyroid-Eye Disease Center, Allegheny-Singer Research Institute, Pittsburgh, PA.

The identity and subcellular localization of the principal extra ocular muscle (EOM) autoantigens and prevalences of the corresponding serum autoantibodies in patients with thyroid-associated ophthalmopathy (TAO) need to be clarified. We have used porcine eye muscle tissue as substrate in an indirect immunofluorescence assay. Several different patterns of antibody binding to EOM tissue antigens were obtained with sera from patients with TAO namely, membrane, cytoplasmic, interstitial and nuclear. Overall, sera from 75% of patients with TAO contained one or more antibodies reactive with EOM compared to 32% of patients with Graves' hyperthyroidism, 38% with Hashimoto's thyroiditis and 16% of normals. All sera which reacted with EOM membrane or cytoplasmic antigens also reacted with the same antigen(s) in other (facial) skeletal muscle. Surprisingly sera from 31% of patients with TAO but only 8% of those with Hashimoto's thyroiditis and no patient with Graves' hyperthyroidism without evident ophthalmopathy, contained antinuclear antibodies (ANA). The most common nuclear fluorescence pattern was the finely speckled type consistent with anti-Sm or anti-RNP antibodies. Anti-interstitial tissue antibodies and ANA cross reacted with all tissues tested. Significant positive correlations were found between (i) EOM dysfunction and ANA (ii) eye disease of < 1 yr duration and membrane reactive antibodies and (iii) eye disease of < 1 yr duration and interstitial tissue reactive antibodies. Although patients with Graves' disease do not usually exhibit other signs or immunologic features of systemic lupus erythematosus or other generalized collagen disorder the finding of high prevalences of ANA and anti-striated muscle antibodies and, less often, anti-connective tissue antibodies, in patients with ophthalmopathy is consistent with it being a collagen-like disorder of the muscle, connective tissue and thyroid. The reason why this inflammatory process is usually limited to these tissues is unclear although cross reaction of ANA with tissue specific proteins in muscle and connective tissue of the orbit and skin is a possible mechanism.

26. P6 EFFECTS OF BRADYKININ AND TSH ON PHOSPHOLIPASE C AND A_2 SYSTEMS IN RETROOCULAR FIBROBLASTS OBTAINED FROM PATIENTS WITH GRAVES' OPHTHALMOPATHY. M.A. Atwa, R.C. Smallridge, I.D. Gist, E.M. Abo-Hashem, M.H. El-Kannishy, and K.D. Burman, Washington Hospital Center and Walter Reed Army Institute of Research, Washington, DC.

We studied the effects of bradykinin (BK) and TSH on phospholipase C (PLC) and A_2 systems in retroocular fibroblasts from Graves' ophthalmopathy patients. BK (1 μM) immediately increased $[Ca^{2+}]_I$ (from 272 $\pm$ 15 nM to 362 $\pm$ 18 nM, Mean $\pm$ SE) and 1,4,5-triphosphate (IP_3) (from 109 $\pm$ 8% to 192 $\pm$ 6 %). U-73122, a PLC inhibitor (3 μM), completely blocked BK-induced $[Ca^{2+}]_I$ mobilization and IP_3 production. BK also stimulated arachidonic acid (AA) release time- and dose-dependently. Removing external Ca^{2+} had no effect on the BK-induced IP_3 production or AA release. U-26384, a PLA_2 inhibitor (5 μM), but not U-73122, blocked BK-induced AA release(P= 0.01). Thus, BK activates PLC and PLA_2 systems independently (i.e., parallel activation). TSH (1 μU/ml) decreased $[Ca^{2+}]_I$ (154 $\pm$ 2 nM) from basal (238 $\pm$ 6 nM), but TSH (10 μU/ml) significantly increased ($p<.05$) IP_3 production and AA release. The TSH induced IP_3 production was Ca^{2+} dependent but TSH induced AA release was calcium independent. U-26384 blocked AA release induced by TSH. U-73122 blocked TSH-induced (10 μU/ml) IP_3 production and partially blocked AA release. These data indicate that: (1) TSH induced PLA_2 activity was partially PLC dependent, a pattern referred to as sequential activation; (2) bradykinin and TSH activate phospholipase C and A_2 systems in retroocular fibroblasts from patients with Graves' ophthalmopathy; and (3) the calcium dependence of these two enzymes differ between the two agonists. Since Graves' patients' IgG has TSH-like biological activities, further studies are required to assess whether these signal transduction pathways could be involved in the pathogenesis of Graves' ophthalmopathy.

27. P7 **PREVALENCES OF ANTIBODIES REACTIVE WITH PIG EYE MUSCLE MEMBRANE ANTIGENS IN PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY.** S.Wengrowicz*, M.Puig-Domingo, J.Soldevila and A. De Leiva. Endocrine Research Laboratory. Hospital de la Santa Cruz y San Pablo, Barcelona's Autonomous University, Barcelona, Spain.

Antibodies reactive with pig eye muscle membrane (PEMM) proteins have been found in some studies but not others, to be related with thyroid-associated ophthalmopathy (TAO). We have studied the prevalences of such antibodies in 27 patients with TAO, 13 Graves' hyperthyroidism and no apparent eye disease (GH) and 12 normal(N) subjects as control. SDS-polyacrylamide gel electrophoresis with PEMM and Western blotting was carried out in patients and healthy control sera. Clinical activity score (CAS) ranging from 0 to 7, and orbital Magnetic Resonance Imaging (MRI) were included in eye assessment. Different bands of reactivity were detected on immunoblotting:

group	n	64 kDa	55 kDa	95 kDa	other[a]
TAO	27	9(33%)	14(52%)	4(15%)	17(63%)
GH	13	3(23%)	1(8%)	0	8(61%)
N	12	2(17%)	0	0	7(58%)

[a]molecular weight ranging from 30 to 140 kDa.

The most frequently detected antibody was that directed against a protein of 55 kDa in 52% unselected TAO patients, and the only significative ($p<0.02$) when compared statistically with GH and N using the x^2 test. Reactivity against 64 kDa protein was found in 8/15 (53%) TAO patients with CAS >=3 and less than 1 year duration. All 27 TAO patients showed eye muscle thickening in MRI. In conclusion: 1) we have found a strong association between TAO and the detection in immunoblotting of antibodies reactive with a 55 kDa PEMM antigen, 2) clinically active and recent eye disease could be related with antibodies reactive with the 64 kDa protein, 3) follow up of GH patients will provide more information about PEMM antibodies' predictive value in early diagnosis of eye disease.

Acknowledgments: S. Wengrowicz,M.D.is a LAIR Foundation Research Fellow, special recognition to J.R.Wall,M.D., Ph.D. from Allegheny-Singer Research Institute, Pittsburgh,PA,USA for introducing the procedure of antibodies measurement.

28. P8 ANTIBODIES CROSS-REACTING WITH THYROGLOBULIN AND ACETYLCHOLINESTERASE IN GRAVES' DISEASE ARE ASSOCIATED WITH OPTHALMOPATHY. G. Philippou[1], D. G. Mappouras[2], A. Souvatzoglou[1] and P. Lymberi[2], [1]1st Endocrine Section, "Alexandra" General Hospital, and [2]Department of Immunology Hellenic Pasteur Institute Athens, GREECE.

In the present study, we analyzed by ELISA (non-competitive and competitive) the ability of sera from 53 patients with Graves' disease (GD) (29 with ophthalmopathy), 20 with Hashimoto's thyroiditis (HT) (none with ophthalmopathy), and 36 healthy controls (CR) to react with acetylcholinesterase (AChE) from *Electrophorus electricus* and human thyroglobulin (Tg). Immunoreactive polypeptides of AChE molecule were detected by western blotting. Furthermore, reactivity of pathological autoantibodies to AChE and Tg was compared to that of induced antibodies raised in rabbits after hyperimmunization with the above antigens. Finally, reactivity of autoantibodies to Tg and AChE was correlated with the presence of ophthalmopathy in GD. Results have shown that: (i) increased IgG anti-AChE activity (21%) was expressed only in GD sera, while increased IgG anti-Tg activity was detected in both GD (51%) and HT (85%) sera; (ii) most of the IgG anti-AChE ELISA-positive sera were found by western blotting to react with a 72 KD protein band, suggestive of the catalytic subunit of AChE; (iii) a significant proportion of IgG anti-AChE positive GD sera was also found to exhibit IgG anti-Tg activity (8/11; 73%). This bi-reactivity was shown, by cross-inhibition and immunoadsorption studies, to be exhibited by antibodies cross-reacting with both Tg and AChE (anti-Tg/AChE activity). Serum anti-AChE activity was effectively inhibited by soluble Tg, while affinity purified anti-Tg antibodies cross-reacted with AChE. IgG anti-Tg/AChE cross-reactivity seems to be a property of pathological autoantibodies since induced antibodies obtained after hyperimmunization of rabbits with Tg or AChE were highly monospecific.; (iv) analysis of clinical data showed that increased IgG anti-Tg/AChE bi-reactivity occurs more frequently in GD patients with ophthalmopathy ($p<0.01$) than in GD patients without ophthalmopathy. Taking into account the presence of AChE in ophthalmic muscle as well as the molecular homology (28%) shared by AChE and C-terminal end of Tg, the above results suggest that serum anti-Tg/AChE cross-reactive antibodies could have pathophysiological implications in the appearance of ophthalmopathy in patients with Graves' disease.

29. P9 RETROBULBAR AUTOANTIGENS AND CELLULAR IMMUNITY IN GRAVES' OPHTHALMOPATHY. G. Kahaly, K. Ochs, C. Hansen, J. Beyer and E. Otto, IIIrd Medical Department, University-Hospital, Mainz, Germany.

Graves´ ophthalmopathy (GO) is based on autoimmune processes that lead to lymphocyte infiltration of the retrobulbar space. In this study, antigenic character of retrobulbar adipose, connective and muscle tissue as well as of cultured fibroblasts and myoblasts were examined. Samples were obtained from GO patients (n=14, 9 fem., age 26-82 years) undergoing orbital decompression surgery. Retrobulbar and abdominal tissue from 7 controls (4 fem., 48-74 y) was investigated, too. Tissues were homogenized and the proteins were separated by SDS-PAGE according to molecular weight. In order to recover the separated proteins in soluble form, an electroelution technique was employed. Twenty-two separated soluble protein fractions were used as antigenic stimuli for autologous peripheral blood lymphocytes (PBL) separated by Ficoll gradient centrifugation. Furthermore, organ specific, retrobulbar T-cells were cloned and tested. Subsequently, the proliferation of T-cells was measured by [^{3}H]-thymidine uptake. A marked T-cell response to protein fractions with molecular weight of 6-10 kD and 19-26 kD was detected ($p<0.001$). These autoantigens were found reproducibly in adipose tissue of 8 out of 9 GO patients, stimulation index (SI) to antigen 6-10 kD 29±4.6 (mean+SEM); 19-26 kD 5±1.4 and in 3 out of 4 patients using retrobulbar eye muscle tissue (SI: 6-10 kD 23±4.2; 19-26 kD 6±2). Using the proteins of cultured fibroblasts as antigen, the autologous PBL from 2 out of 4 tested GO-patients responded, too (SI: 7±2; 4±1.4). Testing cultured retrobulbar myoblasts of a GO patient, a response to the 19-26 kD antigen was found only (SI: 8). Isolated, organ specific activated T cells recognized and responded to these two autoantigens and showed markedly elevated SI (65 and 85 to antigens 6-10 and 19-26 kD, respectively). In response to retrobulbar adipose or muscle proteins, PBL of 2 controls showed proliferations (SI: 16±3.5; 13±2.8), whereas, a response to abdominal adipose or muscle proteins (4 controls) was never found. Thus, two orbital autoantigens reacting with organ specific T-cells were demonstrated and may play an important role in the immunopathogenesis of GO. According to these findings, retrobulbar fibroblast antigens are most likely the main T cell targets.

30. P10 INCREASED SUPEROXIDE DISMUTASE ACTIVITY IN LACRIMAL SECRETIONS (TEARS) FROM PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY. A. Barsouk, B. Fraiture, K.A. Peele, C. Stolarski, M. Hayes, J.S. Kennerdell and J.R. Wall, Thyroid-Eye Disease Center, Allegheny-Singer Research Institute, Pittsburgh, PA.

There has been much interest in recent years in the role of oxygen free radicals as second messengers of various traumatic, inflammatory and autoimmune processes. As free radicals are produced in the course of tissue inflammation, enzymes including catalases, peroxidases and superoxide dismutases (SOD) are activated in order to catalyze the reduction of free radicals and reduce tissue damage. Of these, SOD are the most important. In order to investigate the role of this process in the pathogenesis of the extraocular muscle damage of thyroid-associated ophthalmopathy (TAO), we have measured levels of total SOD activity in the lacrimal fluid (tears) of patients with TAO, and, as controls, patients with other inflammatory eye disorders and age and sex matched normal subjects. Tears were collected using Schirmer test filter papers which were inserted into the conjunctival sac. SOD activity was measured using a xanthine oxidase based assay and total protein by Bradford protein assay. One unit of SOD activity was defined as the amount of protein that inhibits the rate of nitroblue tetrazolium reduction by 50% and total SOD activity as the number of units of SOD per mg total protein. Mean (+/- SE) levels of total SOD activity in tears from 16 normal subjects was 33.0 (+/- 4.1) and from 12 patients with TAO 91.8 (+/- 21.8), the difference being significant (X^2 test $P<0.01$). In 5 patients with other, non immunologic, eye disorders namely, chronic conjunctivitis (3 cases), entropion (1 case) and cataract (1 case), mean (+/-SE) SOD activity was 86.1 (+/- 28.7) and the difference, compared to normals, was also significant ($P<0.01$). Overall tests were positive (SOD activity > mean + 2SD for normals) in 58% of patients with TAO and 40% of patients with other eye disorders. In patients with TAO there was a significant negative correlation between SOD activity and duration of the eye disorder and a significant positive correlation with disease activity measured as an index. Although increased SOD activity in tears is not specific for TAO its measurement may be useful for the early demonstration of ophthalmopathy in patients with Graves' hyperthyroidism.

31. P11 DEVELOPMENT OF A MOUSE MODEL OF THYROID ASSOCIATED OPHTHALMOPATHY

V. L. Nebes and S. M. Longinotti, Allegheny-Singer Research Institute, Pittsburgh, PA 15212 USA

Experimental autoimmune thyroiditis is a well characterized model system for inducing an autoimmune-like disease in animals. After injection with thyroglobulin a characteristic infiltration of thyroid tissue, anti-thyroid antibodies, and hypothyroidism develops. We have induced autoimmune thyroiditis in mice and have tested whether or not this disease is accompanied by development of eye disease. In addition, one set of mice was injected with eye muscle protein extracts as well as thyroglobulin to increase the likelihood that immune reactivity to eye muscle tissue would develop. An additional set of mice was injected with eye muscle protein extracts only and tested for development of cross reactivity to thyroglobulin, the extracellular domain of the TSHR, and extracts of murine thyroid, eye muscle and skeletal proteins. Serum from mice was also tested for changes in levels of thyroid hormone and TSH. Thyroid, eye muscle, and skeletal muscle were examined histologically for infiltration. At 8 weeks after initial injections high titers of antibodies to thyroglobulin and eye muscle membranes were measured but no infiltration of thyroid or eye muscle tissue was found. Further studies are being conducted to examine tissues histologically at earlier time points to determine if these methods will induce eye disease.

32. P12 OCCURENCE AND FEATURES OF OCULAR INVOLVEMENT IN GRAVES PATIENTS WITH ASSOCIATED MYASTHENIA GRAVIS.

M. Marinò, *R. Ricciardi, G. Barbesino, L. Manetti, P. Caturegli, L. Petrone, *B. Rossi, *A. Muratorio, **S. Mariotti. Institute of Endocrinology and *Insitute of Neurology, University of Pisa (Italy); **Endocrinology, University of Cagliari (Italy)

Myasthenia Gravis (MG) and autoimmune thyroid diseases (AITD) are often associated. In a previous study, we observed a peculiar clinical expression of MG when associated with AITD, characterized by higher prevalence of restricted ocular MG and lower prevalence of thymus involvement. Furhtermore we obtained preliminary evidence of a higher prevalence of euthyroid ophtalmopathy in patients with Graves' disease (GD) associated with MG. Aim of the present study was to evaluate the occurence and the features of Graves' ophtalmopathy (GO) in GD associated with MG. To this purpose 27 patients (M=6, F=21; age=20-72) with GD associated with MG were selected (Group A). Control group (Group B) included 387 GD patients (M=73, F=314; age=13-79) without any evidence of associated MG, consecutively seen at our institution. All patients and controls were submitted to exophtalmometry, computerized visual field and retroorbital CT. GO severity was arbitrarily ranked in 3 grades: Grade 1= very mild; Grade 2: mild; Grade 3=severe. MG patients were also submitted to a full neurologic evaluation including assay for circulating AchRAb and CT or MRN of the thymus.

			GO severity		
	GO	EO	1	2	3
Group A	21/27* (77.8%)	4/27** (14.8%)	12/21 (57.2%)	6/21 (28.5%)	3/21 (14.3%)
Group B	218/387 (56.4%)	12/387 (3.1%)	121/218 (55.7%)	55/218 (25%)	42/218 (19.3%)

*$p<0.05$, $\chi 2=3.92$ and **$p<0.02$, $\chi 2=6.44$ *vs* Group B

As shown in the table, a significant higher prevalence of GO and EO was found in Group A. The distribution of the severity of GO did not differ between patients and controls. No difference was observed between GO patients of the two groups for sex, age, interval between the apparence of GO and of hyperthyroidism. With particular regard to MG patients, no difference was observed in the prevalence of GO and EO, as well as in the distribution of GO severity, between patients with ocular and generalized MG and between patients with or without thymic alterations.

In conclusion, this study provides evidence of a higher prevalence of GO and particularly of EO in GD patients with associated MG. A pathogenetic relationship between GD and MG based on shared ocular autoantigens could provide the explanation for this clinical observation.

33. P13 IMMUNOMODULATORY EFFECT OF NICOTINAMIDE ON CULTURED HUMAN ORBITAL FIBROBLASTS FROM PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY.

Y. Hiromatsu, G. Gen, M. Migita, J. Kamachi, Department of Medicine, Kurume University School of Medicine, Kurume, Japan

The activation of fibroblasts in the orbit has been stressed in the development of thyroid-associated ophthalmopathy. In the present study we investigated the effects of nicotinamide, a inhibitor of poly (ADP ribose) synthetase, on cytokine-induced HLA-DR antigen, intercellular adhesion molecule 1 (ICAM-1) and CD44 expression on cultured orbital fibroblasts from patients with thyroid-associate ophthalmopathy. Two to eight passaged cultured orbital fibroblasts were incubated for three days with interferonγ (10-400 U/ml) or TNFα (1-200 U/ml) in the presence of nicotinamide (1-2 mM). The surface expression of HLA-DR, ICAM-1 and CD44 was measured by flow cytometry. Nicotinamide inhibited the induction of both HLA-DR and ICAM-1 expression by cytokines on fibroblasts (23% in the presence of IFNγ v.s. 12% in the co-presence of 20 mM of nicotinamide; 40% in the presence of 10 U/ml of TNFα v.s. 28% in the co-presence of 20 mM of nicotinamide, respectively), but not in case of CD44. Furthermore, nicotinamide inhibited the orbital fibroblasts proliferation assessed as [^{3}H]-thymidine incorporation assays or Cell counting kit. Nicotinamide also enhanced Fas antigen expression on the fibroblasts, which may be potential to mediate apoptosis of fibroblasts (20% in basal condition v.s. 38% in the presence of 20 mM of nicotinamide). Our data suggest that nicotinamide inhibits the cytokines-induced activation of fibroblasts and ,therefore, suppresses the autoimmune reaction in the orbit in Thyroid-associated ophthalmopathy.

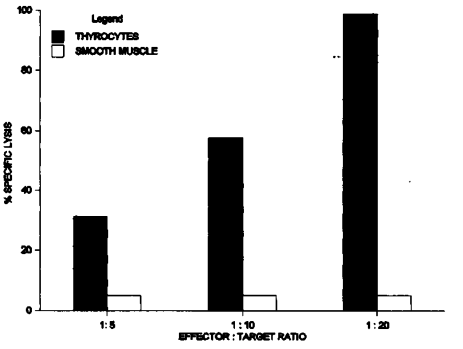
34. P14 **ANTIBODY TO gp39, THE LIGAND FOR CD40 SIGNIFICANTLY INHIBITS HUMORAL RESPONSE FROM GRAVES' THYROID TISSUES XENOGRAFTED INTO SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE.**

E. Resetkova*, K.Kawai*, T. Enamoto*, T.M. Foy**, R.J. Noel** and R. Volpé*. Endocrinology Research Laboratory*, The Wellesley Hospital, University of Toronto, Toronto, Ontario, M4Y 1J3, Canada, and Department of Microbiology**, Dartmouth Medical School, Lebanon, NH 03756, U.S.A.

Experimental evidence suggested that interference with gp39-CD40 interactions may have therapeutic potential in autoimmune diseases. The binding between CD40 expressed on mature B cells and CD40 ligand (CD40L, gp39) transiently expressed on activated CD4$^+$ T (Th) cells further stabilizes the interaction and coordinate the responses of the interacting cells during antigen presentation and is essential for thymus-dependent humoral immunity. To determine the role of interactions between gp39 and CD40 in an established human autoimmune thyroid disease, "*in vivo*" effects of anti-gp39 mAb administration on human Graves' disease (GD) thyroid tissue xenografted into SCID mice was studied. GD thyroid tissue from 2 patients was xenografted into 13 SCID mice (0.8g/mouse). Human IgG levels became detectable in SCID mice one week after xenograftment. Seven SCID mice were sequentially administered with anti-gp39 mAb (250μg/mouse/dose) i.p. every 4 d until the end of experiment. Six control animals were injected with PBS in similar fashion. Blood samples were taken every 2 wks from the tail veins for measuring of humoral response (human IgG, TSAb, anti-TPO and anti-Tg Abs), and thyroid function tests. After 8 wks, animals were sacrificed and thyroid tissue was examined histologically. Humoral response from intrathyroidal lymphocytes was detected and tissue morphology of GD was preserved during eight weeks period in PBS treated SCID mice xenografted with GD xenografts. However, administration of anti-gp39 mAb completely blocked or significantly decreased humoral response in all treated animals. No significant histological changes were associated with administration of anti-gp39 mAb. Degree of lymphocytic infiltration in thyroid tissue xenografts was comparable in both groups. Thyroid function tests were normal in both groups. In spite of profound immunosuppressive effect on humoral response by directly blocking CD40-gp39 interactions "*in vivo*" this did not result in significant deletion of responding Th in thyroid specimen. This divergence of histological vs. humoral responses may be a result of established disease, short duration of study or temporary induction of T-cell tolerance. Further studies will elucidate the role of this molecule.

35. P15 **A PATHOGENIC LYMPHOCYTE LINE FROM SPONTANEOUSLY-OCCURRING THYROGLOBULIN-REACTIVE BB/WOR RAT T CELLS IS CYTOTOXIC.** E. M. Allen, J. N. Thupari. University of Maryland Medical Center and Baltimore Veteran's Administration Medical Center, Baltimore, Maryland, USA.

Spontaneously-occurring thyroglobulin (Tg)-reactive splenic T lymphocytes have been isolated from lymphocytic thyroiditis (LT)-prone NB line BB/Wor rats (University of Massachusetts Medical Center, Worcester, MA, USA) and maintained in longterm culture. The pathogenic potential of this lymphocyte line has been determined by adoptive transfer and cytotoxicity studies. Adoptive transfer was accomplished by injecting 1.0 x 10^5 Tg-reactive lymphocytes into the tail vein of MHC compatible, non-LT-prone BB line BB/Wor rats. Control recipients received tetanus toxoid-reactive lymphocytes which had been maintained in parallel culture. After 14 days, the animals were euthanized and the thyroids examined for the presence of LT. Of the Tg-reactive cell line recipients, 100% developed LT. This was significantly greater than the 20% incidence seen in controls ($p < 0.05$, Fisher's exact test). Cytotoxicity assays were performed by incubating the Tg-reactive lymphocytes with Wistar rat thyrocytes (WRT line, courtesy of Terry Davies, NY) at increasing effector:target ratios in flat-bottomed wells. Control wells contained Wistar rat smooth muscle cells as targets. Target cell lysis was detected by measuring lactate dehydrogenase (LDH) release using the Promega CytoTox kit (CAT #G1760). The results are expressed as % Specific Lysis. As the graph indicates, this Tg-reactive T cell line demonstrated thyrocyte-specific cytotoxicity in a dose-response fashion ($p < 0.05$, linear regression). Smooth muscle cells experienced < 5% lysis at all ratios.



Conclusion: Pathogenic antigen-specific T lymphocytes isolated from nonimmunized LT-prone BB/Wor rats are cytotoxic.

36. P16 **THYROID TRANSCRIPTION FACTOR-1 (TTF-1) REGULATES MAJOR HISTOCOMPATIBILITY (MHC) CLASS I GENE EXPRESSION IN THYROID CELLS AND IS THYROTROPIN (TSH) CONTROLLED.** M-H. Shong, M. Ohta, S-I. Taniguchi, Y. Shimura, H. Shimura, NIH, Bethesda, MD, and Yamanashi School of Medicine, Yamanashi, Japan.

Expression of MHC class I is regulated during development and varies in different tissues; its precise regulation is crucial for the control of the immune response, since abnormally high levels are associated with autoimmune thyroid disease (ATD). TTF-1 is a homeodomain protein which regulates thyroid development and the expression of genes associated with thyroid-specific function, i.e. the TSH receptor (TSHR) and thyroglobulin (TG). Its RNA levels are downregulated by TSH in thyroid cells We have identified a downstream 48 bp silencer, -127 to -80 bp in the MHC class I promoter, whose function depends on a cyclic AMP response element (CRE), -107 to -100 bp, and whose activity is regulated by TSH or methimazole (MMI). Using gel shift assays (EMSA), we show that a protein/DNA complex formed by this silencer involves TTF-1, since it is present in rat thyroid, but not liver cells, and since its formation is inhibited by unlabeled oligonucleotides mimicking the TTF-1 binding sites on the TSHR and TG promoters. Using EMSA, recombinant TTF-1, and oligonucleotides mimicking -127 to -104 bp and -105 to -80 bp of the class I promoter, we identify two TTF-1 binding sites downstream and upstream of the CRE; one is TTF1-specific, the other can also interact with Pax-8. TTF-1 and CRE binding protein, CREB, footprint the region between -120 to -80 bp and -113 to -95 bp, respectively; the overlapping footprints suggest TTF-1 and CREB binding is mutually competitive. Overexpression of TTF-1 in rat thyroid cells increases the activity of a class I-reporter gene chimera containing the TTF-1 sites and the CRE, p(-209)CAT, but not the activity of a chimera without them, p(-68)CAT, nor a chimera with a nonpalindromic CRE mutation, p(-209NPCRE). When TSH decreases class I levels, it coordinately decreases TTF-1 complex formation with the silencer and increases complex formation with 2 Y-box protein (TSEP-1) suppressor sites, one near each TTF-1 element. The TSEP-1 elements are characterized separately; TSEP-1 activity is modulated by interferon and MMI. In sum, TTF-1 regulates expression of the MHC class I gene in thyroid cells by its action on a downstream 48 bp silencer. TTF-1 and class I expression are normally coregulated by TSH; and TTF-1 interactions with this silencer are normally coordinated with TSEP-1. Since TSEP-1 also suppresses MHC class II and since TSEP-1 binding and activity can be perturbed by interferon/MMI, it is suggested the downstream silencer is a region of tissue-specific control, normally regulated by TSH/TTF-1, subject to abnormal regulation in autoimmune thyroid disease, and a site of action for MMI.

37. P17 TSEP-1, A Y-BOX PROTEIN, IS A NEGATIVE REGULATOR OF MAJOR HISTOCOMPATIBILITY (MHC) CLASS I, TSH RECEPTOR (TSHR) AND MHC CLASS II GENES: IT IS A TARGET OF METHIMAZOLE (MMI) ACTION. M. Ohmori, M.H. Shong, G. Napolitano, D. S. Singer, and L.D. Kohn, NIH, Bethesda, MD.

We have cloned a cDNA encoding a Y-box protein, TSEP-1, which binds to the coding strand of the 5'-decanucleotide sequence in the TSHR and shown it suppresses TSHR promoter activity by decreasing constitutive enhancer activity of the cAMP response element (CRE) of the minimal TSHR promoter. TSEP-1 also acts as a suppressor by binding to the coding strand of the S-box of the TSHR, downstream of the CRE. The TSHR S-box and its CCTC/T binding motif is homologous to the S-boxof the MHC class II promoter, where TSEP-1 also acts as a suppressor. TSEP-1 suppression of TSHR and class II is associated with a common structural motif as well as binding motif: two TSEP-1 sites surrounding a CRE. The class I promoter has a downstream 48 bp silencer, -127 to -80 bp, which is just 3' to the interferon response element (IRE), -170 to -161 bp. The silencer has aCRE, -107 to -100 bp, which is critical for its activity. We show that TSEP-1 is a suppressorof class I activity when cotransfected with class I promoter-reporter gene chimeras. Using gel shift assays and oligonucleotide competitors from the TSHR gene, we identify 2 TSEP-1 sites on the coding strand of theclass I promoter, surrounding the CRE. One is 3' to the CRE and within the 48 bp silencer; the other is upstream, between the IRE and CRE. The TSEP-1 sites are in each case associated with thyroid transcription factor-1 (TTF-1) elements whose importance we describe separately. Mutation data indicate TTF-1/TSEP-1 binding to their respective sites is mutually exclusive. Interferon (IFN) increases class I expression in thyroid cells; MMI reverses this. IFN decreases TSEP-1 RNA levels and complex formation with this region of the class I promoter; MMI reverses this. In sum, TSEP-1 is a negative regulator of MHC class I, as well as MHC class II and TSHR gene expresion. In all three genes there is a common structural motif for action: 2 TSEP-1 sites surrounding a CRE, which is a constitutive regulator of gene expression. Like the TSHR, class I interactions with TSEP-1 are regulated by a nearby TTF-1 element. TSEP-1 activity and RNA levels are modulated by IFN and MMI, suggesting it is a critical protein involved in autoimmunity and in the treatment of autoimmunity by MMI. The datafulfill our prediction that TSHR and MHC class I have common regulatory elements and factors; they additionally identify a common regulatory factor in MHC class I, TSHR, and MHC class II gene expression likely to be involved in the control of thyroid self tolerance and the development of autoimmunity.

38. P18 IODIDE AND TPA CAN DECREASE MAJOR HISTOCOMPATIBILITY (MHC) CLASS I GENE EXPRESSION IN THYROID CELLS VIA NF-κB, BUT THEY USE DIFFERENT PHOSPHOLIPASE C (PLC)-ACTIVATED SIGNAL PATHWAYS. S-I. Taniguchi, V. Montani, C. Giuliani, and M. Saji, NIH, Bethesda, MD, and Dept. of Surgery, Johns Hopkins University, Baltimore, MD, USA.

Iodide (1-5 mM) and 1-100 nM o-tetradecanoyl phorbol 13-acetate (TPA) decrease MHC class I RNA levels within 1 hour of addition to FRTL-5 thyroid cells; their effects are over within 24 hours, but exhibit a different time course. In both cases, the mechanism is transcriptional and involves NF-κB. Thus, using nuclear extracts from cells treated with each agent for 1.5 hours and oligonucleotides mimicking the sequence of Enhancer A in the MHC class 1 gene or a generic NF-κB sequence, we show in electrophoretic mobility shift experiments (EMSA) that iodide and TPA decrease formation of a complex, termed Mod-1, involving the p50 subunit of NF-κB and a c-fos family member, fra-2, while simultaneously increasing formation of a complex involving the p50 and p65 subunits of NF-κB. Nevertheless, the action of several inhibitors shows that the mechanism by which iodide and TPA modulate the formation of these complexes is different. The effect of iodide is inhibited by acetylsalicylate, indomethacin, 5,8,11,14-eicosatetraynoic acid (ETYA), and methimazole (MMI), suggesting its effect is via activation of PLC, inositol phosphate (IP), Ca^{++}, and phospholipase A2 (PLA2); this is confirmed by demonstrating iodide modulation of the IP signal in FRTL-5 cells. These inhibitors do not alter the action of TPA at maximal concentrations of ligand. This suggests that activation of PLC results in independent regulation of the same transcriptional factors via the diacylglycerol/Ca^{++} transduction signal which activates kinase C, the TPA target. These data support the conclusion that iodide and TPA modulate class I differently, despite the fact both involve PLC and NF-κB. To localize all the loci on the MHC promoter where PLC activation might modulate transcription, we overexpressed $PLC\beta_1$ and γ_1 in cells cotransfected with various 5'-deletions of the class I promoter linked to a reporter gene. We show that there are multiple PLC target sites. One, between -1000 bp and -465 bp, involves regulation of an upstream silencer/enhancer complex which controls class I levels in different tissues. We also identify downstream loci other than Enhancer A, where iodide/TPA act. One appears to be the downstream 48 bp silencer where TSH regulates the activity of thyroid transcription factor-1 (TTF-1), as reported separately. TPA has no direct effect on TTF-1 complex formation; it does, however, enhance the ability of ATP and basic fibroblast growth factor (bFGF) to decrease TTF-1 complex formation between 1 and 16 hours after FRTL-5 cells are cochallenged by TPA and either ATP or bFGF. Iodide and TPA regulation of MHC class I may, therefore, involve the PLC signal system and common transcription factors, but their pathways differ and may involve additional sites other than Enhancer A. This may reflect the multiple possible effects of PLC activation on the growth and function of thyroid cells and the need to maintain self tolerance in each case.

39. P19 IMMUNOSUPPRESSION OF THYROIDITIS. V. Guimaraes, J. Quintans, M-E. Fisfalen, and L.J. DeGroot, Thyroid Study Unit and Department of Pathology, The University of Chicago, Chicago, IL 60637.

Immunization of mice with 50 μg of human thyroglobulin (TG) in complete Freund's adjuvant (CFA) leads to histologic thyroiditis, production of IgG, IgA, and IgM anti-TG antibodies, and in vitro T cell proliferative responses after incubation with TG. Oral administration of 500 μg of TG at 4 intervals prior to TG immunization and one time afterwards causes an 80% suppression of all responses. The effect is antigen specific, and dose dependent. Feeding TG after immunization produces 40% reduction in responses. Popliteal lymph nodes (PLN) of orally tolerized (T) animals are reduced in size compared to immunized (I) animals. PLN and mesenteric (M) LN of I animals produce IL-2 and IFNγ following in vitro incubation with TG. PLN and MLN of tolerized animals do not proliferate to antigen, do not produce IL-2 or IFNγ, but do produce IL-4 and TGFβ. Mixing in vitro of T and I LNC produces a dose dependent reduction in response when incubated with TG, but no reduction in response with PPD (the antigen in CFA). When T splenocytes are incubated with TG and PPD together, response to PPD is suppressed. I cells can transfer thyroiditis to naive animals, whereas T splenocytes cannot. In this model, oral tolerization produces a dramatic reduction in the immune response. T animals appear to shift from a TH-1 T cell subset response to a TH-2 response. Exposure of MLN to oral TG causes production of a cell which migrates to spleen and PLN; on exposure to antigen it produces IL-4 and TGFβ which suppress responsiveness. Oral tolerization may include some element of T cell deletion or anergy. This model defines an experimental system with relevance to immunosuppression of human autoimmune thyroid disease.

40. P20 ANTIBODY-DEPENDENT CELL MEDIATED-CYTOTOXICITY IN AUTOIMMUNE THYROID DISEASE ; RELATIONSHIP TO THYROPEROXIDASE ANTIBODIES.

P. Rodien, A.M. Madec, H. Bornet, A. Stefanutti, J. Ruf and J. Orgiazzi, INSERM U.197, Faculté de Médecine A. Carrel, 69372 Lyon Cédex 08, France and INSERM U. 38, Faculté de Médecine La Timone, Marseille, France.

We and others have reported antibody-dependent cell mediated-cytotoxicity (ADCC) in up to 70 % of thyroiditis patients and 50 % of Graves' disease patients. Poor correlation between ADCC and thyroperoxidase antibodies (TPO-Ab) has questioned the relationship between cytotoxic antibodies and TPO-Ab, and existence of a new antigen-antibody system has been suggested. We previously reported the use of porcine thyroid cell as a target for ADCC detection. Affinity purified human thyroperoxidase (TPO) was used to challenge ADCC against porcine thyroid cell. Heat inactivated 1/10 diluted sera were preincubated overnight at 4°C with growing concentration of TPO prior to being used in ADCC experiment. ADCC of a pool of 16 cytotoxic sera (Graves' disease patients, TPO-Ab ranging from 120 to 11000 UI/L, mean : 3742 UI/L) could be inhibited in a dose-dependent manner and totally abolished at a TPO final concentration 100 μg/ml. Neither lactoperoxidase nor thyroglobulin at same or higher concentrations (up to 300 μg/ml) had any preventing effect. The 50 % inhibiting TPO concentration, 40 μg/ml, was able to partially suppress ADCC of all sera of the pool tested individually from 40.5±11.4% to 11.4±8.8 (71.8 % decrease), as well as of 14 thyroiditis patient sera regardless of the goitrous or atrophic status from 39±10.4 % to 4.3±5.5 % (89 % decrease), (TPO-Ab: 100 to 14190 UI/L; mean: 4490). TPO-Ab, affinity-purified from patient sera, elicited thyroid specific ADCC from 5 to 35 % at concentration ranging from 0.01 μg/ml to 10 μg/ml whereas same concentration of thyroglobulin-antibodies (Tg-Ab) had no effect. Tg-Ab depleted IgG retained their cytotoxic activity whereas Tg-Ab depleted and TPO-Ab depleted IgG lost it. Conclusion : TPO-Ab are the antibodies responsible for ADCC in autoimmune thyroid disease. This further points to TPO as a major antigen in thyroid autoimmunity.

41. P21 CLONAL T-CELL RESPONSE TO hTSHR-PEPTIDE REVEALED BY RADIO-LABELLED PCR.

Munetoshi Nakashima and Andreas Martin, Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA.

Peripheral blood mononuclear cells (PBMC) from a patient with Graves' disease with extrathyroidal manifestations (including pretibial myxedema and acropachy) were stimulated with a panel of 29 overlapping, synthetic peptides of the extracellular domain of the human TSH-receptor (hTSHR; J. Morris, Mayo Clinic) and showed a significant proliferative T-cell response towards peptide 121-140 (stimulation index = 5.3, t-test, $p<0.05$). To evaluate the clonality of the responding T cells, uncloned T cells from 1-week cultures of PBMC with peptide 121-140 and control peptide were compared for their human T-cell receptor (hTcR) V gene families using a radio-labelled PCR approach and confirmatory sequencing. The radio-labelled PCR is based on the differing lengths of the hTcR complementarity-determining region 3 (CDR3) which is subject to random nucleotide additions and deletions. Hence T-cell clones can have CDR3 regions of different lengths which can be visualized as distinct bands for each hTcR V gene family. The radio-labelled PCRs were performed with 18 Vα and 21 Vß oligonucleotides as forward primers and a ^{32}P-labelled C region oligonucleotide as reverse primer. PCR products were then separated on polyacrylamide gels and exposed. Enhanced bands were only observed in the cultures of peptide 121-140 stimulated T-cell cultures compared to controls. These bands were seen in a limited set of V gene families (Vα14, Vα15, Vß1 and Vß7). Clonality was confirmed by sequencing the cloned Vß hTCR PCR-ligation products of Vß1 and Vß7 (four bacterial colonies per V gene family). Three out of 4 sequences for Vß7 (75%) showed complete identity of their CDR3 regions (6 amino acids) and their J segments (Jß2.1) whereas 4 sequences obtained for the Vß1 family were heterogenous for both CDR3 and J segments. In conclusion, radio-labelled PCR is a practical approach for the rapid identification of peptide-induced, expanded T cells. Our data suggest that the T-cell autoimmune response to thyroid peptide is restricted, but involves both clonal and non-clonal elements.

42. P22 CONGENITAL HYPOTHYROIDISM SCREENING PROGRAMS PROVIDE A SENSITIVE METHOD FOR THE IDENTIFICATION OF POPULATIONS AT RISK FOR ENDEMIC GOITER.

G. Costante, O. Ludovico, M. Nocera, G. Parlato*, E. Schifino, M.F. Marasco, U. Crocetti,C. Capula, S. Filetti. Dipartimento di Medicina Sperimentale e Clinica and *Istituto di Chimica Clinica - University of Reggio Calabria, Catanzaro, Italy.

Evidence of thyroid insufficiency, including elevation of circulating TSH levels, may occur in newborns of women resident in iodine deficiency areas. Several reports have, in fact, documented severe iodine deficiency as a cause of higher newborn recall rate in congenital hypothyroidism screening programs performed by monitoring neonatal TSH levels. However, the recall rate is likely to represent an underestimation of the prevalence of endemic goiter in mild to moderate iodine dificiency areas. Aim of the present study was to analyze data from the congenital hypothyroidism screening program in Calabria, a Southern Italy region in which several foci of iodine deficiency (mean±SD urinary iodine excretion ranging from 47±23.5 to 74.6±28.0 µg/g UCr) have been identified. TSH levels on whole blood at day 5 of life were measured in 21,674 infants (>95 % coverage; 51.1 % males, 48.9 % females) born in the region from January 1st to december 31st, 1993. The cut-off TSH value for recall was established at 20 µU/ml. The recall rate was 0.9 % (n=19). Fourteen newborns, all with TSH values >30 µU/ml, were subsequently diagnosed with congenital hypothyroidism and were excluded from further analysis of the data. The frequency distribution analysis showed that the median neonatal TSH level was 2 µU/ml and the mode (28 % of newborns) corresponded to neonatal TSH values <1 µU/ml. The 95 % TSH cut-off was 10 µU/ml, while 4.5 % of newborns had TSH levels >10<20 µU/ml. The results were also examined in relation to the place of origin of the infants. For this purpose, the region was divided in 22 areas, according to the geomorphological characteristics of the territory. Individual areas comprised 371-1360 newborns. The distribution analysis of neonatal TSH in these areas showed that for neonatal TSH levels ranging from <1 µU/ml to 10 µU/ml, no significant difference could be demonstrated among different areas. On the contrary, the frequency of TSH levels >10<20 µU/ml in individual areas varied from 2.2 % to 7.3 %. Furthermore, a significant correlation ($r=0.814$; $p<0.01$) was observed between the frequency of neonatal TSH values >10<20 µU/ml and the prevalence of goiter in school children, in those areas (n=8) for which epidemiological data were available (total number of school children examined = 9,572). In conclusion, these results indicate that 1) congenital hypothyroidism screening programs performed by neonatal TSH measurement can be used for the identification of the populations at risk in mild to moderate iodine deficiency regions; 2) the frequency of neonatal TSH values >10<20µU/ml provides a good estimation for the prevalence of endemic goiter in such areas.

43. P23 STUDIES OF RE-XENOGRAFTED THYROID TISSUE OF GRAVES' DISEASE (GD) AFTER SOJOURN IN NUDE MICE INTO SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE: THE EFFECT OF ADDING AUTOLOGOUS PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) WITH A SURFEIT OF CD8+ T CELLS. T. Mukuta, G. Arreaza, E. Resetkova, K. Kawai, T. Enomoto, and R. Volpé, Endocrinology Research Laboratory, Wellesley Hospital, University of Toronto, Toronto, Ontario M4Y 1J3, Canada.

To investigate the effect of adding a surfeit of autologous CD8+ cells as a potential immunomodulator in GD, thyroid tissues from 4 patients with GD and 2 normal persons (N) were initially xenografted into Nude mice. Eight wks after xenografting, the now normalized thyroid tissues were retrieved from the Nude mice, and re-xenografted into SCID mice (rTH). 20 x 10^6 (20M) autologous PBMC, or 20M of CD8+-depleted PBMC (non-CD8+ cells, ie., CD4 enriched PBMC), were simultaneously engrafted into SCID mice with rTH. In addition, 20M of CD8+ enriched PBMC (CD8+ doubled cells, prepared to double the percentage of CD8+ T cells compared to that of PBMC) were engrafted into SCID mice with rTH from 2 GD and 2 N; finally, 20M of PBMC + an extra 10M of CD8+ T cells (total 30M of CD8 enriched cells) were engrafted into separate SCID mice with rTH from 2 GD. The re-engraftment of GD or N rTH alone did not result in the detection of human IgG, thyroperoxidase (TPO)-antibodies (Abs), thyroglobulin (Tg)-Ab, thyroid-stimulating Abs (TSAb), or lymphocytic infiltration in rTH. The engraftment of autologous PBMC or non-CD8+ cells from all GD and N into SCID mice with rTH resulted in the appearance and elevation of human IgG; when human IgG, TPO-Ab, Tg-Ab and TSAb were quantitated, GD rTH + non-CD8+ cell - engrafted SCID mice showed higher production of human IgG and each Ab than in GD rTH + PBMC, or GD rTH + CD8-doubled cells, or GD rTH + extra CD8-added cells. Moreover, when CD8+ doubled cells, or extra CD8+ added cells were engrafted to SCID mice with rTH, they showed generally lower production of human IgG and thyroid Abs, compared to SCID mice receiving PBMC engrafted with rTH, despite the fact that 50% more T cells (30 M) were engrafted in the preparations of extra CD8+ added cells. In conclusion, CD8+ T cells from patients with GD, when in excess, appeared to suppress the induction of human IgG, thyroid Abs and TSAb. The CD8+ cells thus are acting as suppressor or regulatory T cells, and might be important in the pathogenesis of autoimmune thyroid disease.

44. P24 **ACTIVATED AND IFN-γ PRODUCING PERIPHERAL AND THYROID DERIVED T CELLS ARE DETECTED IN GRAVES' DISEASE, THYROID AUTONOMY AS WELL AS IN NON-TOXIC MULTINODULAR GOITER**

G. Aust , I. Lehmann*, S. Laue, H-J. Heberling°, Department of Internal Medicine III and *Institute of Immunology and Transfusion Medicine, University of Leipzig; °City Hospital Leipzig, Germany

The relative numbers of activated and IFN-γ producing peripheral blood (PBL) and thyroid derived (TL) lymphocytes were determined using double surface and a novel intracellular paraformaldehyde-saponin labeling technique in flow cytometry. Cells were analyzed from 10 patients with Graves' disease (GD), 8 patients with thyroid autonomy (TA) and 7 patients with non-toxic multinodular goiter (NTG). TL were separated after enzymatic digestion of thyroid tissue.

A maximum of 1 % IFN-γ^+ cells was detected both in PBL and TL. Stimulation using phorbol 12-myristate 13-acetate (PMA, 10 ng/ml) and ionomycin (1 μM) together with monensin (1 μM) for 5 hrs caused a 2 to 3 fold higher number of IFN-γ^+ cells in TL (GD: 48 ± 12 %, TA: 48 ± 11%, NTG: 50 ± 14 %) than in PBL (GD: 15 ± 7%, TA: 16 ± 8%, NTG: 18 ± 11%) of the same patients. There were no significant differences between the three investigated groups. Nearly all IFN-γ^+ TL in patients with GD were $CD3^+$ T cells. The number of $CD16/CD56^+$ $CD3^-$ natural cells (NK) cells among TL was very low (5 ± 4 %). In TL of patients with TA and NTG, which showed a significantly higher number of NK cells, 10 - 20 % of IFN-γ^+ cells were NK cells. In PBL 80 % and in TL of almost 100% IFN-γ^+ cells were antigen primed $CD45RO^+$cells. Surprisingly only 25 - 35 % of IFN- γ^+ thyroid derived T (tT) cells were $CD4^+$.

42 ± 12 % of tT cells in GD, 31 ± 9 % in TA and 34 ± 10 % in NTG expressed the HLA-DR molecule but not the IL-2 (CD25) or the transferrin receptor (CD71). Only 40% of these HLA-DR^+ tT cells showed an intracellular staining with IFN-γ. Half of the HLA-DR^+ tT cells coexpressed the activation antigen CD69, which is present exclusively on mononuclear cells. Two additional experiments were done to exclude the in vitro induction of HLA-DR and CD69 on tT cells in TA and NTG. Using semi-quantitative RT-PCR high levels of CD69 mRNA were detected within the various thyroid tissues. Immunofluorescence double labeling on 4μm cryostate sections of thyroid tissue showed, that HLA-DR^+ T cells were also present in situ.

The detection of activation antigens on tT cells not only in patients with GD but also in TA and NTG suggests failsafe mechanisms such as anergy, suppression or cytokine regulation in so called non-immunogenic goitre. The ability of activated tT cells to cause autoimmunity is influenced not only by their quality but also by their quantity, wich is known to be different in the three investigated groups.

45. P25 **IMPAIRED AUTOLOGOUS MIXED LYMPHOCYTES REACTION IN PATIENTS WITH HYPOTHYROIDISM UNDERGOING HEMODIALYSIS TREATMENT.** T.Tokoro, Y.Ogawa. The 2nd Dept. of Internal Medicine, Kansai Medical University, Moriguchi, Osaka 570, Japan.

We have recently demonstrated the impairment of autologous mixed lymphocytes reaction (AMLR) in patients with autoimmune thyroid disease. Some patients undergoing hemodialysis (HD) treatment reveal hypothyroidism.The reasons for this have still been uncertain , although some explanations such as abnormal cellular immunity , impaired iodine metabolism , and reduced response of the thyroid to thyroid stimulating hormone (TSH) have been proposed.We thus studied the cellular immunity including the AMLR of peripheral blood mononuclear cells (PBMC) from patients with hypothyroidism undergoing hemodialysis treatment (HYPO+HD) , in an attempt to elucidate the possible roles of cellular immunity on the pathogenesis of HYPO+HD. Serum triiodothyronine, free thyroxine, TSH, thyroid-autoantibodies were measured from 6 HYPO+HD patients, 12 HD patients, and 12 normal volunteers (N) . Autologous PBMC were obtained by different gradient centrifugation. 10^6 cell / mL suspended in RPMI1640 medium were cultured at 37℃ for 7 days. After culture, PBMC were stained with fluorescent-conjugated monoclonal antibody (mAb) (anti-CD3,CD4,and CD8 for the determination of T cell subsets and anti-HLA-DR for activated T cell marker) . T cell activation by AMLR was then analyzed on a flow cytometer. In the AMLR, the activation of $CD3^+$ and $CD4^+$ T lymphocytes from HD and HYPO+HD patients were significantly impaired compared to N. The mean values of the activation of $CD3^+$ and $CD4^+$ T cells were lower in HYPO+HD PBMC than those in HD although these differences did not reach significance.The activation of $CD3^+$ T cells from patients with HYPO+HD showed a significant negative correlation with their serum TSH levels ; The impaired activation of $CD3^+$ T cells in the AMLR at the beginning of hemodialysis treatment (time 0) significantly ameliorated after single hemodialysis session (time 240 min) . In conclusion, dialysable uremic subatances and/or hypothyroid state *per se* might be the factors for an abnormality of cellular immunity in HYPO+HD patients.

46. P26 T-LYMPHOCYTES RESPONSE TO *Yersinia enterocolitica 0:9* IN PATIENTS WITH AUTOIMMUNE THYROID DISEASES(AITD). I. Zubaschev, E. Gaspar*, A. Peters, B.E. Wenzel, *Haynal University, Budapest; Cell & Immunobiol.Lab., Dept. Internal Medicine, Med. University Lübeck, Germany.

There is substancial clinical and experimental evidence for an association of a persisting subclinical infection with enteropathogenic *Yersinia enterocolitica(Y.e.)* together with the manifestation of AITD. Moreover, *Y.e.*-antigens show crossreactivity with thyroid antigens and with heat shock protein 72/73. In mice, superantigenicity of *Y.e.* membrane antigen was demonstrated and immunization of BALBc mice with *Y.e.* antigen produced thyrotrophin-receptor antibodies together with the manifestation of hyperthyroidism. In the present study, we investigated the type of the T-lymphocytes response to *Y.e.* virulence antigens in patients with Graves Disease(GD) or Hashimoto's thyroiditis(HT). Mononuclear cells(MNC) were stimulated with *Y.e.* virulence antigens for 72h. Thereafter, the cytokine profile in culture supernatants and the thymidine uptake of MNCs were measured. T and non T-cells were separated, antigenprocessing was blocked by gentle formaldehyd fixation of accesssory cells, T and non T-cells were reconstituted and the proliferative response was measured. In addition, the Vß-repertoire of the T-cell receptors from responding MNCs was determined. *Y.e.*-virulence antigen stimulated the IL-2, IL-10, IFN-γ and TNF-α secretion of T-cells from normals and from patients with AITD. The proliferative response was not dependent on the antigen processing of accessory cells. The Vß-repertoire of TCR was restricted by 30% of the 22 specificities with an enhanced expression of $V\beta_{15/17}$. The proliferative T cell responses of patients were significantly increased in GD-patients($p< 0.05$) and HT ($p< 0.001$), when compared to normal controls. The prevalence of *Y.e.*-antibodies in patients sera was 75%(n=50) compared to 35%(n=300) in normals. Our findings suggest that in vitro *Y.e.*-antigen induces a secondary TH1 response of MNCs from patients with AITD. Further analysis of the interaction of *Y.e.*-antigen with MHC and the Vß-TCR will elucidate wether Y.e. also acts as a superantigen.

Supported by Deutsche Forschungsgemeinschaft, SFB 367/B6

47. P27 Class II expression and function of dendritic cells from thyroid gland draining lymph nodes of the BB rat

Frans G.A. Delemarre, Peter J. Simons and Hemmo A. Drexhage, Dept. of Immunology, Erasmus University, Rotterdam.

The BB rat is a model for spontaneous thyroid autoimmune disease. The first events of disease are an accumulation of class II positive cells with a dendritic morphology and the development of plasma cells with anti-thyroid antibodies in the thyroid draining lymph nodes (TLN). The report on the prevention of diabetes in NOD mice by transfer of dendritic cells (DC) from lymph nodes which drain the pancreas[1], prompted us to study whether this phenomenon also holds for the BB rat with respect to the development of thyroid autoimmunity.

Three weeks old BB rats were kept on an enriched iodine diet to accelerate the process of thyroid autoimmunity. TLN were removed for isolation of DC and for histological studies. Isolated DC were injected subcutaneously into the footpads of 3 weeks old BB rats, which were then put on an enriched iodine diet up to 19 weeks. The development of autoimmune thyroiditis was monitored by screening of blood samples for anti-colloid antibodies on frozen sections of porcine thyroid gland and by determinating of infiltrates in the thyroid gland. Class II expression of isolated DC and DC *in situ* in the TLN was determined.

DC were present in low numbers in the T cell area of the TLN and had a low expression of class II molecules as compared to the Wistar rat. Macrophages were in the majority in the TLN. Upon isolation however TLN DC had a strong class II expression. With regard to the transfer, anti-colloid serum antibodies were detected after 2 weeks of diet in 9% of untreated or PBS-treated recipients. This percentage increased up to 90% after 9-11 weeks. All animals had become already positive for anti-colloid antibodies after 13 weeks of diet. The first rats (9%) treated with TLN DC became positive for autoantibodies after 6 weeks of diet and 71% of these rats had autoantibodies after 19 weeks. Rats which were positive for anti-colloid antibodies had infiltrates in the thyroid glands.

In conclusion, the DC population of the TLN of the BB rat is abnormal with respect to their number and class II expression. Upon isolation DC class II expression is upregulated and transfer of these cells modulates the autoimmune response resulting in a delayed development of anti-colloid serum antibodies in the BB rat.

[1] *M.J. Clare-Salzler et al., J. Clin. Invest. 90, 741, 1992*

48. P28 EXPRESSION OF HLA-DR, THYROID PEROXIDASE AND THYROGLOBULIN ON CULTURED HUMAN THYROID CELLS.Å.K.Rasmussen,M.-L.Hartoft-Nielsen,P.Carayon,U.-Feldt-Rasmussen,K.Buschard,University Depart. of Endocrinology,Rigshospitalet,Bartholin Institute,Copenhagen,Denmark,U38 INSIRM Marseille Medical School,France.

This experiment was conducted in order to investigate human leukocyte antigen (HLA-DR), thyroid peroxidase (TPO) and thyroglobulin (Tg) expression on human thyroid cell surface by an indirect immunofluorescence-activated cell sorter (FACStar IV, Becton-Dickinson). Human paraadenomatous thyroid cells were cultured in monolayers in T-80 flasks and detached by PBS-EDTA and mild mechanic treatment. The cells were rinsed, pelleted and resuspended in 0.3 ml PBS, 0.2% BSA, pH 7.4. The specific antibodies and the fluorescein-conjugated second antibodies were each added for 30 min at 4C, and washed three times in between. Thyrocytes (1×10^5) were analysed by FACS for cell size (forward-angle light scattering) and antigen expression (scatter gated fluorescence reactivity). Data are expressed as percentage fluorescence positive thyrocytes. For controls the cells were stained with irrelevant IgG alternatively the first step antibody was omitted.

HLA-DR was identified in 98% of the cells both with and without 72h TSH stimulation. Tg was expressed both using a monoclonal (MAb) and a polyclonal anti-Tg antibody, and TSH stimulated the expression from 58.9% to 73.2% (n=3). Using MAbs towards different epitopes of TPO (Mab 2,9,15,18,24,30,40,47,53,59,60,64) the TSH stimulated expression was dose-dependent and varied between 5.6% (Mab 47) and 88% (Mab 18). Serum from a patient with Hashimoto's disease also induced a dose-dependent expression of TPO with up to 80% positive stained cells.

FACS analysis of thyroid cells in culture seems to be a useful method for detection of surface antigen expression. Furthermore, the expression of different epitopes during various culture conditions can be disclosed by the use of MAbs.

49. P29 **Intrathyroidal infiltrating lymphocytes (ITL) from patients with Graves' disease (GD) induce functional and histological changes in human thyroid transplants in vivo**

PM Schumm-Draeger[1], K Rippegather[1], Z Abasi[2], C Müller[3], HJC Wenisch[3], G Herrmann[4], KH Usadel[1], Department of Internal Medicine I[1], Centres of Animal Research[2], Surgery[3] and Pathology[4], University Clinics Frankfurt/Main, F.R.G.

Up to now immunological mechanisms leading to autoimmune thyroid disease (AITD) are not yet fully understood. As, according to in vitro studies, ITL are considered to play a major role in the development of AITD, we were prompted to study the effect of ITL in comparison to peripheral blood lymphocytes (PBL) from patients with GD, non toxic nodular goiter (NTG), and toxic adenoma (TA) in the nude mouse xenotransplantation model.

Methods: Immediately after surgery, preparation and characterization of ITL and PBL from 10 patients with GD, NTG, and TA each were carried out and subsequently injected (i.v., 0.2 ml, > 10^3 cells/ul) into 5 athymic nude mice, each bearing transplants of normal human thyroid tissue. Controls received saline only. Two days after the injections of ITL and PBL 1) the ^{131}I-bound thyroxine (T4) was determined in mice to analyse thyroid function, 2) TSH-receptor antibody (TSHRAB) titers in mice sera were measured (Henning Berlin), and 3) histological and immunohistochemical examination of thyroid transplants, with special regard to HLA-class-II and ICAM-1 expression (Dianova/Immunotech) was carried out.

Results: ITL derived from patients with GD are able to induce thyroid hyperfunction, via TSHRAB production, as shown by a significant increase of ^{131}I-bound T4 and of TSHRAB titers, in contrast to controls and those study groups which received ITL or PBL from patients with NTG and TA. Furthermore, only after injections of ITL from patients with GD was a significant increase in the expression of HLA-class-II and ICAM-1 in thyroid transplants observed.

Conclusion: Our in vivo data strongly suggest a most important role of ITL in the development of AITD. Further studies are needed to elucidate the action of different lymphocyte subgroups, as well as of cytokines and specific autoantibodies in vivo.

50. P30 EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND P53 GENES IN THYROID CARCINOMA. Chen Lin, Huai-Yin Shi, Li Rong, General Hospital Of PLA, Beijing, China.

The correlation between the prognosis and abnormal expression of epidermal growth factor receptor (EGFR) and p53 mutations were studied in 52 cases of thyroid carcinomas. Of the 52 cases, 32 were papillary type, 10 were follicular type , 6 were medullary type and 4 were undifferentiated type. The papillary and follicular types were classified as well-differentiated thyroid carcinomas and medullary and undifferentiated types were classified as poor-differentiated thyroid carcinomas. Mutant p53 protein and EGFR simultaneously determined with specific monoclonal antibodies by immunohistochemistry. The results showed that 4 of 6 (66.67%) cases of medullary carcinomas and 3 of 4 (75%) cases of undifferentiated carcinomas were mutant p53 positive, only 3 of 32 (9.38%)cases of papillary carcinomas and 2 of 10 (20%) cases of follicular carcinomas were mutant p53 protein positive . The difference was statistically significant ($p<0.01$) between the well differentiated types and poor differentiated types. 5 of 6 (83.33%) cases of medullary carcinomas and 4 of 4 (100%) cases of undifferentiated carcinomas were EGFR positive, but only 8 of 24 (25%) cases of papillary carcinomas and 3 of 10 (30%) cases of follicular carcinomas present positive staining for EGFR. The difference was statistically significant($P<0.01$) between the well differentiated types and poor differentiated types . These results suggest that the expression of p53 and EGFR were strongly correlated to the differentiation of thyroid carcinoma. Since patients of medullary and undifferentiated carcinomas have poor prognosis clinically than the papillary and follicular carcinomas, it is indicated that p53 and EGFR seem to be significant prognostic parameters for thyroid carcinomas.

51. P31 THYROID CARCINOMA IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS. - REPORT OF TWO CASES AND REVIEW OF THE LITERATURE - Kaoru Kobayashi, Hiroyuki Maeta, Yoshimasa Suzuki, Yoshiyuki Tanaka, Tohru Mori, Chiaki Shigemasa Second Department of Surgery and First Department of Internal Medicine, Tottori University Faculty of Medicine, Yonago, Tottori 683, Japan.

Familial adenomatous polyposis (FAP) including Gardner's syndrome is an autosomal dominant disease characterized by the development of hundreds of colorectal adenomas in young adults. These colorectal adenomas will develop colorectal carcinomas by the fifth decade of life. Extraintestinal cancers have been in association with FAP. These include malignancy of the adrenal gland, biliary tract, pancreas and thyroid gland. Genetic mechanisms of tumorigenesis of colorectal and thyroid neoplasms are now elucidating. Recently, the adenomatous polyposis coli gene was referred as being located on chromosome 5q21. The purpose of this study is to report two cases of thyroid carcinoma associated with FAP, and review the literature with special attention to clinical and pathological features of thyroid carcinoma in those patients. Case 1; A 22-year-old woman underwent total thyroidectomy and cervical lymph nodes dissection. Pathological examination showed approximate 50 lesions of papillary carcinoma in the thyroid gland and no metastatic carcinoma in cervical lymph nodes. Six months after the surgery, total colectomy and ileo-rectal anastomosis were performed because of FAP. The specimen of the colon showed 642 lesions of polyps. Case 2; A 22-year-old woman was diagnosed as a papillary thyroid carcinoma. She had undergone total colectomy and ileo-rectal anastomosis two years before because of FAP. The specimen of the colon had showed 164 lesions of polyps. Total thyroidectomy and bilateral cervical lymph nodes dissection were performed. Pathological examination showed seven lesions of papillary carcinoma in the thyroid gland and no metastatic carcinoma in cervical lymph nodes. To date, 56 cases, including the present patients, of thyroid carcinoma associated with FAP have been reported in the literature. When the pathological diagnosis was mentioned (N=39), papillary thyroid carcinoma accounted for 87.2 % (N=34) and follicular carcinoma for 12.8 % (N=5) of the neoplasms. When the patient's sex was mentioned (N=54), 87.0 % (N=47) were females and only 13.0 % (N=7) were males, giving a female-to-male ratio 6.7 to 1. The average age at which FAP was recognized was 25.7 years (range 14-63, N=36) and at which thyroid carcinoma was diagnosed was 28.9 years (range 16-72, N=51), but only 12 patients were over age 30 and only 5 was over age 40 at the time their thyroid carcinoma was recognized. Thyroid carcinoma was recognized in 66.5 % (N=39) of the patient before age 30. Multiple lesions in the thyroid gland were mentioned in 73.1 % (N=19) of the patients in which information concerning tumor number was provided. In conclusion, thyroid carcinoma in patients with FAP tends to appear in females at a relatively young age, especially before age 30, and present a papillary thyroid carcinoma and multicentric lesions in the thyroid gland. We should pay careful attention to the presence of thyroid carcinoma in a patient with, and total thyroidectomy should be performed because of multicentric lesions in the thyroid gland.

52. P32 COMPARISON OF P53 MUTATION BETWEEN CHINESE AND JAPANESE PATIENTS WITH POORLY DIFFERENTIATED THYROID CARCINOMA. R.X. Chen[1,2], T. Masuda[1], M. Saito[3], H. Naganuma[4], M.Watanabe[1], J.S. Cui[2], J.H. Dong[2] and S. Mori[1], [1]Tohoku University School of Medicine, Sendai, Japan. [2]Norman Bethune University of Medical Sciences, Changchun, China. [3]Akita University School of Medicine, Akita, Japan. [4]Sendai City Hospital, Sendai, Japan.

We investigated 44 patients (15 Chinese and 29 Japanese) with poorly differentiated thyroid carcinoma to compare tumor suppressor gene p53 overexpression and p53 mutation in the ethical groups with completely different life habits and geographical backgrounds. Diagnosis of all cases were histologically done with operative materials, 15 cases from Norman Bethune University of Medical Science in China during 1988-1991, 29 cases from Tohoku University and Sendai City Hospital in Japan during 1984-1992. p53 overexpressions were investigated with immunohistochemical method using a monoclonal antibody (DO7, Novocastra Newcastle, England). We selected morphological particular cells from non-stain sections under a microscope, disintegrated the protein, amplified the intentional DNA by PCR flanking exon5-8, and then separated PCR products with cold-SSCP. The DNA of p53 SSCP suspicious variants was sequenced by ABI 373 sequencer. There was a significant difference of p53 overexpression between China and Japan ($p=0.025$). The p53 positive rate is 93% (14/15, China) and 55% (16/29, Japan) respectively. On the other hand, there were no any significant differences in cold-SSCP and sequence between the 2 groups. The rates of certain mutation were 46.7% in the Chinese (5/15, including 4 cases of exon5, 1 case of exon7 and 2 cases of exon8) and 42.3% in the Japanese (11/26, including 9 cases of exon5, 2 cases of exon7 and 0 cases of exon8). Although we are still doing the sequence for all cases of p53 SSCP suspicious variants, up to date, in the 8 cases examined there have been 1 case each of mutations at codon 162 (ATC-ATA) and codon 175 (CGC-CAC). Our concluded that overexpression of p53 tumor suppressor gene has strong relation with human poorly differentiated thyroid carcinoma, but there are some different gene mutations between the Chinese and Japanese.

53. P33 THYROGLOBULIN, bcl-2 AND p53 IMMUNOREACTIVITY IN POORLY DIFFERENTIATED CARCINOMA VARIANTS OF THE THYROID GLAND. P. Collini, S. Rao, C. Lavarino, F. Rilke, Istituto Nazionale Tumori, Milan, Italy

Bouin- or formalin-fixed paraffin-embedded archival material of 73 cases (22.9%) of poorly differentiated carcinoma (PDC) of the thyroid gland selected from 278 well differentiated and 41 undifferentiated cases of carcinomas of the thyroid gland has been tested for the immunoreactivity for thyroglobulin (Tg) (MAb J7-B49-9), bcl-2 (MAb 124) and p53 (MAb DO7) proteins using microwave oven retrieval. The 73 PDCs were made up of 43 cases of the tall-cell and columnar-cell variants of papillary carcinoma (TC-CC), 20 cases of the insular carcinoma variant (INS) and 10 cases of the variant described by Sakamoto (SAK). The immunoreactivity is shown in the following table:

		PDC	
VARIANTS	TC-CC	INS	SAK
Tg	43/43 (100%)	20/20 (100%)	5/10 (50%)
bcl-2	32/43 (74.4%)	18/20 (90%)	8/10 (80%)
p53	4/43 (9.3%)	4/20 (20%)	4/10 (40%)

The results show a similarly maintained immunoreactivity for Tg and bcl-2 for the three variants, with the exception of an earlier switch-off of Tg than bcl-2 reactivity in the SAK variant. This contrasted with a progressively increasing p53 reactivity from TC-CC toward the SAK variants. The observed data suggest a more aggressive biological behavior of INS with respect to TC-CC variant, and show the SAK variant as being the most aggressive one within the spectrum of PDCs.

54. P34 **Utilization of 'Cold'-SSCP Screening For Clinical Diagnostic Laboratory Mutation Detection in the Human Thyroid Receptor-β and the *p53* Tumor Suppressor Genes**

M. B. Grace[1], A. M. Hruszkewycz[2], R. M. Delgado[2], W. P. Bennett[3] and G. S. Buzard[4]
[1]MCEB/ NIDDK, [2]Clin. Path. Dept.Clin. Cntr., [3]DCE/NCI, [4]PRI/DynCorp NCI-FCRDC,
[1,2,3] National Institutes of Health, Bethesda & [4]Frederick MD.

Traditional clinical laboratory testing can be complemented by molecular diagnostic methods such as single-strand conformation polymorphism (SSCP) analysis. SSCP analysis permits an earlier and indisputable diagnosis of germline mutations in genetic diseases and can also be used in mutation screening for predisposition to and detection of cancer. 'Cold'-SSCP analysis is conducted on commercial minigels under tightly regulated and highly optimized temperature and gel electrophoretic parameters using a recirculating buffer-modified XCell II Mini-Cell (NOVEX, San Diego, CA) with non-isotopic detection of bands. We have successfully applied 'Cold'-SSCP mutational analysis to studies of the ligand-binding domain of the thyroid hormone receptor-β (hTR-β) gene in kindreds with the clinical diagnosis of resistance to thyroid hormone. The method can be applied to somatic mutations in genes such as *p53* which is mutated in thyroid and other neoplasms.

The 'Cold'-SSCP technique has permitted technical developments which make molecular genetic analysis cheaper, faster and easier. A direct comparison was made of the potential clinical applications between three temperature-regulated electrophoresis systems designed for SSCP detection using precast polyacryamide minigels: the PhastSystem (Pharmacia Biotech Inc., Upsala Sweden); the Thermo-flow Mini-Cell (NOVEX, San Diego, CA) and the TherMaster (BioTherm Inc., Falls Church, VA). SSCP analysis was performed for exons 9 and 10 of the hTR-β gene and exons 5 through 8 of the human tumor suppressor gene. Amplicon sizes ranged from 156-398 base-pairs. Preselected optimized amplicon-specific SSCP detection conditions were used. SSCP bands were detected with ethidium bromide, SYBR Green II or silver staining. Silver-staining was the most sensitive method tested and showed single-stranded DNA bands at concentrations as low as 3 ng/μl. Mixing studies of the mutant and wild-type DNAs detected by silver staining on the PhastSystem found detection limits comprised 3% of the total gene copies in a PCR mixture, similar to ethidium bromide staining using the NOVEX system. Recirculating buffer systems provided the highest band resolution and fewest band artifacts resulting from differential and dimensional cooling across the gel. As an adjunct to a more efficient cooling system, precast thin-glass minigels permitted SSCP results in 35 minutes when electrophoresed at 600 Volts, while maintaining a constant in-gel temperature of 26°C; as recorded with an in-gel thermosensor probe. These results demonstrate that the newly developed temperature-controlled electrophoresis systems with non-isotopic staining of DNA in precast gels offer an accurate, convenient and rapid approach for applying SSCP in both the research and clinical diagnostic laboratory.

55. P35 CHARACTERIZATION OF THE *ret*/PTC CHIMERIC INTRONS IN VARIOUS HUMAN PAPILLARY THYROID CARCINOMAS. P.A. Smanik, T.L. Furminger, E.L. Mazzaferri, and S.M. Jhiang, The Ohio State University, Columbus, Ohio.

The *ret*/PTC oncogene, a rearranged form of the *ret* proto-oncogene (*c-ret*), has been detected specifically in a minority of papillary thyroid carcinomas, and may serve as a molecular marker of poor prognosis in these patients. Three forms of the *ret*/PTC oncogene have been identified, in which the *ret* tyrosine kinase domain becomes linked to different amino-terminal sequences. A paracentric inversion of the long arm of chromosome 10 generates the two most common forms, *ret*/PTC-1 and *ret*/PTC-3, where *ret* becomes linked to the H4 or *ele1* gene, respectively. Using an extra-long PCR method, we amplified the chimeric introns, ranging from 1.4 -10 kb, from 4/5 tumors known to contain the *ret*/PTC-1 rearrangement. We also characterized both the chimeric intron and the reciprocal chimeric intron from one tumor containing the *ret*/PTC-3 rearrangement. We localized the breakpoints within the chimeric introns using nested PCR, and determined the exact nucleotide sequence at the breakpoint for each tumor. We also identified *Alu* repetitive sequences in the regions near the breakpoints of these rearrangements. Our results indicate that the breakpoints in *c-ret* occur at sites distributed across intron 11, while breaks in H4 intron 1 appear to occur more frequently at the 5'-end of the intron. In addition, a common feature was observed in the two tumors where we compared the breakpoint sequence to both contributing germline sequences. A three nucleotide match was observed between the *c-ret* and H4 germline sequences in tumor PC-31 (*ret*/PTC-1), and between the *c-ret* and *ele1* germline sequences in tumor PC-9 (*ret*/PTC-3), at the site where the rearrangements occurred. Characterization of the H4 germline sequence at the regions where the breakpoints occurred in additional tumors is currently underway. In conclusion, this study will allow us to develop a genomic PCR method to screen for the presence of *ret*/PTC rearrangement in fine needle aspirates as well as archival paraffin-embedded tissues. As we identify and characterize the *ret*/PTC breakpoints in additional tumors, we will also obtain more information about the mechanism underlying this rearrangement.

56. P36 THYROID CANCER IN THE UKRAINE POST CHERNOBYL T Bogdanova[1], M Bragarnik[1], ND Tronko[1], HR Harach[2], GA Thomas[2], ED Williams[2],
[1]Institute of Endocrinology, Kiev, Ukraine and [2]Department of Histopathology, University of Cambridge, Cambridge, UK CB2 2QQ

Large numbers of thyroid carcinomas have been recorded in Belarus and the Ukraine in children exposed to fallout from Chernobyl. We have analysed the histological features of 45 sequential Ukrainian cases and studied the expression of thyroglobulin (Tg) and calcitonin (Ct) mRNA by in situ hybridisation, and Tg, Ct, met and ret by immunohistochemistry (ICC). We have compared these results to the findings in 78 cases from the UK childhood cancer registry. We have also compared the age and sex incidence of all 114 cases seen in the Institute of Endocrinology in Kiev from 1990-1994 with that of 154 cases registered from the whole of England and Wales from 1963-1992.

The range of morphological patterns seen in both series were similar, in particular both showed two patterns of papillary carcinoma (PC), a solid follicular variant and a classic type, but the relative frequency of the two types differed markedly.

	solid follicular PC	classic PC	other PC	follicular, medullary and other Ca
Ukraine	76%	12%	7%	5%
UK	23%	28%	17%	31%

It can be seen that the major difference lies in the % of SF papillary carcinomas. Ret and met ICC showed some differences between the different types of tumour, but no consistent difference between the two series. In both series over 80% of papillary carcinomas showed ret positivity. Tg ICC and ISH results were also similar in the two series.

Analysis of the place of residence data showed the highest rate of thyroid cancer occurred in the most heavily contaminated regions. Of 18 cases seen during 21 months from an area with the population of about 1 million children, 17 were of the SF papillary type. We suggest that this dominant histological type is related to the aetiological agent involved. There was a clear change in age at operation over time, consistent with a causative event at the time of the Chernobyl accident, and suggesting that the causative agent does not persist in the environment. These findings provide strong suggestive evidence for exposure to radioisotopes of iodine in fallout from Chernobyl as the cause of the considerable increase in incidence of childhood thyroid carcinoma in the Ukraine..

57. P37 THYROID TUMOURS IN PERSONS FROM TWO REGIONS OF RUSSIA AFFECTED BY CHERNOBYL ACCIDENT RADIATION. *M.Bronstein, V.Bogdanov, I.Panteleev, Dept. of Pathomorphology and Dept. of Surgery, Endocr.Sci.Center, Moscow, Russia.*

In 1991-1994 population screening was performed in some areas of Tula and Tambov regions (Russia)affected by Chernobyl radiation. 202 persons suspected of having thyroid tumors were selected for punction biopsy. Age of the patients (pts) was 7.5-69 years (up to 17 years - 28 pts, over 61 - 20 pts); females - 286, males - 6. Adenomas of various structure verified by cytologic investigation were identified in 25 (8.5%) pts, 5 of them were Askenazy-(B)-cell adenomas; in 2 cases adenomas were double-sided. Thyroid carcinomas were found in 9 (3%) pts: 6 (66.6%) of them are papillary carcinomas, 2 (22.2%) - A-cell follicular carcinomas and 1 (11.1%) - B-cell carcinoma. In 30 (10.2%) cases lymphocytic thyroiditis was revealed; in 25 (8.5%) cases were found autoimmune processes on the background of the diffuse goiter (chronic strumitis). All other patients (69.8%) had nodular goiters (solitary or multinodular or diffuse nodular). It noteworthy that adenomas were found out in pts of all ages but carcinomas in pts from 17 years (in adults) and 3.5 times more often in women. In the same period it was performed punction biopsy of thyroid gland in 4651 pts with various thyroid pathology predominantly from Moscow and Moscow region (control group). Adenomas including B-cell adenomas were detected in 418 (9%) pts. Cancers were found in 87 (1.9%) pts. (77 females, 10 males) aged 8-73 years. It is about 55.5% less than in the patients of regions affected by radiation. 50 (57.5%) cancers were papillary, 15 (17.2%) were follicular, 3 (3.4%) - papillary-folicullary. 1 (1.1%) - undifferentiated, 13 (15%) - B-call carcinomas, 5 (5.7%) C-cell carcinomas. Thus, in the persons from radiation affected regions may be stated a tendency to the increase of frequency of thyroid cancers, predominantly papillary from A-cells.

58. P38 EXPERIENCE WITH ORAL IODIZED OIL IN THE REGION OF RUSSIA WITH MILD IODINE DEFICIENCY. N.Sviridenko, N.Mayorova, B.Mishenko, M.Arbusova. Russian Endocrinology Research Centre, Moscow, Russia

Iodized oil is been extensively using for IDD control in areas with severe iodine deficiency. Few data is available on its use in regions with marginal iodine deficiency. Single oral dose of iodized oil (capsull Lipiodol, containing 0.2 g of iodine) was given to 385 village and 452 town children aged 7-14 years in iodine deficient area near Moscow. Goiter prevalence by ultrasonography was varied 31 - 47% in children from town children and 43 - 61% in village children. Medan urinary iodine level was 2.5 - 4.9 mcg/dl in village children and 5.7 - 8.8 mcg/dl in town children. One week after iodized oil administration median urine iodine level was 38.8 mcg/dl, after 2 weeks - 30 mcg/dl, after 3 months - 16 mcg/dl, after 9 month - 11.3 mcg/dl. Goiter prevalence significantly decreased to 18 - 22% in town children and to 29 - 42% in village children. Goiter prevalence in control group of children without iodized oilsupplementation didn't change. Thus, single dose of iodized oil secures adequate iodine supply for at least 9 month in children living in an area with mild and moderate iodine deficiency. Nine month, after iodized oiladministration mean TSH level significantly ($p<0.001$) decreased in the limits of normal range. No acute effect of iodized oil was found on microsomal antibodies. Lipiodol may be an alternative to other methods of iodine supplementation especially in areas close to nuclear power stations to prevent excessive radioiodine uptake in case of accident when iodized salt of good quality cannot be currently obtained.

59. P39 MANAGEMENT OF CHILDHOOD THYROID CANCER FOLLOWING THE CHERNOBYL ACCIDENT. T. Delbot, L. Leenhardt, A. Moutet*, D. Leguillouzic, B. Gameiro, M. L. Simonet*and A. Aurengo, Department of Nuclear Medicine, Pitié Hospital, 83 Bd de l'Hôpital,*Les Enfants de Tchernobyl, 110 bis Avenue d'Italie, 75013 Paris, France.

Morę than 4000 children evacuated from Prypiat (close to Chernobyl) were followed up at the Kiev Franco-Ukrainian Center.This Center has managed 26 cases of thyroid cancer diagnosed among children living in the area of Chernobyl at the time of the explosion. After initial thyroidectomy in Ukraine, 17 of them (9 girls and 8 boys) underwent further investigations in Paris by our own care and 14 of them received radioiodine treatment in our Department. The mean age of these children at the time of the irradiation was 4,4 years (min: 5 months, max: 8 years) and thyroid cancer was discovered after a mean period of 71 months (min: 20, max: 90), consecutively to systematic ultrasound examination in 50%. All tumors were invasive papillary carcinoma associated with metastatic cervical nodes (85%) and lung metastasis (43%). Evidence for lung metastasis was only yelded by total body-scan after therapeutic dose of radioiodine in 2 children. Fourteen children were given in France a therapeutic dose of 50-100 mci of 131 iodine, including those (N= 8) who early received treatments with low doses of radioiodine after initial surgery in Ukraine.The rise in the incidence of childhood thyroid carcinoma, reported in Ukraine and Belarus as early as 1989, will probably amplify during the next decades, according to classic data about radiation-induced thyroid cancers. In this series, the high incidence rate of lung metastasis is of obvious concern. A complete medical management of these cancers by local departments is a difficult challenge for these countries and should be achieved through an international co-operation to establish the equipments required for the administration of high activities of 131 iodine.

60. P40 THYROID CANCER IN CHILDREN AFTER CHERNOBYL NUCLEAR DISASTER. Lidio Baschieri, Alessandro Antonelli, Marco Ferdeghini*, Baldassare Alberti, Marco Puccini§, Giuseppe Boni* and Paolo Miccoli§. Departments of Internal Medicine, of Surgery§ and Nuclear Medicine*, University of Pisa, Italy.

After the nuclear accident in Chernobyl in 1986, the incidence of thyroid cancer in childhood increased sharply in the area of Gomel. 47 children (20 males, 27 females 6 to 16 years of age) from this area, presenting a differentiated thyroid cancer, have been evaluated in our Institutes. Diagnosis (follicular cancer in 4 of them) and initial surgical treatment of the tumor had been carried out in the country of origin between 1989 and 1994. Neck ultrasonography showed the presence of a large unilateral thyroid residue and/or metastatic lymph nodes in 24 of them. 21 of these children underwent completion thyroidectomy and excision or biopsy of enlarged nodes during October 1994. Histology revealed the presence of cancer in 6 of these patients (28.6%). WBS one month after operation led to diagnosis of (radiologically negative) lung metastases in 5 (4 presenting also lymph node metastases and 1 bone metastasis) while the 6th patient presented residual tissue in the thyroid bed. In the remaining 15 patients, WBS showed no uptake in 6, non-radiologically detectable lung metastases in 2 and lymph node metastases in 6 (one patient not yet evaluated). Histological examination showed also mononuclear and lymphocytic infiltration in a high percentage (about 50%), while circulating anti-thyroglobulin and peroxidase antibodies were present in 40% and 30% of the children, respectively. In the 23 patients previously submitted to total thyroidectomy the whole body scan revealed 3 cases of lymph node metastases, 8 with lung metastases (5 also with lymph node metastases) and 9 without evidence of recurrence of the disease. Three patients have been previuosly submitted to hemithyroidectomy and did not present radiologically detectable metastases.

The high prevalence of recurrent disease (25/47 = 53%) and lung metasteses (15/47 = 32%) in this sample suggests an aggressive form of cancer in these children. An unusuallly high prevalence of mononuclear and lymphocytic infiltration is also present. Total thyroidectomy is confirmed to be mandatory for thyroid cancer in children. Reoperation should be taken into consideration in all cases where it has not been carried out during first intervention, because it can be performed with minimal morbidity by experienced surgeons. Completion thyroidectomy allowed to excise residual disease in 28% of patients in this series of children. Moreover, it allowed to detect metastases in 65% of the cases by WBS, even when histology in the opposite lobe was negative (8/14=57%).

61. P41 **THE PROLIFERATIVE RESPONSE OF PORCINE THYROID FOLLICULAR CELLS TO EPIDERMAL GROWTH FACTOR REFLECTS THE AUTOCRINE INVOLVEMENT OF TRANSFORMING GROWTH FACTOR-ß1 AND INSULIN-LIKE GROWTH FACTOR-I.** S. Bidey, J. Soden, *H. Beere and *A. Cowin. Endocrine Sciences Research Group and *School of Biological Sciences, University of Manchester, Manchester M13 9PT, U.K.

Epidermal growth factor (EGF) serves as a potent mitogenic stimulus for thyroid follicular cells (TFCs), which is accompanied by a loss of differentiation expression. The prohferation and functional differentiation of TFCs also reflect the actions of autocrine or paracrine factors synthesised within the thyroid follicle, prominent among which are insulin-like growth factor-1 (IGF-1) and the latent form of transforming growth factor-ß1 (TGF-ß1). IGF-I, acting in synergy with TSH, serves to upregulate TFC growth *in vitro*, while TGF-ß1 is a potent inhibitor. Previous investigations have demonstrated that at high levels of EGF exposure, TFCs show an increased production and activation of TGF-ß1, while treatment with recombinant TGF-ß1 down-regulates autocrine IGF-I synthesis and inhibits mitogen-dependent TFC proliferation. In the present study, using subconfluent porcine TFCs in monolayer, we have investigated the involvement of autocrine TGF-ß1 and IGF-I in determining the effect of EGF on DNA synthesis. Intact follicles were isolated from porcine thyroid tissue by digestion with dispase/collagenase, seeded into 24 well multidishes, and allowed to adhere and flatten during an initial 24h incubation period in Eagles MEM + 5% fetal calf serum. This medium was then exchanged for serum-free Ham's F12 medium containing EGF, and incubation continued for a further 48h. In some cases, cultures also contained precipitating antisera (1:400) to mature TGF-ß1 or IGF-1 or alternatively, in the case of controls, an equivalent dilution of preimmune serum. For the final 24h of incubation, [^{3}H]thymidine (3-HT; 7.4kBq) was added to each culture well, and ^{3}H-labelled TCA-precipitable cellular material subsequently quantified as an index of DNA synthetic activity. In a series of parallel TFC cultures, total IGF-I was determined by radioimmunoassay of cell-conditioned medium, after extraction of IGF binding proteins. Freshly plated TFCs showed a rapid increase in 3-HT incorporation in response to EGF over the range 0-5μg/l. At the highest dose of EGF consistent with stimulation of 3-HT incorporation, the IGF-I content of TFC-conditioned medium was 3-4 fold greater than that in the medium of parallel control cultures, and the response was significantly ($p<0.05$) reduced by IGF-I immunoprecipitation. At EGF doses in excess of 10μg/l, the 3-HT incorporation response plateaued, and in some cases a significant ($p<0.007$) inhibition was observed. In such circumstances, TGF-ß1 immunoadsorption led to a significant ($p<0.0002$) increase in 3-HT incorporation into TFCs, reversing the inhibition observed in non-immunoadsorbed cultures. At lower doses of EGF, consistent with the ascending limb of the 3-HT incorporation response, TGF-ß1 immunoneutralisation also led to a small, though less marked enhancement in DNA synthesis. While confirming, therefore, the close interactive roles of IGF-I and TGF-ß1 in maintaining control of TFC DNA synthesis, the present findings serve to provide evidence that the autocrine production and TFC-directed actions of these factors may also be instrumental in determining the growth-regulating actions of EGF within the thyroid follicular microenvironment.

62. P42 TSH AND cAMP POSITIVELY CONTROL G1/S AND NEGATIVELY G2/MITOSIS TRANSITIONS OF THE CELL CYCLE IN DOG THYROID PRIMARY CULTURES. CORRELATION WITH THE EXPRESSION AND CELLULAR LOCALIZATION OF CYCLIN A AND CYCLIN-DEPENDENT KINASES (CDKs). M. Baptist, F. Depoortere, R. Burikhanov and P.P. Roger, I.R.I.B.H.N., Free University of Brussels, Campus Erasme, B-1070 Brussels, Belgium.

TSH and forskolin, through cAMP, trigger DNA synthesis after a prereplicative lag phase of about 18 hrs in dog thyrocyte primary cultures. Moreover, the co-operation of cAMP-dependent and cAMP-independent mitogens results in a shortening of this prereplicative phase. Analysis of cell cycle kinetics after stimulation by TSH or independently of cAMP by EGF + serum nevertheless revealed a marked lengthening of G2 phase and a delayed appearance of mitoses in the cAMP-dependent cell cycle. The removal of forskolin at late stages of the prereplicative phase prevents cells from initiating DNA synthesis, while a similar forskolin removal once cells have reached S phase, hastens their entry into mitosis. Thus, cAMP positively control a late restriction point just before DNA synthesis, but negatively influences the G2/mitosis transition. Similar G2 delays, by allowing DNA repair are believed to enhance cell resistance to mutagenic treatments and ionizing radiations. In thyroid, they might function to protect cells against mutagenic consequences of the cAMP-dependent H_2O_2 generation involved in thyroid hormone synthesis. The different cell cycle transitions are controlled by the sequential activation and desactivation of a family of CDKs. We have compared the expression, phosphorylation and subcellular localization of cdc2 (CDK1) and CDK2 and their common activator cyclin A in the cAMP-dependent and cAMP-independent cycles of dog thyrocytes using Western blotting and double labelling immunofluorescent microscopy of these proteins with PCNA as a cell cycle marker (Baptist et al., J. Cell Sci. 105, 69 (1993)).

Unlike cyclin A and cdc2, respectively induced by TSH at G1/S and during S phase, CDK2 was already present in quiescent thyrocytes as a cytoplasmic and weakly nuclear protein. TSH induced a previously undescribed nuclear translocation of CDK2, which just preceded DNA synthesis. This nuclear translocation should be necessary for the activation of CDK2 by nuclear cyclins and the nuclear CDK activating kinase. On the other hand, the cAMP-dependent lengthening of G2 phase was associated with a stabilization of the Tyr15-inhibitory phosphorylation of cdc2 and with a comparatively high nuclear accumulation of both cyclin A and CDK2 in some S phase and in the majority of G2 cells. Our observations are consistent with the proposal that high cyclin A/CDK2 activity in G2 could prevent cyclin B/cdc2 activation and delay mitosis.

We hypothesize that the nuclear translocation and accumulation of CDK2 could mediate the positive effects of cAMP on G1/S transition, and as a complex with cyclin A, the negative effects of cAMP on G2/mitosis transition.

63. P43 EFFECT OF EPIDERMAL GROWTH FACTOR ON POLARIZED PROTEIN SECRETION IN FILTER-CULTURED PIG THYROID EPITHELIAL CELLS.

J. Husmark, M. Nilsson, Institute of Anatomy & Cell Biology, Göteborg Univ., Göteborg/Sweden.

Epidermal growth factor (EGF) is mitogenic to thyroid follicular cells in vitro. Along with stimulated growth, EGF is known to strongly but reversibly inhibit thyroid-specific functions. Recent data from pig thyrocytes cultured on permeable filter in Transwell chambers, which unlike most other culture systems promotes a polarized epithelial phenotype, show that basolateral I- uptake and TSH-regulated apical I- efflux prevail during EGF-induced cell proliferation. Also, the polarized morphology and epithelial barrier function of thyrocytes are not abrogated by EGF in this system. To further characterize the filter-cultured pig thyrocytes, we examined the effects of EGF on polarized protein secretion. Confluent and contact-inhibited cell monolayers were pretreated for 48 h with EGF (10 ng/ml), which increased culture DNA content by 30-60%, or TSH (0.1 mU/ml), which did not induce growth. Pretreated cultures were metabolically labelled with [^{35}S]methionine or [^{3}H]leucine for 4-16 hours and analyzed for steady state protein secretion into either of the apical and basal media with immunoprecipitation of thyroglobulin (Tg) and SDS-PAGE/autoradiography. The acute effect of TSH (10mU/ml) on the release of proteins was studied in pulse(2h)-chase(1-4h) experiments.

In untreated cultures, 90% of Tg secretion occurred in the apical direction. Tg as amount of total labeled protein was different in the apical (~60%) and basal (~40%) medium; several non-Tg bands in SDS-PAGE autoradiographs did not appear in the opposite medium. Long-term TSH increased the overall protein secretion, but the relative amounts of Tg and non-Tg were similar. The apical secretion of Tg was acutely stimulated by TSH. In EGF-treated cultures, Tg secretion was significantly reduced in both directions, but mostly to the apical medium. Still, ~70% of secreted Tg appeared apically. Tg secretion was unresponsive to acute TSH. Non-Tg precipitable proteins dominated over Tg in both media. Densitometry of autoradiographs showed that EGF reduced some non-Tg bands but increased others, especially a ~150 kD band released into the basal medium.

In conclusion, although EGF inhibits the secretion of Tg, preferably in apical direction, it is still polarized as is the release of several other secretory proteins. The presence of Tg synthesis and secretion and its unresponsiveness to acute TSH, which in the same cells provokes cAMP-mediated I- efflux, suggest that EGF might impair intracellular transport and/or apical exocytosis of Tg. EGF up- and down-regulates several secretory proteins, the nature of which remains to be identified.

64. P44 ANGIOGENESIS IN THE HYPERPLASTIC RAT THYROID: CONTROL BY FIBROBLAST GROWTH FACTOR (FGF)-2 AND THROMBOSPONDIN (TSP)? *VA Patel, **DJ Hill, *MC Eggo, **GP Becks, *MC Sheppard and A Logan; Departments of Clinical Chemistry and *Medicine, University of Birmingham, B15 2TT and **Department of Medicine, Lawson Research Centre, London, Ontario, Canada N6A 4V2

The trophic and cellular events underlying pathogenic progression to goiter and on to neoplasia are not fully established, but clearly this growth must be accompanied by a co-ordinated neovascularisation. We believe that the angiogenic response may be a determining factor in progression of thyroid pathology and that monitoring the expression of angiogenic/anti-angiogenic factors in the tissue may prove to be a useful prognostic indicator of disease progression. The purpose of this study was to document the time course of the angiogenic response in an *in vivo* model of goiter and to investigate whether this was associated with changes in the local expression of the angiogenic factor FGF-2 and the anti-angiogenic factor TSP. Rats treated with methimazole (MMI) and a low iodine diet (LID) for 1 and 2 weeks quickly became hypothyroid compared with controls and exhibited thyroid hyperplasia (Control, n=10: serum thyroxine (T_4) 66± 4 nmol/l, thyroid weight 5±1 mg/100g body weight, means ± S.D.; Experimental, n=10: T_4 undetectable, thyroid weight 27±4 mg/100g body weight after 2 weeks of treatment). We quantified the abundance of capillaries in thyroids by immunohistochemistry (IHC) using a specific endothelial cell marker, CD31, and morphometric analysis (point counting of immunopositive cells) to reveal that the progression of goiter in the rat thyroid is accompanied by an increase in capillary endothelial cell growth. n=3, Mean±SEM; Endo = endothelial cells; Epi = follicular epithelial cells.

	Control Week 1	LID/MMI Week 1	Control Week 2	LID/MMI Week 2
CD31 Epi:Endo	11.7±1.4	6.4±0.6	14.9±1.9	3.6±0.2
% FGF-2 +ve stroma	18.7±3.3	49.5±2.8	16.7±4.2	69.9±2.0
% FGF-2 +ve Epi	71.6±3.5	100±0.0	79.6±5.3	100±0.0
%TSP +ve stroma	82.3±1.1	29.7±4.4	88.7±2.4	10.1±1.3
TSP Epi:Endo	8.9±0.5	5.3±0.8	8.1±0.8	28±1.7

In control rats FGF-2 was present at low levels in thyroid follicular cells with minimal levels also present in the stroma surrounding blood vessels. During hyperplasia, FGF-2 immunoreactivity increased steadily in both locations, so that by 1 week an increase in immuno-positive stroma was evident and all epithelial cells were positive. By 2 weeks of treatment all epithelial cells remained positive and a higher proportion of stroma became strongly immuno-positive. In controls TSP was localised strongly to the stroma, endothelial cells and all follicular epithelial cells. Following one week of LID/MMI treatment, whilst stromal staining was reduced, strong staining was still present in an increased number of endothelial cells. After two weeks of treatment, immunoreactive TSP had virtually disappeared from all locations. These results show that in the hyperplastic thyroid an increase in FGF-2 and a decrease in TSP accompanies angiogenisis. Hence, there may be an autocrine/paracrine relationship between the expression of FGF-2, TSP and angiogenesis during goiter formation.

65. P45 Selenium deficiency, decreased thyroid epithelial cell proliferation, and thyroid fibrosis. Role of the inflammatory cells and cytokines. Possible involvement of an excess of TGF-ß.

B. Contempre*, O. Le Moine§, J.E. Dumont*, J.-F. Denef#. *IRIBHN and §Service of Gastroenterology, Free Univ. of Brussels; and # Laboratory of Histology, Catholic University of Louvain, Brussels, Belgium.

Selenium deficiency has been proposed as a cofactor to iodine deficiency in the thyroid destruction observed in myxoedematous cretins. In rats, selenium deficiency strongly increases free radicals induced thyroid necrosis, decreases the ensuing epithelial cell proliferation, and promotes thyroid fibrosis. This study was aimed to further clarify the role of selenium deficiency in the thyroid fibrosis and proliferation.

Method: Rats were fed a selenium deficient diet (SE-, 0.005mg/Kg) or sufficient diet (SE+) for five weeks. Goiter was induced by giving 1% NaClO4 in water (I-). Rats were injected i.p. with 1mg NaI twelve hours after perchlorate weaning, and killed 1, 3 or 15 days after the iodide injection. All the rats received i.p. 100 µCi of [^{3}H] thymidine 1 hour before the killing. Thyroids were kept frozen or fixed by glutaraldehyde perfusion. Frozen sections were used for immunohistochemical typing of the interstitial cells, and for TGF-ß detection. The surface occupied by the inflammatory cells was estimated by point counting on frozen sections. Semi-thin (0.5 µm) sections of fixed thyroids were used for counting the number of necrotic thyroid cells, and for the estimation the proliferation index of thyrocytes and fibroblast by counting the [^{3}H] thymidine positive cells.

Results: Se- thyroids showed increased necrosis, increased inflammatory reaction, and evolved to thyroid fibrosis.

1 day after iodide: Necrotic epithelial cells were three times more numerous in the SE- thyroids. Inflammatory cells were not increased as compared to before iodide administration, and occupied ± 10 % of the surface of the gland both in SE+ and SE- thyroids. Epithelial cells were actively proliferating in response to cell necrosis (± 10 %). Epithelial cells and fibroblast proliferation was comparable in SE+ and SE- thyroids. *3 days after iodide:* Necrosis was still more important in SE- thyroids. Monocytes and macrophages represented 18 % and 29 % of the surface of the thyroids in SE+ and SE- thyroids respectively ($p<0.01$). Epithelial cell proliferation was decreased in SE- thyroids (7.5 % in SE+ Vs 3.2 % in SE- ($p<0.01$)), while fibroblast proliferation was increased (10.1 % in SE+ Vs 25.5 % in SE- ($p<0.01$)). In SE- thyroids TGF-ß was detected in macrophages.

Conclusion: One day after iodide, whatever the selenium supply the thyroids showed a similar proliferation burst. Three days after iodide, concomitant to the invasion of inflammatory cells SE- thyroids showed decreased epithelial cell proliferation while fibroblasts proliferated. In SE- thyroids, inflammation is suggested to play a central role in the defective thyroid repair. Excess of TGF-ß could account for both the decreased proliferation of epithelial cells and the increased proliferation of fibroblasts leading to thyroid fibrosis.

66. P46 EFFECTS OF GROWTH FACTORS ON RAF-1, MEK AND MAP KINASE ACTIVITY IN PORCINE THYROID CELLS IN CULTURE. T.Tsushima, H.Murakami, O.Isozaki, H.Demura, K.Shizume and Y.Nozoe. Department of Medicine 2, Tokyo Women's Medical College1, and Institute of Growth Science (K.H, Y.N), Tokyo 162, Japan

Mitogen-activated protein kinases (MAPKs) are serine-threonine kinases that are activaed in resopnse to a wide varitey of growth factors in many types of cells. They are activated by dual phosphorylation on both tyrosine and threonine residues in response to MAP kinase kinases (MAPKK or MEK). This MAPKK is in turn phosphorylated and acivated by MAP kinase kinase kinases (MAPKKK) including raf-1. Recent studies have shown that this pathway plays an important role in regulation of cell growth. We examined whether growth-stimulators in porcine thyroid cells could activate raf-1, MEK and MAPK activities. Porcine thyroid cells were cultured in F-12 medium and exposed to growth factors. Raf-1, MAPK and MAPK activities were determined by their ability of phosphorylate GST-MEK, GST-MAP and myelin basic protein (MBP), respectively. All of EGF, IGF-I, basic FGF and TPA (phorbol ester) stimulated activities of raf-1, MEK and MAPK. Activation was detected within 5 min and peaked at 15-20 min after stimulation. MAPK activation by EGF or IGF-I was also seen in protein kinase C-down regulated cells, but not in cells treated with tyrosine kinse inhibitors. Experiments using antisense oligonucleotide to MAPK mRNA showed that MAPK is involved in thyroid cell growth, but not in iodine metabolism. Growth-inhibitors such as TGF-β, TSH, 8-Bromo cAMP and iodide were without effect on Raf-1, MEK and MAPK activity. These inhibitors did not suppress activation of MAPK cascade stimulated by either EGF, TPA or IGF-I, indicating that MAPK pathway is not involved in growth inhibition by TGF-β or iodide. We conclude that MAPK cascade plays an important role in growth regulation in thyroid cells.

67. P47 TRANSFORMING GROWTH FACTOR-β_1 (TGF-β_1) INHIBITS Na^+/K^+-ATPase EXPRESSION IN FRTL-5 RAT THYROID CELLS.

A. E. Pekary, S. R. Levin, D. Johnson, L. Berg, J. M. Hershman. Endocrinology and Metabolism Section, West Los Angeles VA Medical Center and UCLA Department of Medicine, Los Angeles, CA.

We have recently reported that tumor-necrosis factor-α (TNF-α) induces the synthesis and secretion of TGF-β_1 by FRTL-5 cells (Am J Physiol 268:R808,1995). Because TNF-α is cytotoxic to aged (>40 passages) FRTL-5 cells, and TGF-β secretion is associated with programmed cell death (apoptosis) in vivo, we postulated that TGF-β_1 mediates some of the effects of TNF-α on the function, growth, and viability of FRTL-5 cells. One prominent effect of TNF-α (and TGF-β_1) on FRTL-5 cell function is suppression of iodide uptake. Na^+/K^+-ATPase activity, which drives iodide uptake by thyroid cells, is inhibited by TNF-α (J Invest Med 43:107A,1995). The following experiments explore the role of TGF-β_1 as a possible mediator of the inhibitory effects of TNF-α on Na^+/K^+-ATPase activity. Young (< 20 passages) and aged (> 40 passages) FRTL-5 cells were grown to near confluency in standard FRTL-5 medium containing 2 U/L bTSH. Cells were then treated with various doses (0 to 100 ng/ml) of recombinant human TGF-β_1 for 2 days or with 50 ng/ml TGF-β_1 for various times (0 to 3 days). The Na^+/K^+-ATPase α_1 subunit mRNA level in FRTL-5 cells was at least 10-fold greater than the corresponding level for the α_2, α_3, and β_1 subunits for all experimental conditions used. The Na^+/K^+-ATPase β_1 subunit mRNA level in young cells was reduced 95% by either treatment for two days with 6.25 ng/ml TGF-β_1 or treatment for one day with 50 ng/ml TGF-β_1. The β_1 subunit mRNA level in aged cells was reduced 50% by 2 days of treatment with 6.25 ng/ml TGF-β_1. The α_1, α_2, and α_3 subunit mRNA levels in young cells were reduced 80% by 2 days of treatment with 6.25 ng/ml TGF-β_1. TGF-β_1 doses up to 5 μg/ml had no effect on cell viability in young or aged cells. We conclude that TGF-β can potentially mediate all or part of the inhibitory effect of TNF-α on Na^+/K^+-ATPase expression but not the cytoxic effect of TNF-α in aged cells.

68. P48 EGF BUT NOT IODIDE ENHANCES TGFß PRECURSOR, TGFß-1mRNA AND TISSUE PLASMINOGEN ACTIVATOR IN PORCINE THYROID FOLLICLES EX VIVO.

Bechtner G, Erdmann A, Schopohl D, Rafferzeder M, and R. Gärtner. Medizinische Klinik Innenstadt, Universität München, Germany

Previously it has been shown that iodine enhances TGFß-1 mRNA in human and sheep thyroid cells in vitro. Therefore the known inhibitory effect of iodide on goiter development was concluded to be due to the inhibitory effect of TGFß on thyroid cell proliferation. We now investigated the effect of EGF and iodine on the secretion of TGFß precursor and on TGFß-1 mRNA expression in intact porcine thyroid follicles ex vivo. As it is assumed, that TGFß is activated by plasmin, tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI) secretion from thyroid follicles were also investigated under comparable conditions. Intact porcine thyroid follicles were prepared as previously described and cultured in Medium 199 in suspension. Active TGFß and TGFß precursor content of conditioned medium from thyroid follicles was determined using the mink lung bioassay and Western Blot analysis. TGFß-1 mRNA was detected by Northern-Blotting using a human TGFß-1 cDNA fragment and a human GAP-DH cDNA fragment for control hybridisation. PAI as well as tPA were detected by commercial cholorimetric assay. Immediately after preparation no TGFß-1 mRNA could be detected but occurred after 24h in suspension culture under basal conditions. During EGF (5 ng/ml) stimulation a significant, 3-fold increase of the relative densitiy (TGFß1-mRNA/GAP-DH mRNA) after 8h and 12h with a decrease to nearly basal levels after 26h was found. In contrast no change of the relative density during incubation with even high doses of KI (40 umol/l) was found. In contrast, TGFß-1 precursor release into the medium was enhanced with both, EGF and iodine, but only with EGF active TGFß was found. This may be due to the fact, that only EGF, but not iodine increased tPA concentrations about 4-fold. Activity of PAI is unchanged under all these conditions. We conclude that EGF stimulation of intact follicles results both in an increased TGFß-1 synthesis and TGFß secretion into the medium. Since EGF also increases the release of tPA, which then is able to generate plasmin, this may activate the TGFß precursor. In contrast, iodide only seems to induce TGF-ß precursor release without activating TGFß-1 synthesis or tPA release from porcine thyroid follicles ex vivo.

69. P49 TRANSFORMING GROWTH FACTOR-ß$_1$ (TGF-ß$_1$) IN OVINE THYROID: AUTOCRINE PRESENCE AND EFFECTS ON IODINE METABOLISM AND RELEASE OF INSULIN-LIKE GROWTH FACTOR (IGF) BINDING PROTEINS (BPs). G.P. Becks, J.-F. Wang and D.J. Hill, The Lawson Research Institute, St. Joseph's Health Centre and University of Western Ontario, London, Canada, N6A 4V2.

We previously reported alterations in the expression of TGF-ß$_1$, IGFs, IGFBPs and basic fibroblast growth factor in induced thyroid hyperplasia in rats (J. Endocrinol. 1994, 141:45-57, 142:325-338; Growth Factors 1994, 10:207-222), suggesting the likelihood of multiple growth factor interactions during thyroid growth and function. Further studies were undertaken to characterize and eludicate interactions of TFG-ß$_1$, an acknowledged cytokine inhibitor of thyroid growth and function, and the IGF axis, essential to follicular cell competence and progression, utilizing differentiated ovine thyroid tissues *in vivo* and *in vitro*. Immunocytochemical (ICC) staining for TGF-ß$_1$ was evident over epithelial cytoplasm, demonstrating considerable intra- and interfollicular heterogeneity, with more intense uniform staining in perivascular regions of normal sheep thyroid tissue sections. Similar cytoplasmic ICC TGF-ß$_1$ staining was observed in quiescent and TSH-stimulated primary cultures of ovine thyroid follicular epithelium in serum-free medium. Following acid or heat treatment to activate TGF-ß$_1$-latency associated peptide (LAP) in cell-conditioned culture media (CCM), TGF-ß$_1$ migrated as an approximately 40 kd single band on Western immunoblot analysis, possibly representing monomeric TGF-ß$_1$-LAP or a glycoprotein. *In vitro*, exogenous TGF-ß$_1$ enhanced (0.01 ng/ml) and subsequently inhibited (ED_{50} 1ng/ml) TSH-stimulated ^{125}I uptake and organification in dose-response experiments, which correlated with increasing abundance of IGFBP-2, -3 and -5 in CCM as assessed by ligand blotting with ^{125}I-IGF-II. These effects of high TGF-ß$_1$ concentration were prevented by neutralizing antisera which itself slightly, but consistently, inhibited iodine uptake and organification. Control antisera (chicken IgG) exhibited none of these effects. These data underscore the likely autocrine and paracrine influence of thyroid-derived TGF-ß$_1$ reported now in several species, and suggest a role of constitutive TGF-ß$_1$ in maintaining a differentiated phenotype while confirming an inhibitory effect at higher concentrations. The latter may be due in part to TGF-ß$_1$ effects on the endogenous thyroid IGF-IGFBP system.

70. P50 **PREVALENCE OF SUBCLINICAL HYPOTHYROIDISM IN PATIENTS WITH DOWN'S SYNDROME AND RELATION TO PSYCHOMOTOR DEVELOPMENT.** F Baptista[1], M Palha[2], A Costa[2], P Oom[2], L Sampaio[2], C Freitas[2], H Proença[3], T Condeço[2], L Cotrim[2] S Jorge[2]. Endocrine Unit[1] Child Development Center[2] and Clinical Chemistry Lab[3] Sta. Maria Hospital. University of Lisbon

Thyroid dysfunction (TD) has been described in association with Down's syndrome (DS), especially the presence of subclinical hypothyroidism (SH). However, the management of this situation is not well established. **The aim of this study** was to evaluate the prevalence of TD in patients with DS and to look for differences in psychomotor development in patients with and without SH, which could justify treatment with thyroid hormone in the first group. **Methods**: Forty-three children with cytogenetic diagnosis of DS (20 female and 23 male) referred to the Child and Development Center of our University Hospital in 1994, with ages over 10 months and under 9 years old (3.1± 0.3 years old) (mean±SEM), were enrolled in the study. Twenty-six normal first degree siblings aged 7.4±0.9 were evaluated as a control group. The evaluation of these patients included a full physical examination, psychometric testing using Griffiths scales (General quotient (GQ), Locomotor (A), Personal-social (B), Hearing and Speech (C), Eye and hand co-ordination (D) and Performance (E)) and venous sampling for T4, T3 and TSH. Hormones were measured by a commercial ELISA kit (Boeheringer Mannheim). DS group was subsequently divided into two subsets of patients matched for age, according to thyroid function: normal thyroid function (NTF): (TSH≤4 µU/mL) (n=13); and SH: (TSH> 4 µU/mL) (n=12) and results of psychometric evaluation were compared. **Results** (presented as mean± SEM). DS patients had TSH levels (4.9±0.29) higher ($p<0.001$) than normals (2.5±0.2). Both groups had levels of T3 (176.9±6.3 vs 169.7±5.2) and of T4 (8.6±0.2 vs 8.0±0.2, respectively DS and controls) with no statistical differences. Thirty patients had a TSH level above the upper limit of normal range (SH). The score on scales in Griffiths test on patients with NTF (TSH 2.9±0.2) and with SH (TSH 5.4±0.2) are summarized in the table. We found no statiscally significant differences among these two groups.

	GC	A	B	C	D	E
NTF	57.4±5.0	56.3±5.3	63.9±5.1	54.7±5.1	58.1±5.0	54.7±4.7
SH	59.4±4.8	58.9±5.2	69.2±5.3	55.4±5.6	60.3±5.1	56.4±5.1

Conclusions: Subclinical hypothyroidism is a common situation in patients with DS; however, it doesn't affect psychomotor development. In DS patients with subclinical hypothyroidism, replacement therapy is probably not warranted for psychomotor development reasons.

71. P51 INCREASED THYROID VOLUME IN CHILDREN LIVING IN AN AREA SUBMITTED TO A RECENT IODINE PROPHYLAXIS: A CLUE TO PAST EXPOSURE TO IODINE DEFICIENCY

F. Aghini-Lombardi, L. Antonangeli, T. Rago, AM. Bartolomei*, P. Vitti
Istituto di Endocrinologia, University of Pisa, Pisa *Divisione di Pediatria, Ospedale di San Sepolcro, Italy

Aim of the present study was to investigate the evolution of goiter endemia in the whole schoolchildren population residing in an area of Tuscany (Tiberina Valley) in which urinary iodine excretion was 39 ±21 μg/L (22 as median) in 1985 and 98±57 μg/L (88 as median) in 1993 after a voluntary iodine prophylaxis. Thyroid ultrasound was performed using a portable realtime instrument (Aloka SSD-500, Tokio, Japan), using a 7.5 MHz linear transducer and thyroid volume (TV) was calculated by the rotation ellipsoid model formula. Two thousand six hundred and ninety-three children living in iodine sufficient areas were considered as controls.

The median thyroid volume progressively increased from 3.1 ml in 7 yr old and 9.2 ml in 14 yr old children. In older children (12-14 yr) but not in those under 12 yr, TV was greater than that observed in age-matched controls, while younger children did not differ from controls. In the 12-14 yr old group a highly significant difference in mean values was found. Even excluding subjects with an abnormal TV, older children residing in Tiberina Valley had a median TV higher than that of the age-matched controls, although still comprised in the normal range. (table).

Age (yr)	7	8	9	10	11	12	13	14	12*	13*	14*
TV (median, ml)											
Tiberina Valley	3.1	3.25	3.8	4.3	5.1	6.9	7.8	9.15	6.2	7.5	7.9
p**					0.35	*<0.007*	*<0.001*	*<0.001*	*<0.007*	*<0.001*	*<0.001*
TV (median, ml)											
Control area	3.1	3.3	3.8	4.3	4.7	5.2	6.0	6.4	5.2	6.0	6.4

*subjects with normal TV
** calculated by Student' t Test between mean values ± SD

In conclusion, the results of the present study suggest that a brief exposure to a moderate iodine deficiency causes a subtle enlargement of the thyroid that persists after correction of iodine deficiency. Thus, the knowledge of the previous iodine intake is mandatory to understand the apparent discrepancy between a high goiter prevalence and a borderline normal iodine intake.

72. P52 ULTRASOUND MASS SCREENING OF THE THYROID GLAND IN AN ENDEMIC AREA OF CHINA.

K. Fujimori[1], T. Takaya[1], H. Ohtomo[1], T. Takahashi[1], Y. Taguchi[1], D. H. Zhang[2], X. L. Sun[3], [1]Tohoku University School of Medicine, Sendai, Japan, [2]The 3rd Teaching Hospital of Norman Bethune University, Changchun, China, [3]The 2nd Jilin Provincial Institute for Endemic Disease Control and Research, Jilin, China.

There have been few studies of ultrasound mass screening for endemic goiter in developing countries. From 1991 to 1993, we performed mass screening in an endemic goiter area in Jilin Province, China, to evaluate the effect of iodine supplementation. We measured body height and weight, and thyroid volume. As a parameter of thyroid volume, we use the two dimension Volume Index(VI: VI=1/2ab+1/2a'b', a or a'=maximum width of rt. or lt. thyroid lobe, b or b'=maximum thickness of rt. or lt. thyroid lobe). VI has good positive linear correlation to calculated thyroid volume (r=0.97) and mild positive correlation to height and weight. We examined 1799 people living in the Fengman District where iodine content of drinking water is under 1 μg/L and iodized salt (30mg iodine/1kg salt) had been supplied for thirty years. Their average urinary iodine excretion is 110 μg/L. As a control in the same province, we examined 714 residents living in Sijingzi Village where drinking water has 15 μg/L iodine. We made four groups of female participants: y-F(age 15-29, in Fengman, n=574), o-F(age 30-49, in Fengman, n=450), y-S(age 15-29, in Sijingzi, n=136), o-S(age 30-49, in Sijingzi, n=148). There were no differences among these four groups concerning the mean height and weight. The prevalence of thyroid nodules of y-F, y-S, o-F and o-S was 5.1%, 8.1%, 52.4% and14.9%, respectively. The VI of y-F, y-S, o-F and o-S was 162.8±79.2, 202.0±69.7, 281.4 ±108.3 and 205.3±53.2 (mean±SD), respectively. The nodule prevalence of y-F was similar to that of the control, and that of o-F was significantly higher than that of other three groups. In the Fengman District, nodule prevalence was markedly low under the age of 30 years. The VI of young female was significantly smaller than that of the control. Based on ultrasonographic results, it is considered that iodine supplementation with iodized salt was quite effective to prevent the Chinese people from new endemic goiters in the Fengman District.

73. P53 INCIDENCE OF CONGENITAL HYPOTHYROIDISM (CH): AN INDICATOR OF IODINE DEFICIENT AREAS IN ITALY.

M. Sorcini*, A. Olivieri*, C. Fazzini*, E. Medda*, M.E. Grandolfo*, F. Aghini Lombardi°, P. Balestrazzi°°, G. Giovannelli°° and S. Carta*. *Istituto Superiore di Sanità, Roma; °Istituto di Endocrinologia, Università, Pisa;°°Clinica Pediatrica, Università, Parma-Italy.

Neonatal screening for CH began in Italy in 1977 and then progressively developed covering 99% of live births in 1993. The National Register of CH infants was established in 1987 as a project of the Health Ministry. The aim of the Register is to provide disease surveillance, to monitor efficiency and effectiveness of neonatal screening and to allow identification of possible etiological risk factors in CH. 1095 infants had been recorded in the Register between 1987 to 1993. A mean CH incidence of 1:3200 live births has been estimated, nevertheless several districts showing clearly higher incidence have been observed. Thyroid scan, performed before starting therapy in 55% of infants, showed 20% of total CH newborns with glands in normal position. In the areas with more elevated CH incidence (>1:2000) the percentage of normal glands was higher (31%). In order to investigate whether this higher percentage was associated to occurrence of transient hypothyroidism, the normal gland newborns were compared to those with agenesis and ectopia. The group of infants with normal gland showed significantly lower mean gestational age, weigth and lenght at birth, higher percentage of preterm newborns and a more elevated incidence of respiratory distress syndromes respect to infants with agenesis or ectopia. In the same group frequency distributions of T4 and TSH values at screening and at recall were significantly shifted towards less severe hypothyroidism. In areas with CH incidence >1:2000 the reevaluation of diagnosis, till now performed in 44% of infants with thyroid in normal position, showed 75% of transient hypothyroidism or hyperthyrotropinemia. For some of these areas a mild condition of iodine deficiency had been documented (Carenza iodica e gozzo endemico in Italia. Rapporto 1994.Eds. A. Pinchera, G. Salvatore, G. Faglia, R. Vigneri). The results of the National Register highly evidence that variable degrees of iodine deficiency are still present all over the Country and are responsible of higher incidence of transient neonatal hypothyroidism or hyperthyrotropinemia in some areas.In addition our results further suggest that neonatal TSH screening represents a particularly sensitive indicator of population iodine status and can contribute to identify even mild iodine deficient areas. Thus, the National Register represents an usefull tool for developing of collaborative studies in order to promote diffusion of iodine prophylaxis, still inadequate in Italy.

74. P54 LONGITUDINAL STUDY OF IODINE DEFICIENCY IN NEWBORNS OF THE REUNIFIED PARTS OF THE CITY OF BERLIN

A.Grüters, K.P. Liesenkötter, B. Stach*, H.Willgerodt* and H. Helge. University Childrens Hospital KAVH, Free University Berlin and University Childrens Hospital, Leipzig *, Germany

After the reunificiation of Germany the newborns of the eastern parts of the city of Berlin, belonging to the former GDR, where an effective national iodine supplementation had been instituted, were integrated into the newborn screening program for congenital hypothyroidism in the former West-Berlin. Since 1978 all West-Berlin newborns have been screened for congenital hypothyroidism by determination of TSH in dried blood spots in the same laboratory. The incidence of congenital hypothyroidism was the same in the former eastern (1:2790) and western newborns (1: 3200). However, a striking difference in the frequency of transiently elevated levels (TSH > 15 mU/L) between both parts of the city was observed, being 0.74 % in the western parts compared to only 0.1 % in the former eastern parts.

Therefore a prospective study of the urinary iodine excretion in 750 randomly selected western and formerly eastern newborns was conducted in 1991 and 1993. Higher iodine concentrations in the urine of formerly East-Berlin newborns were found in 1991 and still in 1993, although in the former GDR the nationwide iodine supplementation had been abandoned. In the same newborns TSH levels were significantly ($p<0.006$) lower than in the western newborns (1991: 0.8 vs 1.8 mU/l; 1993: 0.94 vs 1.9 mU/l). There was no difference in the T4 serum levels (1991: 15.3 vs 15.6 µg/dl; 1993: 15.7 vs 16.1 µg/dl).

These data demonstrate that neonatal screening for congenital hypothyroidism with sensitive TSH measurements is not only effective in identifying newborns with congenital hypothyroidism, but is also able to monitor the iodine supply of a newborn population. Furthermore these data demand the rapid correction of the iodine deficit in West-Berlin newborns during this critical period of CNS-development by effective iodine supplementation programs.

75. P55 EARLIEST PREVENTION OF ENDEMIC GOITER BY IODINE SUPPLEMENTATION DURING PREGNANCY.

K.P. Liesenkötter, H. Helge, W. Göpel, B. Stach* and A. Grüters. University Childrens Hospital KAVH, Free University Berlin and University Childrens Hospital, Leipzig *, Germany

An optimal iodine intake is essential for a normal pregnancy and a normal development of the fetus. Germany is an area with a moderate iodine deficiency and therefore goiter in the mothers and newborns and complications due to maternal and fetal hypothyroidism occur with higher frequencies. To monitor the success of iodine supplementation during pregnancy, we investigated at 10-12 weeks of gestation and post partum the thyroid volume, thyroid function, urinary iodine excretion and antibodies to thyroid peroxidase (anti TPO) in 38 mothers, who received 300µg potassium iodine per day. Their data were compared to 70 mothers without iodine supplementation. Furthermore the thyroid volume of the newborns from both groups was determined by ultrasound directly after birth and was examind a second time in the children at the age about 3 years.

As expected, urinary iodine excretion increased significantly after iodine supplementation during pregnancy (53.2 µg/g creatinine (median) at begin of gestation; 104.5 µg/g creatinine (median) postpartum) in the mothers and was elevated in the normal range, corresponding to the WHO-criteria. Likewise the iodine excretion in the newborns of these mothers was significantly higher ($p<0.05$) compared to the controls (77.0 vs. 55,6 µg/g creatinine). No development of hypo- or hyperthyroidism was observed in the mothers or newborns and no anti-TPO developement was found in either group.

In the iodine group the newborns had significantly ($p<0.004$) lower thyroid volumes (median: 0.7±0.3 ml) than those in the control group (median 1.5±1.0 ml). After 3 Years we could reexamine 20 children from the iodine group and 15 children from the control group. Although after birth no iodine supplementation was performed in the infants of both groups, we still found a difference in their thyroid volumes (iodine group: 1.4±0.6 ml; control group 2.3±1.2ml). In this study we could clearly demonstrate, that iodine supplementation during pregnancy, in a moderately iodine deficient area results in a lower size of the thyroid volumes in the newborns already at birth. This effect obviously persists in the first 3 years of live.

76. P56 THE EFFECT OF IODINE DEFICIENCY ON NEONATAL - MATERNAL THYROID RELATIONSHIP.

R. Rajatanavin, L. Chailurkit, K. Suthivechvorakul, P. Winichakoon, P. Tananchai and S. Srinawat. Faculty of Medicine, Ramathibodi Hospital, Mahidol University and The Ministry of Public Health, Bangkok, Thailand.

Recent studies have provided further insight into neonatal-maternal thyroid relationship and indicated that both systems may not act independently. Significant amount of maternal thyroid hormone may cross the placenta in the first trimester and especially when fetal thyroid system is in jeopardy like in congenital absence of the thyroid.

We studied neonatal and maternal thyroid function in two areas in Thailand with borderline (Bangkok) and severe iodine deficiency (Nan Province) to evaluate the effect of environmental iodine availability on such system. The median urinary iodine concentration in Bangkokians was 6.4 (95% CI; 5.5 - 7.6) µg/dl and the goiter rate in school children was 6 %, while the urinary iodine concentration in subjects living in Nan was 24 - 53.4 µg/gmCr and the goiter rate was 22 - 83 % with the presence of endemic cretinism.

In each area, three hundred maternal and cord blood were collected consecutively on neonates at birth whose gestational age were greater than 37 weeks and Apgar score at 5 minutes equal or greater than 8. The sera were assayed for serum T_4, Free T_4 (FT_4), T_3, TSH, and thyroglobulin (Tg) concentrations.

Maternal serum T_3 and TSH concentrations in Nan were higher and the serum T_4 concentration was lower than in Bangkok. Maternal serum T_4 and Tg concentrations were lower than in neonates in both areas. The median neonatal serum TSH level in Nan was significantly higher than in Bangkok [5.5 (95% CI; 5.1 - 5.9) v.s. 3.5 (3.3 - 3.9) µU/ml] while the reciprocal was true for the median serum FT_4 [1.5 (95% CI; 1.5 - 1.6) v.s. 1.7 (1.6 - 1.7) ng/dl] and the T_3 levels [44.0 (95% CI; 42.1 - 45.9) v.s. 58.4 (55.1 - 62.2) ng/dl] and the neonatal serum Tg cencentrations [37.7 (95% CI; 33.9 - 41.1) v.s. 44.2 (40.7 - 45.8) ng/ml].

Furthermore, no parallel increase in neonatal serum Tg concentration was seen with serum TSH concentration in both areas. There was also no significant correlation between neonatal serum Tg level and T_3/T_4 ratio in both areas. Significant correlation between maternal and neonatal TSH and FT_4 levels were found in Nan ($r = 0.18$, $p < 0.001$ and $r = 0.21$, $p < 0.01$, respectively.) while no such correlation was observed in Bangkok.

In conclusion, maternal and neonatal thyroid function at birth correlated more in area of severe iodine deficiency than borderline iodine deficiency. Serum Tg concentration did not rise in parallel with serum TSH cencentration. Furthermore, neonatal serum TSH concentation not serum Tg concentration appears to be a better index for assessment and surveillance of iodine deficiency in the neonates.

77. P57 THE PATHOGENESIS OF ENDEMIC GOITRE IN AN AFRICAN POPULATION

D F Smith, C Darke, A B Parkes and AM Hetherton and J H Lazarus. University College, Dublin, Ireland, University of Wales, College of Medicine, Cardiff and NBTS, Cardiff, Wales, UK

The presence of dietary iodine deficiency in the E. Casamence province of Senegal in West Africa is well established. However, population studies on the prevalence of goitre in the region reveal that despite sharing a common diet, not all subjects develop palpable goitres. The objective of the present study was to investigate iodine status, ultrasound measured thyroid volume, thyroid antibody prevalence and HLA phenotype frequency in a Senegalese population with a view to ascertaining prevalence and pathogenesis of thyroid enlargement. Urinary iodine (UI) excretion was measured by dry ashing in random samples obtained from 71 subjects. Thyroid ultrasound scans were performed on 195 subjects using a 7.5mHz linear transducer. In an attempt to study the pathogenesis of thyroid enlargement, the prevalence of thyroid antibodies microsomal (M) and thyroglobulin (Tg) were carried out in 154 subjects using ELISA techniques. In addition, HLA class II (DR/DQ) typing was performed using the technique of restriction fragment linked polymorphism (RFLP) analysis.

Mean UI excretion of 41± 4.5(SE) μg/g creatinine confirm the iodine deficient status of the study population. The mean thyroid volume of 28.3± 1.2(SE) ml was consistent with endemic goitre; individual values being elevated in 83.1% of females, 50.8% of males and 20.6% of children aged< 13 yrs. Tg antibodies were present in 17.1% and M in 7.2% of 154 patients being studied. Analysis of HLA phenotype frequency, although demonstrating a number of rare DR/DQ haplotypes not seen in Caucasoid populations (DR4,DQ2: DR18,DQ4/DR11, DQ6, DR11 ,DQ5), did not reveal a significant association with either thyroid enlargement or antibody positivity.

Thus the results suggest a primary role for dietary iodine deficiency in producing the widespread thyroid enlargement observed in the study population. Predisposition to such enlargement does not appear to be dependent on HLA associations.

78. P58 THE PREVALENCE OF THYROID DYSFUNCTION IN DIFFERENT GERIATRIC SUBPOPULATIONS FROM A MODERATELY IODINE DEFICIENT REGION. COMPARATIVE CLINICAL AND HORMONAL SCREENING. O.Dohán, I.Szabolcs, Zs.Kovács, J.Gönczi, T.Kákosy*, M.Góth, L.Kovács, G.Szilágyi, F.A.Horster**, Haynal Imre University of Health Sciences; *István Hospital, Budapest, Hungary, **University of Düsseldorf, Germany.

The aim of this study was to investigate the prevalence of thyroid dysfunctions in different geriatric subpopulations from a moderately iodine deficient region and to compare the efficiacy of clinical versus hormonal screening.

Design: A screening study was done on 279 chronically ill geriatric patients (Group I.) and 256 consecutive hospital admissions over 60 years of age (Group II.). The method of clinical screening was different from those used so far: the object was not to search for symptoms of hypo- or hyperthyroidism but to find any sign justifying a further, TSH-based biochemical evaluation.

Results: The rates of overt hypothyroidism, overt hyperthyroidism, subclinical hypothyroidism and subclinical hyperthyroidism newly discovered by the hormonal screening were 2.5%, 0.7%, 3.2% and 5,4% in Group I and 1.2%, 1.9%, 3.9% and 2.0% in Group II. The sensitivities of the clinical screening to suspect overt or overt+subclinical dysfunctions or overt+subclinical dysfunctions+cases with subnormal TSH were 0.82, 0.64 and 0.54 in Group I, and 1.0, 0.7 and 0.49 in Group II (or 0.67, 0.4 and 0.25 if the clinical investigation was done not by an endocrinologist but by the medical attendants). A primarily clinical investigation-based screening would have spared 171/279 TSH estimation in Group I, and 161/256 in Group II but would have missed 2/11 overt, 11/26 subclinical dysfunctions and 25/47 cases with subnormal TSH in Group I. In Group II, 9/15 subclinical dysfunctions and 21/29 cases with subnormal TSH would have been lost by this way.

Conclusions: Our approach of a clinical investigation-based screening was rather efficient and cost saving in suspicion of overt thyroid dysfuntions but failed to detect many cases with subclinical dysfunction and with subnormal TSH. Since there is increasing evidence from the literature on the clinical importance of the subclinical thyroid dysfuntions, the primary screening method should be the hormonal measurement, at least in the elderly sick. The prevalence of overt and subclinical dysfunctions would justify the screening of chronically ill hospitalized geriatric patients.

79. P59 LONG TERM SEQUELAE OF HEARING IMPAIRMENT IN CONGENITAL HYPOTHYROIDISM. W. Walker and J. Rovet, The Hospital for Sick Children, Toronto, Ontario.

Thyroid hormone is required for auditory ontogenesis and thyroid beta receptors are localized in the cochlea. In children with early treated congenital hypothyroidism (CH), mild hearing loss is reported in 10 to 20% of cases. Using a larger cohort of children with CH, we sought to determine the incidence and type of hearing impairment (HI) and its consequences on later language and school-related abilities. From the medical charts of 100 children with CH born between 1976 and 1985 who were participating in a study of long-term outcome following newborn thyroid screening, we retrospectively identified 17 cases (22%) with hearing abnormalities, mostly mild unilateral or bilateral sensorineural hearing loss. Children with CH and HI did not differ from those without HI in early or later language skills. However, those with HI performed significantly worse on all measures of mechanical reading, spelling, and writing throughout the first six grades of school. Regression analyses revealed poorer reading was associated with hearing impairment and initial disease severity, but not ongoing treatment and management factors. Implications of altered acoustic signals and auditory processing at the cortical level for making subtle phonological distinctions when learning to read will be discussed.

80. P60 BREAD IODIZATION FOR MICRONUTRIENT SUPPLEMENTATION IN IODINEDEFICIENT REGION OF RUSSIA.

G.Gerasimov, A.Nazarov, N.Mayorova, A.Schischkina, M.Arbusova, B.Mischenko and I.Dedov.
Russian Endocrinology Research Centre, Moscow, Russia.

Iodine Deficiency Disorders (IDD) are a major public health problem in Russia and other Newly Independent States. Until now no effective control programs are implemented in these countries. Iodization of bread can be an important method to deliver iodine to the target groups, namely children. In Russia the unique system of centralized bread production by big regional mechanical bakeries gives possibility to deliver bread from one site to practically all inhabitants. Bread is historically a major dietary item in Russia which is available to all socioeconomic groups. We studied the effect of iodine repletion by bread produced by the local bakery (60 mg of KI per 100 kg of flour) on thyroid volume and urinary iodine in target groups of 252 schoolchildren aged 9-11 years residing in the small town near Moscow. After 3 months of regular bread consumption (300-500 g daily) median urinary iodine level increased from 4.8 to 12.6 mcg/dl ($p < 0.05$). Before iodized bread 13% of children had urinary iodine level less than 2.0 mcg/dl.Three month later all children had urinary iodine levels higher than 2.0 mcg/dl. Thyroid volume did not change during the observed period. Thus, preliminary data show that bread iodization is a simple, cheap and effective method of supplementation and can be easely implemented in iodine - deficient regions of Russia.

Supported by UNICEF

81. P61 A VARIANT OF ADENOMATOUS GOITER WITH CHARACTERISTIC HISTOLOGY AND POSSIBLY THYROGLOBULIN ABNORMALITY. S. Yoshida, S. Sakane, N. Kobe and N. Ohsawa, Kuma Hospital, Kobe, and First Department of Internal Medicine, Osaka Medical College, Takatsuki, Japan.

We identified 24 patients with a specific type of adenomatous goiter in 2160 patients who underwent thyroidectomy and was diagnosed as adenomatous goiter in 1974 to 1993. The characteristic histology of thyroid gland included the presence of a large goiter, small follicles, scant colloid, and columnar follicular cells containing yellowish-green granules by HE staining. Since the mean weight of resected thyroid gland was heavy (118 ± 60 g : mean $\pm$ SD), we compared the features of this group of 24 patients with those of a thyroid-weight-matched control group of 24 patients with common adenomatous goiters.

The frequency of family history of thyroid disease in this particular group was higher (63 %), while the age at the time of detection of goiter was lower (17 ± 15 yr *vs.* 44 ± 17 yr; $p<0.001$). The thyroid function was mildly decreased [total thyroxine (T4) : 84 ± 21 nmol/L *vs.* 103 ± 18 nmol/L; $P<0.01$, thyrotropin (TSH) : 2.4 ± 2.1 mU/L *vs.* 1.0 ± 0.9 mU/L; $P<0.01$], thyroid radioiodine uptake at 24 hours was significantly increased (49 ± 22 % *vs.* 16 ± 9 %; $p<0.001$), but the serum thyroglobulin concentration was significantly decreased ($33 \pm 51\ \mu$ g/L *vs.* $484 \pm 603\ \mu$ g/L; $p<0.01$). The ratio of serum protein bound iodine to total T4 was greater and the thyroglobulin content of the thyroid gland was low as 1.2 mg/g in the extranodular portion and 0.3 mg/g in the intranodular portion. The thyroid on gross inspection presented characteristic pale orangish-red color, and electron microscopic examination showed abundant lysosomes and colloid droplets.

The etiology of this variant adenomatous goiter is suggested to be defects of thyroglobulin synthesis, resulting in decreased hydrolysis by lysosomal enzymes. This type of adenomatous goiter is able to be distinguished by the clinical and histological findings.

82. P62 LOWERED LEARNING POTENTIAL AND ACHIEVEMENT MOTIVATION DUE TO PROLONGED IODINE DEFICIENCY

BD Tiwari, *LB Tripathi, **MG Karmarkar
Department of psychology, Kashi Vidyapith, Varanasi, India-221002 *Gorakhpur University, Gorakhpur,India-273009, **SBISR, B-16 Mehrauli Institutional area, New Delhi, India-110016

The effect of prolonged iodine deficiency on learning and achievement motivation was studied. 100 male children each matched on age, socio-economic status and formal education were selected from iodine deficient (ID) and not-iodine deficient (NID) villages. Mean UEI (μg/dl) [2.79 ± 0.73(ID); 5.70 ± 0.41 (NID); $p < 0.001$)]; and T4 (nmol/l) [90.36 ± 6.46 (ID); 123.70 ± 15.42 (NID); $p < 0.001$]; concentrations in ID children were significantly low. TSH concentration (mIU/ml) [6.23 ± 0.34 (ID); 4.85 ± 0.28 (NID); $p<0.01$] was significantly high in ID children.

The children were administered maze learning and verbal learning tasks. A test of achievement motivation, which is a psychological force that makes a person to attain excellence in the task he is performing, too was administered. Results showed that ID children were slow learners in maze learning [Errors - $F(1,196) = 170.36$, $p<0.01$; Time - $F(1,196) = 64.61$, $p<0.01$]; and verbal learning [Mean Correct Serial Recall - $F(1,196) = 64.45$, $p< 0.01$]. Also, the rate of learning over trials was superior in younger age group children though the initial performance of higher age group children was better. Achievement motivation in ID group was significantly less [$F(1,196) = 12.56$, $p< 0.01$].

These results suggest that prolonged iodine deficiency leads to subnormal mentation during the developmental stages culminating into reduced learning potential when the opportunites of skill learning are provided. In endemic areas where the community suffers from cerebral hypothyroidism, the socio-psychological stimulation for the development of learning ability and achievement motivation is lacking, the children usually manifest subnormal cognitive functions.

83. P63 HYPOECHOICITY OF THYROID SONOGRAPHY IN CHILD IN IODINE DEFICIENCY AREA CANNOT BE CONSIDERED AS AN INDEX FOR THYROID AUTOIMMUNITY.Mihaela Simescu, M.Varciu, I.Podoba, Gabriela Voiculescu,Emilia Nicolaescu, Mariana Sava, Institute of Endocrinology,Bucharest; District Hospital, Brasov - Romania and Cathedra of Internal Medicine and Endocrinology Bratislava.

The epidemiological study in 12o children aged 6-14 yrs, in a subcarpathian area was performed by Merck Thyromobil to assess the severity of IDD. The study revealed a relatively frequent hypoechoicity in child thyroid whose volume was estimated by ultrasonography. Since a possible connection between this hypoechoicity and the prevalence of thyroid autoantibodies and thyroid dysfunction was assumed Ab antiTPO,Ab antihTg, TSH were determined in three groups of children: group I - 10 children with thyroid hypoechogenity with or whithout goiter: group II - 22 children with goiter and group III - 16 children without goiter or hypoechoicity. Goiter prevalence by ultrasonic measurement of thyroid volume was 2o percent and mean urinary iodine (spot urine sample method) was 5,08 ug/dl in the 120 children. In group I, Ab-TPO were present in one case only: Ab-TPO in none of them: TSH was within normal limits. In group II, Ab-hTg and Ab-TPO showed positive values in two cases; TSH was above normal limits in two cases out of which one with Ab-TPO and Ab-hTg present. In group III, Ab-hTg and Ab-TPO showed negative values, TSH was within normal limits. In 15 parents of the children in group I Ab-TPO and Ab-hTg were negative and TSH was above normal limits in two cases.According with the above data the hypoechoicity in child thyroid in iodine deficiency area can not be considered as a criterion sugesting thyroid autoimmunity.

84. P64 W. Reinhardt, M. Luster, K.H. Rudorff*, Ch. Heckmann*, R. Haase, K. Cissewski, D. Reinwein
Medical Clinic, Division of Endocrinology, University of Essen, D-45122 Essen; Endocrine Outpatient Clinic, D-42103 Wuppertal

Effect of Small Doses of Iodine on Thyroid Function in Patients with Hashimoto's Thyroiditis Residing in an Iodine Deficient Area

Several studies have suggested that iodine may influence thyroid hormone status and perhaps antibody production in patients with autoimmune thyroid disease. Therefore, we evaluated the effect of small doses of iodine on thyroid function and thyroid antibody levels in euthyroid patients with Hashimoto's Thyroiditis living in an area of modest iodine deficiency (70,2 ± 6,8 µg/g creatinine).
38 patients with positive anti-TPO levels and/or moderate to severe hypoechogenic pattern on ultrasound, received 250 µg iodine daily for 6 months (range 4 - 12 months). 37 patients also with positive anti-TPO antibody and hypoechogenecety on ultrasound served as a control group. All patients were TBII-negative.
5 patients of the iodine R_X-group developed subclinical hypothyroidism and 1 patient became hypothyroid. 1 patient became hyperthyroid with a concomitant rise of TBII to 17 U/l. Following iodine withdrawal, this patient became euthyroid. Only 2 patients of the control group became subclinically hypothyroid during the same time period. In 31 patients of the iodine R_X-group and in 35 control patients no significant changes in thyroid function, antibody titers or thyroid volume were observed.
Conclusions: Small amounts of supplementary iodine may induce thyroid dysfunction in patients with underlying Hashimoto's Thyroiditis residing in an iodine deficient area.

85. P65 THYROXINE DETERMINES INSULIN-LIKE GROWTH FACTORS AND IGF-BINDING PROTEIN-3 LEVELS IN PREPUBERTAL BUT NOT PUBERTAL MALNOURISHED CHILDREN IN IODINE-DEFICIENT AREAS. W.M. Wan Nazaimoon, A. Osman, M. Norhazwati and B.A.K. Khalid, Division of Endocrinology, Institute for Medical Research, Kuala Lumpur, MALAYSIA.

Malnourished children in endemic goitre areas have poor physical development which may be due to multiple factors, including thyroxine deficiency and inadequate growth hormone, insulin-like growth factors (IGF) and IGF-binding protein-3 (IGFBP-3). A study was done in remote aboriginal jungle settlements known for iodine deficiency and endemic goitres, involving 49 prepubertal (age 4-6 years) and 28 pubertal (13-15 years). They were assessed anthropometrically and biochemically for T4, TSH, IGF-I and IGFBP-3 levels. Both prepubertal and pubertal children were found to be equally mildly malnourished (mean±SEM, BMI of 14.1±0.22 and 16.8±0.48 kg/m^2 respectively), have low serum levels of IGF-I (74.44±6.58 and 308.4±39.32 ng/ml respectively) and IGFBP-3 (1.63±0.10 and 3.12±0.22 ug/ml respectively) compared to age-matched normals, but were all euthyroid, with normal serum levels of T4 (97.0±3.13 and 86.4±4.3 nmol/L respectively) and TSH (2.79±0.25 and 2.83±0.30 nmol/L respectively). The BMI of prepubertal children correlated significantly only with IGFBP-3 levels ($r = 0.384$, $p<0.01$), while the BMI of pubertal children showed significant correlation only with IGF-I levels ($r = 0.568$, $p = 0.002$). Thyroxine levels showed significant correlation only with the IGFBP-3 ($r = 0.422$, $p = 0.003$) but not with IGF-I levels of the prepubertal children. In pubertal children however, there was no significant correlation of T4 to either IGF-I or IGFBP-3 levels. This study showed that, in mild-malnourished children of iodine-deficient areas, IGFBP-3 proves to be a better biochemical indicator for growth disorder at prepubertal age but not during puberty, when other hormones such as increased sex steroids and growth hormone production are also important.

86. P66 BONE MINERAL DENSITY (BMD) AND PROLONGED THYROID SUPPRESSIVE THERAPY IN PREMENOPAUSAL WOMEN. F. Matteucci, D. Bacciardi, R. Bonini, G. Ciranna, A. De Biase, M. Grosso, A. Lanzillotta, G. Manca, M. Ravani and R. Bianchi. Nuclear Medicine Center of the University of Pisa; Pisa (Italy).

The effect of suppressive doses of L-thyroxine (L-T4) on BMD in premenopausal women constitutes a therapeutic dilemma. With the aim of clarifying the above problem, we have evaluated a group of 36 premenopausal women on long-term suppressive treatment because of normofunctioning nodular goiter. Their age range was 20 to 53 years (43.5 ± 6.8 SEM) and they were on L-tiroxine (L-T4) therapy since a period of 5 to 30 years (6.8 ± 5.2); the daily dosage of L-T4 had not been changed since at least 2 years. BMD was evaluated by means of a computerized double-photon densitometer (Sophos, Sopha, Gadolinum source) at the lumbar spine (L2-L4) and at the left femoral neck. A group of 85 healthy premenopausal women in the same age range represented the control group (C). Thyroid suppression was evaluated by the assay of serum thyrotropin (TSH) (ultrasensitive IRMA, [by CIS], or EIA method, [by Abbott]) both in basal conditions and following stimulation with 200 μg synthetic thyrotropin releasing hormone (TRH). Serum T4 was 12.2 ± 3.5 μg/dL in pts, significantly higher than in group C (8.2 ± 2.4 μg/dL, $P<0.001$); accordingly, basal TSH was significantly suppressed (0.08 ± 0.10 μU/mL *vs* 1.7 ± 0.7 μU/mL in C, $P<0.001$). On the other hand, 19 patients had a completely suppressed TSH response to TRH (maximum increment 0.15 ± 0.16 μU/mL), while 17 had a blunted TSH response (2.13 ± 0.96 μU/mL). Serum osteocalcin (RIA) was 1.89±0.24 ng/ml in patients and 1.90±0.45 in group C (NS). BMD (g/cm^2) was not significantly different in the two groups (1.09 ± 0.02 *vs* 0.99 ± 0.02 at the spine and 0.81 ± 0.03 *vs* 0.74 ± 0.01 at the femoral neck). Finally, there was no correlation between the BMD values and the cumulative dose of L-T4. The results of this study indicate the lack of any significant long-term effect of suppressive L-T4 treatment on BMD in premenopausal women affected by nodular goiter.

87. P67 NO ASSOCIATION EXISTS BETWEEN EVIDENCE OF THYROID FAILURE AND DEVELOPMENT OF ISCHAEMIC HEART DISEASE (IHD) IN A TWENTY-YEAR FOLLOW-UP STUDY OF A COMMUNITY.

W.M.G.Tunbridge, M.P.J.Vanderpump, J.M. French, D.Appleton, F.Clark, J.Grimley Evans and E.T.Young. Department of Medicine, Newcastle General Hospital, Newcastle upon Tyne, UK.

No clear data exist from longitudinal epidemiological studies to support or refute the theory that there is an association between evidence of minor degrees of thyroid failure and IHD via abnormalities of lipid metabolism. The Whickham survey documented a comprehensive range of risk factors for cardiovascular disease as well as the prevalence of thyroid disorders in a representative sample of the community. The aim of the follow-up study was to determine any association between raised serum thyrotropin (TSH) and thyroid antibodies identified twenty years ago and the development of IHD. Outcomes in terms of morbidity and mortality were determined in over 97% of the original sample. Evidence of IHD was sought from a past history of angina or myocardial infarction, standardised WHO chest pain questionnaire, ECG coded by Minnesota code and death certificates. 825 subjects (30%) of the sample had died and of these one third had died from IHD. Of the 1877 survivors, 96% participated in the follow-up. The expected mortality for each age and sex distribution was obtained from national data and used to calculate the relative risk (RR) of death in relation to thyroid antibody status at first survey. The RR (with 95% confidence intervals) in men for all cause mortality was 1.0 (0.5 - 1.4, p = 0.92) and for IHD was 1.3 (0.5 - 2.6, p = 0.55), and in women the RR were for all cause mortality 0.9 (0.7 - 1.0, p = 0.49) and for IHD 1.1 (0.6 - 1.1, p = 0.80). All women identified in the original survey as having either thyroid antibodies or raised serum TSH concentrations (>6mU/L), or both, in the absence of overt thyroid disease (n = 126) were matched with controls for age, sex and smoking history. Neither group had any evidence of previous IHD. In addition, an analysis of both groups identified no significant difference in fasting blood glucose, fasting serum cholesterol and triglyceride, mean arterial blood pressure or body mass index as measured at first survey. Of those with thyroid antibodies and/or raised serum TSH, 26 had died compared with 28 of the controls (p = 0.76), and 32 had evidence of IHD at follow-up (either alive or deceased) compared with 21 controls (p = 0.09). This twenty-year follow-up study has not found any significant association between evidence of thyroid failure documented at first survey with mortality or development of IHD.

88. P68 SCINTIGRAPHIC EVALUATION OF HEART FUNCTION IN REST IN UNTREATED HYPOTHYROIDISM AND HYPERTHYROIDISM.

E. Tielens, M. Pillay, C. Storm, A. Berghout. Depts. of Internal Medicine and Cardiology, Zuiderziekenhuis, and Dept. of Nuclear Medicine, Dr. Daniel Den Hoed Kliniek, Rotterdam, NL.

Thyroid disease affects heart function both directly as well as by secondary cardiac responses to changes in preload and afterload.
The aim of the study was to evaluate cardiac function in rest by multiple gated equilibrium radionuclide ventriculography (MUGA) in combi-nation with simultaneous, continuous recording of blood pressure (BP).
Consecutive outpatients with untreated longstanding hypothyroidism or hyperthyroidism were studied. Patients with atrial fibrillation, cardiac medication, a cardiac history, or a history of recent illness were excluded. MUGA-parameters for systolic function are peak emptying rate(PER), time to peak emptying (TPE) and ejection fraction (EF); diastolic function is evaluated by peak filling rate (PFR) and time to peak filling (TPF). Computerized left ventricular volumes were corrected for differences in gamma-ray attenuation (due to variable chest-heart distance) by means of additional lateral static images. The systolic BP/end-systolic volume ratio ("Pressure-Volume Ratio", PVR), estimates myocardial contractility, relatively independent from after-load. The results are given in the table. Data are given as mean ± s.e.m. In the hypothyroid group, but not in the hyperthyroid patients, correlations were found between T4-PFR (R = 0.65, p < 0.01), T3-PFR (R = 0.54, p= 0.04) and FTI-PFR (R= 0.63, p= 0.01). No other correlation were found between thyroid hormone levels and scintigraphic parameters.

RESULTS	HYPO	HYPER	P-VALUE
	14	13	
Sex (M/ F)	3/11	4/9	N.S.
Age (yrs)	44 (3.6)	46 (3.6)	N.S.
BMI (kg/m2)	26.7(1.1)	24.5(1.1)	N.S.
T4 (nmol/l)	52 (8)	251(13)	<0.001
T3 (nmol/l)	1.43(0.15)	5.5 (0.38)	<0.001
FT4-index	43 (7)	323 (24)	<0.001
TSH (mU/l)	79 (16.0)	<0.10	
HR (bpm)	71 (2)	97 (5)	<0.001
BPSYST (mm Hg)	123 (8)	138 (4)	N.S.
BPDIAST (mm Hg)	77 (3)	76 (3)	N.S.
EF (%)	52 (2)	66 (1.6)	<0.001
PER (1/s)	2.44(0.13)	3.87 (0.14)	<0.001
TPE (ms)	159 (12)	110 (9)	<0.005
PFR (1/s)	2.40(0.20)	4.16(0.33)	<0.001
TPF (ms)	503 (12)	419 (13)	<0.001
EDV (ml)	165 (13)	120 (18)	0.07
ESV (ml)	80 (8)	47 (5)	<0.005
PVR	1.83(0.26)	3.22 (0.4)	0.010

Conclusions: 1) In patients with thyroid dysfunction, marked scintigraphic abnormalities in both diastolic and systolic cardiac function are seen despite the absence of evident clinical cardiac disease. 2) The observed similar increases in both afterload-dependent and afterload-independent parameters for myocardial contractility, taken together with comparable diastolic BP in the two groups but increased pulse pressure in the hyperthyroid group, suggest primary cardiac factors to be responsible for the augmented contractility. 3) In hypo-thyroidism, diastolic dysfunction is most pronounced and correlates with circulating thyroid hormone concentrations. 4) Diastolic hypertension in hypothyroidism was not observed.

89. P69 PREVENTION OF THYROXINE - INDUCED BONE LOSS IN POSTMENOPAUSAL WOMEN - THE EFFECT OF CALCIUM SUPPLEMENTATION AND INTRANASAL CALCITONIN. Annie W.C. Kung, S.C. Yeung. Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong.

Although controversies exist on the possible adverse effect of thyroxine (T4) on bone mass, most studies reported bone loss in estrogen deprived-postmenopausal women taking suppressive doses of T4. We prospectively studied 46 postmenopausal women with carcinoma of thyroid for two years to evaluate the rate of bone loss and to assess whether calcium supplementation or calcitonin was able to decrease the rate of bone loss. All patients were receiving a stable dose of L-T4 (170 ± 60 μg) for more than one year and had TSH levels of ≤ 0.03 mIU/L. The calcium intake was low and averaged 545 ± 129 g/day as assessed by dietary recall. The subjects were randomised into 3 groups: (1) intranasal calcitonin 200 μg daily for 5 days per week plus 1000 mg calcium daily (Calcium Sandoz Forte), (2) calcium alone or (3) placebo. Total body and regional bone mineral density (BMD) were measured by a dural energy X-ray absorptiometry bone densitometer (Norland XR-26) at 6-monthly interval.

The results showed that both group 1 and 2 did not have bone loss while patients in group 3 showed significant bone loss at the end of two years (Lumbar spine 5.0%, hip 6.7%, trochanter 4.7%, Ward triangle 8.8%). There were no differences between group 1 and 2. At 1 year, patients in group 3 already had significantly higher osteocalcin levels compared with those in group 1 and 2. No significant changes were detected in the bone specific alkaline phosphatase levels as well as the urinary hydroxyproline levels. PTH level decreased at 6 months in group 1 but returned to basal state at 12 months onwards.

In conclusion, T4 suppressive therapy was associated with bone loss in postmenopausal women which could be prevented by either calcium supplementation or intranasal calcitonin, although the latter did not provide additional benefit when compared to calcium alone.

90. P70 L-THYROXINE TREATMENT AND BONE DENSITY: RESULTS OF A LONGITUDINAL STUDY. C. Marcocci, F. Golia, E. Vignali, G. Bruno-Bossio, A. Picone, S. Puccinelli, R. Bonacci.
Istituto di Endocrinologia, Università di Pisa (Italy)

The effect of chronic treatment with suppressive doses of L-thyroxine ($L\text{-}T_4$) on bone density (BD) has been evaluated in several cross-sectional studies. Conflicting results have been reported, suggesting that only long term longitudinal studies could clarify this issue. We have evaluated prospectively BD in 2 groups of premenopausal women given $L\text{-}T_4$ in suppressive doses: Group 1 included 10 patients with nontoxic goiter (mean age 34 yr) in whom BD was measured at 6-month intervals after institution of $L\text{-}T_4$ therapy; Group 2 included 19 women treated with total thyroidectomy for differentiated thyroid cancer given $L\text{-}T_4$ for more than 5 yr (mean 11 yr); iPn the latter group BD was measured twice after an interval of at least 12 months (mean 21 months). In all patients the daily dose of $L\text{-}T_4$ was individually adjusted in order to use the minimal amount necessary to suppress TSH (1.8 ± 0.2 μg/kg body weight in Group 1 and 2.5 ± 0.4 in Group 2). BD (g/cm^2) was measured at the lumbar spine (L_2-L_4) and femur (neck, Ward's triangle and trochanter) by dual-photon absorptiometry (Lunar DPX). The results are presented in the Table.

	Group 1				Group 2			
	Basal	6 months	12 months	24 months		1st BD	2nd BD	% /yr
L_2-L_4	1.22±0.11	1.19±0.10	1.21±0.11	1.22±010	L_2-L_4	1.24±0.14	1.22±0.14	-0.96
Neck	0.94±0.11	0.94±0.10	0.97±0.07	0.96±0.10	Neck	0.97+0.10	0.98±0.10	+0.18
Ward	0.87±0.14	0.87±0.16	0.91±0.09	0.86±0.13	Ward	0.93±0.13	0.93+0.14	+0.14
Trochanter	0.75±0.12	0.75±0.12	0.76±0.10	0.75±0.10	Trochanter	0.76±0.09	0.78+0.10	+1.1
FT_4 pg/ml	9.4±2.3	12.8±4.0*	12.9±2.9*	14.4±3.1*	FT_4 pg/ml	15.3±4.6	15.6±3.9	-
FT_3 pg/ml	3.2±0.7	3.6±1.1	3.2±0.8	3.4±0.6	FT_3 pg/ml	3.6±0.18	3.7±0.8	-
TSHμU/ml	0.97±0.45	0.45±0.39*	0.14±0.13*	0.10±0.09*	TSHμU/ml	≤0.07	≤0.07	-

* $p<0.01$ vs basal value

Group1: There was no significant change in BD during the 2-year period; mean FT_4 concentration increased significantly, but FT_3 was within the normal range in all patients. Group 2: The inital BD and the annualizezd rate of bone change at each of the sites did not differ from that of age-matched controls.

In conclusion, our data indicate that in premenopausal women carefully monitored $L\text{-}T_4$ suppressive therapy is not associated with bone loss during the first 2 yr of treatment nor with an accelerated rate of BD change when given chronically.

91. P71 URINARY COLLAGEN CROSS-LINKS EXCRETION CORRELATES BETTER WITH FREE T3 THAN FREE T4 IN PATIENTS WITH UNTREATED HYPERTHYROIDISM. E. C. J. Amarante, T. S. Kasamatsu, J. G. H. Vieira and R. M. B. Maciel. Division of Endocrinology, Department of Medicine, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil.

Thyrotoxicosis is associated with increased bone turnover. The predominance of resorption over formation, resulting in bone loss, appears to be dependent on several factors, including the severity of hyperthyroidism. Urinary pyridinium cross-links are considered to be highly sensitive markers for altered bone metabolism and their excretion have been shown to be increased in hyperthyroidism. We studied 23 untreated patients with clinical and laboratory diagnosis of hyperthyroidism due to Graves' disease (18 females and 5 males, aged 16-55 years, median 33 years) and measured serum FT3 (normal range: 4.6 to 7.8 pmol/L) and FT4 (normal range: 7.6 to 19.7 pmol/L) using kits from Wallac Oy and urinary excretion of Pyridinolin (Pyr) and Deoxypyridinolin (DPyr) in 2 h morning samples using HPLC (normal range for Pyr: 15 to 75 pmol/μmol creatinine; DPyr: 0.8 to 6.0 pmol/μmol creatinine). All patients had undetectable TSH levels (<0.03 mUI/L), high values of FT3, ranging from 9.2 to 84.2 pmol/L (mean ± sd: 46.8 ± 26.6 pmol/L) and high levels of FT4, ranging from 24 to 140 pmol/L (mean ± sd: 65.4 ± 28.5 pmol/L). Urinary excretion of Pyr and DPyr were high in 22/23 patients, ranging from 39 to 624 pmol/μmol creatinine for Pyr (mean ± sd: 236 ± 188 pmol/μmol creatinine) and 3.6 to 58 pmol/μmol creatinine for DPyr (mean ± sd: 19.9 ± 17.3 pmol/μmol creatinine). Spearman's correlations between free thyroid hormones and collagen cross-links were: FT4 and Pyr r= 0.477, p= 0.021; FT4 and DPyr r= 0.582, p= 0.010; FT3 and Pyr r= 0.625, p= 0.002 and FT3 and DPyr r= 0.738, p<0.001. These data indicate a high correlation between free thyroid hormone levels and collagen breakdown. The best correlation obtained with FT3 suggests that its measurement reflects better the clinical impact of hyperthyroidism on the bone.

92. P72 MECHANISMS OF THE IMPAIRED EFFORT TOLERANCE IN HYPERTHYROIDISM AND EFFICACY OF THERAPY. G. Mercuro, M. G. Panzuto, A. Poddighe, M. Leo, R. Cabula*, L. Petrini* and A. Cherchi, Institutes of Cardiology and *Endocrinology, University of Cagliari, Sardinia, Italy

Hyperthyroidism (H) is associated with impaired effort tolerance which is widely believed to be due to decreased cardiac function. More recent evidences suggested a peripheral origin of decreased exercise (E) performance in H, where a decline in the skeletal muscle oxidative capacity, higher plasma lactic acid concentration and lower a-v oxygen difference have been demonstrated.

We studied 14 patients (12 women and 2 men, mean±SEM 37.8±3.7 years) with newly diagnosed H due to diffuse (n=12) or nodular (n=2) toxic goiter in comparison with a control group of 14 age-matched healthy volunteers (N). Rest/E heart rate (HR), blood pressure (BP) and rate-pressure product (HR x systolic BP, DP) were assessed during a progressive, symptom-limited E performed on a bicycle ergometer. Maximal E was defined as the inability to complete a 1-min increment or when the predicted maximal HR was reached. Resting systolic index (SI), cardiac index (CI) and systemic vascular resistance (SVR) were noninvasively estimated by the electrical impedance plethysmography. Ergometric sessions were repeated after 15 days and every month for 1 year from the beginning of therapy.

The clinical symptoms of poor E capacity resulted in early exertional dyspnoea and sense of disconfort. In H the rest/E HR, DP, SI and CI were higher and SVR lower than in N (p<0.01). Patients showed a significant impairment of effort tolerance in comparison with N (518±121 vs 662±89 sec, p<0.01). Correction of H occurred earlier after metimazol treatment (mean 2.2±0.4 weeks) or intranodular alcohol infiltrations (mean 3.8±0.90 weeks) and later after 131 I administration (mean 18.9±4.6 weeks). The results after 1 to 3 months of therapy with metimazol or alcohol showed that although the H was clinically and biochemically corrected, the E performance remained impaired in comparison with the control group (p<0.05) and the ergometric test performed after 1 year of therapy (p<0.05). Conversely, the treatment with 131 I produced a normalization of the physical capacity together with the recovery of euthyroidism.

Present data seem to confirm that the reduced E tolerance in H is of both central and peripheral origin. Particularly, it can presume that a faster correction of H need of a longer period to restore the muscular function, whereas a slow and progressive recovery of thyroid activity allows a gradual restoration of the muscular oxidative capacity.

93. P73 CARDIAC PERFORMANCE AND EXERCISE TOLERANCE ARE IMPAIRED IN PATIENTS ON TSH SUPPRESSIVE THERAPY WITH LEVOTHYROXINE. BENEFICIAL EFFECT OF ADRENERGIC BETA-BLOCKADE.

L. Saccà, B. Biondi, S. Fazio, A. Cuocolo, D. Sabatini, M. Salvatore, G. Lombardi. Internal Medicine, Endocrinology, and Nuclear Medicine, Federico II University, Naples.

Thyrotropin (TSH) suppressive therapy with levothyroxine (L-T4) may cause adverse cardiac effects, including myocardial hypertrophy and diastolic dysfunction. The latter is a well-recognized cause of heart failure, even in the presence of normal systolic function. Therefore, in this study we addressed the question whether long-term TSH suppressive therapy leads to abnormalities of cardiac performance and exercise tolerance.

We studied ten patients (9F and 1M, age 40±5 y) on L-T4 therapy for 3-10 y at a mean dose of 2.31±0.4 μg/Kg/day, presenting with symptoms and signs of adrenergic overactivity, and ten normal control subjects (9F and 1M, age 43±9 y). Maximal exercise capacity was assessed by bicycle ergometer and cardiac performance by radionuclide ventriculography both at rest and during supine exercise at a fixed 75 watts load. In the patient group, the study was repeated after a four-month treatment with the beta-blocking drug bisoprolol (2.5-5 mg/day).

As compared with controls, exercise tolerance was remarkably impaired in the patients as shown by the significantly lower values of peak workload (81±11 vs. 121±17 watts; $p<0.001$) and exercise duration (6.4±0.7 vs. 9.4±1.4 min; $p<0.001$). At rest, the ejection phase indices were not different in the two groups, whereas the peak filling rate was reduced in the patients (2.25±0.7 vs. 3.02±0.3 EDV/s; $p<0.02$). During physical exercise, the left ventricular ejection fraction rose from 58±6 to 65±6% ($p<0.01$) in the control subjects, whereas in the patients it fell from the resting value of 62±7 to 53±8% ($p<0.01$). After beta-blockade, both the peak filling rate (2.62±0.5 Vd/sec) and the ejection fraction response to exercise (62±12%) increased significantly ($p<0.05$). Also peak workload (94±17 watts) and exercise duration (7.1±1 min) were improved by beta-blockade ($p<0.05$).

The data indicate that systolic performance on effort is impaired in patients on L-T4 therapy with evidence of adrenergic overactivity. This may explain their reduced exercise tolerance. Beta-blockade induces a partial correction of cardiac dysfunction and the reduced exercise capacity.

94. P74 **EVIDENCE FOR A LONG-STANDING CALCIUM DEFICIENCY AFTER TREATMENT FOR HYPERTHYROIDISM**

E. Nyström, K. Stenlöf, L. Sjöström, L.-E. Tisell, P.-A. Lundberg, A. Michanek, G. Berg, G. Lindstedt, The Thyroid Unit, Depts of Endocrinology, Medicine, Surgery, Oncology and Clinical Chemistry, Sahlgren's University Hospital, Göteborg, Sweden

Introduction: Thyroid hormones increase bone metabolic activity, and hyperthyroidism is associated with an increased risk of osteoporosis, particularly in postmenopausal women. We have studied different aspects of bone turnover in eight subjects with hyperthyroidism (4 males, 4 premenopausal females) before as well as one year after treatment (levothyroxine initiated at apperance of biochemical evidence for hypothyroidism). The subjects were on a regular diet with no extra calcium supply.

Methods: Bone mineral density and body composition were determined by dual energy X-ray absorptiometry (DEXA, DPX-L Lunar). Serum was analyzed for ionized calcium, intact parathyroid hormone (Nichols Institute Diagnostics), osteocalcin (CIS International), aminoterminal propeptide of procollagen-III (Behring) as well as thyrotropin and thyroid hormones (total and free T4 and T3) by Kodak Amerlite methods, and 24-h urine collections were analyzed for calcium.

Results: Table shows mean (SD). Free T4 (pmol/L) and TSH (mIU/L) showed expected changes. After one year all subjects increased in body weight (kg). Bone mineral content (BMC, kg) and total bone calcium (TBC, kg) tended to increase; however, urinary calcium was still low after one year (u-Ca, mmol/24h) in 5 individuals. Ionized calcium (Ca^{2+}, mmol/L), osteocalcein (Osteo, μg/L) and procollagen-III peptide (PIIIP, kU/L) decreased and parathyroid hormone (PTH, ng/L) increased ($p<0.05$ or less).

	FT4	TSH	Weight	BMC	TBC	U-Ca	Ca^{2+}	PTH	Osteo	PIIIP
Pre	86(41)	<0.02	67(13)	2.8(0.6)	1.1(0.2)	4.6(1.5)	1.33(0.05)	16.8(12)	18(8.0)	1.5(0.44)
Post	17(5.7)	3.6	78(12)	3.2(0.6)	1.2(0.2)	4.1(2.3)	1.22(0.05)	42.8(13)	9.6(3.7)	0.7(0.06)

Conclusion: Pronounced changes in calcium homeostasis are observed after therapy for hyperthyroidism. The low urinary Ca and increased mean PTH indicate a negative calcium balance still one year after therapy, and the results raise the question whether (most) hyperthyroid patients might benefit from additional calcium during the year following therapy.

95. P75 IMPROVEMENT OF SKELETAL MUSCLE FUNCTION AND KINANTHROPOMETRIC PROFILE AFTER TREATMENT OF HYPERTHYROIDISM.

M.Vaisman, ACL.Nobrega, CGS.Araujo, JDM.Nunes, FD.Oliveira, MO.Annarumma. - Div. Endocrinology and Cardiology, HUCFF, Univ. Fed. Rio de Janeiro, Brasil.

In order to determine whether the improvement in muscle performance is only due to an increase in muscle mass (lean body mass), nine patients (7W/2W:age 39yrs) with clinical and laboratorial diagnosis of Graves' disease were evaluated before and after 182±93 days of clinical treatment with antithyroid drugs.Anthropometric mesasurements included: height, Body Weight (BW), right-side skinfolds (triceps, subscapular, biceps, iliac crest, supraspinal, abdominal, front thigh, and medial calf), circumferences (arm relaxed, arm flexed and tensed, forearm, wrist, chest, waist, gluteal, thigh, calf, and ankle), and bone width (distal humerus and femur). Muscle performance was measured by maximal static contration (MAA) and a constant force equal to 30% of max until fatigue as an index of muscle endurance (END).Three muscle groups (movements) were tested: proximal lower limb (hip flexion), distal lower limb(foot dorsiflexion), and distal upper limb (handgrip). An index of muscle mass was obtained from the sum of skinfold-corrected circumferences of arm, thigh and calf. Body weight changed from 53.4±3.2 to 58.2±2.9 Kg (P=0.004) and sum of corrected circumferences from 84.7±3.5 to 154.9±69.0 cm (P=0.017). MAX and END of all movements increased significantly after treatment, even if standardized for the sum of skinfold-corrected circumferences: hip flexion - MAX: 22.2±3.4 to 33.4±5.1 g. cm^{-1} and END: 0.5± 0.2 to 1.2± 0.4 kg.s.cm^{-1},foot dorsiflexion - MAX: 13.2± 2.1 to 28.9± 2.6 g.cm^{-1} and END: 1.0± 0.3 to 2.7± 0.6 kg.s.cm^{-1}, handgrip - MAX: 23.6± 2.4 to 32.1± 2.0 g.cm^{-1} and END 1.3 ±0.1 to 2.2± 0.3 kg.s.cm^{-1}. In conclusion the treatment of hyperthyroidism increased BW mostly from recovery of lean body mass (skeletal muscle mass), and improved muscle performance, which resulted from an enhanced intrinsic muscle function as well as from a greater muscle mass.

96. P76 **EFFECTS OF SHORT-TERM THYROXINE TREATMENT ON BONE METABOLISM IN HEALTHY VOLUNTEERS**. W.S. Huang and W.L. Chen. Department of Nuclear Medicine, Tri-Service General Hospital, Taipei, Taiwan, and Thyroid Research Laboratory, VA-UC Irvine Medical Center, Long Beach, California.

We investigated the short-term effects of thyroxine treatment on biochemical markers of bone turnover in 16 healthy young men (age 20.3 ± 1.7 years, BMI: 21.6 ± 2.1 Kg/M^2). Serum parameters of bone formation (osteocalcin, OC and carboxyterminal propeptide of type I procollagen, PICP) and bone resorption (cross-linked carboxyterminal telopeptide of type I collagen, ICTP and the urinary excretion of deoxypyridinoline, D-Pyr) were measured before and after 7 days of treatments with thyroxine (400 μg/day).

Serum concentrations (pre- vs. post-T_4 therapy, mean ± SD) of T_3 (126 ± 11 vs. 188 ± 13 ng/dl) and T_4 (6.9 ± 1.2 vs. 12.1 ± 1.9 μg/dl) increased significantly ($P < 0.001$) and TSH decreased markedly (1.64 ± 0.8 vs. 0.06 ± 0.02 mU/L, $P < 0.001$). Markers of bone formation showed variable responses with a small but insignificant increase. In contrast, parameters of bone resorption uniformly increased (serum ICTP: 4.5 ± 1.9 vs 5.9 ± 2.2 μg/L, $P < 0.01$; urine D-Pyr: 42.4 ± 19.6 vs 86.6 ± 25.8 nmol/mmol creatinine, $P < 0.001$).

In conclusion, the increases of serum type I collagen cross-linked carboxyterminal telopeptide (ICTP) and urinary excretion of deoxypyridinoline (D-Pyr) suggest a rapid stimulation of bone resorption by short-term administration of thyroxine and underlie the long-term effects of hyperthyroidism on bone metabolism. Parameters of bone resorption may serve as sensitive markers to monitor the effects of thyroid hormone replacement therapy on bone metabolism.

97. P77 BONE DENSITY IN TREATED THYROTOXIC AND HYPOTHYROID WOMEN RECEIVING THYROXINE THERAPY. W Evans[2], F Ammari[1], R Pettit[2], D Coleman[2], D Sandeman[1], R John[3]. Departments of Medicine[1], Medical Physics[2], and Medical Biochemistry[3], University of Wales College of Medicine, Cardiff, U.K.

It is known that hyperthyroidism is associated with a reduction in bone mineral density (BMD) and there has been considerable debate as to whether thyroxine (T4) therapy is also accompanied by low BMD.

We have measured BMD by dual energy x-ray absorptiometry in 25 women (mean age 56.3 ± 10-5 SD yrs) receiving T4 for primary hypothyroidism and in 25 (mean age 58.0 ± 9.2 SD yrs) receiving T4 for post 131I hypothyroidism. The two groups were matched for weight (67.9 vs 62.4kg), height (1.60 vs 1.61m), T4 dose/day (127 vs 111µg), duration of therapy (9.0 vs 9.7 yrs) smoking habits and equal number of pre and postmenopausal subjects in each group. Mean FT4 was higher (30.3 ± 1.47 se pmol/l) in the hypothyroid compared to the hyperthyroid group (26.6 ± 1.23 se pmol/l). There were no differences between the two groups in bone mineral density of the spine (1.008 vs 0.956g/cm²) and of hip (0.90 vs 0.84g/cm²). However, although there was no statistical difference between the two groups in BMD compared to a reference age matched population (Z score), the Z score for both the hypothyroid (+0.834) and hyperthyroid (+0.63) group for the spine were significantly raised ($p<0.05$). There were no differences between BMD Z scores for the hip between the two groups and neither Z score was different from normal.

It is concluded that, in this population receiving T4 replacement there is no evidence of reduced bone density either in pre or postmenopausal women irrespective of the aetiology of the hypothyroidism. The effect of hyperthyroidism resulting in reduced bone density has not been continued during the course of management. In both groups there may even be a modest increase in spinal bone density on T4 therapy.

98. P78 LEFT VENTRICULAR CONTRACTILITY MONITORING IN HYPERTHYROIDISM DURING THE TREATMENT. G.Kotova, F.Burumkulova, Russian Endocrinology Research Center, Moscow, Russia.

Many clinical manifestations in hyperthyroidism are due to the hyperdynamic cardiovascular state. This state is characterized by sinus tachycardia and increased myocardial contractility. Specific antithyroid therapy is accompanied by normalization this situation. We have used Echocardiography to evaluate various parameters of left ventricular (LV) contractility in 19 untreated patients with Graves'disease (mean age 33,7±4,8 yr., mean duration of disease 7,6±1,6 months, free thyroxine (FT4) level 61,1±9,5 pmol/L. Normal range of FT4:10-25 pmol/L). All patients had normal electrocardiograms excepting sinus tachycardia at the time of diagnosis. Patients with preexistent heart disease were excluded from the investigation. Parameters including heart rate (HR), stroke volume (SV), cardiac output (CO) and LV ejection fraction (EF) were measured at rest before the antithyroid drugs treatment (15-30 mg methimazole) and when the patients were clinically and chemically euthyroid (FT4 17,2±2,6 pmol/L). We have also studied the prevalence of mitral valve prolapse among this group. The following results were found:

Parametrs	Hyperhyroidism	Euthyroidism	
HR (beats/min)	106±6,1	78,1±5,7	p<0,05
SV (ml)	89,3±5,9	64,8±5,4	p<0,05
CO (l/min)	9,4±0,7	5,0±0,6	p<0,05
LV EF (%)	74,5±2,3	64,4±2,5	p<0,05
mitral valve prolapse (mm)	8,1±1,3	4,2±1,0	p<0,05

Sinus tachycardia was found in 17 cases (89,5%) and was fully reversible in every patients. Parameters of LV contractility in hyperthyroidism were significantly greater than in euthyroidism and correlated with FT4 level. There were 5 cases (26,3%) of mitral valve prolapse and its degree was significantly reduced when the euthyroidism was achieved. Conclusion: echocardiographic measurement of LV contractility could used for dynamic control of antithyroid treatment efficiency.

99. P79 Evidence for an effect of prior hyperthyroidism on the pattern of bone mineral density as assessed by indices of trabecular and cortical bone. J.C. Krakauer, M.D. and Michele Kochan, Henry Ford Hospital Medical Center, West Bloomfield, MI, and Osteoporosis Testing Centers, Inc., Southfield, MI, USA.

Bone loss in hyperthyroidism is stated to be modest overall and to largely affect cortical bone but the pattern of effects on bone mineral density (BMD) has not been well established. In primary hyperparathyroidism there is also recent evidence for overall normal BMD and even augmentation of trabecular bone structure.

Patients and Methods: Patients were referred for BMD measurements over a 9 month period. Dual Xray absorptiometry was performed at the lumbar spine (LS), femoral neck (FN) and proximal- 33% radius (PR) and results expressed as % of age/gender/weight/height expected normals (LUNAR Corporation, DPX-L). Each patient filled in an osteoporosis screening questionnaire. Data are presented below as mean ± s.d. Statistical analysis was performed with commercial software (Poly Software International, 1994) with un-paired and paired t-tests as appropriate. (significance for $p<0.05$)

Main Results: Data on men were excluded (4.2% of total). 311 cases were available for study. 16 had a history of hyperthyroidism (hHT), 14 of hyperparathyroidism (hHPT) and 2 (excluded from further analysis) of both conditions. Using commonly published site specific composition estimates and % matched values, indices were defined for cortical bone, CBI = (0.4 * LS + 0.5*FN + 1.0*PR)/1.9 and for trabecular bone TBI = (0.6*LS + 0.5*FN)/1.1.

	Age years	Weight kg	Height cm	L2-4 LS %	FN %	PR %	CBI %	TBI %	paired t test CBI vs TBI
hHT,n = 16	66.1±13.4	59.3±10.6	157.4±5.5	87.7±24.8	87.9±12.6	86.1±16.2	86.9±14.6	87.8±16.6	0.6
hHPT,n=14	60.2±12.1	74.5±18.5	158.1±7.0	95.0±13.5	93.1±13.5	84.7±12.3	89.1±11.1	94.2±12.6	0.004
neither(NT) n=279	59.2±11.7	66.2±14.1	158.4±7.9	97.4±14.1	95.6±12.7	90.1±12.4	93.1±10.2	96.6±11.8	0.0001
un-paired t	ns	0.03 HPT vs HT&NT	ns	ns	ns	ns	ns	ns	p <

Conclusions: In retrospective analysis patients with hHT were found to have evidence for a difference in the pattern of regional BMD when compared to patients with hHPT or neither condition. Full multivariate and prospective study will be required to confirm the utility of bone composition indices in detecting the trace of hyperthyroidism on the skeleton.

100. P80 **BONE TURNOVER IN PATIENTS WITH THYROID DYSFUNCTION.** Birgitta Norstedt-Wikner, Per Bjellerup, Anders Kallner, [1]Ove Tϕrring. Department of Clinical Chemistry and [1]Molecular Medicine, Karolinska Hospital, Stockholm, Sweden

Background: Thyroid hormones has a regulatory effect on bone turnover. Thyroid dysfunction may alter bone turnover by changes in formation or resorption or both. To evaluate the impact of thyroid dysfunction on bone turnover, serum markers indicating formation and resorption respectively, was monitored in patients with thyroid dysfunction.

Method: Bone turnover in untreated patients with primary thyroid dysfunction and euthyroid subjects were evaluated by measurements of osteocalcin (OST) as a marker for boneformation and type-I telopeptide (ICTP) as a marker for bone resorption.

Results: Median values and SEM in parenthesis for S-Osteocalcin (μg/L) and S-ICTP (μg/L).

Diagnosis	S-OST	S-ICTP	S-OST/S-ICTP
Euthyroid (n=13)	19 (2.1)	4.0 (0.5)	4.75
Hypothyroid (n=6)	17 (2.1)	3.3 (0.7)	5.15
Hyperthyroid (n=23)	36 (3.3)	7.0 (0.9)	5.14
Ref. interval	10-40	1.8-5.0	

Conclusion: A slight decrease in both formation and resorption is seen in hypothyroid patients whereas as marked increase is seen in hyperthyroid patients. The index between S-Osteocalcin and S-ICTP is similar in all three groups indicating that formation and resorption are equally affected by changes in thyroid hormone concentrations.

101. P81 **Transgenic mice bearing human mutant thyroid hormone β1 receptor (TRβ1): a model of resistance to thyroid hormone (RTH) associated with fat loss and hyperactivity.** **R Wong, DI Kutler, VV Vasilyev, YT Ting, M Willingham*, SY Cheng & BD Weintraub. National Institutes of Health, Bethesda, MD 20892; *Medical University of South Carolina, South Carolina.**

RTH is a syndrome characterized by refractoriness of the pituitary and peripheral tissues to the action of thyroid hormone and is caused by mutations in the TRβ gene resulting in reductions in the affinity of TRβ1 for T3. Patients commonly have attention-deficit hyperactivity disorder (ADHD) with or without other phenotypic features including markedly reduced weights. Serum T4 is generally 2-3 fold higher than normal and TSH is inappropriately normal. We have established the first in vivo model of the disease with which to study T3-dependent gene regulation as well as to develop an animal model of ADHD. Transgenic (TG) mice were produced bearing a naturally-occurring potent dominant negative human mutant TRβ1, PV linked to the human β-actin promoter. Previously we reported that TG PV mice weighed significantly less than their non-transgenic (NTG) littermates and displayed thyroid function test anomalies consistent with pituitary resistance. Mutant receptor protein was detected using a specific antibody designed against the unique frameshifted carboxyterminus of PV. We now chose to focus on 3 families, A, D and G with low, high and high expression of mutant:endogenous (M:E) TRβ1 mRNA respectively, as determined by quantitative RT-PCR. Firstly, TG mice of family A did not weigh less than their NTG littermates whereas those of families D and G did. This corresponded to a M:E ratio in white adipose and brown adipose tissues of TG mice of family A of 1:5 and 1:7 respectively; and in families D and G of 5:1 and 3:1; and 19:1 and 2:1 respectively, together with visible reductions of white adipose tissue. Secondly, the behavioral phenotype of TG male mice of families D and G was characterized by a paradoxical response to methylphenidate-induced hyperactivity, not present in male TG mice of family A. This finding correlated with a M:E ratio in the brain of 1:4 in TG mice of family A compared to 6:1 and 7:1 in families D and G. Methylphenidate (MP), a dopamine reuptake blocker with a similar mechanism of action to amphetamine, is widely used clinically for the treatment of ADHD. On MP, TG mice displayed reductions in vertical movements ($p=0.03$), decreased stereotypic behavior time ($p=0.03$) and decreased total distance travelled relative to baseline ($p=0.04$) compared to NTG mice. In summary, M:E ratios of PV mRNA in adipose tissue and brain correlated with the phenotypes of reduced weight in association with fat loss and a paradoxical behavioral response to MP. Moreover, this constitutes the first description of a TG model of a disease related to mutations of the nuclear receptor superfamily and will be useful in the study of fat cell biology as well as ADHD.

102. P82 HISTONE ACETYLATION INFLUENCES THYROID HORMONE AND RETINOIC ACID-MEDIATED GENE EXPRESSION. P.Garcia-Villalba, A.Jimenez-Lara, A.I.Castillo and A. Aranda. Instituto de Investigaciones Biomedicas. CSIC. Madrid. Spain.

The organization of genes into nucleosomes and higher order chromatin structure has important implications for gene expression. Histones are subjected to covalent post-translational modifications e.g. acetylation which cause changes in the structure of nucleosomes and influence transcriptional regulation. To investigate the effect of histone acetylation on hormone dependent gene expression we examined the influence of butyrate, which induces histone hyperacetylation, on the activity of CAT plasmids containing the rat growth hormone (GH) promoter. Thyroid hormone (T3) and retinoic acid (RA) receptors regulate transcription of the rat GH gene through binding to a common hormone response element (HRE) in the promoter. CAT activity increased by 8 and 4-fold after incubation with 1 nM T3 and 1 μM RA, respectively, and this response was estimulated 3-4 fold further in the presence of 0.25 mM butyrate. This concentration of butyrate did not influence basal CAT levels. The effect of butyrate was mimicked by trichostatin A, a highly specific inhibitor of histone deacetylases. Trichostatin A produced a dose-dependent increase of CAT activity in the absence of ligands, and at low concentrations (between 5 and 200 nM) potentiated the effect of T3 and RA. These compounds also increased the hormonal response of constructs in which the HRE was ligated to heterologous (MMTV and TK) promoters. In contrast to these results obtained in conditions of partial histone hyperacetylation, under conditions of global histone acetylation (obtained with >1mM butyrate), basal activity of the GH promoter increased by 6-8 fold and the effect of T3 and RA was no longer observed. Over-expression of T3 receptors was able to counteract the stimulation of the basal CAT levels caused by butyrate. This suggests that, in the absence of ligand, the binding of hormone receptors could locally alter the chromatin structure and inhibit gene expression. This inhibition is totally overcome when the hormone binds to the receptor. As in the case of butyrate, high concentrations of trichostatin A (>400 nM) blocked T3 and RA-dependent activation. This inhibition was also observed with a CAT construct that contains the prolactin promoter. T3 and RA increased by 3-4 fold the activity of the prolactin promoter and high concentrations of butyrate or trichostatin A inhibited this response.

These results show that chromatin structure plays an important role in hormone-mediated transcriptional regulation and suggest that different degrees of histone hyperacetylation may induce an altered configuration of the regulated promoter, facilitating or preventing the access of the receptors or other transcription factors to the hyperacetylated nucleosome.

103. P83 T3 ACTIONS AND T3 RECEPTORS IN THE T3-SENSITIVE MURINE OB 17 PREADIPOCYTE CELL LINE ; INTERFERENCES EXERTED BY RETINOIDS AND 1,25-DIHYDROXYVITAMIN D3.

A. Dace, C. Lenoir, C. Martin, J. Bonne, M. Teboul, R. Planells, J. Torresani.
INSERM Unité 38, Biochimie Médicale, Fac. Médecine, 27 Bd J. Moulin, 13385 Marseille Cédex 5 France.

As we previously reported, in the differentiating Ob 17 preadipocyte, T3 is necessary at an early step for terminal adipose differentiation. The Ob 17 cells contain T3 receptors (TR) which appear to be exclusive c-erb A α gene products. The TR abundance is rapidly down-regulated by T3 with a maximal depletion of approx. 50 % at 1.5 nM, the optimal concentration for emergence of adipose phenotypes. We further reported a fast and marked depletion of TR (max. 80 %) in these cells after application of all-trans retinoic acid (tRA), with an IC 50 at approx. 0.5 μM, and an additivity of tRA and T3 effects. In the 0.5-10 μM conc. range, tRA inhibited terminal adipose differentiation provided an addition at an early step ; moreover, the efficient tRA concentrations were lower when 1.5 nM T3 was present simultneously. These results then evoked : 1/ a role for a marked depletion of TR in the inhibition exerted by tRA on adipose differentiation ; 2/ a possible link between TR depletion and changes in some interferences between the closely related TR and RAR whether liganded or not, (and probably, at both protein and/or gene levels). In this investigation line, it was of interest to comparatively analyze, in the Ob 17 cells, the action of ligands activating other closely related receptors : 9 cis-RA (cRA) for the RXR and RAR, and 1,25-dihydroxyvitamin D3 (VD) for the VDR.

The cells were cultured in DMEM + 10 %. FCS, with insulin (17 nM) ± T3 (1.5 nM) added at confluence and maintained over 2-3 weeks, this allowing the emergence of a series of adipose phenotypes (triacylglycerol accumulation, lipogenic enzymes such as glycerol-3-phosphate dehydrogenase GPDH...). When added at confluence, tRA at low conc. (<100 nM) was inactive or a moderate amplifyer of differentiation, whether 1.5 nM T3 was added or not, and an inhibitor at >100 nM. Conversely, cRA and VD markedly amplified the differentiation and GPDH activity (up to 4-fold) within a physiological conc. range (0.1-100 nM and 0.01-0.25 nM respectively), while becoming inhibitors at higher conc.. The increments brought by low conc. of cRA and VD were attenuated by T3. Paralleling these effects, tRA, cRA and VD all depleted the TR, rapidly (1/2 disappearance time of approx. 7,6 and 11h respectively) and markedly (up to 80 % of initial level), with IC50 of approx.500, 300 and 0.25 nM respectively. The TR depletion was reversed after agent withdrawal and increased by 1.5 nM T3. These agents only partially decreased the abundance of c-erb A α gene transcripts (total and α1), which suggests that TR depletion also possibly involve post-translational events.

The above results indicate that : 1/ several ligands that activate closely related and interacting receptors, are able to markedly down-modulate the level of at least one of these receptors, the TR ; 2/ optimal differentiation of Ob 17 cells occurs when TR abundance is partially decreased this suggesting a possible impairment by an excess of TRα.

104. P84 THE INHIBITION OF RETINOID X RECEPTOR RESPONSIVENESS BY THYROID HORMONE RECEPTORS REQUIRES HETERODIMERIZATION AND IS ISOFORM-SPECIFIC.

Amy L. O'Donnell, Dept. of Medicine, SUNY at Buffalo and VAMC, Buffalo, NY.

Thyroid hormone receptors (TRs) and retinoid X receptors (RXRs) interact in transient transfection studies to affect the transactivation of their respective target genes. I have previously presented data showing that TRβ1 completely inhibits the 9-*cis* retinoic acid (9-*cis* RA) responsiveness of RXRα and RXRγ through the retinoid X response element (RXRE) from the cellular retinol-binding protein type II (CRBPII) gene in JEG-3 cells. Others had shown that TRα1 inhibits the 9-*cis* RA responsiveness of RXRα in NIH3T3 cells using the same RXRE. I have since presented data demonstrating that the effect of T3Rs on the 9-*cis* RA responsiveness of RXRs is dependent both on the cell type and on the RXR isoform used in the studies. Experiments with COS cells revealed that T3Rβ1 inhibits the 9-*cis* RA response of RXRγ by 55% compared to a complete inhibition in the JEG cells. In contrast to the JEG cell data, the response of RXRα to 9-*cis* RA was not inhibited by coexpression of T3Rβ1 in the COS cells. The goal of my current studies is to further characterize the specificity and the mechanism of the TR-RXR interaction in the regulation of 9-*cis* RA responsive genes. For these experiments, COS cells were transiently transfected with a CAT reporter plasmid containing two copies of the RXRE from the CRBPII gene. Transfections also included expression vectors for RXRγ alone or in combination with expression vectors for TRβ1 or TRα1. Some transfections included a vector expressing a TRβ1 containing a mutation at amino acid 300, which I have previously characterized and shown to be defective in interaction with thyroid hormone receptor auxiliary protein (subsequently shown to be RXR). In this series of experiments, there was a 70% inhibition of the 9-*cis* RA response of RXRγ upon cotransfection with wild-type TRβ1, but there was no inhibition of this response upon cotransfection of the mutant TR. These results suggest that the inhibition of 9-*cis* RA responsiveness by TRβ1 requires heterodimerization between the TR and the RXR. Surprisingly, there was also a lack of inhibition of the 9-*cis* RA response upon cotransfection of TRα1 with RXRγ. Thus, this inhibition is not only specific for the RXR isoform tested in the transfection studies and the cell type used for the studies, but is also specific for the TR isoform.

105. P85 PLASMINOGEN ACTIVATOR INHIBITOR TYPE-1 IS REGULATED BY THYROID HORMONES IN HUMANS. A Bennet, F Brousset, S Dumoulin, P Sié, PJ Caron. Service d'Endocrinologie, CHU Purpan et Rangueil, Service d'Hémostase, CHU Purpan, Toulouse, France.

Plasminogen activator inhibitor type-1 (PAI-1), an inhibitor of fibrinolysis and a risk factor of myocardial infarction is dependent on metabolic and hormonal factors. It is known that fibrinolysis is affected by thyroid dysfunction but the effect of thyroid hormones on PAI-1 is unknown in humans. PAI-1 levels were evaluated in premenopausal women who did not use oral contraceptive pills. PAI-1 was measured using a kit (Spectrolyse pL) provided by Biopool (Sweden). Plasma samples were collected at 09.00h from euthyroid, hyper and hypothyroid women, before and after treatment with carbimazole (mean dose 15 ± 2 mg/day) or levothyroxine (mean dose 2.3 µg/kg/day).

	n	Age years	BMI kg/m^2	FT4 pg/ml	FT3 pg/ml	TSH mU/l	SHBG mg/l	Chol mmol/l	PAI-1 U/ml
Euthyroid	47	30±1	22.5±0.4	10.4±0.2	3.8±0.1	1.84±0.12	4.04±0.27	5.03±0.15	8.1±0.9
Hyper	13	32±3	21.4±0.8	48.6±7.2^b	24.5±4.9^b	0.05±0.01^b	14.68±2.12^b	3.50±0.26^b	12.7±2.1^b
Hypo	16	37±2^b	23.7±1.1	4.3±0.8^b	2.3±0.4^b	65.1±14.7^b	3.55±0.41	5.92±0.18^b	4.6±0.7^a

^{a} $p < 0.05$, ^{b} $p < 0.01$ versus euthyroid women

PAI-1 levels are increased in hyper ($p < 0.05$) and decreased in hypothyroid ($p < 0.05$) women. PAI-1 levels are correlated with thyroid hormone concentrations (FT4 : r= +O.396, $p < 0.01$) ; FT3 : r= +0.424, $p < 0.01$) as well as with the concentrations of peripheral index of thyroid hormone action (cholesterol : r= -0.365, $p < 0.01$; SHBG : r= +0.247, $p < 0.05$). During treatment, PAI-1 levels decrease (5.48 ± 0.61 U/ml, $p < 0.01$) in hyperthyroid and increase (10.42 ± 1.68 U/ml, $p < 0.02$) in hypothyroid women. In treated women, PAI-1 levels are not significantly different from those of euthyroid women. In conclusion, plasma PAI-1 level is regulated by thyroid hormones in humans. Like cholesterol and SHBG, PAI-1 appears to be a peripheral index of thyroid hormone action. Changes of PAI-1 level should be taken into account when studying the pathogenesis of cardiovascular events during thyroid dysfunction and their treatment.

106. P86 RETINOIC ACID MODULATES T_3-RESPONSIVENESS BY ALTERING THE LEVEL OF MAXIMUM STIMULATION IN HepG2 CELLS. TC Crowe, NM Loidl, NL Cowen, JW Barlow. Ewen Downie Metabolic Unit, Alfred Hospital, Commercial Rd., Melbourne, Australia, 3181

Previous experiments from our laboratory suggested that all-*trans* retinoic acid (RA) modulates T_3-responsiveness of the hepatoma cell line, HepG2. We now report a more detailed investigation of the interrelationship between T_3 and RA in these human cells. **Methods:** Cells were incubated for 4 days in serum-free medium with T_3 and/or RA or 9-*cis*RA. Responses measured were stimulation of secreted sex-hormone binding globulin (SHBG, by IRMA) or inhibition of secreted thyroxine-binding globulin (TBG, by change in B/F ratio of [^{125}I]-T_4, charcoal separation). Concomitant mRNAs were measured by Northern analysis. **Results:** T_3 induced a dose-responsive increase in SHBG secretion which was maximal (206±24% of untreated, ±SD, n=11) at 10 nM and half-maximal at 0.36±0.16 nM T_3. Parallel changes were seen in SHBG mRNA. RA and 9-*cis*RA, up to 100 nM, induced a slight fall in SHBG secretion to 79±9% and 88±9% respectively. In cells incubated with T_3, 0-10 nM, and RA, the response was significantly attenuated. At T_3, 10 nM, together with RA, 3, 10 or 100 nM, the maximal response (±SD, n≥3) was reduced to 193±24, 151±5 and 132±30% respectively. With T_3 and 9-*cis*RA, 100 nM, maximal stimulation was 169±20%. Importantly, the effective half-maximal concentration of T_3 in the presence of either retinoid up to 100 nM was unchanged at 0.3 nM T_3. In addition, the inhibitory effect of RA could not be overcome even with T_3 concentrations up to 300 nM. The threshold for the effect was between 0.3 and 1 nM with half-maximal inhibition at 30 nM. 9-*cis*RA was ~ 10-fold less potent. Similar changes were seen in SHBG mRNA levels. No effect was observed with vitamin D or clofibrate either alone or combined with T_3. Conversely, T_3 reduced TBG secretion and TBG mRNA production with maximal suppression to 33±6% (n=4) of control at 10 nM. RA alone did not significantly affect TBG secretion but augmented the down-regulation of TBG by T_3, although with reduced potency compared to the SHBG response. T_3 and RA alone or in combination had no effect on either secreted total protein or albumin and did not influence the concentration of nuclear T_3-binding sites. **Conclusions:** (i) retinoids modulate maximal but not half-maximal responses to T_3 in HepG2 cells (ii) this effect is specific for retinoids with RA>9-*cis*RA (iii) retinoids may influence a factor necessary for consummate effects of T_3 on gene *trans*-activation.

107. P87 INTERACTION BETWEEN NUCLEAR PROTEINS AND LIGAND-BINDING POSITIVE MUTANT TRβ IS DIFFERENT FROM THAT OF WILD-TYPE TRβ.

A. Sakurai, H. Kobayashi, M. Katai, Y. Itakura, K. Ichikawa and K. Hashizume.
Department of Geriatrics, Endocrinology and Metabolism, Shinshu University School of Medicine, Matsumoto, Japan.

Resistance to thyroid hormone (RTH) is a syndrome caused, in most cases, by dominant effect of mutant TRβs which have impaired ligand binding activity, and thus have reduced or undetectable transactivation function. We have recently reported a mutant TRβ which was identified in subjects with familial RTH (*Thyroid* 4: Supl. S-84, 1994). This mutant, R243Q, had normal ligand binding activity, but its transcriptional regulatory function was impaired and furthermore it showed dominant negative effect on the function of wild-type TRβ. We speculated that this discordance between ligand binding and transactivation on R243Q might be due to impaired conformational transition and/or impaired interaction with various transcription factors upon ligand binding. To verify this hypothesis, we examined effects of T3 and nuclear proteins on hydrophobicity of TRs using two-phase partitioning analysis. Using this system, we have previously reported that TR changes its conformation or electrostatic properties upon ligand binding (*J Endocr* 119:431,1988, *Mol Cell Endocrinol* 70:175,1990). ^{35}S-labeled TRs were prepared by *in-vitro* translation and unincorporated [^{35}S]Methionine was removed by Sephadex G-50 and hydroxylapatite. Programmed lysates were added to turbid mixtures of PEG-6000 and Dextran T-500, mixed gently by shaking, and two phases were separated by centrifuge. Radioactivity in upper (PEG-rich and more hydrophobic) and lower (Dextran-rich and less hydrophobic) phases were counted. Both wild-type TRβ and R243Q did not show any detectable conformational transition upon T3 binding in this system. However, when TRs were pre-incubated with HepG2 nuclear extract, wild-type TRβ partitioned more preferentially into lower phase upon T3 binding. This is probably due to ligand-dependent association/dissociation with other nuclear proteins since this change was not observed when heat-inactivated nuclear extract was used. R243Q, on the other hand, showed only minimum conformational transition by T3 and nuclear extract. These results indicates that the ligand-dependent interaction between TR and nuclear proteins is altered by amino acid substitution at 243, which may cause discordance between T3 binding and transactivation function of R243Q.

108. P88 EVIDENCE OF TRIIODOTHYRONINE (T3) BINDING TO PURIFIED MICROTUBULE COMPONENTS IN CHICK EMBRYO BRAIN. A Giguère, C. Beaudry, D. Bellabarba. Laboratoire d'endocrinologie, Faculté de médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada.

Thyroid hormones have a profound effect on brain maturation and differentiation. Their lack during a critical period of development causes irreversible lesions of the neuronal networks. This is due to abnormal neurite growth which may result from an inadequate microtubule assembly. In order to understand how thyroid hormones may affect this process, we studied their interaction with various microtubule components during embryogenesis. Purified microtubules were prepared from chick embryo brain by the method described by Fellous et al (Eur J Phar, 1979, 110, 365). Two cycles of assembly/disassembly were used to obtained highly purified microtubules. From this preparation we separated tubulin and microtubule associated proteins (MAPS) fraction, using a phosphocellulose column. The tubulin fraction contained also tau (τ) protein, which could be extracted by heating at 100°C for 5 min. Whole microtubules bound T_3 with positive cooperativity. However saturation analyses at 4°C with the tubulin fraction showed a specific binding of T_3 with a high affinity (Kd of 4 nM and Bmax of 0.2 pmoles of T3/mg of protein). Relative affinity for T_3 analogues was as follows: $T_3 = T_4 > dT_3 >> rT_3$ and Triac, thus indicating that this binding site was different from the nuclear and synaptosomal receptor. Using anti-tubulin antibodies we precipitated 17% of the bound T3 with anti-tubulin α and 15% with anti-tubulin ß. τ fraction also bound T3 with similar features, whereas the MAPS fraction did not. These results demonstrate that T3 binds to microtubule components, such tubulin fraction and τ, which are essential elements for the polymerization and assembly of microtubules. Therefore T3 could directly stimulate the polymerization of microtubule, thus allowing the intensive neurite growth needed during the critical period of brain development.

109. P89 *IN VITRO* BINDING OF IODOTHYRONINES AND ANALOGS TO THE ANGIOTENSIN RECEPTOR OF BOVINE ADRENAL CORTICAL MICROSOMES. C. Horst, C. Wolff, T. Chen, and H. Rokos, Henning Berlin GmbH, Research, Berlin, Germany.

Most metabolites of thyroid hormones (TH) are believed to be devoid of biological activity. Recently, a strong and selective TSH-suppressing effect of 3,5-diiodo-L-thyronine (3,5-T_2) was found [1]. 3,5-T_2 is almost as potent as T_3 in decreasing TRβ_2 mRNA in GH3 cells [2]. In rat mesangial cells (kidney derived), 3,5-T_2 showed an angiotensin-like contraction at 10^{-9} M, whereas T_3 and T_4 were ineffective [3]. This prompted us to study the influence of various TH derivatives on the angiotensin system.

Bovine adrenal cortical microsomes, reportedly containing the angiotensin II (Ang II) receptor type AT_1, were prepared by differential centrifugation. Competitive binding studies were performed with 125-iodine-saralasin, a known Ang II receptor antagonist for 2 hours at room temperature.

50 % of tracer displacement (IC_{50}) was achieved at 15 μM Ang II in our experimental conditions. TH analogs with the acetic acid side chain showed the highest binding: triiodothyroacetic acid (triac) at 120 μM, tetraiodothyroacetic acid at 150 μM, 3,3'-diiodothyroacetic acid at 220 μM. The most effective of the natural thyronines was 3,3'-diiodo-L-thyronine (3,3'-T_2) with an IC_{50} of 380 μM. T_4 showed an IC_{50} of 650 μM, wheras T_3 was similar to 3,5-diiodothyroacetic acid at about 800-900 μM. In view of the positive results with the mesangial cells, the low binding of 3,5-T_2 (> 1 mM) was unexpected. 3'-Isopropyl-3,5-diiodo-L-thyronine (ipT_2) showed a good binding with an IC_{50} of 270 μM. Binding of the D isomers was lower, 3,3'-D-T_2 at 650 μM. Low or no binding was shown by monoiodothyronines, thyronine, 3'-isopropyl-3,5-dimethyl-L-thyronine, and other derivatives.

The binding pattern of the thyroid hormone derivatives to AT_1 is different from their binding to the nuclear T_3 receptors. Ang II is an octapeptide with a tyrosine at position 4. We found that some thyronine analogs (as single though complex amino acids) are bound almost as effectively as Ang II, the IC_{50} is only 8 times lower for triac. These findings suggest a possible involvement in pressure regulation through AT_1; functional studies are now in progress.

Ref. 1. Horst C, et al. J Endocrinol 1995; 145: in press; ETA 1993, #127. 2. Ball S, et al. ATA 1994, #100. 3. Rob PM, et al. ATA 1989, #137.

110. P90 NORMALIZATION OF CARDIAC CONTRACTILITY AND MYOSIN HEAVY CHAIN GENE EXPRESSION WITH TRIIODOTHYRONINE DURING CHRONIC ENERGY RESTRICTION. H.L. Katzeff, S. Powell and K Ojamaa. North Shore University Hospital - Cornell University Medical College. Manhasset, New York.

Chronic energy restriction is associated with a decrease in serum triiodothyronine (T3) levels in both humans and rodents. Impairment in cardiac contractility and alterations in cardiac myosin heavy chain (MHC) gene expression are also observed during energy restriction. To study the role of T3 in nutritionally-induced alterations in cardiac function and gene expression we measured cardiac MHC mRNA content and several parameters of left ventricular function in young adult female rats after 28 days of chronic energy restriction without or without T3 supplementation (6 μg/kg BW/day). Serum T3 levels are linearly related to the reduction in energy intake ($p<0.01$) The proportion of α MHC mRNA to total MHC mRNA was inversely related to body mass ($r=0.83$; $p<0.001$) and serum T3 ($r=0.77$; $p<0.001$) during underfeeding. The α MHC mRNA content of chronically energy restricted hearts was normalized with T3 supplementation. Measurements of the rate of left ventricular contraction were performed in isolated perfused rat hearts. The rate of +dP/dt was decreased 13% ($p<0.05$) during 50% chronic energy restriction and remained normal with T3 supplementation + energy restriction. Left ventricular relaxation time ($\tau_{1/2}$) increased 18.7% ($p<0.05$) and the rates of left ventricular relaxation (-dP/dt) decreased 19.3% ($p<0.05$) during energy restriction and were normalized with T3 supplementation. These data indicate that alterations in cardiac contractility and gene expression are responsive to T_3 and suggest that the nutritionally induced decline in serum T3 is responsible for the functional and genetic alterations in cardiac tissue during chronic energy restriction.

111. P91 PROTEIN KINASE C ACTIVATION IS ESSENTIAL FOR POTENTIATION BY THYROXINE OF INTERFERON-γ-INDUCED HLA EXPRESSION. F.B. Davis, H.R. Thacore, H.-Y. Lin, and P.J. Davis, Division of Molecular and Cellular Medicine, Department of Medicine, Albany Medical College and Stratton Veterans Affairs Medical Center, Albany, NY, and Department of Microbiology, State University of New York School of Medicine and Biomedical Sciences, Buffalo, NY.

We have shown that L-thyroxine (T_4) and 3,3',5-L-triiodothyronine (T_3) potentiate the expression of the human leukocyte antigen, HLA-DR, induced by human interferon-γ (IFN-γ). Potentiation by T_4 of the expression of HLA class I molecules induced by IFN-γ has also been demonstrated. In the present studies, we investigated the signal transduction pathways involved in the potentiating effect of T_4 on the expression of HLA class I and II molecules induced by IFN-γ. Confluent HeLa cells grown in 6-well trays were refed with hormone-depleted fetal bovine serum-supplemented medium and treated with 100 IU/ml of recombinant IFN-γ in the presence or absence of 10^{-7} M T_4 for 2 days at 37 C. During incubation, different modulators were added to investigate the signalling mechanisms involved in the induction of HLA molecules and the potentiating effect of T_4. Cells were harvested and lysed, and proteins separated by electrophoresis. The proteins were transferred to Immobilon membranes for immunoblotting using mouse anti-human HLA-DR α-chain or HLA class I-specific antibody and peroxidase-conjugated rabbit anti-mouse IgG. Immunoblots were visualized by chemiluminesence. A specific protein kinase C (PKC) inhibitor, CGP41251, (0.5 nM to 25 nM) inhibited the expression of HLA class I and II molecules when the inhibitor was added simultaneously with T_4 and IFN-γ. However, in the absence of T_4, no inhibition of the IFN-γ effect was seen. The PKC activator, phorbol 12-myristate 13-acetate (PMA) (10^{-7} M) reversed the inhibitory effect of CGP41251 in the presence of IFN-γ and T_4. The phospholipase C inhibitor, U73122 (1 nM to 1000 nM) and phospholipase C and D inhibitor, neomycin (0.01 mM to 10 mM), did not alter the ability of T_4 to potentiate the induction of HLA-DR expression by IFN-γ. These observations suggest that thyroid hormone modulates the expression of Class I and II HLA through a PKC-dependent pathway.

112. P92 METHOXATIN (COENZYME PQQ) IN MITOCHONDRIAL COMPLEX I: A TARGET FOR THYROID HORMONE ACTION. P.M. Gallop, J. Mah, M.A. Paz, R. Flückiger, †P. Martin and *P.J. Davis, Children's Hospital, Harvard Medical and Dental Schools, Boston, MA, †Ciba-Geigy, Basel, Switzerland and *Albany Medical College, Albany, NY.

Methoxatin (PQQ, pyrroloquinoline quinone) is a redox coenzyme first found in certain bacterial dehydrogenases. PQQ is also present in animal cells and biological fluids (Flückiger et al., Methods Enzymol., in press, 1995) and appears to be an essential nutrient for young mice. In mitochondria, PQQ participates in the passage of reducing equivalents from NADH to ubiquinone and is a coenzyme of NADH:ubiquinone oxidoreductase. A tricarboxylic anion, PQQ is in a reversible charge-transfer complex with a cationic partner, not yet identified. This complex is likely to be a target for L-thyroxine (T_4), which appears to release endogenous PQQ from the above complex, thereby accelerating electron and proton transfer from NADH to ubiquinol, the substrate for complex III. We have shown that agents which sequester endogenous PQQ (rotenone, aryliodonium compounds and N-methyl-phenyl-pyridinium [MPP^+]) are complex I inhibitors. We propose that iodothyronines are redox-reactive agents that release endogenous PQQ from a [PQQ^-:antiPQQ^+] charge transfer complex, when present in their reduced redox state as anionic phenylates. They may also sequester anionic PQQ when oxidized to their cationic quinoid oxonium state. In the present studies, diaphorase activity of isolated rat liver mitochondria (100 mM KCl, 10 mM $MgCl_2$, 10 mM P_i, 10 mM Tris-HCl, pH 7.4) was increased 71% by PQQ (4 μM) and 30% by T_4 (60 μM) in the presence of the NAD-linked substrate, α-ketoglutarate. Mitochondrial buffer [Mg^{2+}] at 10 mM precipitated physiologic concentrations of T_4 (10^{-7} M). The increase in diaphorase activity promoted by T_4 was concentration-dependent at 5-60 μM (5-30%) in the presence of 10 mM Mg^{2+}. Diaphorase activity was only marginally affected by PQQ and T_4 in the presence of the FAD-linked substrate, succinate, the electron source for mitochondrial complex II. Inhibition of complex I by the PQQ-sequestering agents, rotenone and iodoniums was reversed by adding PQQ (4 μM) or T_4 (60 μM in the presence of Mg^{++}). This demonstrates that T_4 may modulate mitochondrial respiration by interacting with the complex I cofactor PQQ and thus influence electron transport.

113. P93 DISTINCT RESPONSES OF SERUM THYROTROPIN CONCENTRATION TO ADMINISTRATION OF TRIIODOTHYRONINE AND TRIIODOTHYROACETIC ACID IN RESISTANCE TO THYROID HORMONE. S.Ueda, M.Ito. First Department of Medicine, Osaka Medical College, Takatsuki, Japan.

Patients with resistance to thyroid hormone (RTH) have inappropriately increased secretion of TSH from the pituitary, and the administration of T3 to the affected patients shows a reduced response in the suppression of serum TSH concentration. Since previous reports documented a successful treatment with 3,5,3'-triiodothyroacetic acid (Triac) in some patients with RTH, we have examined the response of serum TSH to Triac administration in 3 patients with RTH, 3 patients with TSH secreting pituitary adenoma (TSHoma), and 12 normal subjects. The data was compared with that of T3 administration. All subjects received a single oral dose of 1.4 mg of Triac or 75 μg of T3, and serum TSH levels were determined up to 24 hours (7 times of blood drawal).

By T3 therapy , percent changes from the 100% of basal value in serum TSH in RTH, TSHoma, and normal subjects were; at 2 hour, 92±1(mean±SD)%, 93±7%, 78±12%; and at 24 hour, 57±9%, 71±7%, 18±7%, respectively. On the other hand, by Triac therapy, the percent changes of serum TSH were; at 2 hour, 67±3%, 90±4%, 66±11%; and at 24 hour, 78±6%, 90±11%, 38±11%, respectively. Statistical analysis showed that the percent decrement at 2 hour by T3 administration were significantly different between the patients with RTH and normal subjects, while it was not different by Triac administration.

The data suggest that the responsiveness to T3 and Triac is different in RTH, which might explain the successful results of Triac treatment in some patients with RTH.

114. P94 **IMPORTANCE OF AMINO ACID RESIDUES 33-44 OF THE ALPHA SUBUNIT IN THE SECRETION, RECEPTOR BINDING AND BIOACTIVITY OF HUMAN TSH.** M. Grossmann, J. E. Tropea, J. A. Dias*, H. Xia‡, D. Puett‡, N. Teh and M. W. Szkudlinski, MCEB, NIDDK, National Institutes of Health, Bethesda, MD, *Wadsworth Center, Albany, NY (NIH Grant HD-18407) and ‡Dept. of Biochemistry, University of Georgia, Athens, GA.

Amino acids 33-44 of the common alpha subunit of the glycoprotein hormone family have been shown to play a role in heterodimer formation or receptor binding of hCG. Moreover, synthetic peptides encompassing this region inhibited the receptor binding of bovine TSH. In the present study, we therefore investigated the role of individual residues within this region for human TSH (hTSH) secretion, receptor binding and bioactivity in the context of the intact hTSH molecule. We used site-directed mutagenesis to change the following amino acids: $\alpha Phe^{33} \rightarrow Ala$, $\alpha Arg^{35} \rightarrow Ala$, $\alpha Ala^{36} \rightarrow Glu$, $\alpha Pro^{38} \rightarrow Asp$, and, in a composite mutation, αArg^{42}-Ser^{43}-$Lys^{44} \rightarrow Ala^{42}$-$Ala^{43}$-$Ala^{44}$. These mutations were chosen because they have previously been shown to reduce drastically hCG heterodimerization or receptor binding. Wild type (wt) or mutant αcDNAs were transiently coexpressed with a human TSHβ minigene in CHO-K1 cells. Quantitation of the recombinant hTSH variants was based on three different immunoassays. Mutating $\alpha Pro^{38} \rightarrow Asp$ virtually abolished hTSH secretion, and changing $\alpha Ala^{36} \rightarrow Glu$ as well as αArg^{42}-Ser^{43}-$Lys^{44} \rightarrow Ala^{42}$-$Ala^{43}$-$Ala^{44}$ reduced secretion to approximately 60%. Secretion of $\alpha Phe^{33} \rightarrow Ala$ and $\alpha Arg^{35} \rightarrow Ala$ was comparable to wt hTSH. In a receptor binding assay using porcine thyroid membranes, the binding of mutant αArg^{42}-Ser^{43}-$Lys^{44} \rightarrow Ala^{42}$-$Ala^{43}$-$Ala^{44}$ was profoundly reduced, whereas the binding of mutants $\alpha Phe^{33} \rightarrow Ala$, $\alpha Arg^{35} \rightarrow Ala$ and $\alpha Ala^{36} \rightarrow Glu$ was slightly decreased. Furthermore, the ability of mutants αArg^{42}-Ser^{43}-$Lys^{44} \rightarrow Ala^{42}$-$Ala^{43}$-$Ala^{44}$ and $\alpha Arg^{35} \rightarrow Ala$, but not $\alpha Phe^{33} \rightarrow Ala$ and $\alpha Ala^{36} \rightarrow Glu$, to induce cAMP production in CHO cells stably transfected with the human recombinant TSH receptor (JP09, kindly provided by Dr. Vassart) as well as FRTL-5 cells bearing an endogenous rat TSH receptor was significantly reduced. In summary, we have identified the role of specific amino acids within residues 33-44 of the common alpha-subunit to be important for secretion, binding and bioactivity of hTSH. The comparison of the relative role of these individual residues in hTSH to findings in hCG indicates previously unrecognized differences in the structural requirements for heterodimer secretion and receptor binding among the members of the glycoprotein hormone family. In particular, in comparison to hCG, αAla^{36} is much less important for heterodimer secretion, and αPhe^{33} as well as αArg^{35} are less critical for receptor binding of hTSH.

115. P95 **UPTAKE OF TETRAIODOTHYROACETIC ACID (TETRAC) AND ITS EFFECT ON TSH SECRETION IN CULTURED RAT ANTERIOR PITUITARY CELLS.** M.E. Everts, R. Docter, H. van Toor and G. Hennemann. Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands.

The low serum level of T_3 and/or T_4 during non-thyroidal illness (NTI) is not accompanied by a rise in serum TSH. Recently, it was proposed that a shift in T_4 metabolism during NTI from deiodination to conjugation and alanine-side chain alteration may result in metabolites with a TSH-suppressing effect. One of those is triiodothyroacetic acid (Triac) (1), which is rapidly taken up by the pituitary (2), and has a profound TSH suppressing effect in vitro (2) and in vivo (3). Like Triac, Tetrac reduces the TSH response to TRH (3) and was also suggested to contribute to suppression of TSH secretion during NTI (1). We compared the uptake of [^{125}I]Tetrac with that of [^{125}I]T_4, and the effects of unlabeled Tetrac, Triac, and T_4 on TRH-induced TSH release in cultured anterior pituitary cells. Cells were isolated from adult male euthyroid rats, and cultured (500,000 cells/well) for 3 d in MEM with 10% fetal calf serum (2). Incubations were performed at 37 C without or with TRH (100 nM), Tetrac, Triac or T_4 (0.01 nM-1 μM) in MEM/0.5% BSA. The free fractions were Tetrac: 0.005%, Triac: 0.5%, and T_4: 0.5%. Uptake was measured with [^{125}I]Tetrac ($2x10^5$ cpm; 240 pM)) or [^{125}I]T_4 ($1x10^5$ cpm; 175 pM) in MEM/0.1% BSA. In this medium, the free fractions of Tetrac and T_4 were 6-fold higher than in 0.5% BSA. Exposure of cells to 100 nM TRH for 2 h, stimulated TSH release by 89-133% ($P \leq 0.001$). Tetrac at concentrations of 1, 10, 100, or 1000 nM reduced the TSH response to TRH by 45%, 47%, 70% and 82% (all $P<0.025$; n=6-9). The effect of 100 nM Tetrac was similar to that of 10 nM T_4 or 0.01 nM Triac. Because the ratio of the free fractions of the three compounds was 1:100:100, this implied that Tetrac was 10 times more effective than T_4, and 100 times less effective than Triac. Uptake of [^{125}I]Tetrac and [^{125}I]T_4 showed a steep phase up to 1 h of incubation and then leveled off. After 24 h, uptake of Tetrac amounted to 1.16 ± 0.16 (n=6) fmol/pM free Tetrac and that of T_4 to 0.145 ± 0.01 (n=6) fmol/pM free T_4 ($P<0.001$), i.e. as large as that of Triac and T_3, respectively (2). Within this period, 15% of [^{125}I]Tetrac or [^{125}I]T_4 was deiodinated to [^{125}I]Triac or [^{125}I]T_3. Unlabeled Tetrac (10 nM-10 μM) did not affect the 15-min uptake of [^{125}I]T_4, suggesting that Tetrac is taken up by a different transport system as that for T_4. The free Tetrac fraction in serum of NTI patients was increased 2-10 fold, and the production rate (4) 2-fold, which will result in a substantial rise of the free Tetrac concentration. In summary: i) uptake of Tetrac in pituitary cells is similar to that of Triac; ii) Tetrac is deiodinated to Triac and both reduce the TSH response to TRH with higher potency than T_4. As also during NTI, the free serum Tetrac concentration will be very low, it is not likely that Tetrac plays a major role in TSH suppression during NTI. However, the unique pathway for Tetrac uptake may be of significance in this respect.
1) Med Hypotheses 1993,40:38-43; 2) Endocrinology 1993,135:2700-2707; 3) Acta Endocrinol 1979,92:455-467; 4) J Clin Endocrinol Metab 1980,50:712-716

116. P96 EFFECT OF THYROID HORMONES ON RAT PITUITARY CONTENT OF NEUROMEDIN B (NB) CORRELATED WITH THYROTROPIN (TSH) SECRETION. T.M.Ortiga-Carvalho, C.C.Pazos-Moura. Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brasil.

We had suggested earlier (PNAS 89:3035,1992), that NB, a bombesin-like peptide has a role as an inhibitory paracrine/autocrine regulator of TSH secretion. It has been reported that hypothyroid rats have diminished NB mRNA content in the pituitary (Endocrinol.130:1829,1992). Here, we studied the time course of the effect of thyroid hormone administration to hypothyroid rats on the anterior pituitary content of NB. All groups, except one (euthyroid-C), received 0.03% methimazole in the drinking water/3weeks. In experiment I, thyroxine (T_4)-0.8μg/100g B.W. or triiodothyronine (T_3)-0.4 μg/100g B.W. were injected s.c. dayly, for 3 or 5 days or as a single injection 24 or 6 h before sacrifice. In experiment II, T_4, (0.8μg/100g B.W.) was given 6, 3, 1 or 1/2 h before sacrifice. NB, extracted from tissue by boiling it in acetic acid, was measured by RIA, using a highly specific antiserum, as described previously with modifications (Neuroscience 15:1217,1985). Serum (s) TSH was measured by specific RIA (NIDDK). Pituitary NB content was significantly increased ($p<0.005$) above that of the euthyroid group after 6h of T_4 administration and in all T_3-treated groups. Other T_4-treated groups had higher NB content than hypothyroid not treated (hypo) and similar to euthyroid rats (C: 201±18; Hypo: 64±12; T_4= 6h: 340±41; 24h: 241±27; 3d: 155±33; 5d:157±28; T_3= 6h: 548±52; 24h: 338±40; 3d: 318±30; 5d: 326±28 fmol/mg ptn). Maximal NB values at 6h after thyroid hormones came together with the acute inhibition of TSH secretion (C: 1.1±0.1; Hypo: 35±2; T_4= 6h: 8±1; 24h: 16±3; 3d: 5±1; 5d: 3±0.5; T_3= 6h: 1.5±0.4; 24h: 9±2; 3d: 1±0.2; 5d: 1.3±0.1 ng/ml). In experiment II, as early as 1/2h after T_4 injection pituitary NB content was significantly increased while sTSH was similar both compared to hypo group. The peak response to T_4 was at 3 hours after its injection, when NB content maximally increased ($p<0.001$ vs. all groups) and sTSH decreased to the level of normal rats (*NB*=Hypo: 45±8; 1/2h: 223±15; 1h: 203±48; 3h: 383±31; 6h: 224±30 fmol/mg ptn; *sTSH*= Hypo: 31±3; 1/2h: 26±3; 1h: 31±2; 3h:1.3±0.1; 6h: 3.7±0.6 ng/ml). We conclude that NB synthesis is rapidly induced by thyroid hormones and it might be involved in the acute inhibition of TSH secretion in response to replacement of thyroid hormones in hypothyroidism.
Financial support: FINEP, CNPq, CEPG-UFRJ.

117. P97 EFFECT OF GASTRIN-RELEASING PEPTIDE (GRP) AND GRP ANTAGONISTS ON TSH SECRETION FROM RAT ISOLATED PITUITARIES. C. V. Santos and E.G. Moura, Department of Physiological Sciences, Biology Institute, State University of Rio de Janeiro, R.J., Brazil.

It was shown that GRP inhibited "in vivo" TSH release. To evaluate if GRP acted physiologically at the pituitary level we design two separated "in vitro" experiments: 1) Effect of GRP : hemipituitaries of 3 months old male Wistar rats were separated in 5 groups (8-9/group): Two controls (CA and CB) and three GRP-10 treated groups (10^{-9} M, 10^{-7} M and 10^{-5} M). Each hemipituitary was incubated in 1.0 ml of 199 medium, pH 7.4, at 37° C. After 1 hour incubation an aliquot was removed for measurement of basal TSH and then, TRH (50nM) was added in all groups except control A, and incubated for 30 more minutes. Each hemipituitary was homogenized in PBS/BSA, pH 7.6 for measurement of pituitary TSH. 2) Effect of GRP antagonists - It followed the same procedure of the experiment described above, but instead of GRP we used 3 different GRP antagonists at the concentration of 10^{-5} M. TSH was measured by radioimmunoassay using NIDDK kits. One-way ANOVA followed by the Newman-Keuls test was employed for statistical analysis. A $p<0.05$ was considered significant. We showed that GRP acted directly at the pituitary gland, inhibiting in 50% basal TSH and blocking TRH-stimulated TSH release at the concentration of 10^{-5} M, but not at the lower concentrations (10^{-7} and 10^{-9} M), which, however, blocked the decrease of TSH pituitary content that was observed after TRH. Incubating the glands with two antagonists of GRP (d-Phe^{8}-GRP and Gly^{6}-GRP) we showed an increase in 50% of basal TSH secretion. The amount of TSH released in response to TRH did not differ significantly with both antagonists. TRH decreased the intrapituitary TSH content in both the control group and Gly^{6}-GRP, but this effect did not occur in the group incubated with d-Phe^{8}-GRP. Another antagonist (Ala^{6}-GRP) did not change TSH secretion. This result suggests the existence of different subtypes of GRP receptors in the anterior pituitary gland. We suggest a physiological role of GRP or other locally produced bombesin-like peptides in the control of TSH release.
(Supported by: CNPq, CAPES and SR2-UERJ).

118. P98 EFFECTS OF TRH AND DOPAMINERGIC RECEPTOR BLOCKADE ON CIRCULATING TSH BIOACTIVITY IN NORMAL SUBJECTS. L. Persani, E. Giammona, and G. Faglia, *Institute of Endocrine Sciences, Ospedale Maggiore IRCCS and Centro Auxologico Italiano IRCCS, University of Milan, Milan, Italy.*

TRH and dopaminergic receptor blockade induce TSH release from the pituitary acting through two distinct intracellular mechanisms. Although it is well known that different TSH amounts are secreted in response to these two different stimuli, no data are available about the bioactivity of the released TSH molecules. We have, therefore, measured TSH bioactivity (TSH-B) in serum pools from normal subjects (4 males and 5 females) collected at baseline (n=9), 20-60 min (n=4) and 120-180 min (n=9) after TRH (200 μg, bolus iv), or 30-60 min after dopaminergic receptor blockade by sulpiride injection (25 mg, im; n=3). TSH immunoreactivity (TSH-I) was measured by ultrasensitive Delfia®. TSH-B was evaluated as cAMP accumulation in cultured Chinese Hamster Ovary cells transfected with recombinant human TSH receptor (JP-26, kindly supplied by Dr. G. Vassart, Brussels) or FRTL-5 cells. The sensitivity of the two bioassays ranged between 1.5-2.5 mU/L, with intra- and interassay CV <15 and 25%, respectively. TSH was extracted from sera by a monoclonal antibody (TSH recovery: 88-97%), eluted from the antibody in Guanidine-HCl (2M, pH 3.2), and dialysed and concentrated by filtration. As both serum and immuno-concentrated TSH displayed an identical immunological potency and parallelism with standard curve, results are expressed as TSH B/I ratio (mean±SD of three different dilutions bioassayed in triplicate). TSH circulating 20-60 min after TRH showed B/I ratios similar to those found at baseline (0.6±0.1 *vs* 0.9±0.6, *P*=NS). TSH molecules circulating 120-180 min after TRH displayed B/I ratios significantly lower than those observed at baseline in 8 subjects (1.2±0.6 *vs* 0.7±0.1, $P<0.03$), and significantly higher in one (2.8±0.5 *vs* 1.6±0.6, $P<0.01$). Sulpiride released TSH isoforms displaying B/I ratios similar to those observed at baseline in 2/3 subjects (0.7±0.3 *vs* 0.6±0.1 and 0.6±0.2 *vs* 0.5±0.1), while significantly enhanced B/I ratios were found in the same subject who displayed a discrepant pattern after TRH.

In conclusion, these preliminary data indicate that the peak of immunoreactive TSH observed after different stimuli (i.e., TSH contained in secretory granules as ready releasable pool) may be constituted by molecules similar to those circulating in the same subject at baseline. Newly synthesized TSH molecules with different biological properties appear to be released 2-3 hours after TRH injection. Variations in the glycosylation pattern of secreted molecules might explain the observed changes in TSH B/I.

119. P99 EFFECTS OF METOCLOPRAMIDE ON FASTING-INDUCED TSH SUPPRESSION. M.H. Samuels, P. Kramer, D. Wilson, Oregon Health Science Univ, Portland, OR and Univ of Texas HSC at San Antonio, TX.

Short-term caloric deprivation leads to suppression of TSH secretion, primarily by reducing TSH pulse amplitude. In order to determine whether increased dopaminergic tone plays a role in this fasting-induced decrement in TSH secretion, 11 healthy young subjects (9 men, 2 women) underwent four studies: 1) a 48h infusion of 0.9% saline (BASELINE); 2) a 48h metoclopramide (MCP) infusion at 30 ug/kg/h (MCP); 3) a 56h fast (FAST); and 4) a 56h fast plus MCP at 30 ug/kg/h during the final 48h of the fast (FAST + MCP). During the final 24h of each study, TSH was measured in serum samples collected every 15 min, following which a standard TRH stimulation test (250 ug of TRH) was performed. TSH pulses were located by Cluster analysis, and variables were compared by two-way ANOVA (fasting x MCP) with repeated measures on both factors. Results are means ± S.E.M. * = $p<.05$.

	BASE	MCP	FAST	FAST + MCP
mean TSH (mU/L)	1.89±.26	2.38±.28 *	1.10±.25 *	1.47±.20
TSH pulse freq.	12±1	13±1	13±1	14±1
TSH pulse amp. (mU/L)	2.23±.30	2.70±.30 *	1.26±.29 *	1.70±.23
day (0800-2000)	1.68±.31	2.10±.22 *	1.18±.26 *	1.44±.26
noct. (2000-0800)	2.76±.34	3.39±.43 *	1.31±.32 *	1.76±.28
TRH response (mU/L) (peak - baseline)	14.8±2.1	14.9±2.2	8.5±1.2 *	9.9±1.8

MCP infusions increased mean TSH levels and TSH pulse amplitude by 21-26% in the nonfasting state, and by 22-35% in the fasting state. There was no additional effect of MCP on TSH levels during fasting compared to the nonfasting studies, and MCP did not reverse fasting-induced decrements in TSH response to TRH. There were no changes in TSH pulse frequency during any study. Thus, increased dopaminergic tone is unlikely to play a significant role in the suppression of pulsatile TSH secretion during fasting.

120. P100 **EFFECTS OF HUMAN RECOMBINANT INTERLEUKIN-1 BETA (IL-1ß) ON TSH RELEASE BY CULTURED RAT ANTERIOR PITUITARY CELLS.** F.W.J.S. Wassen, E.P.C.M. Moerings, and M.E. Everts. Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands.

Recent evidence indicates that cytokines, in particular IL-1ß and tumor necrosis factor α (TNF-α), may act as mediators in the pathogenesis of non-thyroidal illness (NTI). In vivo experiments in rats and humans showed a decrease in serum T_3 and/or T_4 without a concomitant rise in serum TSH after administration of these cytokines (1,2). It is, however, not yet clear if the cytokines exert their effects directly on the hypothalamic-pituitary-thyroid axis or indirectly by inducing a systemic illness. We therefore investigated the effect of IL-1ß on TSH release from anterior pituitary cells. Cells were isolated from adult male euthyroid rats and cultured for 3d in MEM with 10% fetal calf serum. Incubations were performed at 37 C in MEM/0.5% BSA when [^{125}I]T_3 (50,000 cpm; 50 pM) was used or in MEM/0.1% BSA in case of [^{125}I]T_4 (100,000 cpm; 175 pM) (3). When IL-1ß (100 pM) was added during 3d of culture, TSH release declined to 86 ± 1 % (n=27; P<0.001) of the control value, i.e. cells cultured in absence of IL-1ß. The presence of 100 pM IL-1ß during culture did not alter total cell-associated [^{125}I]T_3 activity after 15 min, 1 and 4 h of incubation. Nuclear binding of [^{125}I]T_3 after 2 h of incubation was 20 ± 2 % (n=4) in control cells and 14 ± 1% (n=3) in cells cultured with 100 pM IL-1ß (P<0.025), indicating that the diminished TSH release during culture in presence of IL-1ß is not due to increased nuclear occupancy of T_3. Exposure of cells for 4 h to IL-1ß (1, 10, 50 and 100 pM) reduced TSH release by 27% (n=6; P<0.01), 29% (n=6; P<0.01), 39% (n=6; P<0.005) and 38% (n=6; P<0.001) respectively. In a separate series of experiments, the effect of IL-1ß on thyroid hormone uptake in pituitary cells was examined. Acute addition of IL-1ß (1-100 pM) did not affect the 15-min uptake of either T_3 or T_4 (n= 9-20). These results indicate that there is an inhibitory effect of Il-1ß on the TSH secretion. This inhibitory effect is not due to increased thyroid hormone uptake or nuclear T_3 binding in the pituitary cells. Whether IL-1ß interferes with the TSH release process and/or with TSH synthesis in the pituitary is currently being investigated.

1) Endocrinology 1992, 131: 2139-2146; 2) J Clin Endocrinol Metab 1990, 71: 1567-1572; 3) Endocrinology 1994, 134: 2490-2497

121. P101 EFFECT OF FEMALE SEX STEROIDS ON RAT THYROTROPIN (TSH) SECRETION *IN VITRO*.
R.M.Moreira, P.C. Lisboa, F.H. Curty and C.C.Pazos-Moura. Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brasil.

It has been shown that there are sex related differences on the regulation of TSH synthesis and secretion. However, the precise role of sex steroids on TSH secretion is still highly unknown. Here, we studied the *in vitro* TSH secretion from pituitaries of ovariectomized rats treated or not with sex steroids hormones. Adults rats were received the following treatments: Control *sham*-operated; Ovariectomized (OVX) not treated; and in different series of experiments: OVX+17-ß-estradiol benzoate (E): 0.7 or 1.4 or 14 μg/100g B.W., s.c./day/10 days; OVX+progesterone: 250μg and 2,5 μg/100g B.W./s.c./day/9days; or OVX+medroxiprogesterone acetate: 250μg/100g B.W./day/10 days. After three weeks of ovariectomy, the rats were sacrificed, their hemi anterior pituitaries incubated in Krebs-Ringer bicarbonate, pH 7.4, 37° C in an atmosphere of 95%O2/5%C02 to evaluate basal and TRH (50 nM)-stimulated TSH release and also TSH content in glands homogenates. TSH was measured using a specific RIA (NIDDK). Basal TSH release from OVX rats were decreased in 2 out of three experiments ($p<0.05$) and the TRH-stimulated TSH release was decreased in OVX groups ($p<0.05$) in all experiments. The low dose of the estrogen (E) did not reverse those alterations of OVX rats.The intermediary E dose, which was able to maintain a normal uterine weight, reverted them partially, since basal and TRH-stimulated TSH release responses increased in relation to OVX but still they were statistically different from control. Conversely, the highest dose of the estrogen induced a further decrease in basal and TRH-stimulated TSH release compared to ovariectomy alone, being highly decreased compared to control ($p<0.05$). Also, pituitary content of TSH which was not affected by ovariectomy, was decreased in these group receiving the highest dose of estrogen. We conclude that estrogen has a biphasic effect: in physiological concentration seems to be necessary to maintain the normal intrapituitary pool of TSH and the response to TRH, while it has an inhibitory action in pharmacological dose. Conversely, medroxiprogesterone induced approximately 120% increase in pituitary TSH content ($p<0.005$) as well as a major ($p<0.05$) increase in basal and a minor in TRH-stimulated TSH release. These important stimulatory effect probably was not mediated by progesterone receptors since progesterone itself had no effect.
Financial support: FINEP, CNPq and CEPG-URFJ

122. P102 **ISOSMOLAR ETHANOL OR UREA STIMULATE HYPOPHYSIOTROPIC TRH SECRETION FROM BOTH THE HYPOTHALAMIC PARAVENTRICULAR NUCLEI (PVN) AND MEDIAN EMINENCE (ME).**
M. Nikodemova, V. Strbak, S.E. Greer, and M.A. Greer, Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia, and Oregon Health Sciences University, Portland, OR, USA.

The regulation of secretion of "thyrotropin-specific" brain TRH which originates in the PVN parvocellular cells and is released into the primary portal plexus in the ME is poorly delineated. Increasing attention is being focussed on cell volume changes, especially cell swelling, as primary events which can profoundly affect cell functions. Cell swelling is readily produced by a rise in the extracellular concentration of small molecules which rapidly permeate the plasmalemma if these molecules are applied in an isosmotic medium. Ethanol is one of the most permeant of such molecules, followed closely by urea. Some reports have suggested alcohol abuse or renal failure affect pituitary-thyroid function. We therefore evaluated the ability of ethanol and urea, at concentrations seen clinically in vivo, to stimulate TRH secretion from both PVN and ME. Young adult male rats were decapitated and the ME and PVN rapidly separated under 10X magnification while keeping the tissue moist with 4C Locke's sol (285 mOsm), the extracellular medium used in all experiments. Previous experiments established the anatomical accuracy of our technique. Tissue at 37C was: a) pooled from 6 rats per experiment, perifused in a 0.2 ml chamber and 0.5 ml fractions collected every min, or b) incubated separately from individual rats in 150 μl in Eppendorf tubes and the fluid changed every 30 min. Addition of 80 mM ethanol or urea in isosmolar medium resulted in a significant increase in TRH secretion from both PVN and ME. The same concentration added directly to the medium to produce a hyperosmolar solution depressed basal secretion. TRH release was not due to non-specific toxicity since there was a prompt return to basal secretion upon removal of the permeant molecules from the medium and secretion induced by depolarizing 56 mM K^+ was the same at the beginning and end of the experiments. Removing Ca^{2+} from the medium did not depress secretion induced by ethanol or urea but abolished that induced by K^+. There was no significant qualitative difference between PVN and ME in the effect of the various conditions on TRH secretion.
Conclusions: Isosmolar ethanol or urea stimulate TRH secretion from both the PVN and ME by a mechanism which is not dependent on Ca^{2+} influx. Cell swelling at the originating cell bodies as well as swelling at the terminal nerve processes at the primary portal capillaries thus can stimulate TRH secretion. This mechanism may be involved in vivo in pathophysiologic effects of ethanol or urea on pituitary-thyroid function. Physiologic changes in either intra- or extracellular osmolyte concentration produced by variations in metabolic or physical activity similarly may play a role in the normal nyctohemeral and ultradian fluctuations in TSH secretion.

123. P103 POTENTIAL THYROID HORMONE RESPONSE ELEMENTS (TREs) WITHIN THE DNA SEQUENCES OF GLYCOSYLTRANSFERASES: IMPLICATIONS FOR THE HORMONAL MODULATION OF TSH GLYCOSYLATION. J.A. Magner and J.B. Menke, Section of Endocrinology, Department of Medicine, East Carolina University School of Medicine, Greenville, NC 27858

Hypothyroid rodents and humans secrete not only more TSH, but also different TSH isoforms having altered oligosaccharides. The increased sialylation of TSH in the hypothyroid state alters important biological properties of TSH, such as its metabolic clearance rate. Within oligosaccharides, sialic acid is bound to galactose. Prior studies have shown that thyrotrophs in hypothyroid mice increase their content of α-2,6-sialyltransferase (STase) mRNA by 170%, and of β-1,4-galactosyltransferase (GT-ase) mRNA by up to 440%. In preparation for gel shift experiments, we reviewed the published sequences of rat α-2,6-STase (Wang et al, Glycobiology 1:25, 1990) and of murine β-1,4-GTase (Harduin-Lepers et al, J Biol Chem 268:4348, 1993) to search for potential TREs. For the α-2,6-STase, two potential TREs were located in the genomic region upstream of exon 1 at -794 and -18, while potential binding sites for the transcription factors DBP, AP-1, AP-2, and HNF-1 were present at positions -217, -130, -97, and -57, respectively. In addition, three potential TREs were located in the region upstream of exon A/B at -580, +25, and +126, while a putative CCAATT box, SP-1 site, AP-4 site, and TATAAA box were at positions -154, -132, -32 and -23, respectively. It previously has been established that multiple promoters are a principal mechanism by which different STase mRNAs predominate in different tissues, and the presence of potential TREs in the two relevant regions of STase genomic DNA raises the possibility that thyroid hormone might influence that process. For the β-1,4-GTase, a potential TRE was present at -1326, near three possible glucocorticoid response elements, multiple AP-2 and SP-1 binding sites, and one CREB site. The presence of these potential TREs lends credence to the concept of thyroid hormone transcriptional control of these glycosyltransferases.

124. P104 **TIME-DEPENDENT RECOVERY OF TSH SECRETION AFTER WITHDRAWAL OF LEVOTHYROXINE SUPPRESSIVE THERAPY: ASSESSMENT BY 3RD GENERATION TSH-ASSAY.** L.Duntas[1,3], D.K.Nelson[2], B.M.Grab[1], K.Hatzimichail[3]. [1]Dept.Internal MedicineI, University Clinic of Ulm, Ulm, Germany, [2]The Genesee Hospital, University of Rochester, NY, USA and [3]Hellenic Laboratory of Complex Systems, Drama, Greece

The recent availability of more sensitive TSH-assays improves our capability to detect depressed TSH levels and to explore analytically the recovery of pituitary function after withdrawal of long term suppressive treatment. **AIM:** To evaluate the dynamics of pituitary and thyroid secretion patterns in patients with diffuse or nodular nontoxic goiter after cessation of prolonged suppressive treatment with levothyroxine. **METHODS:** 16 pts (23-61 yrs) with fully suppressed TSH (<0.03 μU/ml) participated in the study. Serum concentrations (SC) of T_4, T_3, TSH (3rd Generation ICMA assay, LUMItest-TSH, BRAHMS, GmbH, Berlin, Germany, with a functional sensitivity of 0.035 μU/ml) and thyroglobulin (Tg) were measured before and at weekly intervals for eight weeks after stopping suppressive treatment with T_4, which was performed for at least 6 months. **RESULTS:** After withdrawal of suppressive medication the mean levels of serum T_4 concentrations decreased rapidly to 83% of basal by first week (W_0:10.9±1.1; W_1:9.0±0.8 μg/dl, mean±SD) and to 77% of basal by W_2 (8.4±0.7 μg/dl). T_4-SC remained at these levels until the end of the study (W_8: 8.9±0.7 μg/ml). T_3-SC also decreased rapidly (12%) at the beginning (W_0:147±13; W_1:131±13 ng/dl), decreased further at W_2 (125±12 ng/dl), and remained roughly at this level till W_8 (130±12 ng/dl). TSH was fully suppressed (0.03 μU/ml) at W_0 and W_1, with a slow rebound from W_2 (0.05±0.01 μU/ml) to W_3 (0.06±0.01 μU/ml), to W_4 (0.08±0.02 μU/ml), till the end of the study (0.7±0.06 μU/ml; (W_8)). Tg was not markedly altered during the study (W_0:9±1.1; W_8:8±0.9 ng/ml). **CONCLUSIONS:** Our results show that recovery of pituitary function after withdrawal of T_4 suppression is a gradual phenomenon. TSH secretion remains depressed at least two weeks after recovery of T_4. 3rd Generation TSH-assay enables us to follow the time-course recovery of fully suppressed TSH.

125. P105 HEAT STRESS UNCOUPLES CYTOSOLIC CALCIUM AND THYROTROPIN RELEASING HORMONE EFFECTS ON PROLACTIN SYNTHESIS AND SECRETION IN GH_3 CELLS. R.J. Galloway, J.M. Carrick, I.D. Gist, C.U. Fisher, and R.C. Smallridge. Walter Reed Army Institute of Research, Washington, D.C. 20307.

In a previous study we showed that heating GH_3 cells (45°C/15 min) induced heat shock protein 72 (HSP-72) and an uncoupling of the normal direct relationship between increases in cytosolic free calcium ($[Ca^{2+}]_i$) and prolactin (PRL) secretion. We studied this system to better characterize this uncoupling. $[Ca^{2+}]_i$ was measured by Indo-1 AM spectrofluorometry, PRL mRNA by dot blot analysis, and PRL secretion (2 hr) by RIA. Results: $[Ca^{2+}]_i$ acutely increased in response to heating as previously shown, remained elevated for 90 min, and returned to baseline within two hours. The $[Ca^{2+}]_i$ chelator, BAPTA(100μM) and the phospolipase C (PLC) inhibiter, U-73122(3μM; Upjohn Co) completely prevented this heat induced $[Ca^{2+}]_i$ rise, but had no effect on the inhibition of PRL secretion. Protein kinase C (PKC) inhibition (H-7) or stimulation (PMA), and calcium channel blockade (verapamil/nifedipine) failed to block the heat induced $[Ca^{2+}]_i$ rise or prevent PRL inhibition. After heat, TRH stimulated a normal rise in $[Ca^{2+}]_i$ but did not affect PRL inhibition. PRL mRNA decreased from baseline, reached a nadir at two hours, and recovered within 8 hours. Summary: 1) Nonlethal heating of GH_3 cells increased $[Ca^{2+}]_i$ but inhibited PRL release and decreased prolactin mRNA. 2) PRL inhibition was not prevented by inhibition of PLC or voltage dependent Ca^{2+} channels. 3) Chelation of $[Ca^{2+}]_i$ (BAPTA) or PLC inhibition (U-73122) completely blocked the heat induced Ca^{2+} rise but did not affect PRL secretion. 4) Although the effect of TRH on $[Ca^{2+}]_i$ was not altered by heating, it no longer stimulated prolactin release. Conclusion: Heating uncouples the normal direct relationship between increases in $[Ca^{2+}]_i$ and PRL secretion probably via action at the gene level.

126. P106 **A NATURALLY OCCURRING INHIBITOR OF THYROID HORMONE UPTAKE INTO CELL NUCLEI.** M. Lakshmanan and S. Benvenga, Dept. of Medicine, CWRU, Cleveland, OH and Dept of Endocrinology, University of Messina, Messina, Italy.

L-Carnitine (L-C) [3-hydroxy-4-N-trimethylammoniobutanoate], a naturally occurring amino acid, was reported to antagonize thyroid hormone (TH) effects in animals (Rotzsch & Strach, 1958). The principal function of L-C which is ubiquitous in mammalian tissues is to serve as a co-factor for mitochondrial transport of long chain fatty acids. L-C is actively transported into cells, Km about 5 uM in fibroblasts to about 5 mM in hepatocytes where it is synthesized. We studied the effect of L-C and the isomer D-C on radiolabeled T3 (*T3) and T4 (*T4) transport into whole cells (WHO) and nuclei (NUC) of three cell lines - human skin fibroblasts (HSF) and hepatoma cells (HepG2) and mouse neuroblastoma cells (NB41A3). Incubation of cells with *TH and 0.01 M L-C or D-C for 1 h at 37C produced a small inhibition of WHO uptake, 6 to 12%. The effect was dose dependant from 0.001 to 0.05 M with 0 to 42% inhibition. The inhibition occurred from 1 to 120 min of co-incubation but was absent in cells pre-incubated with L-C or D-C, then washed and incubated with *TH alone. As expected because liver cells synthesize and export L-C, this effect was most disparate in the HepG2 cells. In co-incubation experiments NUC uptake was more inhibited than WHO uptake.

PER CENT INHIBITION OF NUCLEAR T3 OR T4 UPTAKE IN NB41A3 CELLS BY L-C
(x ± S.D., n = 3)

L-C mol/L	.001	.005	.01	.05
*T3	13 ± 3	17 ± 3	31 ± 5	71 ± 8
*T4	20 ± 4	56 ± 6	76 ± 7	91 ± 10

The effect was not due to a direct action at the nucleus because there was no inhibition of *TH binding to isolated nuclei by L-C and *L-C did not bind to isolated nuclei or to nuclei in whole cell incubations. Amino acids transported into cells by system L and its subsets have been shown to inhibit TH transport into cells and conversely TH inhibits the transport of system L amino acids. A number of system L and basic amino acids and TH did not appear to affect the transport of L-C. In conclusion, C inhibits TH uptake by cell nuclei after the plasma membrane but before the nuclear envelope. C may therefore be an unique naturally occurring substance that may antagonize TH action by blocking its access to nuclear receptors.

9:00 A.M. 127. TREATMENT OF EUTHYROID SICK SYNDROME IN PATIENTS UNDERGOING CARDIAC SURGERY. J.D. Klemperer, M. Gomez, K. Ojamaa, K. Krieger, I. Klein.. Department of Cardiothoracic Surgery, New York Hospital-Cornell University Medical College, New York, N.Y.

Cardiopulmonary bypass (CPB) results in a euthyroid sick state with transient depression of serum T3 levels. In light of the marked effects of thyroid hormone on cardiovascular hemodynamics, and with the recent observation that T3 may act acutely as an inotropic agent, the effects of T3 treatment were studied in patients undergoing CPB. 143 high risk patients undergoing CPB were randomized to either T3 (n=71) or placebo (n=72) treatment groups in a prospective, double blind fashion. Patients with history of thyroidal illness were not enrolled. T3 was administered as a 0.8 mcg/kg i.v. bolus at the time of aortic cross-clamp removal followed by an infusion of 0.113 mcg/kg/hr for 6 hours, and clinical, hemodynamic, and serologic responses were serially recorded. The table below describes changes in total serum T3 levels (ng/ml) in the two treatment groups.

Group	Preop	CPB	Rx +30 min	Rx +6 hrs	Rx + 24 hrs
T3	0.81± 0.21	0.47±0.18*	4.21±0.78#	4.43±1.03#	0.68± 0.37
Placebo	0.81± 0.23	0.51±0.16*	0.37±0.14*	0.51±0.18*	0.45±0.13*

*p<.01 vs. preop, # p<.0001 vs. placebo

The half life of administered T3 was decreased by greater than 2 fold compared to non-CPB treated controls. No adverse effects of T3 administration were noted. The incidence of supraventricular and ventricular arrythmias as well as mean heart rates did not differ significantly during the treatment period. While T3 treatment enhanced cardiac performance, it did not significantly alter perioperative mortality (4% vs 4 %), need for inotropic support (55% vs 56%), or the length of ICU stay (49.7±36 vs 53±35 hours). We conclude that pharmacologic reversal of the low T3 state associated with CPB can be safely accomplished but does not alter overall clinical outcome in the high risk patient.

9:15 A.M. 128. HEART RATE, HEART RATE VARIABILITY, AND ARRHYTHMIAS IN PATIENTS RENDERED SUBCLINICALLY HYPERTHYROID. F.S. Keck, S. Wieshammer, G. Grossmann, A.Ch. Schäuffelen and Ch.-F. Wolf, University of Ulm, Germany.

The effect of subclinical hyperthyroidism on heart rate variability and on the incidence of arrhythmias was studied in 11 patients without any evidence for heart disease (mean age 40 ± 13 years (mean, SD)), who had had total thyroidectomy and radioiodine treatment for thyroid cancer 41 ± 36 months before the study. All patients were studied on three occasions, once when subclinically hyperthyroid on long-term thyroxine replacement therapy, once when short-term hypothyroid 6 weeks after ceasing thyroxine and 2 weeks after ceasing triiodothyronine for the purpose of screening for metastases, and once when euthyroid under reinstituted thyroxine therapy. The thyroxine dose was increased stepwise by intervals of constant thyroxine dose of at least 5 weeks. Heart rates were assessed by 24-hrs.-Holter ECG (2 leads, PR 3, Prodigy, KardioData, Northboro, Mass., USA). Heart frequency profiles were calculated and beat-to-beat variability was analyzed (1 PDP 11-45, Digital Equipment Corp., Maynard, USA). Thyroid state was assessed by serum concentrations of T_4, T_3 and TSH before and after TRH. The thyroid state was considered subclinically hyperthyroid when the TRH test was blunted and the T_4 and T_3 serum concentration were within the (upper) normal range. There was no difference in the incidence of supraventricular or ventricular arrhythmias under all 3 thyroid states. Twenty-four hours mean heart rates in the subclinically hyperthyroid (86.9 ± 6 min^{-1}) and in the hypothyroid state (76 ± 10 min^{-1}) did not differ significantly from the euthyroid state (81.8 ± 9 min^{-1}, Friedman test followed by Wilcoxon-Wilcox test). The diurnal heart rate profiles were parallel under all 3 thyroid states. Mean maximum heart rates were unchanged in the subclinically hyperthyroid (141 ± 12 min^{-1}) and in the hypothyroid state (132 ± 12 min^{-1}) as compared to the euthyroid state (136 ± 12 min^{-1}). In order to diminish any possible influences of mental and/or physical activity, mean minimum heart rates from the 10 min. interval of lowest heart rate during bedtime were calculated; these values were found not to differ significantly in the euthyroid and in the subclinically hyperthyroid state (60 ± 11 min^{-1} and 62 ± 5 min^{-1}), while they were found to be decreased in the hypothyroid state (54 ± 8 min^{-1}, $p < 0.05$). As compared to the euthyroid state, zero beat-to-beat variation occurred significantly more often in the hypothyroid state (39.3 ± 13 % vs. 34 ± 11% of all heart beats registered, $p < 0.05$) and was unchanged in the subclinically hyperthyroid state (33.1 ± 12%). When beat-to-beat calculated heart rate differences exceeded 2 beats/min., heart rate variability was significantly ($p < 0.05$) attenuated in the hypothyroid state, while it was unchanged in the subclinically hyperthyroid state as compared to the euthyroid state. Focusing the 10 min. interval of lowest heart rate during bedtime, no differences in heart rate variability could be detected under all 3 thyroid states. We conclude that heart rate, heart rate variability, and the incidence of arrhythmias are not significantly affected when passing from the euthyroid to the subclinically hyperthyroid state.

9:30 A.M. 129. DETERMINATION OF SERUM IL-6 CONCENTRATION IS USEFUL TO DIRECT THE CHOICE OF TREATMENT OF AMIODARONE-INDUCED THYROTOXICOSIS (AIT). L. Martino, S. Brogioni, L. Grasso, L. Manetti, G. Scarcello, A. Burelli, F. Bogazzi and L. Bartalena, Ist.di Endocrinologia, University of Pisa, Italy

AIT can occur in the absence (type II) or presence (type I) of an underlying thyroid disorder. Type II AIT is probably due to a destructive process, of which the markedly increased serum IL-6 concentration seems to represent a useful marker. Type I AIT (developing on nodular goiter or latent Graves' disease) is a form of hyperthyroidism precipitated by iodine overload and usually associated with slightly increased serum IL-6 levels. In this study we further evaluated serum IL-6 levels and their relationship with the effects of treatment in 9 patients with type I AIT (5 men, 4 women, age range 52-72 yr, mean 64) and 9 patients with type II AIT (6 men, 3 women, age range 35-83 yr, mean 57). Median serum IL-6 levels (normal values <100 fmol/L) were: type I AIT 140 fmol/L (range 96-176), type II AIT 686 fmol/L(range 272-1129), p<0.004. Four type II patients were treated with methimazole (MMI,30 mg/day initial dose) and potassium perchlorate (KClO4,1 g/day for 20-40 days) for 1-4 months, but remained hyperthyroid with high IL-6 levels. Two of them and the remaining 5 patients of this group were treated with methylprednisolone (Pred, 40 mg im, gradually tapered and withdrawn over a period of 3 months) and had a dramatic reduction and normalization of IL-6 values within 3-12 days, which paralleled (and often preceded) the normalization of free thyroid hormone levels. One patient subsequently became hypothyroid. Type I AIT patients were given MMI and KClO4 . Five type I AIT patients became euthyroid in 28-60 days. In two patients serum thyroid hormone levels markedly increased during treatment concomitantly with a marked rise in serum IL-6 levels from 112 to 532 fmol/L and from 125 to 286 fmol/L, respectively: in both cases the addition of Pred promptly restored normal thyroid hormone and IL-6 levels. Another patient of this group, given Pred together with MMI and KClO4, showed an increase in both thyroid hormone and IL-6 concentrations when the dose of Pred was reduced from 40 to 20 mg/day after 2 weeks of treatment: the increase of the daily Pred dose to 40 mg was then associated with the prompt normalization of biochemical parameters.

In conclusion: i) IL-6 is a marker of amiodarone-induced thyroid destruction; ii) A destructive process can also occur in type I patients: IL-6 determination can unravel these forms; iii) Amiodarone-induced destructive thyrotoxicosis responds well to glucocorticoids but poorly to combined MMI+KClO4 treatment; iv) An exacerbation can occur when the dose of steroids is decreased, implying that treatment must be prolonged and the dose must be tapered with caution.

9:45 A.M. 130. **ORGAN SPECIFIC EFFECTS OF TIRATRICOL (TRIAC).** S.I. Sherman[1,2], M.J. Smith[1], W. Zoghbi[2], M.D. Ringel[3], and P.W. Ladenson[3], [1]U.T. M.D. Anderson Cancer Center, [2]Baylor College of Medicine, and [3]Johns Hopkins University School of Medicine, Houston TX and Baltimore MD, USA.

We have previously reported that tiratricol (T_3A) has enhanced hepatic and skeletal actions when co-administered with L-thyroxine sodium (LT_4), perhaps due to greater specificity for ß thyroid hormone receptors. A randomized, double-blind, placebo-controlled trial has been initiated to define the organ specific effects of monotherapy with T_3A. While receiving LT_4 to suppress TSH to <0.1 mU/L and following a 2 month controlled dietary lead-in period, 24 athyreotic patients undergo a baseline physiologic evaluation. They are then randomly assigned to receive either 1) T_3A, 24 µg/kg bid, or 2) LT_4, 1.9 µg/kg qAM and a placebo qPM. The daily dose of study drug is modified until TSH levels are again <0.1 mU/L, and the physiologic evaluation is repeated after 2 months on the final study drug dose. Comparison is made between the results of baseline physiologic testing and the study drug evaluation. Eight patients have completed the protocol. Compared to their baseline on LT_4, patients taking T_3A had significantly lower serum concentrations of total cholesterol (-17%), LDL cholesterol (-26%), and apoprotein B (-16%); and higher levels of sex hormone binding globulin (36%). Serum osteocalcin levels and urinary pyridinoline excretion trended higher, 24% and 57%, respectively. Patients on T_3A had significantly lower resting metabolic rates (-9%), but no change in their weight. Mean overnight pulse rate and systolic time intervals, including the pre-ejection period/left ventricular ejection time ratio (PEP/LVET), did not change. Patients in the control LT_4 group had no evidence of changes in any parameter. These preliminary results from the first controlled trial of monotherapy support the conclusion that T_3A is more active than LT_4 in the liver and bone, relative to the pituitary gland.

Parameter	T_3A	LT_4
Study Drug Dose, µg/kg/d	48.1	2.0
Δ Weight, kg	-0.2	0.85
Δ Metabolic Rate, cal/min/kg	-1.8 ***	-0.5
Δ Total Cholesterol, mg/dL	-37 ***	-2
Δ LDL Cholesterol, mg/dL	-33 **	-3
Δ Sex hormone binding globulin, nmol/L	14 **	-7
Δ Pyridinoline, pmol/µmol creat	29	0.3
Δ Osteocalcin, µg/L	2.3 *	-1.35
Δ PEP/LVET, msec/msec	0.015	0.028

Significance compared to baseline: * P < 0.1, ** P < 0.05, *** P <0.01.

9:00 A.M. 131. **GENETICALLY TRANSMITTED GOITROUS HYPOTHYROIDISM DUE TO A DEFECT IN THYROGLOBULIN FOLDING** P. Kim, O. Kwon and P. Arvan, Beth Israel Hospital, Boston, MA

One important cause of complete or partial thyroid dyshormonogenesis leading to congenital hypothyroidism and goiter, is thyroglobulin (Tg) deficiency. We believe that in many instances, this illness is due to defective intracellular transport of Tg to the lumen of thyroid follicles. In previously published studies of homozygous *cog/cog* mice containing a genetic defect causing hypothyroid goiter that maps near or at the Tg locus, most iodoproteins have been found to be unusually small, although formation of some 19s Tg has been reported. Thyrocyte ultrastructure from the mutant mice has strongly suggested that this illness represents a new member of the recently-defined family of endoplasmic reticulum (ER) storage diseases, defective for tissue-specific protein export.

By pulse-chase with radioactive amino acids followed by reducing SDS-PAGE, we have found that all newly synthesized Tg in the mutant mice is of normal size. Further, all Tg molecules receive N-linked glycans indistinguishable from those of normal mice, based on endoglycosidase digestion. Nevertheless, secretion of Tg from mutant mice appears undetectable. Surprisingly, at all chase times, labeled Tg in the thyroid lobules of mutant mice remained sensitive to digestion by endoglycosidase H, indicating that it never arrived in the Golgi complex for carbohydrate modification. Independent experiments confirmed that Golgi carbohydrate processing enzymes were indeed active. To further explore the phenotype, we employed 2D-PAGE analysis of radiolabeled Tg folding intermediates. Remarkably, no Tg dimers appeared to form in the ER of mutant mice, even at chase times when a large fraction of Tg from normal mice was homodimeric and exported to the Golgi complex. Presumably in response to ER accumulation of mutant Tg, there was induced expression of ER molecular chaperones, as demonstrated by immunoblot analysis. These chaperones, including BiP/GRP78, GRP94, as well as protein disulfide isomerase (a catalyst of Tg folding) and several others, now appeared as dominant thyrocyte proteins even by Coomassie blue staining. Moreover, immunoprecipitating with an antibody to BiP, significant amount of mutant Tg was co-precipitated even at very late chase times, reflecting persistent presence of Tg that is retarded and inefficient in acquiring a mature tertiary and quaternary structure. Interestingly, it has been reported that at older ages, untreated *cog/cog* mice develop a sufficient thyroid mass to achieve biochemical euthyroidism. We propose that in addition, physiological stimulation to maximal chaperone levels may serve to promote export of a small fraction of Tg that is properly assembled and hormonogenic. We conclude that the primary defect in these mice is a mutation in the coding sequence of Tg which greatly inhibits protein dimerization and ER export.

9:15 A.M. 132. **MULTIMERIZATION OF THYROGLOBULIN (TG) BY COVALENT CROSS-LINKING: A UBIQUITOUS PHENOMENON AND A PREREQUISITE FOR THE STORAGE OF TG IN OSMOTICALLY INERT FORM.** U. Berndorfer, Y. Saber and V. Herzog, Institut für Zellbiologie der Universität Bonn, Ulrich-Haberland-Str. 61a, 53121 Bonn, FRG.

Extracellular storage of thyroglobulin (TG) in the follicle lumen is an important prerequisite for maintaining constant levels of thyroid hormones in the circulation. The high concentration of TG in the follicle lumen raised the question wether specific intermolecular interactions may exist which allow tight molecular packaging. The application of specific separation techniques distinct from the procedures for the preparation of soluble TG resulted in the isolation of intact lumenal contents from the thyroids of various mammalian species including man. These preparations consisted of large spherical translucent globules (50-500µm) which were composed mainly of TG and which represented in form and surface characteristics a precise replica of the corresponding follicle lumena. The protein content and the volume of single globules were determined showing protein concentrations up to 750 mg/ml. The volume of such globules varied between 0.0015 and 0.015 µl. Hence, these globules contained between 10^{11} and 10^{13} thyroglobulin molecules in a multimerized state. Surprisingly, the globules from bovine thyroids were completely insoluble indicating the covalent cross-linking of TG by intermolecular nondisulfide bonds (J. Cell Biol. 118, 1071, 1992). The presence of transglutaminase in the follicle lumen of bovine thyroids suggests that part of these covalent cross-links may be mediated by the action of this enzyme. Human thyroid globules were insoluble in SDS but completely dissociable by treatment with DTT and SDS indicating that TG in human globules is covalently cross-linked by intermolecular disulfide bridges. In addition, cultured human thyrocytes were found to secrete protein- disulfide-isomerase (PDI) which was iodinated indicating that it is released via the apical cell surface. In addition, PDI could be detected by immunoblot analysis of solubilized human thyroid globules demonstrating its integration into the globule matrix. Partial dissociation of human thyroid globules caused a variable but dramatic increase in their volume indicating the osmotic effect of high concentrations of single TG molecules. We conclude that different mammalian species follow different strategies to cross-link TG and that this multimerizing process is a general phenomenon and an important means to allow storage of TG at high concentrations in osmotically inert form. (Supported by SFB 284 and by Fonds der Chemischen Industrie)

9:30 A.M. 133. ANALYSIS OF THYROID PEROXIDASE-CATALYZED IODINATION AND COUPLING MECHANISMS. Alvin Taurog and Martha L. Dorris. Univ. of Texas Southwestern Medical Center, Dallas, Texas

Thyroid peroxidase (TPO) catalyzes two very different types of reaction in the thyroid, iodination and coupling. We have recently presented evidence for a radical mechanism in the coupling reaction (ABB 315:82,1994). Iodination, on the other hand, is generally considered to be a 2-electron reaction, although a radical mechanism has also been proposed (Vitam. Horm. 39:175,1982). Compound I of TPO exists in 2 isomeric forms. One form resembles Cpd I of horseradish peroxidase, in which one of the two oxidizing equivalents exists as a porphyrin π-cation radical. The other form resembles Cpd I of cytochrome c peroxidase, in which one of the two oxidizing equivalents exists as an amino acid radical (protein radical form). It has been proposed (ABB 242:41,1985) that iodination is mediated by the π-cation radical form and coupling by the protein radical form. However, observations in our laboratory (ABB 315:90, 1994) raised the possibility that TPO-catalyzed coupling is mediated, at least partly, by the porphyrin π-cation radical form. In the present study, we compared catalytic iodination and coupling by two peroxidases with closely related crystal structures (JBC 268:4429, 1993)- cytochrome c peroxidase (CcP) and lignin peroxidase (LiP, kindly provided by Dr. Michael H. Gold). The overall fold and active site regions of these 2 peroxidases are very similar. However, because of replacement of critical Trp residues in CcP by Phe, LiP, in contrast to CcP, forms a Cpd I of the porphyrin π-cation radical type. In iodination systems containing $^{125}I^-$, BSA, and either glucose-glucose oxidase or directly added H_2O_2, CcP was completely inactive over the pH range, 3.5-7.0. LiP, on the other hand, displayed very significant iodinating activity at pH 3.5. These results support the following conclusions: 1) peroxidase-catalyzed oxidation of iodide involves simultaneous transfer of 2 electrons from iodide to a porphyrin π-cation radical form of the enzyme, and 2) the protein radical form does not catalyze this reaction, probably because the 2 oxidizing centers are separated by an appreciably greater distance than in the π-cation radical form. CcP and LiP were both quite active in catalyzing the coupling reaction at pH 7.0, in the presence of a low conc. of free diiodotyrosine, but LiP displayed significantly greater activity than CcP. This demonstrates that in two very similar peroxidases, coupling is better mediated by the porphyrin π-cation radical form. These observations with CcP and LiP raise the possibility that, under catalytic conditions, coupling with TPO may also involve the porphyrin π-cation radical form of Cpd I. Supported by NIDDK 03612.

9:45 A.M. 134. CHARACTERIZATION OF THE SODIUM/IODIDE SYMPORTER OF THE THYROID GLAND. Orlie Levy, Ge Dai and Nancy Carrasco. Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, 10461.

The accumulation of I^- in the thyroid is mediated by the Na^+/I^- symporter, an intrinsic plasma membrane protein located in the thyroid follicular cells. I^- transport into the thyroid is the first step in the biosynthesis of thyroid hormones T_3 and T_4. Thyroid stimulating hormone (TSH) stimulates all aspects of thyroid function, including I^- transport. Functional membrane vesicles have been prepared from rat thyroid cells in culture (FRTL-5 cells), in which kinetic parameters, driving forces, and regulation of I^- transport have been analyzed. In an effort to identify the symporter a ^{125}I photolabeling method has been developed to label thyroid polypeptides with high affinity for I^-. Membrane vesicles prepared from thyroid and other cells were incubated in the presence of ^{125}I and irradiated with a mercury arc lamp. Polypeptides were then electrophoresed and visualized by autoradiography. Significantly, only polypeptides from I^--transporting cells were labeled, suggesting a strong link between polypeptide labeling and the ability of cells to transport I^-. The mechanism of the photolabeling reaction is thought to involve a charge transfer complex between the imidazolium ion from a histidine side chain and iodide. NH_2-terminal sequencing information has been obtained for a 90 kDa ^{125}I photolabeled polypeptide, which was identified to be the chaperone phosphoprotein calnexin. The identity of calnexin was confirmed by immunoprecipitation of the polypeptide with anti-calnexin antibody (generously provided by Dr. A. Helenius, Yale University). In the thyroid, calnexin assists in proper folding of thyroglobulin. Importantly, TSH has been found to stimulate calnexin expression in the thyroid, both in culture and *in vivo,* as revealed by immunoblot analysis with anti-calnexin antibody. Na^+/I^- symport activity has been expressed in *Xenopus laevis* oocytes. Functional screening of thyroid cDNA libraries in the oocyte system has led to increasing positive, perchlorate-sensitive Na^+/I^- symport activity signals as the number of cDNA clones per screened pool has been reduced.

10:30 A.M.
135. INCIDENCE AND MECHANISMS OF HEARING LOSS IN PATIENTS WITH RESISTANCE TO THYROID HORMONE (RTH+). F. Brucker-Davis, A. Pikus, D. Ishizawar and M. Skarulis. National Institutes of Health, Bethesda, Maryland, USA.

Sensorineural deafness was one striking sign in the first kindred with RTH reported by Refetoff et al, but has only been reported subsequently on an anecdotal basis. Furthermore, hearing impairment has been observed in both congenital and acquired hypothyroidism, suggesting an important role of thyroid hormone (TH) in development and function of the auditory system. RTH is a heterogeneous syndrome, usually transmitted in an autosomal dominant fashion and caused by mutations in the T3-binding domain of the TH receptor β; mutant receptors exert a dominant negative effect on normal receptors; however, the first kindred was an exception, the genetic defect being an homozygous deletion of the TH receptor β. Within the inner ear there is a different ontogenic distribution of TH receptors: the β are restricted to the cochlea, while the α are ubiquitous. Therefore, it has been speculated that the β receptor has a pivotal role in the cochlear development. To assess the incidence of hearing impairment in RTH and to define its mechanisms, we have studied 82 RTH+ patients (48 children, 34 adults) and compared them with 55 (22 children, 33 adults) of their unaffected relatives (RTH-). Audiologic examination included standardized psychoacoustic studies of puretones and speech sensitivity using speech reception thresholds (SRT), and biomechanical measurements of middle ear integrity using tympanometry and acoustical reflexes. History of ENT infections, noise exposure and speech impairment was recorded. RTH+ had a higher incidence of ear infections (66% vs 28%, $p<0.0001$) and speech problems (35% vs 15%, $p<0.05$), while the incidence of significant noise exposure was equally frequent (43% vs 56%). 21% of RTH+ had abnormal SRT (one fourth representing a significant handicap) vs none of RTH-, $p<0.0005$. Stepwise regression analysis showed that RTH was the only parameter contributory for hearing loss ($R2=0.124$), while ENT infections, noise exposure, age or maternal status for RTH did not contribute. Furthermore, exonic location of mutation had no influence on the incidence of hearing loss. In addition, more RTH+ had abnormal tympanometry (34% vs 12% $p<0.01$) and abnormal acoustic reflexes (39% vs 19%, $p<0.05$). In RTH+ with hearing loss, 50% had abnormal tympanometry, reflecting predominant middle ear pathology and 25% had isolated abnormal acoustic reflexes suggesting inner ear pathology. In conclusion, hearing loss is a more significant problem in RTH than previously recognized. 75% are caused by middle ear problems, including ear infections, but 25% are caused by a separate sensorineural mechanism. These results support the hypothesis of specific effect of mutant β receptor on hearing.

10:45 A.M.
136. FAMILIAL RESISTANCE TO THYROID HORMONE (RTH) NOT LINKED TO DEFECTS IN THE THYROID HORMONE RECEPTORS (TR) α OR ß GENES. R.E. Weiss,[1] Y. Hayashi,[1] T. Nagaya,[2] K.J. Petty,[3] Y. Murata,[2] H. Seo,[2] and S. Refetoff,[1] Departments of Medicine and Pediatrics, University of Chicago, Chicago, IL[1]; Institute of Environmental Medicine, Nagoya University Nagoya, Japan[2]; and Department of Medicine, Southwestern Medical School, Dallas, TX.[3]

RTH is an inherited syndrome of variable hyporesponsiveness to thyroid hormone. In more than 100 families characterized at the molecular level, RTH was associated with mutations in the TRß gene. These mutant receptors show dominant impairment of transactivation usually in association with reduced binding affinity for T_3. RTH linked to a defective TRα has not been identified. The proposita, presenting typical biochemical features of RTH, was identified 15 years ago when aged 11.5 years. She had mental retardation (IQ 67) and required 1,000 μg T_4 or 400 μg T_3 to suppress her serum TSH. Her parents and 5 siblings were clinically and biochemically normal. The proposita had two children from fathers of different ethnic origin. At birth both had high T_4 (54, 33 μg/dl) with TSH of 26 and 27 μU/ml. At 1 year of age, 300 μg of L-T_4 or 100 μg of L-T_3 were required to suppress their serum TSH level and to induce peripheral tissue responses. Thus, the inheritance of their RTH was dominant. To determine the etiology of RTH, the TRß gene was sequenced using genomic DNA from white blood cells and mRNA from skin fibroblasts. No mutations were detected in 8-20 templates covering the entire coding region. A putative abnormality in the TRα was excluded by linkage analysis based on polymorphism of CA repeats within the TRα gene, showing that each of the affected children inherited a different TRα allele from the mother. The possibility that RTH in this family is caused by a defective cofactor interacting with the TRs was explored. Nuclear extracts from fibroblasts were analyzed by Far Western blotting probed with normal TRß and by gel shift assay using an inverted palindromic TRE with added normal TRß. By both methods, the patients showed a unique TRß/nuclear protein complex which was absent or present only in reduced amounts in extracts from 5 normal subjects and from 6 RTH subjects with different TRß mutations. It is thus concluded that genetic defects not involving the TR genes can cause RTH.

11:00 A.M. 137. A STUDY OF SUSCEPTIBILITY GENES OF HLA CLASS II ANTIGEN IN GRAVES' PATIENTS WITH METHIMAZOLE-INDUCED AGRANULOCYTOSIS. H. Tamai, T. Sudo, S. Matsubayashi, A. Kimura and T. Sasazuki, Department of Psychosomatic Medicine (H.T., T.S., S.M.), Faculty of Medicine, and Department of Genetics, Medical Institute of Bioregulation (A.K., T.S.), Kyushu University, Fukuoka 812, Japan.

Methimazole (MMI) has been widely used for the treatment of Graves' disease; however, an important and serious side effect is agranulocytosis, with an incidence of 0.1-0.3%. The present study was undertaken to clarify HLA-linked genetic factors involved in the pathogenesis of Graves' patients with agranulocytosis. Twenty-two patients (4 males and 18 females, ages 22-65 yr) and 317 healthy Japanese controls were examined by DNA typing using the PCR-SSOP method. Furthermore, serum granulocyte autoantibodies were tested for and found during the acute phase in 2 patients. The frequency of HLA-DR8 was increased compared to controls ($p<0.001$). The frequencies of DRB1*0803, DRB1*1501 and DPB1*0501 were also increased by DNA typing. The increased frequencies of DRB1*0803, DRB1*1501 and DPB1*0501 in the patients were statistically significant when the corrected P value (pc) was applied ($pc<0.001$, $pc<0.01$ and $pc<0.01$, respectively). As in a previous DPB1 analysis, we demonstrated a strong positive association with HLA-DPB1*0501 in Japanese Graves' patients. On the other hand, in this study DRB1*0803 and DRB1*1501 were not significantly increased in patients with Graves' disease who did not suffer from agranulocytosis. Therefore, we observed a strong association between development of MMI-induced agranulocytosis and specific HLA class II alleles such as DRB1*0803 and DRB1*1501.

In conclusion, MMI-induced agranulocytosis complicated with antithyroid drug therapy for Graves' disease shows a strong association with HLA class II antigens: DRB1*0803 and DRB1*1501. Moreover, we found granulocyte autoantibodies in patients serum samples. These observations suggest that the occurrence of MMI-induced agranulocytosis in Graves' patients undergoing antithyroid drug therapy might be due to an autoimmune mechanism.

11:15 A.M. 138. OCTREOSCAN IN THE EVALUATION OF ACTIVE GRAVES' OPHTHALMOPATHY. R. Moncayo, I. Baldissera*, R. Metzler*, C. Decristoforo, and G. Riccabona. Department of Nuclear Medicine and *Ophthalmology, University of Innsbruck, Austria.

The correct evaluation of the degree of clinical activity of Graves' ophthalmopathy is a fundamental question for therapeutic decisions. The recent introduction of the indium-labelled somatostatin analog octreotide (OctreoScan ®, Mallinckrodt) provides the theoretical possibility of investigating the presence of activated T-cells, as an indirect indication of an immunological process (1). The aim of the study was to evaluate the ability of this new scintigraphic method to detect active eye disease as compared to a purely clinical evaluation (2). A group of 46 patients were included in the study, 8 patients were studied more than once (total n= 4). The scan was done within 3-months after clinical symptoms in 46 cases, whereas in 8 cases the duration of the disease was > 1 year. Planar and SPECT images were done 2 hours after the i.v. application of 111 Mbq of the 111-Indium-labelled Octreotide. There was a significant correlation between both evaluation methods ($r=0.45$, $p<0.01$; Table). All patients with highly active disease were clearly identified by both procedures. Tracer accumulation was localized to the retroorbital space. In 18 patients with negative or intermediate clinical activity the scan was also positive, thus disclosing a significant number of cases with active disease. Octreoscan also provided a higher number of positives as compared to CT or MRI. Positive scans were also seen even under immunosuppressive therapy with steroids and/or cyclosporin-A, indicating persistent active disease. Patients with disease of long duration were negative. We conclude that the octreotide scan constitutes an objective tool in the evaluation of Graves' ophthalmopathy, both at initial stages as well as during treatment. The tracer accumulation in the retrobulbar space suggests an on-going immunological process in early disease.

Clinical Score	Negative Scan	Positive Scan
Negative	7	8
Intermediate	9	10
Positive	0	20

(1) Eur. J. Clin. Invest 1994; 24:91-99. (2) Br. J. Ophthalmol. 1986; 73:639-644.

11:30 A.M. 139. FURTHER STUDIES ON THE COURSE OF GRAVES' OPHTHALMOPATHY (GO) FOLLOWING RADIOACTIVE IODINE (RAI) ADMINISTRATION. L. Bartalena, C. Marcocci, F. Bogazzi, G. Bruno-Bossio, M.L. Tanda, G. Vanni, E. Dell'Unto, E. Martino and A. Pinchera, Istituto di Endocrinologia, University of Pisa, Italy

Conflicting results have been reported on whether RAI may influence GO. We addressed this problem by carrying out a prospective, randomized, controlled study, which enrolled 450 pts with Graves' disease and slight or absent GO, randomly allocated in three groups: RAI group (n=150, 121 F, 29 M, age range 16-85 yr, mean 44) treated with RAI alone (4-19 mCi, mean 11); RAI+glucocorticoids (GLUC) group (n=145, 117 F, 28 M, age range 15-69 yr, mean 42; 5 pts lost from follow-up) treated with RAI (5-32 mCi, mean 12) and GLUC (0.4-0.5 mg prednisone/Kg BW/day initial dose, then gradually withdrawn over a 3-month period); Methimazole (MMI) group (n=148, 120 F, 28 M, age range 16-77 yr, mean 41; 2 pts lost from follow-up) treated with MMI for a 18-month course. There was no substantial difference in the duration of hyperthyroidism and GO in the three groups, nor in the degree of ocular involvement. Pts were evaluated every 2 months for 12 months. There was no difference in the outcome of RAI treatment in the two groups treated with RAI. Persistent hyperthyroidism or hypothyroidism after RAI were promptly corrected. MMI group: 3 pts (2%) showed an improvement of GO, 4 patients (2.7%) had a progression (slight in three cases, moderate to severe in one case). RAI group: GO did not change in 127 pts, worsened in 23 (15.3%) 3-6 months after RAI administration, with soft tissue changes (palpebral edema in 21, conjunctival hyperemia in 19, chemosis in 17, caruncle edema in 19), lid retraction in 11, lagophthalmos in 10, eye muscle dysfunction with diplopia in 14. Mean Hertel readings increased from 16.5 to 18.7 mm. Worsening was transient in 15 pts, but persistent, requiring treatment with orbital irradiation and high-dose GLUC, in 8 pts (5.3%); 8 of the 23 pts had no eye signs prior to treatment. RAI+GLUC group: GO did not change in 95 pts; no patient had an exacerbation; an improvement occurred in 50 pts (34.5%), involving soft tissue changes (palpebral edema in 40, conjunctival hyperemia in 45, chemosis in 39, caruncle edema in 34), lid retraction in 35, lagophthalmos in 10, eye muscle dysfunction with diplopia in 19. Mean Hertel reading decreased from 17.5 to 15.1 mm.

In conclusion: i) RAI treatment may be associated with an exacerbation of GO; ii) GLUC given concomitantly to RAI may cure slight GO and prevent RAI-associated exacerbation of GO; iii) MMI treatment does not influence the course of GO.

11:45 A.M. 140. **RECURRENCE OF GRAVES' DISEASE IS INDEPENDENT OF THYROXINE ADMINISTRATION AFTER MEDICAL THERAPY.** A. Doufas, G. Mastorakos, J. Mantzos and D.A. Koutras. Endocrine Unit, Department of Clinical Therapeutics, Athens University, Evgenidion Hospital, Athens, Greece.

Thyroid-stimulating hormone (TSH) secretion has been incriminated in the past for the exacerbation of production of antibodies to TSH receptor (TRAb) in Graves' disease. It has been, therefore, suggested that thyroxine administration during and post-antithyroid drug treatment may decrease both the production of TRAb and the frequency of recurrence of hyperthyroidism. We studied the role of T_4 or T_3 on the recurrence rate of Graves' disease when these hormones are administered after the antithyroid drug therapy. Fifty patients with Graves' disease (13 males, age=48,7±14,7 years, mean±SD, 37 females, age=44,5±13,6) were followed-up for 12 months after successful medical treatment with carbimazole (final dose 5 mg/d) and 25 μg T_3 for 18 months. During the follow-up period, patients received daily either 100 μg T_4 or 25 μg T_3 or placebo after random assignment into three groups and were evaluated every 3 months. We measured the TRAb levels with a radioimmunoassay at the beginning and at the end of this period. The rate of recurrence of the disease was evaluated at 12 months of the follow-up period by survival analysis (Cox's test). Thirteen out of 20 (65%), 5 out of 14 (35%), and 5 out of 16 (31%) patients receiving T_4, T_3, and placebo, respectively, had recurrence of hyperthyroidism ($p<0.05$). Patients who relapsed had significantly higher TRAb levels than the rest of the patients at the beginning of the follow-up period ($p<0.05$, ANOVA). Interestingly, although placebo-treated patients had a significantly lower recurrence rate than the T_4-treated patients they had significantly higher plasma levels of TRAb at the end of the follow-up period ($p<0.05$, ANOVA). We conclude that, although thyroxine administration after successfull antithyroid drug treatment of Graves' disease keeps TRAb plasma levels at their initial values, it does not decrease but actually increases the recurrence of hyperthyroidism as compared to the T_3 or placebo. High TRAb levels at the end of antithyroid drug treatment but not during follow-up period may predict recurrence. The results of multivariate analysis about the other clinical and biological factors studied will also be reported.

10:30 A.M. 141. **THYROID HORMONE REGULATES THE EXPRESSION OF LAMININ IN THE DEVELOPING RAT CEREBELLUM.** A.P. Farwell and S.A. Dubord. Molecular Endocrinology Lab, University of Massachusetts Medical School, Worcester, Massachusetts, USA.

In the rat cerebellum, migration of neurons from the external granular layer (EGL) to the internal granular layer occurs postnatally and is dependent upon the presence of thyroid hormone (TH). In hypothyroidism, many neurons fail to complete their migration and die. Key guidance signals to these migrating neurons are provided by laminin (LM), an extracellular matrix protein that is fixed to the surface of astrocytes in the molecular layer (ML) of the cerebellum. Expression of LM in the brain is developmentally timed to coincide with neuronal growth spurts. We have previously shown that TH regulates the extracellular orientation of LM on astrocytes in culture. In this study, we examined the effect of TH on LM expression in the cerebellum. Neonatal hypothyroidism was induced by giving propylthiouracil (PTU) in the drinking water (2 mg/L) to pregnant dams beginning on d17 of gestation and continuing throughout the neonatal period. Animals were sacrificed from birth to P20 and the content and regionalization of LM in the cerebellum was analyzed. Growth of the PTU-treated rats was >2-fold less than that of the euthyroid rats (weight gain 1.3 g BW/d vs 3.1 g BW/d). In the euthyroid rat cerebellum, LM content increased 2-5-fold from P0-P4, plateaued from P4-P12 and then increased steadily to P20. LM staining first appeared in the ML on P4 and the intensity of LM staining increased to maximal levels by P10. By P14, LM staining was lost from the ML and was confined to the vasculature. The EGL decreased steadily in the euthyroid rat, with few cells remaining in the EGL by P14. In contrast, LM content in the PTU-treated rat cerebellum remained at basal levels, ~4-fold lower than in the euthyroid rat, from P0-P10 and then increased steadily to P20. LM staining appeared in the ML from P8-18. Maximal LM intensity in the ML was observed on P14 and was ~50% less than the maximal intensity of LM staining in the ML of the euthyroid cerebellum. The EGL still contained abundant cells at P20 in the PTU-treated rats. In summary, LM expression in the ML of the hypothyroid rat cerebellum is markedly delayed and is much less abundant than in the euthyroid cerebellum. These data suggest that TH-dependent regulation of LM in the rat cerebellum plays a major role in the profound derangements of neuronal migration observed in the cretinous brain.

10:45 A.M. 142. IDENTIFICATION OF RC3/NEUROGRANIN-EXPRESSING, THYROID HORMONE-SENSITIVE NEURONS IN THE RAT FOREBRAIN. M. A. Iñiguez, B. Morte and J. Bernal. Instituto de Investigaciones Biomédicas, CSIC, Madrid, Spain.

Thyroid hormone is an important epigenetic factor in brain development. As in other organs and tissues, T3 most likely acts in the brain by binding to nuclear receptors and regulating gene expression. In the past few years a number of genes have been shown to be under thyroid hormone control. One of these genes encode RC3/neurogranin, a calmodulin-binding, protein kinase C substrate, expressed mainly in the cerebral cortex, striatum, and hippocampus. RC3 has been implicated in the generation of long term potentiation, the prevalent model of memory in vertebrates, and in other postsynaptic events involving calcium as a second messenger. We have shown that expression of RC3 is under thyroid hormone control in both developing and adult rats. In this work we have studied the effect of thyroid hormone deficiency and replacement on the expression of RC3 mRNA in the rat brain by in situ hybridization.

Normal, hypothyroid, and T4-treated rats were used. In situ hybridization was performed in cryostat sections of frozen brains which had been perfused with 4% paraformaldehide. In situ hybridization was performed using digoxygenin-UTP labeled riboprobes using as template the coding region of RC3 cDNA.

In normal rats RC3 expression at postnatal days 5 (P5) and 10 occurs mainly in layers II-V of cerebral cortex and the CA fields of the hippocampus. This early pattern was not altered by fetal-onset hypothyroidism. From P10 to P15 RC3 mRNA decreased in layer V, and increased in layer VI, the retrosplenial cortex, the caudate-putamen nucleus, and the dentate gyrus. This late pattern was impaired by hypothyroidism. Thyroxine treatment increased RC3 expression in the regions affected. In the caudate-putamen of normal rats RC3 was expressed from P5-P10 following a ventro-lateral to dorso-medial gradient. This gradient was absent in hypothyroid rats which displayed little hybridization, suggesting that the developing striatum is a major target of thyroid hormone. The striatum was also thyroid hormone-sensitive in young adult animals. To our knowledge this is the first report which identifies in situ changes of a brain-specific mRNA after manipulations of the thyroidal status. The changes induced by thyroid hormone deficiency on RC3 expression could be relevant to understand the phenotype of neurological cretinism.

11:00 A.M.
143. **EVIDENCE FOR CIRCADIAN VARIATIONS OF THYROID HORMONE CONCENTRATIONS AND TYPE II 5'-IODOTHYRONINE DEIODINASE ACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE RAT**

A. Campos-Barros, A. Musa, A. Flechner, C. Hessenius, U. Gaio, M. Eravci, H. Meinhold and A. Baumgartner°. Dept. of Nuclear Medicine and Psychiatric Clinic°, Klinikum Benjamin Franklin, Free University of Berlin.

Previous reports have indicated the existence of nictohemeral rhythms in the activity of type II 5'-iodothyronine deiodinase (5'D-II) in rat pineal gland (Tanaka et al. 1987, Murakami et al. 1988) and frontal cortex (Guerrero et al. 1988). We recently also reported on topographically specific, daytime-dependent variations in brain 5'D-II activity of control rats maintained under a normal 12:12 LD schedule (Campos-Barros et al. 1994). In order to further characterize the apparent circadian variations of thyroid hormone metabolism in the rat CNS we have investigated in this work the rhythmicity of local T_3 generation by measuring 5'D-II and type III 5-deiodinase (5D-III) activities and tissue T_4 and T_3 concentrations in regions of the rat brain over a 24-h period. Groups (n=6) of male euthyroid Sprague-Dawley rats (300-350 g) kept under a LD 12:12 schedule (lights on 6 am) were killed by decapitation in 4-h intervals over a 24-h period. Brains were rapidly removed and dissected. T_4 and T_3 concentrations were determined in tissue extracts by RIA as described by Morreale de Escobar et al. (1985). 5'D-II and 5D-III were determined by measuring the release of radioiodide from [5'-^{125}I]T_4 and [5-^{125}I]T_3 (courtesy of R. Thoma, Henning, Berlin), respectively. One-way ANOVA of the data revealed significant ($p < 0.001$) diurnal variations of 5'D-II in frontal cortex *(cf)*, hypothalamus *(hyp)*, cerebellum (*crb*), midbrain *(mid)*, hippocampus (*hip*), amygdala (*amy*), septum (*sp*), and brain stem (*bs*). In most of the studied regions 5'D-II rose after lights off, reaching a zenith at the second half of the dark period (3-5 am) and a nadir between noon and 4 pm. In the *cf*, however, both maximum and minimum 5'D-II values were clearly delayed (7 am and 7 pm, respectively) with respect to the other brain areas. Periodic regression analysis (Fourier) revealed a significant sinusoidal rhythm ($0.71 < r < 0.98$) with amplitudes *(A)* of approximately + 30 % of the daily mean value and a period *(T)* of 24 h ± 1 in all above mentioned regions. With respect to 5D-III only the *amy*, *hip*, and *str* exhibited significant daily variations ($p < 0.01$). However, periodic curve fitting was statistically significant only in the *amy* (A = +12.1% of daily mean value; acrophase (Φ) at 9 am ± 1 h and T = 14 h, r = 0.87). Tissue T_4 and T_3 concentrations were also determined in the hypothalamus and cortex frontalis of a second group of animals killed under the same conditions. Both iodothyronines exhibited significant but not sinchronic sinusoidal variations (A = 12-30 %; T = 24 h) in both brain regions. In the *hyp*, T_4 peaked between noon and 1 pm (+ 20 % of the daily mean value) and reached the lowest value at the middle of the dark period (00-1 am). In *cf* a similar, slightly delayed pattern of variation was found with a maximum (+30 %) at the end of the light period (4 pm) and a minimum at the end of the active period (4-5 am). Whereas cortical T_3 concentrations approximated the pattern of variation of 5'D-II in this region (Φ = 10 am), the hypothalamic T_3 appeared to be negatively correlated to its respective 5'D-II levels, reaching a maximum at the nadir-time of hypothalamic 5'D-II (i.e. 4-5 pm). Our results demonstrate the existence of circadian rhythms of 5'D-II activity and thyroid hormone concentrations in several regions of the rat brain. As in the pineal gland, local production of T_3 by 5'D-II was 1.5-2 fold increased during the dark period of the day in most of the studied areas. For all parameters under investigation however, the patterns of variation observed were (in part) regionally specific, suggesting that different regulatory mechanisms could be involved in the generation of the detected rhythms.

11:15 A.M.
144. **SUPERAGONISTS OF RECOMBINANT HUMAN TSH PROVIDE A MODEL OF RATIONAL DESIGN OF GLYCOPROTEIN HORMONE ANALOGS: SITE-SPECIFIC BOVINIZATION OF THE ALPHA SUBUNIT INCREASES IN VITRO AND IN VIVO BIOACTIVITY.**

M.W. Szkudlinski, N.G. Teh, M. Grossmann, J.E. Tropea, J. East-Palmer, C. DaCosta, N.R. Thotakura and B.D. Weintraub, MCEB, NIDDK, NIH, Bethesda, MD, USA.

Human TSH (hTSH) and bovine TSH (bTSH) share high homology in the α (70%) and β (86%) subunits. We have observed that bTSH is more active than pituitary or recombinant hTSH both *in vitro* and *in vivo*. Our studies involving dimerization of hTSH and bTSH subunits indicated that the higher activity of bTSH is dependent primarily on the bovine α-subunit. Based on comparison of α-subunit sequences and the hypothesis that the positively charged residues may be important in receptor binding activity, we introduced selected amino acids present in bovine α-subunit into the sequence of human α-subunit using the PCR-based megaprimer method of site-directed mutagenesis. The following substitution mutants of human α-subunit were studied: Thr11-Lys, Gln13-Lys, Pro16-Lys, Gln20-Lys, Gln50-Pro and Arg67-Lys. The only conservative replacement was at position 67 (Arg-Lys). Wild-type (WT) and mutant α cDNAs were transiently coexpressed with a WT hTSH β minigene in CHO-K1 cells. αWT/TSHβ and its α TSH mutants in conditioned medium were quantitated with 3 different immunoassays. Secretion of αThr11-Lys/TSHβ, αGln13-Lys/TSHβ and αPro16-Lys/TSHβ were 2-fold higher than αWT/TSHβ; the other mutants were secreted at the level comparable to the αWT/TSHβ, indicating that this set of evolutionary justified mutations did not impair synthesis or secretion of the TSH molecule. A receptor binding assay using thyroid membranes revealed the following order of potencies: αPro16-Lys/TSHβ (5-fold greater affinity than WT) > αGln20-Lys/TSHβ > αGln13-Lys/TSHβ > αThr11-Lys/TSHβ > αWT/TSHβ = αGln50-Pro/TSHβ = αArg67-Lys/TSHβ. The *in vitro* bioassays including stimulation of cAMP in CHO-JP09 cells expressing hTSH receptor and growth assay in FRTL-5 cells showed the same order of potencies as observed in the binding studies; αPro16-Lys/TSHβ was again 5-fold more active than αWT/TSHβ. There were no significant differences in the metabolic clearance rate between mutants and αWT/TSHβ. However, injection of αPro16-Lys/TSHβ, αGln20-Lys/TSHβ and αGln13-Lys/TSHβ in mice increased serum T4 significantly higher than the αWT/TSHβ. Thus, we localized at least 3 residues in the β1 strand and *L*1 loop of the α-subunit which were probably critical in the modulation of TSH bioactivity during evolution. Our data supported by homology modeling of hTSH suggest that the region (aa 11-20) of the α-subunit forms a previously unrecognized domain important in receptor binding. Moreover, by introducing positively charged lysine residues normally present in other species into this region we have generated the first superagonists of hTSH which display major increases in both *in vitro* and *in vivo* bioactivity, and which may have potential use in the diagnosis and treatment of thyroid carcinoma. Such an approach may help to generate superagonists of other glycoprotein hormones.

11:30 A.M. 145. Asparagine of the Highly Conserved $NP(X)_{2-3}Y$ Sequence in the Seventh Transmembrane Domain of G Protein Coupled Receptors is Important for Binding and Agonist-Induced Internalization of the Thyrotropin-Releasing Hormone (TRH) Receptor. J.H. Perlman, E. Geras-Raaka, W. Wang and M.C. Gershengorn, Cornell University Medical College-New York Hospital, New York, NY, USA

In general, heptahelical G protein coupled receptors (GPCRs) undergo rapid agonist-induced internalization and domains in the seventh transmembrane helix and carboxy-terminal tail of GPCRs appear to signal this type of internalization. The sequence $NPX_{2-3}Y$ (Asn-Pro-Xaa-Xaa-Tyr or Asn-Pro-Xaa-Xaa-Xaa-Tyr) of the seventh transmembrane domain is highly conserved among members of the GPCR family and the conserved Tyr is critical for internalization of the β_2-adrenergic receptor but not for internalization of the gastrin-releasing peptide receptor. We substituted Ala for the conserved Tyr and Asn residues in TRH-R and assayed binding and internalization in intact cells expressing wildtype (WT) or mutant receptors. Binding and internalization of a mutant receptor in which Tyr of NPVIY of TRH-R was substituted by Ala were not different from WT TRH-R. Therefore, the conserved Tyr of NPVIY of TRH-R is not essential for binding nor for internalization of TRH-R. The similar phenotype regarding internalization of TRH-R and gastrin-releasing peptide receptor may reflect the fact that both are receptors that are coupled predominantly to a GTP binding (G) protein that activates the phosphoinositide pathway whereas β_2-adrenergic receptor couples to the G protein that activates adenylyl cyclase. In contrast, a mutant receptor in which Asn of NPVIY of TRH-R was substituted by Ala exhibited decreased binding affinity (23-fold lower than that of WT TRH-R) and decreased internalization (64% of WT TRH-R). Thus, the conserved Asn is important for binding and internalization. We suggest that agonist occupancy of TRH-R may cause a conformational change around a helical kink at Pro of the NPVIY sequence resulting in a positional change of Asn that is required for optimal binding and internalization. To our knowledge, this is the first demonstration of the importance of the conserved Asn of $NPX_{2-3}Y$ for agonist-induced internalization of a GPCR.

11:45 A.M. 146. TRH GENE EXPRESSION IN THE ANTERIOR PITUITARY: EVIDENCE FROM TRANSPLANTATION STUDIES FOR *IN VIVO* HYPOTHALAMIC INHIBITION. I.M.D. Jackson, B. Monfils, J. Kile and R.B. Todd, Division of Endocrinology, Brown University, Rhode Island Hospital, Providence RI, USA.

We have previously reported that TRH mRNA and peptide show a steady rise within the anterior pituitary (AP) gland in long-term monolayer culture, and that glucocorticoids enhance TRH gene expression in this location, most likely by a transcriptional mechanism. In contrast, TRH levels in the intact AP *in vivo* at the time of sacrifice are low. Such findings suggest that the AP *in vivo* is under inhibitory control from the hypothalamus, and that long-term culture *in vitro* in essence represents a state of hypothalamic disconnection. To test this hypothesis, APs were transplanted under the kidney capsule of donor rats and the content of TRH peptide in the ectopic vs. eutopic AP with or without glucocorticoid treatment was determined. Adult Fisher F344 male rats served as recipients and F344 male 15 day postnatal pups were donors. In those animals receiving transplants, 5 APs were placed under each kidney capsule. Dexamethasone (DEX) was administered by subcutaneous implantation of a slow-release tablet which would expose the tissues to a calculated concentration of at least 10^{-6}M. Four groups of animals were studied: (i) controls (C) , (ii) DEX treated controls (DEX/C), (iii) transplant recipients (Tp) and (iv) DEX treated transplant recipients (DEX/Tp) (n = 5 or 6 per group). At the time of sacrifice (34 days), both ectopic and eutopic pituitary tissues were rapidly removed for TRH determination by RIA. Evidence for functional viability of the grafted pituitaries was shown by the increased serum levels of prolactin (PRL) in the Tp group, with suppression of PRL content in the eutopic pituitary of these animals. The transplanted pituitaries, when exposed to glucocorticoids (DEX/Tp group), showed a substantial increase in TRH expression with levels 16 fold that of untreated grafted pituitaries (Tp group) (115±9 vs. 7±1 fmol; $p<0.01$). In contrast, the mean TRH content of the AP from the DEX/C group was not significantly different from the level found in the C group (18.3±3.1 vs. 11.5±3.1 fmol; $p>0.05$). These findings indicate that the AP, when removed from hypothalamic regulation and then exposed to DEX, shows a dramatic increase in TRH peptide content, in contrast to the eutopic pituitary of normal animals. This reponse to DEX in the grafted AP is analagous to that found in monolayer culture. Conclusion: TRH gene expression in the eutopic AP *in vivo* is likely subject to inhibitory tonic regulation by the hypothalamus, which prevents glucocorticoid stimulation.

147. P1 CLINICAL SIGNIFICANCE OF MEASUREMENTS OF ANTITHYROGLOBULIN AND ANTIPEROXIDASE ANTIBODIES IN THE DIAGNOSIS OF HASHIMOTO'S THYROIDITIS; COMPARISON WITH HISTOLOGICAL FINDINGS. K. Kasagi, T. Kousaka, K. Higuchi, S. Sasayama, S. Miyamoto, T. Misaki and J. Konishi. Department of Nuclear Medicine and Clinical Pathology, Kyoto University, Kyoto, JAPAN.

Measurements of antithyroglobulin and antimicrosomal antibodies have widely been performed for the clinical diagnosis of autoimmune thyoid diseases. The present study was designed to compare these antibody titers with histological findings of the thyroid in patients with diffuse goiter who were suspected of having Hashimoto's thyroiditis. One hundred and ten euthyroid or hypothyroid patients (10 males and 100 females) at the age of 48±15(SD) year old were studied for the measurement of antithyroglobulin and antimicrosomal or antiperoxidase antibodies by a haemagglutination technique (Fujirebio, Tokyo, Japan ; TGHA and MCHA, respectively) and by a newly developed radioassay (RSR Ltd, Cardiff, UK; TgAb and TPOAb, respectively). The antibody titers were compared with histological findings obtained by needle biopsy. The histological diagnosis was made by two experts in thyroid pathology. The diagnosis of Hashimoto's thyroiditis was given to 83 patients. The remaining 27 patients were diagnosed of having simple goiter. When the diagnosis of Hashimoto's thyroiditis was based on detectability of these antibodies, sensitivity, specificity and accuracy of each assay was 96.4 % (80/83), 96.3 % (26/27) and 96.4 % (106/110) for TgAb; 73.5 % (61/83), 96.3 % (26/27) and 79.1 % (87/110) for TPOAb; 44.6 % (37/83), 96.3 % (26/27) and 57.3 % (63/110) for TGHA; and 65.1 % (54/83), 96.3 % (26/27) and 72.7 % (80/110) for MCHA, respectively. In 55 patients with negative TGHA and MCHA, the TgAb positivity was more closely associated with the pahological diagnosis of Hashimoto's thyroiditis than the TPOAb positivity was (sensitivity, specificity and accuracy: 89.7 % (26/29), 96.2 % (25/26) and 92.7 % (51/55) for TgAb and 27.6 % (8/29), 96.2 % (25/26) and 60.0 % (33/55) for TPOAb, respectively). In conclusion, the newly developed radioassay for TgAb has the advantage of providing the most accurate diagnosis of Hashimoto's thyroiditis, and thus may be helpful for the elimination of chances of unnecessary biopsies.

148. P2 CRYSTALLISATION OF THE HUMAN AUTOANTIGEN, THYROID PEROXIDASE FOR STRUCTURAL ANALYSIS. A Gardas, M K Sohi, B J Sutton and A M McGregor. Medical Centre Postgraduate Education,Warsaw, Poland, Department of Medicine and The Randall Institute, King's College, London, UK

Thyroid peroxidase (TPO) is the 105Kd molecular weight, membrane bound target autoantigen in lymphocytic thyroiditis in man. Several epitopes of TPO recognised by autoantibodies and autoreactive T cells have been identified by us and others. Knowledge of the three dimensional structure of this autoantigen will provide the basis for understanding (i) the mechanism of action of TPO in the generation of thyroid hormones, and (ii) the nature of the autoantigenic epitopes recognised by autoantibodies and T cells in autoimmune thyroid disease in man.

Structural determination by X-ray crystallography requires milligram quantities of highly purified protein. We have purified over 50mg of full length TPO from Graves' thyroid tissue by a combination of monoclonal antibody affinity chromatography and hydrophobic, ion exchange and lectin chromatography to give preparations of ≥ 95% purity. A screen of crystallisation conditions yielded small, but highly birefringent crystals of TPO by the hanging drop vapour fusion technique. It is likely that heterogeneity within TPO preparations such as the existence of two alternatively spliced forms and glycosylation differences are limiting the size of the crystals. To circumvent this, the larger form of the molecule (TPO-1) has been expressed in insect cells using recombinant baculoviruses and both Graves' and recombinant TPO have been deglycosylated. Crystallisation trials of these homogeneous preparations of TPO are now underway. The crystallisation of a membrane autoantigen derived from human tissue represents a significant step towards understanding the role of this enzyme at a molecular level in patients with autoimmune thyroid disease.

149. P3 ESTIMATION OF INDUCTION TO HUMAN ANTI-HUMAN THYROGLOBULIN AUTOANTIBODY PRODUCTION LED BY ANALYSIS OF B CELL EPITOPE. M.Horimoto[1], Y.Nakajima[1], T.Suzuki[1], and S.Mori[2], Section of Internal Medicine, Kobe City General Hospital, Kobe[1], and Second Department of Internal Medicine, Kansai Medical University, Osaka[2], Japan

《Introduction》 Autoantibodies would recognize several epitopes on an autoantigen if they were induced by suppresser T cell deficiency or antigen-driven immunity due to autoantigen-specific helper T cell as well as polyclonal B cell activation because one antigen has several different antigenic regions or epitopes, whereas autoantibodies recognize a single universal epitope which has cross-reacted with one of several epitopes on an non-self antigen in case they are induced by molecular mimicry. 《Materials and Methods》 Two IgG-class human monoclonal anti-human thyroglobulin autoantibodies (anti-hTg aAbs), [A] and [B], were secreted by hetero-hybridomas between human lymphocytes from patients with CT and a mouse myeloma cell-line, X63-Ag8.653. Human anti-hTg aAb positive sera were obtained from seven patients with CT. IgGs were purified from serum or culture supernatant using chromatography on columns of Protein A-agarose. Anti-hTg aAbs were determined by measuring radioactivity of ^{125}I-hTg bound to tubes coated with these IgGs. Epitope analysis of anti-hTg aAbs was done by determining interaction between liquid-phase IgGs and solid-phase IgGs bound to ^{125}I-labelled hTg. 《Results》 Bindings of non-reduced ^{125}I-hTg to tubes coated with IgGs from the patients' sera or the hybridomas'culture supernatants were 10 to 50% of total counts, while bindings of reduced ^{125}I-hTg to these IgGs were all less than 3%, suggesting that human anti-hTg aAbs recognized a conformational region(s) of hTg. Total bindings of ^{125}I-hTg to tubes coated with IgGs from patients' sera were from 10 to 50% of total counts. The bindings were inhibited in a dose-dependent manner by pre-incubation of ^{125}I-hTg with liquid-phase IgGs of [A] or [B] and resulted in plateau phases of almost complete (both more than 70%) inhibitions of total bindings by addition of either [A] or [B], and of complete inhibitions by the addition of a combination of both [A] and [B]. Moreover, when ^{125}I-hTg was incubated in tubes coated with either [A] or [B], bindings of ^{125}I-hTg to these tubes showed plateau phases at complete inhibitions by addition of the same IgG ([A] to [A] or [B] to [B]) as well as both [A] and [B], and at almost complete (both more than 65%) inhibitions by the addition of the opposite IgG ([A] to [B] or [B] to [A]). On the other hand, addition of IgG from normal control sera or mouse monoclonal anti-hTg Ab showed no inhibition. These results suggest that human anti-hTg aAbs in most patients with CT recognized only one 'universal'determinant cluster of the epitope which was common in the patients, though precise epitopes were not exactly identified. 《Conclusion》 Human anti-hTg aAbs in most patients with CT recognized a single conformational region of hTg and production of anti-hTg aAbs appeared to be induced by some form of molecular mimicry or other unknown mechanisms, but the evidence appears to suggest that it was not caused by suppresser T cell deficiency, antigen-driven immunity due to autoantigen-specific helper T cell, or polyclonal B cell activation.

150. P4 **EFFECTS OF THE TREATMENT OF HYPERTHYROIDISM ON THE LEVELS OF EYE MUSCLE AUTOANTIBODIES IN GRAVES PATIENTS.** **A. Boucher, G. Emond, M. Richard, N. Griffiths, H. Beauregard and R. Comtois, Louis-Charles Simard Research Center, Montreal, Canada.**

Thyroid-associated ophthalmopathy (TAO) is related to Graves hyperthyroidism. ^{131}I treatment has been thought to aggravate eye disease and one hypothesis is the release of thyroid/eye muscle (EM) antigens (Ag) which would stimulate autoimmunity. This study was designed to compare the effects of ^{131}I and propylthiouracile (PTU) on antibody (Ab) levels against EM. Twenty-seven patients with Graves were treated for hyperthyroidism (^{131}I:14, PTU:13). Titers of EM Abs and TSI (Kronus) were measured before and 4, 8, 12 weeks (W) after treatment. The measure of EM Abs was done against pig EM membranes which were run on a 7% SDS-PAGE and transfered onto nitrocellulose. Western Blotting was done with patients serum diluted 1/25 to 1/51200. Ag/Ab complexes at the level of 64 kDa were detected with a goat IgG (1/3000). EM Abs titers were determined as the last dilution giving a band. Results of the changes in Ab levels are expressed in the table:

W	^{131}I: A/EM Abs	^{131}I: TSI	PTU: A/EM Abs	PTU: TSI
0	1/9992.8±1/17216	32.7±34.0	1/9830±1/18524	23.4±17
4	1/17216±1/19024	195.9±495.2	1/791.6±1/745.2	26.9±24.3
8	1/8327.3±1/16029	227.7±179.8	1/627.3±1565.8	32.4±24.6
12	1/525±1/340.3	204.5±152.3	1/1025±1/1707.6	23.2±26.4

There was no difference in pre-treatment levels of both EM Abs and TSI levels between the two groups of treatment ($p=0.7$). ^{131}I induced an increase in EM Abs (W4, $p<0.02$) which returned to T0 levels after. TSI increased at 8W ($p<0.01$) and remained high (W12, $p<0.01$). PTU induced a sustained decrease in EM Abs titers (W4:$p<0.02$, W8: $p<0.005$, W12: $p<0.05$). TSI did not decrease significantly with PTU ($p=0.42$). This suggests that the treatment of hyperthyroidism may influence autoimmunity against EM Ags.

151. P5 THYROID PEROXIDASE IS AN AUTOANTIGEN IN NON-OBESE DIABETIC MICE WITH THYROIDITIS. A.S. Motani, P. Arscott, M.R. Shen, L. Hennessey, M. McInerny* and J.R. Baker, Jr. University of Michigan Medical School, Ann Arbor, Michigan and *University of Toledo, Toledo, Ohio.

Animal models of autoimmune diseases may provide insights into the pathogenesis of human disease. The non-obese diabetic mouse (NOD) spontaneously develops a number of different autoimmune diseases, including thyroiditis. No specific antigen has been implicated in the development of thyroiditis in these animals. In contrast, the development of diabetes in these mice has been related to the recognition of autoantigens, such as glutamic acid decarboxylase, similar to those important in human disease. Therefore, studies were undertaken to determine if thyroid peroxidase, an autoantigen associated with human thyroiditis, is recognized by these mice. The size of mouse thyroid makes it difficult to use native TPO to evaluate antigenicity. The cDNA for mouse TPO (mTPO; a gift of Dr. Sachiya Ohtaki, Miyazaki Medical College, Japan) was used for the expression of recombinant mTPO. mTPO cDNA was initially inserted into the plasmid vector pCMV (Invitrogen) which contains a T7 polymerase promoter sequence. This plasmid was used to express recombinant, ^{35}S-labeled mTPO in an *in vitro* transfection system (Promega TNT™ T7 Coupled Reticulate Lysate System). The expressed protein was then used in immunoprecipitation experiments to screen for the presence of autoantibodies to mTPO in the sera of mice with thyroiditis. Serum from 3 of 10 mice examined with histologic thyroiditis immunoprecipitated mTPO; those precipitating the antigen were over six months old and appeared to have diffuse disease by histology. To confirm these findings, a truncated mTPO cDNA coding for the extracellular domain of the TPO protein was inserted into the pGEX-ML expression vector and the resulting plasmid was used to transform HB101 bacteria. The recovery of the recombinant GST-mTPO fusion protein was aided by solubilization of the bacterial pellet in sarkosyl/Triton X-100, and the protein was then isolated by preparative SDS-PAGE. Western blots confirmed the reactivity to mTPO in these animals, with anti-TPO autoantibodies identified in a higher percentage (50%) of mice than were observed in the immunoprecipitation studies. These results indicate that TPO is an autoantigen that is recognized by NOD mice with thyroiditis. Further studies examining T cell reactivity to TPO will help to determine if recognition of this antigen contributes to the pathogenesis of thyroiditis in NOD mice.

152. P6 **DETECTION OF ANTI-THYROGLOBULIN AUTOANTIBODIES WITH A HIGHLY SENSITIVE IRMA IN HASHIMOTO'S THYROIDITIS AND NON TOXIC NODULAR GOITER**
G. Barbesino, M. Marinò, L. Petrone, G. Bendinelli, T. Giacomelli, L. Grasso, L. Chiovato
Institute of Endocrinology, University of Pisa (Italy)

A highly sensitive IRMA for circulating anti-thyroglobulin antibodies (TgAb) has recently been developed (TgAb IRMA BIOCODE, Sclessin-Belgium). In this method, serum samples are incubated in Tg-coated tubes and the binding of specific antibodies is revealed by the addition of a ^{125}I-Protein A tracer, which mainly binds to the Fc fragment of IgGs. Results are read out of a standard curve calibrated using the MRC standard 65-93 (6.25-4000 IU/ml).

In the present study this highly sensitive IRMA was employed to search for TgAb in sera from three groups of patients: 1) 23 patients with typical Hashimoto's thyroiditis (HT) (M=2, F=21; age range=13-72) with (n=19) or without circulating thyroperoxidase antibodies (TPOAb); 15 patients with non toxic nodular goiter associated with focal thyroiditis (FT) (as assessed by circulating TPOAb) (M=1, F=14; age range=26-78); 3) 20 patients with non toxic nodular goiter (NTG) without circulating TPOAb (M=4, F=16; age range=29-64). All patients sera included in the study were negative for TgAb as assessed by passive agglutination or ELISA techniques. Sera from 37 age- and sex-matched normal subjects were used as control. All control sera were negative for TgAb and TPOAb. In the highly sensitive IRMA for TgAb the results of control sera ranged from 1 to 13 IU/ml. Values exceeding 14 IU/ml were considered positive for TgAb. Using the above cut-off TgAb were detected in sera from 9/23 patients with HT (range=15-92 IU/ml; mean±SD=39.7±26.6 IU/ml), from 4/15 patients wit FT (range=24-41 IU/ml; mean±SD=32.2±9 IU/ml), and from 6/20 patients with NTG (range=17-95 IU/ml; mean±SD=52.8±31.4 IU/ml). The prevalence of TgAb was 39.1% in patients with HT, 26.6% in patients with FT, and 30.0% in those with TNG. Mean levels of TgAb did not differ betwwen patients with HT, FT and NTG.

To ascertain whether the positivities detected in the highly sensitive IRMA were really due to TgAb, inhibition experiments were carried out. To this purpose 25 µl of serum were incubated with purified human Tg (45 µg/ml) or, as control, with bovine albumin (45 µg/ml) in the assay buffer (total volume=1 ml) for 12 h at 4°. At the end of the incubation, serum-human Tg or serum-bovine albumin mixtures were added to test tubes. All sera with TgAb values >14 IU/ml were significantly inhibited by the addition of human Tg, but not by bovine albumin (TgAb mean±SD: serum only=42.1±23.7 IU/ml; serum+Tg=7.8±7.3, $p<0.0005$ *vs* serum only; serum+bovine albumine=45.7±22.2, p=NS *vs* serum only). No significant inhibition was produced by human Tg coincubation in control sera.

In conclusion our data show that, using a highly sensitive IRMA, it is possible to detect TgAb present at low levels in sera that would have been considered negative with passive agglutination or ELISA techniques. Using this method 39.1% of previously "seronegative" patients with HT had circulating TgAb. Similarly, TgAb were deceted in 26.6% of previously "seronegative" patients with FT and in 30% of those with TNG. Since TgAb detection can be inhibited by the addition of Tg, this IRMA provides a really specific assay. Therefore, our study demonstrates that in HT and FT patients TgAb are more frequently detectable than what appeared until now. An excessive leakage of Tg from NTG could explain the finding of a high frequency of low levels of TgAb in these patients.

153. P7 **PROBING THE EPITOPE RECOGNITION OF THYROID PEROXIDASE AUTOANTIBODIES IN SERA OF PATIENTS WITH AUTOIMMUNE THYROID DISEASE.**

B.Czarnocka, D.Pastuszko, U.Malczewska, A.Gardas, Dept.Clinical Biochemistry, Med.Ctr.Postgraduate Education, Warsaw, Poland.

Serum autoantibodies to thyroid peroxidase (TPO), previously known as the thyroid microsomal antigen are a specific diagnostic indicator for autoimmune thyroid disease (AITD). They are polyclonal and reactive to different epitopes on the TPO molecule. Previously we generated and characterized 13 murine monoclonal IgG-class antibodies (mAb) to native TPO. These mAbs interact with four antigenic domains on TPO :A,B,C and D. Region A and B delineated by mAbs:2,9,47,60 and B:15,18,59 and 64 are preferentially recognized by TPO autoantibodies (aAb) of AITD patients' sera. Antigenic regions C and D are recognized by TPO aAb less frequently. These mAbs have been used to probe the epitope recognition by TPO aAbs in 75 AITD patients' sera. The competition enzyme-linked immunosorbent assay confirmed our previous data, that TPO aAbs react predominantly with epitopes for mAbs discriminating antigenic regions A and B, and to much lesser extent C and D. The inhibition of 13 mAbs binding to the antigen by sera tested devided on the basis of aAbs titre (see table) revealed that virtually all sera tested contained a high proportion of TPO aAbs reactive to mAb 9 epitope irrespective of aAbs content. The inhibition of mAb 9 binding was 92.9±14.8% (mean±SD).

	Inhibition of mAbs binding (%); (mean ± SD)												
TPO autoantibodies titre	2	9	47	60	15	18	59	64	24	1	30	40	53
1/2000 n=6	16.75±4.7	86±11.5	0	25.2±10.3	9±15.59	16±11.8	64.7±11.2	18±11.85	10±4.3	9.2±3.6	15.2±4.5	4.7±4.5	3.3±4.7
1/4000 n=5	13.5±7.4	69.4±9.7	0	25.5±10.9	2.5±5.6	18.2±12.3	54.5±6.6	15±10	7.6±2	10.5±6.5	17.7±5.5	9±6.4	5.8±7.2
1/8000 n=9	38.3±26.3	91.6±14.7	2.9±4.1	43.2±25	12±2.6	15.2±12.3	58±6.8	20.8±21.9	8.7±5.7	10.6±6.4	18±4.7	6.9±4.3	3.7±4.8
1/16000 n=10	25.3±24.6	73.2±30.8	7.3±10.3	40±20.6	15±17	30.8±14.4	63.3±12.3	22.5±18.5	9±6.3	9.15±6.3	19.3±6	9.9±4.4	6.7±5
1/32000 n=6	60.5±20.4	100	7.2±5.3	70.4±8.6	52.7±22.7	52.2±29.8	86.4±8.6	58.5±20.6	19.3±4.8	14.2±5.3	22.8±5.3	6.7±3	4±4.1
1/64000 n=9	67.6±25	97.3±5.5	15.3±8.3	77.6±22.7	63.8±20	74±25.2	87.8±12.3	63.8±19.8	22.6±2.9	13.4±3.8	21.9±6.5	8.5±4.4	6±4.4
1/128000 n=12	76.9±20.5	100	19.2±8.2	86.4±18.5	76.5±14.8	90.6±11.8	96.4±3.4	75.5±20.2	22.8±6.8	18.1±6.6	34.3±8.7	12.3±8	14±7.3
1/256000 n=8	83.5±16.8	100	24.1±4.6	88.3±17.8	88.1±12.8	95.8±8.6	98±3.3	88.3±10.5	31.8±6.5	22.1±5	35.5±6.7	10.9±3.7	18.7±4.9
1/512000 n=10	81.1±14.3	100	25.6±8.6	81.8±2.5	92±12.9	96.8±5.8	95.6±2.5	90.3±8.2	34.2±9.3	24.5±9.3	35.4±15.7	14.4±6.5	23.1±7.1

The inhibition curves of individual sera with mAb 9 and mAb 2 (region A) and mAb 15 and mAb 59 (region B) showed that TPO aAbs reactive to mAb 9 epitope are a major fraction of total TPO aAbs. The $ID_{50\%}$ of mAb 9 binding to TPO could be detected at aAbs concentration 10 to 15 fold lower as compared to mAb 15, and 3 to 10 fold lower than for mAb 2 and 59. In summary, the data indicate that TPO aAbs response in AITD patients is highly restricted and predominantly directed to one "epitope" mapped by mAb 9. The majority (80-90%) of TPO aAbs were reactiv to this particular epitope.

154. P8 **INTERACTIONS OF THYROCYTES ISOLATED FROM TOXIC AND EUTHYROID GOITERS WITH ANTITHYROID AUTOANTIBODIES.**

V.I. Kandror, S.I. Krajnova, N.Yu. Sviridenko and E.N. Bazarova, Endocrinology Research Center, Academy of Medical Sciences, Moscow, Russia.

Interactions of thyrocytes isolated from tissue of diffuse toxic and paranodular tissue of euthyroid mononodular goiters ("thyrotoxic cells" and "normal cells", respectively) with blood sera of patients with autoimmune thyroid diseases (Graves' disease and Hashimoto thyroiditis) have been studied. It was found that in the presence of complement 40% of Graves' patient sera having a cytotoxic effect on normal cells did not produce such an effect on thyrotoxic cells. The cytotoxic effect of Hashimoto patient sera did not depend on source of the cells. The binding of antibodies present in tested sera by immobilized thyrotoxic cells was significantly less ($p < 0.001$) than by immobilized normal cells. This difference was not due to initial occupation of thyrotoxic cell surface by corresponding antibodies. The preliminary experiments with preincubation of normal cells with TSH and T3 allow us to suggest that cell stimulation and thyroid hormone excess *per se* might cause a certain reduction of thyrocytes interactions with antithyroid autoantibodies.

155. P9 THYROID ANTIBODY PREVALENCE IN SCHOOLCHILDREN: GEOGRAPHICAL DISTRIBUTION AND RELATIONSHIP TO ENDEMIC GOITER IN THE MEDITERRANEAN ISLAND SARDINIA.

A. Loviselli, M.A. Cambosu, F. Velluzzi, P. Mele, A. Balestrieri, S. Mariotti. Endocrinology, University of Cagliari, Cagliari, Italy.

The epidemiology of thyroid autoimmunity in children is poorly known, as well as its relationship with endemic goiter. In the present study we report thyroid antibody prevalence in relation to endemic goiter and thyroid function in 6507 schoolchildren aging 7-14 yr. Survey was carried out in 25 towns or villages from central and southern Sardinia, including several areas with mild to moderate iodine deficiency. Goiter was assessed by palpation and confirmed by echography; antibody tests included anti-microsomal antibody (anti-M) until 1990 and anti-thyroid peroxidase antibody (anti-TPO) by RIA thereafter. Anti-M and anti-TPO titers ≥1/400 and ≥200 U/ml were respectively considered as positive tests. Data from three main geographic areas (provinces of Nuoro [NU], Cagliari [CA] and Oristano [OR]) were grouped together. As shown in the Table, there was a progressive increase of positive antibody prevalence form NU to CA to OR (covering eastern, southern and western areas, respectively), with the highest prevalence in OR. Individual "hot spots" of thyroid antibody prevalence were also observed in some villages throughout the 3 provinces. Interestingly, a similar behavior has recently been described in the same Sardinian regions for islet cell antibody prevalence. Thyroid antibody was unrelated to goiter and to the occasional observation of borderline increased serum TSH.

Province	Towns or villages (n.)	Children examined (n.)	Goiter prevalence (%)		Anti-M(TPO) prevalence (%)		Serum TSH 5-10 U/L (%)	
			mean	range	mean	range	mean	range
NU	12	1755	25.6	17-48	2.1	0-7.3	0.72	0-7.0
CA	7	3059	15.7*	11-27	3.4	0.7-5.0	1.1	0-6.7
OR	6	1693	23.4	13-49	4.9**	4.1-8.7	1.3	0-7.9

* $p<0.0001$ vs NU and OR; ** $p<0.0001$ vs NU (by $\chi 2$ test)

In conclusion, this large survey discovered wide areas as well as small "hot spots" with a relative increase of thyroid antibody prevalence in Sardinia schoolchildren. Thyroid antibody and goiter prevalence were unrelated, suggesting the involvement of genetic and/or environmental factors independent from iodine deficiency in triggering autoimmunity. Thyroid function was generally well preserved in this age range, but follow-up longitudinal studies are needed to ascertain the proportion of antibody-positive children subsequently developing thyroid function abnormalities.

156. P10 CLINICAL FEATURES AND THYROID FUNCTION OF AUTOIMMUNE THYROIDITIS WITH ISOLATED ANTIBODIES AGAINST THYROGLOBULIN OR THYROID PEROXIDASE. N.Kobe, J.Takamatsu, S.Yoshida, Y.Yamano, A.Fukao and K.Hirai. First Department of Medicine, Osaka Medical College, Takatsuki, and the Kuma Hospital, Kobe, Japan

Patients with autoimmune thyroiditis (AIT) commonly have antibodies against both thyroglobulin (TGAb) and thyroid peroxidase (TPOAb), but some patients are noted to have only TGAb or TPOAb in their serum. This study aimed to investigate the characteristics of clinical features and thyroid function in patients with AIT who possess only TGAb or TPOAb. The levels of the antibodies in serum were determined by newly developed kits: TGAb by RIA (RSR Ltd., UK), and TPOAb by EIA utilizing the recombinant TPO (Nissui Pharmaceutical, Japan), both remarkably high in not only sensitivity but also specificity. Among 401 patients with AIT, 315 patients (79%) had both TGAb and TPOAb (group A), 75 patients (18%) had isolated TGAb (group B), and 11 patients (3%) had isolated TPOAb (group C). The data of randomly selected 40 patients from group A and 40 patients from group B, and all 11 patients from group C were compared.

Age and female/male ratios were not different among the three groups of patients. The prevalence of familial predisposition of AIT were also not different among the three groups. The volume of thyroid measured by ultrasonography in groups A and B were 42 ± 29 ml(mean $\pm$ SD) and 57 ± 42 ml, respectively, and smaller in group C as 27 ± 16 ml($p<0.05$). The association of nodular changes, most of which were adenomatous nodules, in group A,B and C was found in 9%, 53%(greater for $p<0.01$), and 20%, respectively. The frequency of low echogenecity in group A,B and C was 69%, 26%, and 0%, respectively. The incidence of hypothyroidism was 58%, 23%, and 45%, respectively.

In conclusion, patients with AIT with isolated TGAb frequently have adenomatous nodule, and hypothyroidism is less frequent. The incidence of AIT with isolated TPOAb is extremely rare, and the affected patients usually have smaller goiters and hypothyroidism probably due to the in vivo activity of TPOAb.

157. P11 THYROID PEROXIDASE (TPO.Ab) AND THYROID ENLARGEMENT IN BREAST CANCER

PPA Smyth , DF Smith , A. Zbar, MJ Murray, EWM Mc Dermott & NJ O'Higgins. Endocrine Laboratory, Depts of Medicine & Surgery, University College Dublin, & St. Vincent's Hospital, Dublin, Ireland.

Although evidence of extremes of thyroid dysfunction increasing breast cancer risks is difficult to obtain, the impression persists of some link between the two. An association between breast cancer and Hashimotos Thyroiditis has been proposed although this is disputed. In the present study antithyroid peroxidase antibodies (TPO.Ab) were measured using a highly sensitive direct radioimmunoassay in 150 consecutive patients with breast cancer and 50 with benign breast disease (BBD). Thyroid volume was measured by ultrasound using a 7.5mHz linear transducer. Volumes of > 18.0ml were termed enlarged. Of those with breast cancer, 2 were hyperthyroid, 5 hypothyroid and 16 had nontoxic palpable goitres. Patients with BBD included 3 hyperthyroids, 1 hypothyroid and 4 with nontoxic goitre. TPO.Ab were present at titers of 0.4 -25.1 U/ml MRC Standard 65/93 (normal reference < 0.3 u/ml) in 54/150 (36.0%) of patients with breast cancer and were also present in 17/50 (34.0%) of those with BBD (range 0.4-147.0U/ml) compared to 14.8 % of a control female population. Thyroid volume measured by ultrasound was enlarged (>18.0ml) in 56/150 (37.3%) of patients with breast cancer and in 13/50 (26.0%) of patients with BBD. This compares to thyroid enlargement of between 6.3 and 8.6% in age-matched female control populations. The association between TPO .Ab positivity and thyroid volume showed that in Breast Cancer 27/56 (48.2%) of those with enlarged volumes were TPO.Ab positive compared to 27/94 (28.7%) of those with normal volumes ($p<0.01$). This finding was even more pronounced in BBD in which TPO.Ab were positive in 9/13(69.2%) with enlarged thyroid volumes compared to 8/37(21.6%) with normal volumes ($p<0.005$).The demonstration of a high prevalence of TPO.Ab positivity, as well as thyroid enlargement, in patients with breast disease suggests an apparent underlying autoimmune thyroid disturbance. This finding provides strong evidence for an association between abnormal breast and thyroid cellular growth stimulation in the study groups which may reflect the presence of common growth stimulators or receptor sites.

158. P12 DIFFERENTIAL REGULATION OF GENE EXPRESSION IN THE SAME CELL BY THE INTERACTION OF THYROID HORMONES WITH THE THYROID RECEPTOR β1 (TRβ1).

Puymirat Jack and J.H. Dussault. Department of Medicine and Molecular Genetics, CHU Laval Research center, 2705 Blvd Laurier, Ste-Foy, Quebec, Canada.

Cellular responsiveness to thyroid hormones is conferred through specific nuclear receptors. Studies of thyroid hormone-responsive genes have demonstrated multiple levels of control, including rate of transcription, regulation of mRNA stability, and regulation of protein half-life. In the present study, we propose to determine whether thyroid hormones regulate gene expression at different levels in the same cell by the interaction with one TR isoform. To answer this question, we used neuroblastoma cells that overexpress the TRβ1 (Lebel et al., Proc. Natl. Acad. Sci, 1994, 91:2644) and we studied the effect of 3,5,3'-triiododothyronine (T3) on the level of expression of the transcription factor NGFI-A, and acetylcholinesterase (AChE) genes, both genes are regulated by T3 in the brain.

T3 treatment of these cells increased by 3-fold the levels of NGFI-A mRNA after 6 hr of treatment with maximal effect occuring after 24 hr of treatment. The effect of T3 is inhibited by the simultaneous treatment of cells with T3 and actinomycine D (5 μg/ml) suggesting that this effect occurs at the transcriptional level. The trancriptional effect of T3 on NGFI-A was further demonstrated by run-on measurements with nuclei isolated from cells maintained for 1 and 24 hr in the presence of T3. T3 increased by 3-fold the rate of transcription of NGFI-A gene after 1 and 24 hr of treatment which is in accord with the changes in mRNAs levels. No effect of protein-serine kinase inhibitors (H7), protein-tyrosine kinase inhibitors (Genistein), and phosphatase inhibitors (okadaic acid OA, vanadate) was observed on the effect of T3 on the rate of transcription of NGFI-A gene, indicating that phosphorylation events are not involved in this effect. On the other hand, T3 treatment of these cells with T3 increased AChE activity and its mRNA after a lag period of 24 to 48 h, and these levels increased through stabilization of the transcripts by T3. T3 had no effect on the transcriptional rate and the processing of AChE gene. H7 inhibited the T3-induced accumulation in AChE activity and its mRNAs, whereas OA potentiated the effect of T3. No effect of H7 and OA was observed on the transcriptional rate of the AChE gene. Finally, treatment of cells with T3 stimulated cytosolic serine/threonine but not tyrosine kinase activities, and this effect precedes the effect of T3 on AChE mRNAs. No effect of T3 on NGFI-A and AChE gene expression was observed in cells that overexpress the TRα1.

These results indicate that T3 controls the expression of different genes at different levels in the same cell through an interaction with a specific TR isoform.

159. P13 ASSESSMENT OF DYSLEXIA IN SUBJECTS WITH RESISTANCE TO THYROID HORMONE.

P Hauser, F Brucker-Davis, E Wiggs. *Baltimore VA Medical Center and University of Maryland School of Medicine, Baltimore, MD, and National Institutes of Health, Bethesda, MD, USA.*

We have previously reported that subjects with resistance to thyroid hormone (RTH), an autosomal dominant disease caused by mutations in the human thyroid receptor-beta gene (hTRß) on chromosome 3, had a 10-fold higher risk of attention deficit hyperactivity disorder (ADHD) than their unaffected family members. Furthermore, the mean IQ scores of subjects with RTH were over 10 points lower than those of unaffected family members for verbal, performance and full scale IQ, as well as for reading and arithmetic achievement tests. However, few subjects with RTH had a full scale IQ score under 70, the upper limit for a diagnosis of mild mental retardation.

In the present study, we systematically assessed our RTH families for the presence of dyslexia and slow learning, common learning disabilities of childhood, using validated criteria defined by Fletcher and Shaywitz. Intelligence was evaluated using the age-appropriate Wechsler scale and Wide Range Achievement Test. Subjects were diagnosed dyslexic if their reading achievement test score (actual ability) was 22 IQ points less than either their verbal or performance IQ score (intellectual resources). However, if the verbal or performance IQ score was less than 80 then the subject was considered to be a slow learner and therefore not diagnosed dyslexic. The study sample consisted of 69 RTH affected subjects and 54 unaffected family members. The two groups did not differ in age or gender ratio.

Nineteen percent (13/69) of RTH subjects compared to 19% (10/54) of unaffected family members met criteria for dyslexia (p=n.s.). However, 33% (23/69) of RTH subjects compared to 13% (7/54) of unaffected family members were slow learners (p=0.01). Fifty-nine percent (41/69) of RTH subjects compared to 19% (8/54) of unaffected family members met criteria for ADHD (p=0.001).Among RTH subjects with dyslexia, 85% (11/13) had a co-diagnosis of ADHD, whereas among unaffected family members with dyslexia, only 20% (2/10) had a co-diagnosis of ADHD (p=0.01). Although language articulation disorders have been reported to be significantly more common in subjects with mutations in exon 9 than in those with exon 10 of the hTRß gene, in the present study the frequency of dyslexia was the same for both groups. Since affected and unaffected family members have similar environmental and genetic backgrounds except for a mutant hTRß gene, our data suggest that RTH is not associated with dyslexia, but is associated with the diagnosis of slow learning and is specifically associated with ADHD. Furthermore, the data emphasize the importance of systematically assessing poor school performance in children with RTH in order to ensure appropriate treatment.

160. P14 **DETECTION OF WIDESPREAD DISTRIBUTION OF β2 THYROID HORMONE RECEPTOR mRNA IN RAT BRAIN USING cRNA IN SITU HYBRIDISATION HISTOCHEMISTRY.**

M. Li, S.C. Boyages. Department of Endocrinology, Westmead Hospital, Westmead 2145, Sydney, Australia.

The developmental effects of thyroid hormone are mediated through specific thyroid hormone receptors (TR) and in the rat, 4 c-erbA related cDNAs (α1, α2, β1, β2) have been described. Initial studies using Northern blot analyses suggested restricted expression of the TRβ2 mRNA to the anterior pituitary, but subsequent PCR and in situ hybridisation (ISH) studies revealed limited expression in selected brain areas. Recent studies using antibodies directed against the TRβ2, however, have demonstrated widespread distribution of immunoreactive TRβ2 in extrapituitary rat tissues, including brain. The reasons for the detection of substantial levels of TRβ2 protein with a poor ability to detect mRNA by Northern blot analyses remain poorly understood. The aim of this study was to determine whether using a β2 specific cRNA probe, directed at the entire length of the β2 specific region (-56bp to 495bp), to perform ISH would improve detection of TRβ2 mRNA in adult rat brain. A secondary aim of the study was to identify selected areas of rat brain that express TRβ2, which should provide clues as to the direct regulatory role of TRβ2 gene products in brain development. Fifteen micron coronal sections of the rat brain, through telencephalon, diencephalon, cerebellum and brain stem, were cut on a cryostat and collected on gelatine coated slides. cRNA probes were created by inserting the TRβ-2 specific segment at the XbaI site of pGEM3Z. ^{35}S-UTP labelled cRNA probes were transcribed from the templates with T7 (antisense) or SP6 (sense) RNA polymerase. *In situ* hybridisation using cRNA probe was based on the procedures of Fremeau et al (1986); sections were apposed to Hyperfilm βmax and exposed for 1 week. We found widespread distribution and intense expression of TRβ2 mRNA in diverse areas of grey matter, including hippocampus (dentate gyrus and C1,2,3), cerebral cortex (predominantly layer 3), arcuate nucleus, medial geniculate nucleus, tegmental bundle, medial lemniscus, Purkinje layer of the cerebellum, and several brain stem nuclei. In conclusion, we have developed a highly sensitive and specific method to demonstrate TRβ2 mRNA expression in adult rat brain. The present findings are in agreement with immunoreactive TRβ2 studies of rat brain, and argue against the presence of an unidentified T3-binding protein to explain the previous discordant results of TRβ2 mRNA and protein studies. In addition, the specificity of distribution of TRβ2 to certain brain nuclei, particularly those involved in hearing, implies a specific functional role of this receptor and provides a physiological basis to understand the effects of hypothyroidism on brain development.

161. P15 MATERNAL-NEONATAL IODINE DEFICIENCY DECREASES MYELIN BASIC PROTEIN (MBP) IMMUNOREACTIVE STRUCTURES IN THE STRIATUM (STR) WHICH MIGHT AFFECT CORTICAL-SUBCORTICAL PROJECTIONS. JR Martinez-Galan §, P Pedraza #, M Santacana-Altimiras §, F Escobar del Rey#, G Morreale de Escobar#, A. Ruiz-Marcos §. Cajal Institute (Dr. Arce 37) § ; Instituto de Investigaciones Biomédicas (Arturo Duperier 4) #, CSIC & UAM. Madrid, Spain.

In man severe maternal iodine deficiency results in neurological damage of the progeny, involving the STR, and due to com bined maternal-fetal low T4, leading to low fetal brain T3. Serum T4 is very low in neurological cretins, but T3 is normal and prevents clinical hypothyroidism. Term fetuses from rats fed a low iodine diet (LID) have low cerebral T4 and T3, but during lactation brain T3 of the pups is almost normal. This is due to a small increase of iodine availability through milk, which slightly improves serum and brain T4 (1). This neonatal period corresponds to phases of brain development which in man occur in utero, when brain T3 is low. To avoid normalization of brain T3 in rat pups, we supplemented LID with trace amounts of KClO4 (0.005 % of diet) which interferes with the uptake of small amounts of iodine by the mammary gland, and , presumably, with its increased supply through milk (3).

Normal Wistar rats were fed pellets (C group), LID with 10 µg I / day (group I), LID alone (group II), or LID + 0.005% KClO4 (group III) for 3 months before mating. Pups were killed 10 days after birth, to measure T4 & T3 in tissues (1), or perfused for fixation. The STR region, at the anterior commissure level, was cut in 50 µm sections and immunocytochemically processed using Myelin Basic Protein (MBP) as primary antibody, the superficial density (S. Dens.) of MBP-positive processes being quantified (3) in the dorso-lateral (DL), ventro-lateral (VL), dorso-medial (DM) and ventro-medial (VM) STR. The S. Dens. (and myelination) was higher in the lateral as compared to the medial STR regions, a result consistent with the fact that the cortico-subcortical bundel of fibers crosses the STR laterally. Results in the C and I groups were similar and pooled (C+I), and compared to groups II and III . See the Table for the DL and VL regions, and liver and brain T4 and T3. Results for the DM and VM were similar. MBP-positive processes were markedly decreased in group III, both versus group II and C+I; they also decreased in pups on LID alone (VL), as compared to C+I. The decrease was related to that of brain T3. Concentrations of T3 did not decrease in other tissues, and the body weight and appearance of the pups was similar to that of the C+I euthyroid pups.

Marked maternal-neonatal iodine deficiency affects myelination of STR structures, including important cortical-subcortical fibers. even if pups as a whole are not T3 deficient . Experiments are in progress to determine whether these alterations are irreversible.

Group	Liver (ng/g) T4	T3	Brain (ng/g) T4	T3	DL S. Dens.	VL S. Dens.
C+I	16.3	1.9	4.4	3.7	91	48
II (LID)	11.8 a	1.7	1.8 a	3.3	68	21 a
III- LID+KClO4)	5.0 a,b	1.6	0.6 a,b	2.2 a,b	22 a,b	4 a,b

a Difference significant versus C+I; b significant versus II (ANOVA)

Grants: FIS 93/0160 and FIS 92/0888.

1) Escobar et al. Endocrinology 121:803, 1987; 2) Ben Chaouaha-Chekir et al. Path Biol 31:675,1983.3) Weibel E.R. Stereological Methods. Vol 1. p. 109. AcadPress. 1979.

162. P16 EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR m-RNA AND PROTEIN IN HUMAN THYROID NEOPLASIA. E.Y.Soh, Q.Y.Duh, M.G.Wong, D.M.Young, H.D. Epstein, R.F*. Grossman, S.A. Sobhi, A.E. Siperstein and O.H. Clark, Surgery and Pathology* Service, UCSF/Mount Zion Medical Center and Veterans Affairs Medical Center, and Department of Surgery and Pathology University of California, San Francisco, California

Several factors control angiogenesis. Vascular endothelial growth factor (VEGF) is a endothelial specific mitogen that induces endothelial cell proliferation and capillary hyperpermeability. Thyroid cancers, especially follicular carcinomas, are vascular tumors and metastasize via the blood vessels, whereas papillary thyroid cancers usually metastasize via the lymphatics. Expression of VEGF has not been previously demonstrated in thyroid tumors. We investigated the presence of VEGF m-RNA and protein in thyroid cell lines (FTC-133, FTC-236, FTC-238, TPC-1, XTC-1, MTC-1.1, MTC-2.2 and FRTL-5), and in human thyroid tissues (papillary thyroid cancer, follicular thyroid cancer, medullary thyroid cancer, Hurthle cell carcinoma, anaplastic cancer, follicular thyroid adenoma, multinodular goiter, Hurthle cell adenoma and Graves' disease one each) by Northern blotting, immunohistochemistry and ELISA. For Northern blotting, total RNA were isolated from each thyroid cell line and from normal and tumor tissue from the same patients. The human VEGF cDNA insert from the pBluescript II KS was labelled with ^{32}P dCTP to a specific activity of 1.5 x 10^6 cpm/ml DNA using the *redi*Prime DNA labeling system. For immunohistochemistry, paraffin blocks or frozen section tissues were used. Cell lines were grown on chamber slides and fixed with acetone. Immunohistochemistry was performed using affinity purified antibodies to human VEGF peptides and an avidin-biotin peroxidase method.

Results: Messenger RNA encoding VEGF was detected in all thyroid cell lines and tissues studied. VEGF protein were found in the cytoplasm of thyroid cells and vessels. VEGF protein expression was higher in the malignant thyroid tissues than the benign thyroid tissues and also higher in the tumor tissue than the adjacent normal tissue from the same patients.

Conclusion: These studies document that VEGF is present in normal, hyperplastic and neoplastic thyroid tissues. The presence of increased VEGF in neoplastic thyroid tissue suggests that it may play an important role in thyroid tumor growth and will enable clinical correlative studies with cellular proliferation, invasiveness, and potential for anti-VEGF or angiosuppressive therapies.

163. P17 mRNA EXPRESSION OF RBI 1 - AN IODINE DEFICIENT RAT BRAIN SUBTRACTED CLONE. R KHER, N CHATTOPADHYAY, A MANDAL, J VIRMANI, P JAISWAL, A MITHAL, MM GODBOLE. SANJAY GANDHI PG INSTITUTE OF MEDICAL SCIENCES, LUCKNOW 226 014, INDIA. *NATIONAL BOTANICAL RESEARCH INSTITUTE, LUCKNOW 226 001, INDIA.

We have recently used a membrane immobilized subtraction procedure to isolate a putative thyroid hormone responsive cDNA. Euthyroid rat brain (EUT) `tester' cDNA was used for hybridization with `driver' RNA from iodine deficient rat brain (ID) and the subtracted cDNA was cloned in pBluescript KS(+). One of the subtracted clones designated as RBI 1 (Rat brain iodine deficient derived clone) shows >50% reduction of mRNA expression in ID RNA as compared to EUT RNA in a dot blot hybridization system. Northern analysis with RBI 1 in developing rat brain (fetal day 19 to post natal day 11) demonstrates two major transcripts of 4.0 and 2.0 kb size, which remained unaffected by age and thyroidal status. However, a minor 1.3 kb low abundance transcript is expressed in a developmental pattern from fetal day 19 to post natal day 3, whereafter the transcript is not detected in EUT brain. However, this transcript is not detected in ID brain at all the ages studied. The two major transcripts encoded by RBI 1 are expressed in various EUT brain regions as well as other non-brain tissues viz., heart, liver, kidney and lung. The 1.3 kb transcript is widely expressed in various brain regions, but its expression is blunted/abolished in other non-brain tissues. Southern hybridization analysis reveals a single gene encoding the three transcripts, generated probably through an alternate splicing mechanism. In conclusion: 1) the ubiquitous presence of the two major transcripts all through the study point to thyroid hormone-independent expression of these transcripts; 2) non-detection of the brain-specific minor transcript in ID tissues may indicate thyroid hormone-dependent expression of this low abundance class transcript; and 3) developmental factors may be responsible for the abolished expression of the minor transcript beyond day 3 of post natal life.

[This work is a part of Ph.D. thesis of Rajesh Kher; supported by a grant from DST, INDIA (SP/SO/B-66/88)]

164. P18 EVIDENCE THAT TYPE III DEIODINASE IN RAT ASTROCYTES IS A SELENOPROTEIN. F. Courtin, M. Ramaugé, S. Pallud, J-M Gavaret, A-M Lennon and M. Pierre. INSERM U-96, 80 rue du Gal Leclerc - 94276 Le Kremlin-Bicêtre cedex, France.

Whereas type I deiodinase has been identified as a selenoprotein, the question as to whether the type III deiodinase (D-III), the enzyme responsible for thyroid hormones inactivation, is also a selenoprotein, remains open. The cerebrocortical D-III is decreased in selenium deficiency. The recent identification of a D-III from tail of Xenopus Laevis, as a selenoprotein led us to examine the effects of selenium on D-III of rat astrocytes.

Astrocytes were obtained from cerebrocortical hemispheres of 2-day old rats, cultured to confluence with 10 % fetal calf serum and further cultured in DMEM / Ham's F-12 changed every day. D-III activity was measured on cell sonicates. During selenite depletion, the TPA-induced D-III was three to ten-fold reduced in about 4 days. 30 nM selenite addition increased TPA-induced D-III as soon as 2 hours (two-fold stimulation). Maximal induction of D-III (reaching ten-fold stimulation) was observed after 6 hours treatment with selenite. The effect of selenite was not observed in the presence of cycloheximide (5 µg/ml). Similar effects of selenite addition were observed when D-III was induced by cAMP, FGF, retinoic acid or T_3. These effects of selenium on D-III in rat astrocytes strongly suggest that D-III in rat astrocytes is a selenoprotein as the D-III from tail of Xenopus Laevis.

165. P19 3,3',5-TRIIODOTHYRONINE EFFECTS ON ASTROCYTE DIFFERENTIATION DURING BRAIN DEVELOPMENT. F. Lima, A. Trentin, D. Rosenthal, C. Chagas and V. Moura-Neto; Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

· T3 induces "in vitro" differentiation of astrocytes derived from developing rat cerebral hemispheres, mesencephalon and cerebellum. Astrocytes from embryonic (E18), newborn (P0), and post-natal (P10) rat brains were cultured until confluence in DMEM-F12, containing 10% fetal calf serum. The medium of the confluent culture was changed to DMEM-F12 without FCS for 3 days and then to T3 (50 nM) containing medium or conditioned medium (CM), again for 3 days. These media were changed daily except after the third T3-treatment (or CM) day. Conditioned media (CM) were collected from some cultures 4-5 days after the last T3-containing medium change. T3 treatment produced morphological changes in E18 and P0 astrocytes from cerebral hemispheres and mesencephalon, inducing the appearance of long processes, and a decrease in the cell body of the formerly flattened astrocytes. T3 treatment did stimulate cerebellar P0 astrocyte proliferation but did not produce any morphologic changes. However, cerebellar P10 T3-treated astrocytes did change in morphology, showing short stubby processes, as well as long ones similar to those seen in the other cerebral cultures. The astrocyte cultures show similar morphological changes when conditioned media is used instead of T3-containing media, but they appear 1-2 days earlier. A more than two-fold increase in the expression of glial fibrilar acidic protein (GFAP), a specific astrocyte intermediate filaments component, was found by immunoblotting in protein extracts from all P0 astrocytes that changed form in response to T3 treatment; GFAP expression changes were inexpressive in cerebellar proliferating T3-treated P0 astrocytes. Our results show that astrocytes are not homogeneous in respect to their responsiveness to T3. Furthermore, the fact that CM produces a similar response to T3, but in less time, suggests that the T3-treatment might induce secretion of growth factors, as well as synthesis of GFAP, by the astrocytes. The growth factor(s) might then, by autocrine effect, induce astrocyte growth and differentiation in the developing brain.

Supported by FINEP, CNPq and CEPG/UFRJ.

166. P20 BATCH TESTING OF NEWBORNS IN EIGHT ASIAN CAPITAL CITIES REVEALS A MARKED SHIFT IN THYROTROPIN LEVELS TO HIGHER LEVELS. G.F. Maberly, W. May, R. Houston, F. van der Haar, and K. Sullivan. Rollins School of Public Health, Emory University, Atlanta, GA, USA.

Since the World Summit for Children, New York 1990 where more than 70 Heads of State declared elimination of iodine deficiency by the year 2000 as a priority public health problem, much progress has been made towards national salt iodization. A common misconception is that iodine deficiency affects only remote, rural, obviously goitrous populations. While goitre may be the most visible evidence of iodine deficiency, this clinical sign is just the "tip of the iceberg" of the consequences. These include; lower cognitive capacity, increased fetal and infant mortality, poorer growth and birth defects. This misconception has led to attempts to target interventions, such as iodized salt to only the areas thought to be affected.

This study was conducted to evaluate newborn TSH testing as a method to assess IDD in urban populations. TSH levels among newborns in hospitals in eight Asian cities (Kuching, Manila, Islamabad, Quetta, Lahore, Karachi, Bishkek and Osh) from four countries (Malaysia, Philippines, Pakistan and Kyrgyzstan) with known iodine deficiency problems were determined using sensitive whole blood assays. Results found prevalences of high TSH (>5mU/L serum units) ranging from 32-80% compared to a prevalence of 3% usually found in iodine replete areas. According to WHO population standards, urinary iodine levels in school children living in these cities were low, as were levels in the pregnant women prior to birth. In some cities, however, goitre rates in school children were only 4%, less than the WHO cut point for iodine deficiency as a public health problem. These findings suggest that the developing brains of newborns are unprotected from the detrimental effects of iodine deficiency in these Asian cities. These findings also suggest that the nature and magnitude of the iodine deficiency problem has often been seriously underestimated. These results, along with other information, support the importance of directing intervention efforts to both urban and rural populations. Collection of blood samples dried onto filter paper from a small number of newborns for TSH analysis provides a method to rapidly assess the prevalence of iodine deficiency and can be used for advocacy purposes to promote and sustain elimination programs.

167. P21 PSYCHOEDUCATIONAL OUTCOME IN CHILDREN WITH EARLY-TREATED CONGENITAL HYPOTHYROIDISM. J. Rovet, R. Ehrlich, D. Altmann, The Hospital for Sick Children, Toronto Ontario, Canada.

Newborn screening for congenital hypothyroidism (CH) is successful in preventing mental retardation. However, children so identified who are treated early and adequately are still at risk for subsequent subtle language, neuromotor, and cognitive impairment. Poorer school performance is often reported. We describe here our findings with 52 third grade and 42 sixth grade children with CH, who are being followed in a long-term prospective study of outcome following screening. Children were assessed on multiple tests of reading, spelling, writing and arithmetic achievement as well as class behavior ratings by teachers. Classmates (27 grade 3, 49 grade 6) served to control for teaching effects and siblings (18 grade 3, 20 grade 6) for family background factors. Results at grade 3 indicate that CH were significantly outperformed in Arithmetic and Reading Comprehension but not in Spelling, Writing, or Mechanical Reading. There were fewer differences at grade 6. Parents and teachers (blinded to CH child) both reported greater inattention. CH was associated with an increased risk of severe but not mild learning disability. Poorer skill in language arts was associated with more severe and longstanding hypothyroidism whereas poorer arithmetic and attention were associated with higher levels of T4 at time of testing. Implications of early diagnostic and treatment methods as well as later management for optimal school functioning will be discussed.

168. P22 HYPERPLOLACTINAEMIA AND PITUITARY LESIONS IN PRIMARY HYPOTHYROIDISM.

T.Tchernova, V.Kandror, N.Goncharov,
National Endocrinology Research Centre, Moscow, Russia.

The main objective of our research was to analyze groups of patients with primary hypothyroidism (PH) by comparing those with the presence or absense of coexistent hyperprolactinaemia (HP).We have examined 30 women, aged 16-45: twenty (group A) had PH without HP and ten (group B) had PH with HP .The structural changes of the pituitary (enlargement,microadenomas) were found on CT and MRI scans in 15% of patients of group A and in 80% in the patients of group B (p<0,01). One patient of group B showed an "empty sellae". Patients of both groups were of the same age, had equal basal TSH level and duration of the disease. During the functional test with metoclopramide(MCP)(2 ml.,iv), the maximal relative increase of the PRL level from the basal value was most marked in group A, where the increase was 11-fold in comparison with 7,8-fold in control group.In group B the PRL level increased by 2,9-fold from the basal level. Therefore, we suggest that these two groups vary in the intensity of dopaminergic reaction to lactotrophes. In group A this effect was apparently higher than in group B. Thus, we conclude that such factors as duration of the disease and basal TSH level do not affect the presense of coexistent HP.This fact as well as the results of MCP test show, that both forms of PH (with or without coexistent HP) are of different pathogenetic entities. HP in PH is often associated with the existence of pituitary lesions (enlargement, microadenomas or "empty sellae" syndrome).

169. P23 **IODISED OIL IMPROVES BRAIN FUNCTION IN EUTHYROID IODINE DEFICIENT CHILDREN.**

P.Mirmiran,A.Sarshar,M.Nafarabadi and M.Kimiagar. Endocrine Research Center, Shaheed Behesthi University of Medical Sciences, Tehran, I.R. Iran.

In order to evaluate effects of iodised oil on various parameters of iodine deficiency disorders, 139 schoolchildren aged 6 to 14 years of a village near Tehran were randomly divided into control (C) and iodised (I) groups. Group I (n= 69), received 1ml containing 480mg iodide (Lipiodol) and group C (n=70) were given 1m saline. Grades of goiter (WHO classification) and serum T4, T3, TSH and RT3 uptake were measured at 0, 1, 2, 3 and 4 years after intervention. Psychomotor age was estimated before and at 3 years by Bender Gestalt test, IQ was estimated before and at 5 years by Raven test, and hearing threshold was assessed before and at 3 years by pure tone audiometer. Urinary excretion of iodine was 21.0±11.2 μg per gm of creatinine before, increasing to high levels after injection in I and reaching 98±21 by 3 years. There was gradual decrease in severity of goiter (grad 2 from 10 to 7 and grade one from 72 to 58%) in I; while in C grade 2 increased from 3 to 29% by 4 years. In I serum T4 was 8.3±1.9, 10.0±3.4, 11.2±2.0, 10.0±2.2 and 8.4±2.1 μg/dl, before and 1, 2, 3 and 4 years after injection. Corresponding TSH values were 3.0±1.5, 1.5±0.9, 1.0±0.9, 1.0±0.8, 1.5±1.2 μU/ml respectively. There were no significant changes in T4 and TSH in C. Serum T3 and hearing threshold did not change in either groups. Differences between psychomotor and chronological ages were -2.2±1.8 and -2.0±1.2 (NS) before, and -2.4±1.9 and -3.4±1.9 years (P<0.05) at 3 years in I and C respectively. IQ was 97±9 and 97±6 before and 111±8 and 98±11 (P<0.001) at 5 years in I and C recspetively.

It is concluded that iodised oil injection while decreasing goiter severity and increasing thyroxine production, may improve IQ and prevent further psychomotor dysfunction in apparently normal and euthyroid iodine deficient schoolchildren.

170. P24 INHIBITION OF TYPE II-DEIODINASE BY OXYDATIVE STRESS IN RAT ASTROCYTES.

S. Pallud, M. Ramaugé, A-M Lennon, F. Courtin, M. Pierre and J-M Gavaret.. INSERM U-96, 80 rue du Gal Leclerc - 94276 Le Kremlin-Bicêtre cedex, France.

Thyroid hormones are essential for a normal development and function of the central nervous system. T_3 is the active form of thyroid hormones locally produced by 5'-deiodination of T_4 catalyzed by type II deiodinase (D-II). Since the oxydative stress is suspected to play a role in some brain pathologies (Ischemie-reperfusion, Alzheimer, Parkinson, HIV..), we asked if oxydative stress could affect thyroid hormones metabolism in the brain. In a first investigation, we examined if oxydative stress had an effect on D-II in astrocytes.

Astrocytes in culture were obtained from cerebrocortical hemispheres of 2-day old rats, cultured to confluence with 10 % fetal calf serum and further cultured in DMEM / Ham's F-12 changed every day. D-II activity was measured on cell sonicates. H_2O_2 had no apparent effect on basal level of D-II. cAMP-stimulated D-II was inhibited by H_2O_2 (0.25 mM) after a lag period of 30 min. D-II activity attained 20% of the stimulated value after 5 h H_2O_2 treatment. The inhibition was maximal (95 %) after 8 h treatment and remained unchanged until 24 h. Cell culture in presence of selenium (30 nM) or addition of N acetyl-cysteine (2 mM) together with H_2O_2 partly protect D-II against inhibition by H_2O_2. In summary, the oxydative stress could inhibit or prevent the induction of the enzyme responsible of the T_3 production in astrocytes. A possible role of glutathion peroxydase remains to be established. The decrease in brain T_3 concentration by oxydative stress could participate to pathologies implicating this stress.

171. P25 CLINICAL, NEUROPHYSIOLOGICAL, CT AND MR FINDINGS IN ENDEMIC CRETINISM.

A. Antonelli, S. C. Boyages*, JP Halpern§, F. Falaschi^, F. Sartucci°, M. Ferdeghini°°, M. Mascalchi^, G. Tognoni°, S. Venturi, M. Sturniolo, C. Kotopulos, P. Fallahi, L. Murri°, L. Baschieri. Departments of Internal and Nuclear°° Medicine, Radiology^ and Neurology°, University of Pisa, Italy; Departments of Endocrinology* and Neurology§, Westmead Hospital, Westmead, Australia.

Endemic cretinism is the most svere manifestation of dietary iodine deficiency. Two forms of the syndrome are traditionally described: neurological and myxoedematous. The nature and the extent of the neurological deficit found in endemic cretinism was investigated in 11 cretins from continental Italy; 6 from Central Apennines (Montefeltro) (goiter prevalence 55%; mean urinary iodine level 39 μg/g creatinine) and 5 from South Italy (San Lorenzo Bellizzi) (goiter prevalence 65%; mean urinary iodine level 37 μg/g creatinine). In these areas neurological cretinism is more prevalent than myxoedematous forms. Clinical and biochemical features of patients with hypothyroid (4) and neurological (7) cretinism were studied. The hormonal profiles of the two types of cretinism were clearly different. Nevertheless all myxoedematous cretins had some neurological disorders (hyperreflexia, increased muscle tone, disorder of gait, Babinski sign, hypoacusia) that were similar to those present in neurological cretins. Neurophysiological examination revealed: 1) motor evoked potentials were abnormal in 4 cases (1 hypothyroid, 3 neurological); 2) somatosensory evoked potentials were abnormal in 2 cases (neurological); 3) brainstem auditory evoked potentials were abnormal in 5 cases (2 hypothyroid, 3 neurological); 4) event related potentials (P300) were abnormal 7 cases (4 hypothyroid, 3 neurological). In conclusion, neurophysiological abnormalities were similar in neurological and hypothyroid cretins. Cerebral and petrosal computed tomography and cerebral magnetic resonance showed basal ganglia calcification in 2 cases (hypothyroid), mild cerebral atrophy in 9 cases (2 hypothyroid, 7 neurological), focal cerebral atrophy in 3 cases (1 hypothyroid, 2 neurological), mild cerebellar atrophy in 1 case (neurological), empty sella in 1 case (hypothyroid), alteration of pneumatization of mastoid and ethmoid in 2 cases (neurological). The presence of basal ganglia calcification was confined to cretins with severe hypothyroidism. Otherwise cerebral CT and MR demonstrated only minor abnormalities which did not contribute to the localization of the clinical deficits. These findings suggest that neurological damage is very similar in all forms of endemic cretinism reflecting a diffuse insult to the developing fetal nervous system. Furthermore these data support the hypothesis that the primary pathophysiologic event in the different types of endemic cretinism is represented by maternal and fetal hypothyroidism, while differences may be explained by the extent and duration of post-natal hypothyroidism.

172. P26 EFFECTS OF ACTIVELY INDUCED THYROGLOUBLIN IMMUNITY IN THE VASCULAR DEPOSITION OF AMYLOID PROTEINS IN THE BRAIN OF RABBITS.

H.T. Blumenthal, B.N. Premachandra, R.G. Naidu, and I.K. Williams. VA Med. Ctr. and Washington Univ, St. Louis, MO.

In our serial studies of pathological changes in animals with actively induced thyroglobulin (Tg) immunity, we have previously reported a variety of lesions in peripheral tissues. In the current investigations in Tg immune animals we note the deposition of amyloid in cerebral vessels, i.e. cerebrovascular amyloidosis (CVA). This lesion (CVA) is an invariable concommitant in Alzheimer's disease and its induction utilizing a thyroid protein is of particular interest since it has been difficult to induce this lesion experimentally. Six 3 month old albino rabbits were immunized with human thyroglobulin (0.1%) emulsified (1:1) in Freund's complete adjuvant. The rabbits received 1 ml intramuscular injections for 3 weeks and were sacrificed six months after the final injection. The control animals received intramuscular injection of Freund's complete adjuvant alone on the same schedule as the experimental animals. All experimental animals demonstrated circulating Tg antibodies (1/3645-1/10,935). After sacrifice representative tissue specimens from the brain were fixed in 10% formalin or gluteraldehyde (2% in PBS) respectively for conventional light and electron microscopic studies. Three of the six experimental animals showed hyaline deposits in small intracerebral vessels. The deposits stained positively with Congo red and demonstrated birefringence when viewed under polarized light (indicative of amyloid protein). The vascular lesions also showed a thickening and spliting of the basement membrane and when the deposits were viewed under higher magnification (60,000 X) fibrillar deposits were evident and consistent with the fibrillar structure of amyloid. Vasculitis was also present showing the typical crystalline structures associated with amyloid. The presence of perivascular lymphocytic infiltrate as earlier noted may suggest that the blood-brain barrier may be compromised in Tg immune rabbits, thus allowing for the egression of amyloid precursors into the brain parenchyma. The observations are of particular significance as they highlight the importance of Tg induced immunological events in the pathogenesis of this brain lesion. Investigations are in progress to determine the molecular composition of brain amyloid in an attempt to relate the Tg-immune process to amyloidosis.

173. P27 ENDOGENOUS TRIIODOTHYRONINE (T3) LOCALIZATION IN FIBER TRACTS AND NEURONAL CELL GROUPS OF RAT FOREBRAIN IS REDUCED BY ACUTE ADMINISTRATION OF A SPECIFIC LOCUS COERULEUS (LC) LESIONING AGENT. J.T.Gordon,C.B.Rozanov and M.B.Dratman, Medical College of Penna. and VA Medical Center, Phila. PA.

T3-immunohistochemistry (IHC) in rat brain reveals heaviest staining in the LC, seen as clumps within perikarya and cell processes. In LC terminal fields, neurons (of which cell nuclei are most prominent) and surrounding neuropil are strongly labeled. DSP-4 is a chemical lesioning agent, which acts initially through irreversible destruction of LC presynaptic terminals, resulting in rapid loss of norepinephrine in LC terminal fields. Extensive permanent loss of LC axons and neurons follows slowly. We have reported that in coronal sections through the hippocampus, DSP-4 causes marked loss of T3 localization in dentate gyrus, in cingulate gyrus of cortex, and in arcuate and dorsomedial hypothalamic nuclei. We now report results of light microscopic examination of coronal sections of rat brain thru forebrain, rostral to hippocampus, prepared for T3-IHC at 3 and 7 d following DSP-4 (50 mg/kg i.p.). Brains of untreated controls were processed simultaneously and identically. Alternate sections of brain were subjected to tyrosine hydroxylase (TH)-IHC. In sections anterior to the fornix, loss of T3 reaction product was observed in selected cell groups of the pyriform cortex. At the level of the fornix, T3 staining was altered in pyramidal cells of layer V and markedly reduced in corpus callosum, anterior commissure and optic chiasm. At the same time, there were no observed changes in TH localization. Conclusion:The data indicate that intact presynaptic LC terminals are a prerequisite for normal T3 localization in LC terminal fields. DSP-4 induced reduction in T3 localization may result from an inability of destroyed terminals to deliver T3 to those fields, in keeping with the concept of the LC system as a conduit for T3 in brain.

174. P28 CHARACTERIZATION OF THYROID HORMONE RECEPTOR (TR) IN HUMAN HEPATOMA CELL LINES AT DIFFERENT STAGES OF DIFFERENTIATION K-H Lin, Chang Gung College of Medicine and Technology, Kwei-San, Taoyuan, Taiwan, R.O.C.

To understand the role of TRs in hepatocarcinogenesis, we characterized the TRs in human hepatocarcinoma (HHC) cell lines. Using reverse transcription-polymerase chain reaction, we isolated the full length of TRα1 and TRβ1 cDNA from HA22T, Mahlavu, Sk-Hep-1, HepG2 and J7 cell lines. Using plasma proteins as markers, we showed that HA22T, Mahlavu, Sk-Hep-1 are poorly differentiated, HepG2 is well differentiated and the degree of differentiation of J7 is between the two. TR proteins were prepared by *in vitro* transcription and translation and their binding activity to the thyroid hormone, 3,3', 5-triiodo-L-thyronine (T3) and to the hormone response elements (TREs) were evaluated. Except for the TRβ1 proteins of HA22T which had a molecular weight 2-4 Kd larger, the TRs from other cell lines had a similar molecular weight as the TRs from the control cell lines. Analyses of T3 binding data indicate that TRα1 from J7 cell line has lost its T3 binding activity. The binding affinity of TRβ1 from poorly differentiated HA22T and SK-Hep-1 cells was about 4- to 12-fold stronger than that in well-differentiated HepG2 (Ka=7.9, 24.3 vs. $1.9 \times 10^9 M^{-1}$). To analyze the possible mutation of TRβ1 gene in five HHC cells, single-strand conformational polymorphism was performed. Bands due to altered conformations were observed in J7 cells. The TRβ1 c-DNA from J7 cells was sequenced and confirmed that the methionine 329 was changed to valine in Exon 10 in the E domain. However, this mutation did not affect its T3 binding activity ($Ka=2.6 \times 10^9 M^{-1}$). Nuclear extracts were isolated from HHC cells to determine their interaction with *in vitro* translated TRβ1 binding to TREs (Pal, DR4, F2) by gel retardation experiments. Nuclear extracts alone did not bind to TREs indicating that very little endogenous TRs were present. However, each cell line showed its unique retarded band pattern after exogenous TRβ1 was added. Plasmids containing TREs and chloramphenicol acetyltransferase gene (CAT) were transfected into four HHC cells. Analyses of the CAT activity indicate that endogenous TRs transactivated the three TREs in a T3 dependent manner. However, the dose response curves of poorly differentiated HA22T and SK-Hep-1 are different from that of well-differentiated HepG2 cells by using the palindromic TRE. These results indicate that TRs are expressed in HHC cells. However, they have anomalous transcriptional activity. Identification of the molecular basis of the anomalous characteristics of TRs will lead to further understanding of their role in liver carcinogenesis.

175. P29 DETECTION OF CD44 VARIANT USING A NEW METHOD TO EXTRACT RNA FROM FINE NEEDLE ASPIRATION BIOPSIES OF THYROID TUMORS. T. Takano, H. Sumizaki, S. Umena and N. Amino, Department of Laboratory Medicine, Osaka University Medical School, Suita, Osaka, Japan

Aspiration Biopsy Cytology (ABC) is often used for the diagnosis of thyroid tumors. In some clinical situations, however, a more simple and objective method is needed for the exact diagnosis. Gene diagnosis using RNA in thyroid tumor cells obtained from fine needle aspiration biopsy (FNAB) can be a useful method for such purpose, although it usually accompanies many laborious laboratory works. On the basis of these considerations, we tried to establish a more simple method to obtain RNA from FNABs. A syringe with a 22G needle was used for FNAB. After preparing the sample on a slide glass for cytological daignosis, leftover cells inside the needle were lysed and washed out with denaturing solution containing 4M guanidine thiocyanate, 25mM sodium citrate (pH 7.0), 0.5% sarcosyl and 0.1M 2-mercaptoethanol and then stored at 4C. Total RNA was extracted by modified acid guanidinium- phenol-chloroform extraction followed by synthesis of cDNA. This sample provides enough amount of RNA for RT-PCR analysis for up to 20 times even when RNA was extracted from the leftover cells. Using this cDNA, thyroglobulin and CD44 cDNA were amplified by PCR. We found RNA was stable at least for one week at 4C in denaturing solution, that freed us from the laborious work of immediate RNA extraction. However, samples stored at room temperature or for more than two weeks showed the smaller amount of the final PCR products, which proved the degradation of RNA. By using this method, we examined the expression of CD44 variant, which is often overexpressed in malignant cells, by RT-PCR using RNA obtained by 20 FNABs (cytological diagnosis: class II -class III), although we failed to detect the variant form in these samples. However, using a small amount of RNA (50ng) extracted by the same method from thyroid tissues, we can detect CD44 variant in two out of eight papillary carcinomas, one out of one follicular carcinoma. On the contrary, we could not detect the variant form in 9 normal thyroid tissues or 3 follicular adenomas. It is possible that CD44 variant only expressed in undifferentiated carcinoma cells. Thus, detection of CD44 variant by RT-PCR using RNA obtained by FNABs can be a useful method for the detection of thyroid carcinoma cells.

176. P30 TSH SUPPRESSION IN THE COURSE OF DIFFERENTIATED THYROID CANCER. *C. Jaffiol, JP. Daurès, P. Pujol, N. Nsakala, J. Guerenova, L. Baldet, D. Vannereau, J. Bringer. Department of Endocrinology and Epidemiology, University Hospital of Montpellier, France.*

A few documented reports confirm the benefit of TSH suppression on long term follow-up of patients with differentiated thyroid cancer. The aim of this study was to compare the relapse free survival (RFS) of thyroidectomized patients according to the level of TSH suppression.

141 patients, 114 women and 27 men (mean age 44.4 y./old), were thyroidectomized for differentiated thyroid cancer (papillary 54.3 %, follicular 29.3 %, mixed 17 %). 126 patients with a thyroid remnant received one or several radioiodine ablative doses. TSH suppression was evaluated by TRH test until 1986, thereafter by a second generation RIA assay. The follow-up included a twice yearly check-up involving clinical examination, neck ultrasonography, X-ray of the chest, plasma Tg and TSH assessment. The mean duration of follow up was 94.5 months. 106 patients were treated with L-T4 (mean daily dose : 171 µg/day). 26 patients with poor tolerance to L-T4 received a combination of L-T4 with 3,5,3'-triiodothyroacetic acid (Triac, mean daily dose=800µg) allowing a significant reduction of L-T4 (mean daily dose : 145 µg/day, $p<005$) and an improvement of TSH suppression (mean TSH decreased from 1.6 to 0.2 mU/l, $p<0.05$).

To evaluate the influence of TSH suppression on RFS, multivariable analysis were performed using different cut-off values of TSH. The TSH level of each patient was defined as the mean value of the annual TSH assessments. No statistical differences were observed when the cut-off values of 0.1 or 0.5 mU/l of TSH were analyzed, although there was a trend towards a better RFS in patients with TSH less than 0.5 mU/l ($p=0.08$). Two groups of patients matched for age and TNM stages were also considered : ***group 1 :*** patients with all annual TSH values <0.10 mU/l ($n = 30$) ; ***group 2 :*** patients with all annual TSH values > 1 mU/l ($n = 15$).The RFS was shorter in group 2 (median:112.4 months) than in group 1 (median:235.5 months, $p < 0.01$ according to Kaplan Meier and Log Rank test).

In conclusion, a high level of TSH suppression may be associated with a longer RFS in patients with differentiated thyroid cancer.

177. P31 GROWTH CHARACTERISTICS OF HUMAN ANAPLASTIC THYROID CARCINOMA CELLS TRANSFECTED WITH A HUMAN THYROTROPIN RECEPTOR cDNA. N.-E. Heldin, B. Gustavsson, A. Hermansson, A.-C. Andersson, L. Grimelius, J. Bergh* and B. Westermark, Dept. of Pathology and *Oncology, University Hospital, S-751 85 Uppsala, Sweden.

The role of thyrotropin (TSH) in the regulation of thyroid growth has been a matter of dispute for the last decades. Experiments *in vivo* and *in vitro* have suggested a complex network of both positive and negative growth signals. In the present study we have transfected a human anaplastic thyroid carcinoma cell line (C 643) with a TSH receptor (TSHR) cDNA in order to investigate the effect of a forced expression of TSHR on the growth characteristics when grown both *in vitro* and *in vivo* in nude mice.

The C 643 cells, lacking endogenous expression of TSHR, were transfected with a human TSHR cDNA in an expression vector (pcDNA1/neo-TSHR-WT) and grown in the presence neomycin (G-418). Out of the 24 neomycin resistant clones tested, four clones (clone 3, 5, 15 and 16) were found to both bind ^{125}I-TSH and respond to a TSH stimulation with an increased level of cAMP. However, the vector transfected control clones (clone C, G, H and K) did not bind ^{125}I-TSH nor responded to TSH.

The influence of the regained TSHR expression on growth *in vitro* was studied by measuring the effect of TSH on the incorporation of ^{3}H-thymidine (^{3}H-TdR) and on cell number. Addition of 10 mU/ml of TSH resulted in a decreased incorporation of ^{3}H-TdR (30 % inhibition) and also in a reduced cell number (15 % inhibition) in the TSHR-expressing cells, however this was not found in the control cells. Stimulation with forskolin (10 μM) led to a growth inhibition both in the control and TSHR-transfected cells.

In order to investigate if the expression of a functional TSHR protein C 643 cells could influence the *in vivo* growth, cells were injected subcutaneously into NMRI nude mice. To manipulate the endogenous level of TSH, animals were given propyltiouracil (PTU; high TSH level), T4 (low TSH level) or no treatment. C 643 cl. 3 (TSHR positive) and C 643 cl. K cells were injected as well as untransfected C 643 cells. Only the TSHR-expressing C 643 cl. 3 cells grew and formed tumors in the mice. There seemed to be a TSH induced inhibition of tumor growth in the C 643 cl. 3 mice, since tumors in mice treated with PTU grew after a longer take rate and with a slower growth rate. In the analysis of the tumors we found the tumors in the PTU-treated animals to be softer compared to the hard and fibrotic tumors in the T4 and control animals. Staining for collagen content (Sirius red staining) showed a lower amount of collagen in the PTU-tumors.

In summary, a forced expression of TSHR in the human anaplastic thyroid carcinoma cell line C 643 led to TSH-induced growth inhibition in cells grown *in vitro*. The effect appeared to be mediated by cAMP since a growth inhibition was also induced by forskolin. Furthermore, the finding of a slower growth rate of TSHR-expressing cells in PTU-treated animals indicates a negative role of TSHR on growth also *in vivo*.

178. P32 **VISUALIZATION OF NON-MEDULLARY THYROID CANCER BY [In-111-DTPA-D-Phe1]OCTREOTIDE (OCT) SCINTIGRAPHY (OCTREOSCAN).** PTE Postema, WW de Herder, JC Reubi, HY Oei, HJ Bruining, EP Krenning. University Hospital Rotterdam, The Netherlands and University of Berne, Switzerland.

Visualization of medullary thyroid carcinoma has been described by Octreoscan. In vitro studies showed inhibition of TSH-stimulated 3H-thymidine incorporation in thyroid follicles (human cancer cell-lines) by somatostatin, suggesting presence of somatostatin receptors.

Visualization of non-medullary thyroid carcinoma by Octreoscan was studied in 21 consecutive patients. 2 patients had anaplastic, 7 had follicular (1 primary), 8 had papillary (5 primary) and 4 papillary cancer in the past. Planar anterior images were prepared 24 h after iv injection of 220 MBq OCT and compared with total body I-123-scintigraphy (after 4 weeks L-thyroxine withdrawal) or I-131-scintigraphy 1 week after I-131-therapy (Iscan). Hotspots were scored visually and the scintigrams were compared with each other and with CT-scanning or bone-scintigraphy.

All primary differentiated cancers were Octreoscan and Iscan positive. Metastasized lesions: see table. Anaplastic cancers were also Octreoscan positive. 3 patients in remission had negative Octreoscan results. In vitro studies showed specific binding of [I-125-Tyr-3]octreotide on follicular cancer cells, indicating expression of somatostatin receptors.

METASTA-SIZED	I SCAN (+)	OCT SCAN (+)	OCTREOSCAN(+)/ISCAN(-) LESIONS			OCTREOSCAN(-)/ISCAN(+) LESIONS		
			N	proving metastasis	extra	N	proving metastasis	extra
Foll 6/7	5/6	5/6	4/6	2/4 bone	2/4 bone	3/6	1/3 bone	2/3 lung
Pap 3/8	2/3	1/3	1/3	1/2 bone	-	2/4	2/2 lung	-

Octreoscan can visualize primary and metastasized non-medullary thyroid cancer. It reveals new or more metastatic lesions than conventional Iscan in some patients, especially bone metastasis in follicular cancers. This maybe of advantage in patient follow-up screening because L-thyroxine has not to be withdrawn. However, lung metastases were missed in some patients. It is unknown at present whether L-thyroxine withdrawal will improve Octreoscan results. (Radiolabeled) octreotide may provide a future (radio)therapeutic option in cancers which do not store radio-iodine.

179. P33 DIPEPTIDYL PEPTIDASE IV (DPP IV / CD26) ACTIVITY STAINING AS A SPECIFIC DIAGNOSTIC ASSAY FOR DIFFERENTIATED THYROID CARCINOMA

Tetsuji Tanaka*, Kazumi Umeki†, Ikuo Yamamoto†, Sachiya Ohtaki† Department of Obstetrics & Gynecology, Osaka City University Medical School, Abeno-ku, Osaka, Japan*. Department of Laboratory Medicine, Miyazaki Medical College, Kiyotake, Miyazaki, Japan†.

Objectives: The aim of this study is to examine if dipeptidyl peptidase IV (DPP IV / CD26) can be a novel histochemical marker for differentiated thyroid carcinoma. **Methods:** DPP IV (CD26) mRNA expression in thyroid tumors was evaluated using northern blot analysis, by comparing with three proto-oncogenes, c-met, c-erbB-2, and EGF-R, all of which are reported to have increased mRNA expression in thyroid carcinomas. Three DPP IV (CD26) assays, northern analysis, immunohistochemical analysis, and activity staining analysis, were compared to evaluate their specificity to differentiated thyroid carcinomas. Thyroid tissue from more than 200 patients with various thyroid diseases were also examined by immunohistochemical analysis and activity staining analysis. Southern analysis was performed to investigate if thyroid carcinoma involve some major defects of DPP IV (CD26) gene. **Results:** Northern blot analysis of thyroid tumors revealed that DPP IV (CD26) is a more specific marker of papillary carcinoma and follicular carcinoma than the above three proto-oncogenes. The comparative study of three DPP IV (CD26) assays clearly showed that DPP IV (CD26) enzyme activity staining is the most specific assay for differentiated thyroid carcinoma, yet the easiest to perform. With activity staining, DPP IV (CD26) was detected in all 52 papillary carcinomas and in all 5 follicular carcinomas, while 58 cases with Graves' disease were all DPP IV (CD26)-negative. The comparative study of northern blot analysis and activity staining also revealed a positive correlation between DPP IV (CD26) mRNA expression and staining intensity of enzyme activity. Southern blot study showed no gene amplification or major translocation of the DPP IV (CD26) gene in papillary carcinomas. **Conclusions:** Ectopic expression of DPP IV (CD26) in differentiated thyroid carcinomas is thought to be partly caused by increased DPP IV (CD26) mRNA transcription. We find that DPP IV (CD26) activity staining is a simple specific assay which should be added to usual pathological examinations in order to distinguish differentiated thyroid carcinomas from benign thyroid diseases.

180. P34 A NOVEL MONOCLONAL ANTIBODY JT-95 SPECIFICALLY REACTS WITH SIALYL FIBRONECTIN OF THYROID CARCINOMA.

H. Takeyama, N. Kimura*, T. Hosoya, N. Shinozaki, K. Uchida, A. Yamashita, S. Nagahara, T.Fukushima, N. Shioya, S.Nakano, H. Kurihara, N. Akiba, K. Itsubo, Jikei University School of Medicine, Tokyo, Japan. * Faculty of Engineering, Soka University, Tokyo, Japan.

By immunizing of mouse with membrane fraction of human thyroid carcinoma(papillary carcinoma), we produced a monoclonal antibody(MoAb) JT-95, which allowed us to characterize a new tumor associated antigen. This molecule was strongly expressed in differentiated thyroid carcinomas confirmed by immunohistochemical staining. MoAb JT-95 reacted to 95/100(95%) cases of papillary carcinoma, 3/4(75%) of follicular carcinoma. On the other hand, benign diseases of thyroid gland, adenoma 0/39(0%), adenomatous goiter 1/21(4%), hyperthyroidism 0/8(0%), chronic thyroiditis 3/8(38%), had low reactivities. MoAb JT-95 did not react to normal thyroid tissues. This molecule was confirmed as a Mr. 250,000 daltons protein by Western blotting using membrane fractions of thyroid carcinoma. We also applied MoAb JT-95 to sera of thyroid carcinoma patients to detect this molecule. A single band was revealed at a Mr. 105,000 in sera of patients(reported at ETA in 1993). For further examination, this molecule was purified, and the structure was investigated.
<Materials and Methods> The antigen was gathered, and purified from culutured supernatant of thyroid carcinoma cell line(SW1736) by affinity columns using MoAb JT-95. 1. Amino acid sequences of the antigen were detected by protein sequencer(ABI). 2. The antigen and fibronectin were subjected to electrophoresis and reacted with MoAb anti-fibronectin(DAKO), and MoAb JT-95 using Western blot analysis . 3. The antigen was treated with syalidase, and also reacted with anti-fibronectin antibody and JT-95 by Western blotting.
<Results> 1. The antigen of JT-95 was confirmed as a kind of fibronectin from amino acid sequence. 2. MoAb JT-95 strongly reacted with the antigen, but did not reacted with normal fibronectin. 3. Specific affinity between JT-95 and the antigen dissapeared after syalidase treatment.
<Consideration> From these data, we conclude that the antigen of JT-95 is a modified fibronectin,---- syalyl fibronectin. Syalyl fibronectin defined by JT-95 may have a strong relation to carcinogenesis of differentiated carcinoma, since MoAb JT-95 reacted with thyroid carcinomas specifically, and no or only low reactivities were observed in normal thyroid gland and benign thyroid diseases by immunohistochemical study.

181. P35 **DETERMINATION OF MALIGNANCY IN THYROID NODULES BY FACTOR ANALYSIS OF SPECTRAL AND DYNAMIC STRUCTURES WITH SIMULTANEOUS DUAL-ISOTOPE : THALLIUM-201 AND IODINE-123**

B.O.Helal*,, F. Frouin**, D. Leguillouzic*, E. Cosson*, M. E. Pueyo, F. Archambaud* and R. Di Paola**. Nuclear Medicine Bicêtre*, U66 INSERM Villejuif**. France**

We have previously demonstrated that Factor Analysis of Spectral and Dynamic Structures (FASDS) in a simultaneous dynamic study with thallium-201 and iodine-123 delineated thyroid nodules accurately and estimated the thallium uptake and its kinetics.
The aim of this study was to verify in a larger group the sensitivity and specificity of the method in the diagnosis of thyroid nodule malignancy. Seventy patients (55 females and 15 males) were investigated and their diagnoses confirmed by histological examination. The acquisition was performed with a gamma-camera equipped with a pinhole collimator.The patients were given simultaneously an IV injection of ^{123}I (7,4 MBq) and ^{201}Tl (37 MBq). A double word list-mode which records spatial, energy and time photon coordinates was acquired. Data processing allows the estimation of the spectra of each isotope and their spatial and dynamic distributions.
Two groups were defined : 28 patients with thallium nodule uptake (6 carcinomas and 22 adenomas) and 42 patients without uptake (42 macrofollicular adenomas). To decrease the number of false positive the thallium kinetics was investigated by Factor Analysis of Dynamic Structures (FADS). Three kinetics and associated images were estimated. The two first concern the vascular and thyroid distributions. In all cold benign thallium nodules, the third kinetic was associated with an extrathyroidal distribution. In the opposite carcinomas were always associated with a high nodular accumulation. For hot thallium benign nodules, 7 (embryonal, fetal, Hürthle adenomas) were associated with nodular concentration and 15 (mainly macrofollicular adenomas)with extranodular and nodular distribution.
The simultaneous double-isotope investigation shows the distribution of both isotopes under similar acquisition conditions. As thallium-201 accumulates in both tumoral and normal tissues, and iodine-123 only in normal tissues, the nodule could be delineated with accuracy. FASDS estimates the thallium uptake and its kinetics in the nodule and in the diagnosis of thyroid nodule malignancy, if the sensitivity is identical (100 %) the specificity is increased (65,6 % to 89 %).

182. P36 VALUE OF TECHNETIUM (Tc)-99m-HYDROXYMETHYLENE DIPHOSPHONATE (HMDP) BONE SCAN SUPPLIMENTED WITH THALLIUM (Tl)-201 SCAN IN DETECTING BONE METASTASES FROM DIFFERENTIATED THYROID CARCINOMA.

M S Alam, R Takeuchi, T Misaki, S Miyamoto, Y Iida, K Kasagi, J Konishi
Dept. of Nuclear Medicine, Kyoto University, Kyoto, Japan.

Prognosis of patients with bone metastases from differentiated thyroid carcinoma is known to be poor. Bone scan so far has been thought to be less efficient in detecting such metastases due to the less malignant nature of the thyroid cells. Our present study is aimed at the re-evaluation of usefulness of Tc-99m-HMDP bone scintigraphy and the adjuvant effect of Tl-201 scan on the total detectability. We studied 57 bone lesions in 24 post-thyroidectomised patients(17 female, 7 male;13 papillary, 11 follicular carcinoma).Thyroidal origin of the lesions was proved by positive Iodine(I)-131 uptake. In I-131 negative lesions histological proof or the absence of other tumour markers was considered. Possibility of stress fracture was ruled out by X-ray or CT scan.All the scintigraphs were evaluated by 6 nuclear medicine experts.Out of the 57 lesions 41 (72%) were positive and 16 were negative in bone scan. But of the 16 negative bone scan sites 11 showed abnormal accumulation of Tl-201, resulting in increased sensitivity of 91%(52/57). In conclusion we have found bone scan supplimented with Tl-201 scan to be a sensitive and easy method for detecting bone metastases from differentiated thyroid carcinoma. This approach is recommended specially in patients having high thyroglobulin level, showing high probability for metastases.

183. P37 THYROID CANCER AND CONCURRENT HYPERTHYROIDISM

M. Auersperg, M. Hočevar, M. Us-Krašovec, T. Movrin. Institute of Oncology, Ljubljana, Slovenia.

Background: Data on the incidence of concurrent thyroid cancer and hyperthyroidism vary from less than 1% to 20%.

Aim of this study was to determine the incidence of thyroid cancer with concurrent hyperthyroidism in Slovenia - an iodine deficient area in the central Europe.

Patients and methods: Records of 933 patients with thyroid cancer treated at the Institute of Oncology Ljubljana, Slovenia from 1972-94 were reviewed. In 43 patients (32 females, 11 males, aged 16-84 years) hyperthyroidism occurring simultaneously with cancer was diagnosed. All patients had morphological diagnosis. In 31 patients cytology and histology were performed, in 5 only histology and in 7 patients with distant metastases only cytology from the primary tumor was performed. There were 23 follicular, 13 papillary, 2 insular, 3 anaplastic, 1 Huertle cell and 1 medullary cancer. Out of 43 patients 10 had Grave's disease, 1 Hashimoto thyroiditis, 17 toxic multinodular goiter, 15 toxic adenoma. In 35 out of 38 patients the indication for surgery was supported also by cytological diagnosis. In 38 patients with cytology performed, the cytological diagnosis was follicular cancer in 11, papillary in 7, cancer type non specified in 3, anaplastic in 2, suspicious for cancer in 5, follicular neoplasm in 5 and goiter in 3 patients. In 21 patients cancer was in scintigraphically cold and in 21 in hot area. In one patient the cancer was too small to be shown on scintigram. Beside other relevant tests in 38 patients T_4 and T_3 were determined as well. An elevation of T_3 and T_4 was found in 19 whereas T_3 alone was elevated in 19 (in 17 follicular and in 2 papillary cancers).

Results and Conclusion: The incidence of concurrent hyperthyroidism and cancer in this seties of patients was 4,6 %. Cancer was more common in toxic nodular goiter than in Grave's disease. An elevation of T_3 alone was more common in follicular cancers than in other forms ($p<0,05$). Cytology was a valuable preoperative diagnostic method. Half of cancers were scintigraphically hot. Therefore also hot not only cold nodules should be examined by cytology.

184. P38 MIBI-Tc FOR DETECTION OF RECURRENCES IN A PATIENT WITH HURTLE CELL CARCINOMA.

J.L. Wémeau, D. Huglo, S. Coequyt, S. Petit, J.F. Cussac, M. Lecomte-Houcke, B. Maes and Ch. Proye. Centre Hospitalier Régional et Universitaire, Lille, France.

Hürtle cell (oxyphilic cell type) carcinoma is an uncommon form of follicular neoplasm, considered as relatively aggressive and usually not taking up ^{131}I. MIBI-Tc was used to detect the parathyroid adenomas, specially those with an important intake of oxyphilic cells. This led us to test the MIBI-Tc in a patient with a multirecurrent oxyphilic cell type carcinoma of the thyroid.

A 17-year-old girl was operated on in 1982 on account of a cold nodule of the thyroid isthmus, considered as a benign Hürtle cell neoplasm. In 1988 and 1992 local neck recurrences were operatively removed. Finally near-total thyroidectomy was performed and the patient was treated with 100 mCi of ^{131}I, since the diagnosis of recurrent Hürtle cell carcinoma was made on basis of clinical story and pathologic data (namely invasion of blood vessels). In spite of therapy with thyroid hormone and TSH levels < 0,010 mU/l, small local recurrences occured in 1993 and 1994. Thyroglobulin levels were 7.4 and 9.2 ng/ml. After discontinuing levothyroxine therapy, no ^{131}I nor Thallium uptake was present on the cervical and total body scans. However MIBI-Tc was concentrated within the local recurrence 1 and 3 hours after injection.

MIBI-Tc could be an useful procedure for survey of the Hürtle cell carcinomas in patients with enhanced thyroglobulin levels and negative ^{131}I scintigram.

185. P39 THE USEFULNESS OF THE CONCANAVALIN A-THYROGLOBULIN AFFINITY TEST FOR THE DIAGNOSIS OF THYROID CANCER. Masayuki Maruyama, Ryoji Kato, Akira Sugenoya, Shinya Kobayashi, Yoshio Kasuga, Department of 2nd Surgery, Shinshu University School of Medicine, Matsumoto.

It has already reported that thyroglobulin (Tg) derived from thyroid neoplasms might be different biochemically and structurally compared to that of normal thyroid. We evaluated the usefulness of a method employing the affinity of Tg for Concanavalin A (Con A) for diagnosing thyroid carcinoma using puncture fluid of thyroid tumor. In 5 patients who were postoperatively diagnosed histologically as having papillary carcinoma of the thyroid, results of preoperative puncture aspiration cytodiagnosis were compared with those of the measurement of Con A affinitive Tg. Tg in each puncture fluid was measured by enzyme-linked immunosorbent assay (ELISA). After its concentration was adjusted to about 0.1 mg/ml, Tg was reacted with Con A (0.1 mg/ml), and centrifuged to remove Con A-bound Tg. The remaining unbound Tg (ub-Tg) fraction was measured by ELISA. Results were expressed as the percentage of the amount of ub-Tg to the amount of Tg before addition of Con A. Based on the results of our preliminary evaluation, 10.5% or more ub-Tg was considered to be positive. Cytodiagnosis showed class V in 4 of the 5 patients and class IIIb in the other 1. Measurement of Con A affinitive Tg revealed positive findings in 3 patients, of whom 1 showed class IIIb. Treatment of cancer Tg with Con A showed an increase in ub-Tg. This procedure may be useful as an adjunctive method for the diagnosis of thyroid cancer.

186. P40 PROGNOSTIC FACTORS IN MEDULLARY THYROID CARCINOMA (MTC): A CLINICOPATHOLOGICAL STUDY ON 53 PATIENTS. M.E. Dottorini *, A. Assi***, M.Sironi*** and G. Sangalli**. * Nuclear Medicine Department and ** Pathology Department General Hospital Busto Arsizio, Italy and ***Pathology Department General Hospital Legnano, Italy.

The aim of this study was to evaluate a series of clinicopathologic features as prognostic factors in MTC. From our caselist of 85 patients with a pathological diagnosis of MTC, we selected 53 cases (32 females and 21 males; 44 sporadic and 9 familial MTC; mean age 46.22 ± 12.63 yrs, median: 46 yrs, range: 18-83 yrs.) operated on in our Hospitals in the period 1958-1993 and for which paraffin-endebbed specimens were available. All pathologic specimens were reviewed at the same time by three pathologists. The pathological factors evaluated were: histotype (Albores-Saavedra Classification); pT, pN, M, stage of disease at diagnosis (1988 UICC Classification), necrosis, argirophilia, amyloid stuff, calcitonin (CT), carcinoembryonic antigen (CEA), thyroglobulin (Tg), calcitonin gene-related peptide (CGRP), chromogranin A, chromogranin A PHE5, neuron-specific enolasis, synaptophysin. Other factors evaluated by a retrospective analysis were: age, sex, familiarity, extension of surgical intervention, external radiotherapy, chemotherapy, 131-I therapy, post-surgical CT and CEA. All patients were followed-up by direct clinical examinations, serum CT and CEA assay, and others imaging procedures when indicated. The mean follow-up was 5.72 ± 5.49 yrs. (range 1-24 yrs). Each variable was evaluated by Kaplan-Meyer survival curves and these compared by log rank test. The variables with a significance level <0.05 were then evaluated by multivariate analysis with a Cox proportional hazard model. 10- year and 15-year cause-specific survival were 70% and 59%, respectively. At the time of the survey 23 patients (43.4%) had died, 20 of them due to MTC and 3 for unrelated causes, 16 (30.2%) were alive free of disease, 3 (5.7%) were alive with metastases and 6 (11.3%) were alive with serum CT high levels, but not evident metastases. 5 patients (9.4%) were reported to be alive, but had been lost at clinical follow-up since more than 2 yrs. Distant metastases were diagnosed in 8 patients in the course of the follow-up (7 out of them died after 0-12 yrs), and cervical lymph-node metastases or local recurrence of the neoplasm in 4 patients (3 out of them died after 1-9 yrs.). Stage of disease at diagnosis, pT, pN, M, post-surgical CT, CGRP and necrosis resulted to be significant prognostic factors for survival at univariate analysis, but only the stage was still significant at multivariate analysis. In conclusion, the stage of disease at diagnosis is the most powerful prognostic factor for MTC, while survival was not affected by the majority of available immunohistochemical markers.

187. P41 Subnormal TSH levels modulate serum Thyroglobulin (Tg) in patients with Differentiated Thyroid Cancers (DTC). CA Spencer, C. Wang, RB Guttler, S. Fatemi, JS LoPresti. Dept of Med. USC, Los Angeles, CA 90033.

High dose L-T4 therapy is used to decrease the trophic influence of TSH on DTC. Since tumors differ in their degree of differentiation and sensitivity to TSH, it is important to individualize the therapeutic goal of suppression therapy (normal vs. subnormal TSH) in order to minimize the detrimental effects of chronic subclinical hyperthyroidism on heart and bone. Since Tg biosynthesis and secretion is TSH dependent, the serum Tg level provides a probe for assessing TSH biologic activity on tumors. This study evaluated serum TSH/Tg relationships in DTC using 3rd. and 4th. generation TSH assays, and controlling non-TSH factors (thyroid mass and damage) that influence serum Tg levels. Three studies of TSH-dependent serum Tg changes were made:-1) The temporal pattern of serum Tg responses to TSH was studied using recombinant TSH (rhTSH) in 10 DTC patients. 2) The acute Tg responses to endogenous TSH stimulation following T3 withdrawal (T3 W/D) was studied in 2 DTC (foll. + mets) and 2 normal subjects. 3) Batchwise serum TSH/Tg relationships were studied in 17 DTC patients (basal Tg = 609 ± 977(sd), range 1.5 to 3100 μg/L), before and > 2 months after 26 L-T4 dose changes. Patients were selected to be >2 years after total thyroidectomy and >6 months after their last RAI Rx. to minimize damage effects on serum Tg levels. Percent serum Tg changes were measured following L-T4 dose-induced changes in serum TSH between the very low (VL = < 0.05 mU/L), low (L = 0.05-0.4 mU/L), normal (N = 0.4-4.0 mU/L) or high (H= >4.0 mU/L) range. RESULTS: 1) There was a 24 hour lag between rhTSH administration and the initiation of a serum Tg response. 2) Before T3 W/D, serum TSH was < 0.02 mU/L and serum Tg detectable in all subjects. A detectable Tg increase was seen 9 days after T3 W/D (when TSH was N) in normals, and 4 days (when TSH was VL) in DTC. 3) Percent serum Tg L-T4 induced changes, relative to control are tabulated below:

TSH change	Control Tg ± sd(range) μg/L	Tg change % control ± sd (range)	n
L to VL	684 ± 843 (23-1640)	-19 ± 9 (-9 to -34)	5
N to L	576 ± 1147 (1.5-3100)	-53 ± 24 (-6 to -85)	10
L to N	1346 ± 1106 (79-2120)	+40 ± 11 (+28 to +47)	3
L to H (T3 W/D)	402 ± 871 (5.1-2490)	+491 ± 977 (+232 to +1589)	8

CONCLUSIONS: 1) rhTSH responses confirmed earlier studies showing a lag in serum Tg response to TSH-stimulation. 2) Acute serum TSH increases in the VL range initiate serum Tg increases in DTC but not normal subjects. 3) Chronic TSH changes ranging from very low (<0.05 mU/L) to high (> 4 mU/L) appear to promote a biologic response in DTC, as judged by changes in serum Tg levels. It is not known how these serum Tg responses relate to the mitotic potential of differentiated thyroid tumors.

188. P42 SOMATIC MUTATION OF THE RET PROTO-ONCOGENE IN SPORADIC MEDULLARY THYROID CARCINOMA ARE NOT RESTRICTED TO EXON 16 AND ARE ASSOCIATED WITH TUMOR RECURRENCE.. C. Romei, R. Elisei, I. Ceccherini*, E. Molinaro, F. Mancusi, G. Romeo*, and F. Pacini. Istituto di Endocrinologia , University of Pisa, Pisa and *Genetica Molecolare, Istituto G. Gaslini, 8 Genova, Italy.

Germline point mutations in exon 10, 11, and 16 of the RET proto-oncogene have been identified as causative in multiple endocrine neoplasia type 2 and in familial medullary thyroid carcinoma (MTC). Somatic point mutations of the same gene, exclusively associated with codon 918 of exon 16, have also been reported in few cases of sporadic MTCs. We analyzed the blood and tumor DNA of 20 patients with sporadic MTC and of 6 patients with primary parathyroid adenoma (PTA) for point mutations at exon 10, 11, and 16 of the RET proto-oncogene by restriction analysis of the PCR-amplified product. A Cys 634->Tyr mutation was found in both the tumoral and blood DNA of one patient, indicating that he was affected by an hereditary form of MTC, erroneously considered sporadic. In the other 19 patients with MTC, somatic mutations of RET were found in 9 cases (47.3%). In five cases the mutation affected exon 16 (Met 918->Thr) and in 3 cases exon 11 (Cys 634->Arg in two and Cys 634->Trp in one). Screening of exon 10 by restriction analysis failed to detect point mutations, however the PCR product of this exon showed the presence of an extra-band in the tumoral DNA of one patient, which by sequence analysis was confirmed to represent a 48 bp deletion. Clinical data showed that 7/9 (77.7%) of the patients whose tumor carried RET mutation had tumor recurrence and/or increased serum calcitonin concentrations in the post-surgical follow-up as opposed to 1/10 (10%) of the patients without mutations ($p<0.02$ by X^2 analysis). No RET mutation was found in the tumoral DNA from parathyroid adenomas. Our findings indicate that somatic RET mutations (mainly point mutation, but also deletion) are frequently found in sporadic MTC, may affect not only exon 16 but also exon 10 and 11, and are associated with less favourable clinical outcome.

189. P43 CHARACTERIZATION OF RET ONCOGENIC ACTIVATION IN MEN2 INHERITED CANCER SYNDROMES. S. Xing, P. A. Smanik, E. L. Mazzaferri and S. M. Jhiang, The Ohio State University, Columbus, Ohio.

The *ret* proto-oncogene (c-*ret*) encodes a receptor type tyrosine kinase with an unknown ligand. Recently, germline mutation of c-*ret* was found to be associated with inherited cancer syndromes: multiple endocrine neoplasia type 2A (MEN2A), MEN2B, and familial medullary thyroid carcinoma (FMTC). Mutations found in MEN2A and FMTC patients were mostly located in the extracellular Cys-rich region of c-*ret*, whereas in MEN2B patients, a specific single point mutation changed Met^{918} to Thr^{918} in the tyrosine kinase domain of c-*ret*. To understand the biological function of the mutated *ret* proteins in the tumorigenesis of MEN2 syndromes, MEN2A/*ret* and MEN2B/*ret* cDNA clones were generated by site-directed mutagenesis from a c-*ret* cDNA clone. The NIH/3T3 stable transfectants expressing c-*ret*, MEN2A/*ret* or MEN2B/*ret* were then established and characterized. Our data shows that NIH/3T3 cells expressing MEN2A/*ret* or MEN2B/*ret* acquired a higher proliferation rate, exhibited a transformed morphology, formed colonies in soft agar, and formed tumors in nude mice. In contrast, the NIH/3T3 cells expressing c-*ret* did not show any change in proliferation rate or morphology compared to the parental NIH/3T3 cells, did not form colonies in soft agar, and have not formed tumors in nude mice after 2 months post-injection. Downregulation of homologous or heterologous gap-junctional intercellular communication (GJIC) has been reported to play an important role in the tumorigenicity of cell lines transformed by various oncogenes. Therefore, homologous GJIC in the NIH/3T3 transfectants was examined by scrape loading-dye transfer assay. Our results showed that GJIC of the transformed cells expressing MEN2A/*ret* or MEN2B/*ret* was maintained or up-regulated compared to the NIH/3T3 cells expressing c-*ret* or the parental NIH/3T3 cells. Currently, correlation of the GJIC function with the expression level and/or cellular localization of a gap-junction protein, connexin-43, is being investigated by Western blot and immunostaining. In conclusion, our results indicate that both MEN2A/*ret* and MEN2B/*ret* act as dominant oncogenes, and the homologous GJIC in the NIH/3T3 cells expressing MEN2A/*ret* or MEN2B/*ret* is maintained or up-regulated.

190. P44 MEDULLARY THYROID CANCER - PROGNOSTIC FACTORS AND THE ROLE OF EXTERNAL BEAM RADIATION THERAPY. J Brierley, R Tsang, W Simpson, M Gospodarowicz, S Sutcliffe, T Panzarella.

Records of 73 patients with medullary thyroid cancer were reviewed to assess prognostic factors and the role of external beam radiation therapy (RT).

Patients were treated between 1954 and 1992. The median age was 49 years (range 15-85), M:F ratio 1.6:1, and the median follow-up was 7.9 years (2.5-34.6). The primary tumor size was <1 cm in 10%, 1-4 cm in 53%, and >4 cm in 37%. Multifocality was noted in 32%, and 26% had metastasis at presentation. Eight patients presented with inoperable tumors, 40% had gross and 37% microscopic residual disease post-thyroidectomy. Extraglandular extension (EGI) was present in 56%, and 74% had pathologically involved lymph nodes. Treatment was by total or near total thyroidectomy in 45 patients, 37 had a lymph node dissection. Forty-six patients were irradiated, the dose of radiation ranged from 20 Gy to 75.5 Gy median 40 Gy, treatment time median was 28 days and the median number of fractions was 20.

The overall cause specific survival (CSS) was 70% and 51% at 5 and 10 years respectively. In a univariate analysis, the following factors predicted for lower CSS: Age as a continuous variable ($p=0.03$), male gender ($p=0.01$), presence of distant metastasis ($p<0.001$), lymph node involvement ($p=0.01$), gross residual disease ($p<0.001$), tumor size > 4 cm ($p=0.03$), EGI ($p<0.001$), vascular invasion ($p=0.007$), diarrhea ($p<0.001$), and abnormal postoperative calcitonin ($p=0.02$). On multivariate analysis only the presence of EGI and of gross residual disease were significant.

There was no difference in locoregional relapse free rate between patients receiving RT or not, but in 40 high risk patients (microscopic residual disease, EGI, lymph node involvement), the locoregional RFR was 86% at 10 years for 25 patients with post-op RT, and 52% for those with no post-op RT ($p= 0.049$).

We therefore continue to advise RT in patients at high risk of locoregional relapse.

191. P45 MEDULLARY THYROID CARCINOMA - SPORADIC OR FAMILIAL? B.G. Robinson[1,2,3], D.J. Marsh[1], D.L. Learoyd[1] and S. Andrew[1]. Departments of [1]Molecular Genetics and [2]Endocrinology, Royal North Shore Hospital and [3]University of Sydney, Sydney NSW Australia

There have been recent advances in the understanding of the molecular genetics of medullary thyroid carcinoma (MTC). MTC is present in three distinct clinical entities. Familial medullary thyroid carcinoma (FMTC) is characterised by MTC alone. The clinical presentations of multiple endocrine neoplasia type 2A (MEN 2A) include MTC along with phaeochromocytomas and in some cases parathyroid hyperplasia. If in addition mucosal neuromas, a Marfanoid habitus and sometimes ophthalmic and gastrointestinal abnormalities are present, MEN 2B is diagnosed. Germline mutations in codons 609, 611, 618, 620 and 634 in exons 10 and 11 of the RET proto-oncogene have been shown to be associated with MEN 2A and FMTC. A mutation in codon 918 has been associated with MEN 2B. Somatic mutations in cases of sporadic MTC have also been identified in codons 768, 883 and 918 of the RET proto-oncogene. In this study, we examined 32 paraffin-embedded archival sporadic MTCs for the presence of these somatic mutations. DNA extracted from these tumours was amplified by the polymerase chain reaction (PCR) and analysed by restriction endonuclease digestion. Exons 10 and 11 were sequenced to exclude "germline type" mutations. None were found. Mutations in tumour tissue were identified at codon 918 in 66% of samples (21/32) and at codon 883 in 3% of samples (1/32). No mutations were found at codon 768. In 31% of samples (10/32), no mutation was found. These findings suggest that analysis of the RET proto-oncogene in tumour DNA is a useful adjunct to the analysis of germline DNA for RET mutations. A decision analysis of the role of germline and somatic RET proto-oncogene testing in the management of new cases of MTC will be presented.

192. P46 ^{99m}Tc SESTAMIBI SCANNING IN RECURRENT MEDULLARY THYROID CARCINOMA. D.L. Learoyd[1,5], P. Roach[2], G. Briggs[3], L. Delbridge[4], A. Poole[4], E. Wilmshurst[5] and B.G. Robinson[1,5], [1]Molecular Genetics Unit, Kolling Institute of Medical Research, Departments of [2]Nuclear Medicine, [3]Radiology, [4]Surgery and [5]Endocrinology, Royal North Shore Hospital and University of Sydney.

The presence of recurrent medullary thyroid carcinoma (MTC) is readily determined by post operative measurement of serum calcitonin levels. Many procedures have been proposed to assist in the localisation of recurrent disease, the most sensitive being venous sampling. This procedure however, is invasive and complicated following surgery. MTC often metastasizes to the mediastinum and visualisation of this area using CT or MRI is problematic. CT scanning is particularly insensitive in the upper anterior mediastinum following a previous central neck dissection, due to the presence of extensive scar tissue. We have evaluated the role of scanning with ^{99m}Tc-sestamibi in 8 patients with recurrent MTC and calcitonin values between 2000 and 62000 ng/L. In 6 patients with calcitonin values greater than 6000 ng tracer uptake was demonstrated in at least one area. Sestamibi enabled visualisation of two palpable tumor nodules of less than 1 cm in two patients. Sestamibi uptake was also demonstrated in two patients in the mediastinum when CT scans of this area were normal and histological confirmation of MTC was obtained in one of these. Another patient had an area of sestamibi uptake in the neck which was normal on CT scanning. In two patients with advanced metastatic disease CT and sestamibi scans were concordant in localising the majority of metastatic lesions but there were additional sites where CT scans were more sensitive than sestamibi scans. The avid hepatic uptake and excretion of this isotope makes visualisation of hepatic metastases difficult but in one patient they could be seen as "filling defects" in the liver. We conclude that ^{99m}Tc sestamibi scanning has an important role in localisation of recurrent MTC in some patients particularly where mediastinal disease is present and not detectable on CT scanning.

193. P47 A NEW SCHEDULE IN THE TREATMENT OF ADVANCED MEDULLARY THYROID CARCINOMA

G. Lupoli, E. Cascone, F. Arlotta, G. Vitale, V. Nuzzo, E. Rampone, G. Lombardi
Depatment of Molecolar and Clinic Endocrinology and Oncology-"Federico II" University, Naples-Italy

Considerable interest exists for the use of octreotide, somatostatin analogue, in the treatment of advanced medullary thyroid carcinoma (MTC). Octreotide is an important adjuvant for the relief of clinical symptoms in a considerable proportion of patients affected by MTC. Recombinant interferon (rIFN) has some therapeutic effects on neuroendocrine tumors with distant spreads. The aim of this study was to evaluate the therapeutic accurancy of octreotide and aIFN2b in patients with advanced MTC, using an original schedule. Seven patients with sporadic MTC, 3 men and 4 women, aged from 32 to 56 years, had been treated previously with total thyroidectomy and lymphadenectomy. They had unresectable local-regional and/or distant metastases (2 patients had mediastinal metastases, 1 patient had pulmonary metastases, 2 patients had hepatic and skeletal metastases, 2 patients had mediastinal and pulmonary metastases). 4 patients reported the development of intractable diarrhoea and 1 patient presented flushing. All patients had persistently elevated plasma CT and CEA levels. The staging procedures performed starting and during therapy included physical examination, hormonal profile (FT3, FT4, TSH), tumor markers (CT, CEA), 111 InOct Scintigraphy, echography, total body computerized tomography and/or magnetic resonance. Daily doses of 150 mcg octreotide were administered by means of 3 subcutaneous injections for 6 months. aIFN2b was administered intramuscularly in doses of 5.000.000 U.I. thrice weekly. In 2 patients therapy was stopped because of aggravation of preexisting diarrhoea and toxicity of IFN treatment. Preexisting diarrhoea in 3 patients and flushing in 1 patient significantly ameliorated during the treatment. A maximal decrease of CT was reached to 44% of the initial level in 1 patient after 1 month and to 15, 70, 65. 62% in 4 patients after 3 months. After the third month, CT levels started to increase moderatly. In all patients CEA levels also decreased to 70, 42, 47, 60, 50% of the initial levels after 6 months of treatment. Preexisting diarrhoea in 3 patients and flushing in 1 patient significantly ameliorated during treatment. No significant changes of size metastases were observed. In conclusion, our study suggests that the combination of octreotide and IFN can be recommended in MTC advanced, with an initial decrease of CT values and a permanent reduction of CEA levels. This new schedule was well tolerated, and the quality of life greatly improved.

194. P48 MEDULLARY CARCINOMA OF THE THYROID ASSOCIATED WITH MULTIPLE NON-ENDOCRINE TUMOURS: A MOLECULAR STUDY.

M. Alevizaki, L. Sarika, K. Karaiskos, E. Anastasiou and A. Souvatzoglou. 1st Endocrine Section, ALEXANDRA Hospital and Department of Medical Therapeutics Athens University, (Director, Professor D. A. Koutras), Athens, Greece.

We present two patients who had medullary carcinoma of the thyroid (MTC) associated with a further two non-endocrine tumours. To exclude the possibility that these might represent MEN "variants", a molecular study of the MEN2 "gene" was performed. Patient A, a farmer, 60 years old, presented with MTC metastatic to the regional lymph nodes. He had been operated for fibrosarcoma at the age of 30. He also had multiple subcutaneous nodules ranging 1-6 cm in diameter. A biopsy showed that these included lipomas and angiolipomas. There was no family history of MTC. One of his sisters had been operated for a follicular and multifocal papillary thyroid carinoma; screening and tissue staining for calcitonin (CT) was negative. Patient B, 65 years old, a concierge, presented with MTC and liver metastases; he had been operated for a malignant melanoma at the age of 50 and for a renal adenocarcinoma at the age of 60. His family history was negative for MTC or other cancers. Both patients had negative tests for phaeochromocytoma. Stimulation tests for CT in their 1st degree relatives were normal. In both patients genomic DNA was extracted from peripheral lymphocytes and screened for the mutations of the RET oncogene which have been reported in MEN2 syndromes. Exons 10, 11 and 16 of the RET protooncogene were amplified by PCR and studied by sequencing (exons 10 and 11) and by RFLP analysis (exon 16). No mutations were detected in any of the positions where MEN2 mutations have been found. Tumour DNA was also studied; no somatic mutation in Met918, which has been reported in a substantial percentage of sporadic MTCs, was detected. We conclude that patients with MTC and multiple non-endocrine tumours may not carry any of the MEN2 mutations, which are apparently related to tumour development in tissues of neuroectodermal origin.

195. P49 **DISTANT METASTASES IN DIFFERENTIATED THYROID CARCINOMA. MULTIVARIATE ANALYSIS OF PROGNOSTIC VARIABLES.** C. Schvartz, S. Theobald, M.J. Delisle and the Thyroid Cancer Group of Champagne-Ardenne. Institut Jean Godinot, Reims, France.

We carried out a study about 83 Patients with Distant Metastases (PDM) from a cohort of 1012 patients with differentiated carcinoma diagnosed between 1970 and 1992 and notified to the hospital-based registry (HBR) of the Regional Cancer Center. These 83 PDM represented 5% from the HBR papillary carcinoma and 14% of those with follicular cancer (7 minimal invasive, 39 invasive). Thirteen percent of the PWM were diagnosed before the diagnosis of carcinoma, 46% were discovered in the first year after diagnosis, 27% between 1 and 5 years and 14% after 5 years. At the time of first diagnosis of metastases, 53% of the PDM presented lung involvement only, bone involvement only in 26% and 21% had multiple involvement. Five and 10 years after the diagnosis of metastases the overall mortality rates were equal to 51% and 69% respectively.

By univariate analysis, using specific death as end point, age at diagnosis, Tp4 according to the TNM classification, histological type, delay between date of diagnosis of thyroid tumor and date of diagnosis of the metastases, metastases extent, pattern of lung or bone involvement, radioiodine uptake (RAI) of the metastases were found as statistical significant pronostic factors. According to a multivariate analysis using the Cox's model, delay between date of diagnosis of the thyoid tumor and date of diagnostic of the metastases ($p=0.0001$), involvement of multiple organ sites or macronodular pulmonary metastases ($p=0.009$), histological type ($p=0.05$) and Tp4 ($p=0.05$) were found independently associated with cancer death.

All the 16 PDM (without regard to the age) diagnosed only by the presence of RAI uptake were still living after a median follow-up of 12 years. The highest risk of cancer death (100% at 5 years) was found in the 11 patients (without regard to the age) which metastases were diagnosed before thyroid tumor.

196. P50 DNA, RNA AND PROTEIN ANALYSES OF MUC1 MUCIN IN BENIGN ADENOMA AND PAPILLARY CARCINOMA OF THE THYROID

I.Bièche*, E.Ruffet**, A. Zweibaum ***, R. Lidereau *, B.Franc **
*Department of Oncogenetic, René Huguenin Center, 92211, Saint-Cloud, **Department of Pathology, Ambroise Paré Hospital, 92100, Boulogne-Billancourt, *** INSERM U178, Paul Brousse Hospital, 94805, Villejuif, France.

Differentiating between benign and malignant follicular neoplasms of the thyroid is still a major problem for surgical pathologists. The MUC1 mucin distributes among a variety of epithelial tissues and we have previously demonstrated by immunohistochemistry its presence at the apical part of follicular thyroid cells. This study aimed to elucidate whether MUC1 gene alteration and/or expression correlated to the thyroid tumor progression. We collected fresh thyroid tissue specimens from 13 patients : including 9 macrofollicular adenoma, 4 papillary carcinoma and the normal thyroid tissue for each patient. To determine the integrity and the expression of MUC1 mucin gene, specific antibody and cDNA probe were used for Southern blot DNA, Northern blot RNA and immunohistochemical protein analysis in all tissues tested.

Over-expression of MUC1 RNA and protein, in the absence of DNA alteration, was observed in the 4 papillary carcinoma. MUC1 RNA and protein were similarly expressed in 12/13 adenoma, compared with the normal adjacent tissue. Only one adenoma shows an increase MUC1 mucin expression. However this adenoma is the only one among the 13 studied neoplastic tissue specimens which shows a DNA amplification of the MUC1 gene. Immunostaining of the corresponding formalin-fixed paraffin embedded tissue sections detected MUC1 mucins at the apical domain of follicular cells. In adenoma and carcinoma expression of MUC1 mucins was more irregular than in normal tissue. The intensity of the staining increased in all papillary carcinoma. Some tumor cells in carcinoma showed reactivity in their cytoplasm.

These preliminary findings suggest that different mechanisms can affect the expression of the MUC1 gene. Additional studies are warranted to define the diagnostic and pronostic significance of the deregulated expression of mucin MUC1 gene in thyroid cancer.

197. P51 **K-RAS AND P53 ONCOGENE MUTATIONS IN THYROID NEOPLASMS.**

S. SUZUKI, M. ONDA, S. OHKI, H.. AMI, H. SAKUMA, K. KIMAN,A.TSUCHIYA, R. ABE, The Department of Surgery II ,Fukushima Medical College, Fukushima,Japan

Initiation and /or progression of cancer correlate with the abnormalities in some oncogenes is well known as the theory of multistep pathway of tumorgenesis. Thyroid cancer consists of the different biological characteristics of well differentiated carcinoma(papillary and follicular),and anaplastic carcinoma. The anaplastic transformation occured in papillary carcinoma which had the long past history has been occasionally experienced. Therefore, thyroid neoplasm has the interesting biological behavior. We have investigated the abnormalities of K-ras and p53 genes on thyroid neoplasms.

Tumor materials were collected from 29 patients with thyroid neoplasms(11 follicular adenomas,13 papillary carcinomas, 1 follicular carcinoma, 1 medullary carcinoma and 3 anaplastic carcinomas)undergoing thyroidectomy at Fukushima Medical College Hospital. The abnormalities of K-ras and P53 genes were analysed by a combination of polymerase chain reaction-single strand conformation polymorphism(PCR-SSCP) analysis and direct sequencing. Mutations in exons 1,2 of K-ras gene and 5, 6, 7 and 8 of the p53 gene were examined.

No point mutaions of K-ras and p53 were found in all cases. But some delation of p53 area were shown in 1 adenoma(9.1%), 3 papillary carcinomas(23.1%) and 1 anaplastic carcinoma(33.1%).

In thyroid neoplasms, there are no prominent genetic instability detected in colorectal cancer. It seems that thyroid carcinoma is stable from genetic point of view. But p53 might be slightly associated with progression in thyroid cancer.

198. P52 IODIDE AND CHLORIDE CHANNEL OF THE THYROID. A.Yoshida and C.Shigemasa, First Department of Internal Medicine, Tottori University,Yonago,Japan.

Thyrocytes accumulate iodide (I) from the blood and transport the I^- to the colloid space, where it is oxidized and organified to mono- and diiodothyrosine on thyroglobulin. I^- channel has been considered to be a possible mechanism of I^- secretion into the colloid space. But there has been no electrophysiological report concerning the I^- channel. Fluid secretion into the colloid space is very important for the follicle formation. The mechanism of fluid transport is unknown. Recently a physiological and pathological role of chloride (Cl) channels has been suggested in many tissues. It is possible that a Cl^-channel in the apical thyroid cell membrane might regulate fluid transport into the colloid space. In the present study, we identify an I^- and Cl^- channel in the membrane of continuously cultured FRTL-5 thyroid cell line. Single channel recording were performed with cell attached membrane patches. When FRTL-5 cells were perfused with a tyrode solution containing KCl , NaCl or NaI and 1 mU/ml of thyroid stimulating hormone (TSH) in the pipette, single channel current was observed. Phorbol 12-myristate 13-acetate had no effect on channel activity, whereas dibutyryladenosine cyclic monophosphate was able to activate the channel. These results indicate that TSH induced the electrophysiological change through the TSH-cAMP-A kinase system. The mean single channel conductance calculated from the slope of the current-voltage relationship with NaCl in the pipette ;was 94 ± 6.5 pS, with KCl in the pipette; it was $95.3\ \pm5.5$ pS, with NaI in the pipette; it was $44.3\ \pm9.5$ pS (mean$\pm$SD ;n=4) . There are no significant difference in the characteristics of channel current in the presence of KCl or NaCl in the pipette . In all cases, the kinetics of the channels were characterized by long openings interrupted by long lasting closing events in the order of sec. Gluconate can not replace chloride or iodide and the channel is impermeable to Na^+,K^+ and tetraethylammonium ions. These results indicate that the channel, we identified, has high unitary conductance and selectivity for $Cl^->I^-$. We suggest the channel is important for the transport of I and Cl ions across the apical membrane into the colloid space and is important for hormone synthesis and follicle formation.

199. P53 IDENTIFICATION OF ANION EXCHANGER AE2 IN THYROID AND ITS ROLE IN IODIDE LOSS.

A. Fanelli, D. Tombaccini, S.Q. Doi, M.C. Baggio, and E.F. Grollman. NIDDK; National Institutes of Health, Bethesda, MD 20892-1800

The chloride/bicarbonate exchanger (band 3) in erythrocytes is inhibited by DIDS (4,4'-diisothiocyano-2,2'-disulfonic acid stilbene). Disulfonic stilbene derivatives however were found to increase iodide accumulation in rat thyroid cells (FRTL-5). Replacement of chloride in the external medium with gluconate did not change the effect of DIDS, and suggests a direct involvement of this compound in iodide transport in these cells. But the iodide pathway affected by stilbenes appears to be independent of sodium iodide uptake; DIDS' action is therefore postulated to be at the site of iodide loss from the cell. Based on these studies there is a DIDS-binding protein in thyroid involved in iodide loss.

Two additional approaches were used to identify the DIDS-binding protein in thyroid: the first involved cloning a band 3- related cDNA from FRTL-5 cells, while the other used protein immunostaining. In the first approach, a FRTL-5 library was screened with murine band 3 cDNA, provided by H. Lodish. Based on sequence analysis, the cloned cDNA was identified as AE2. RNA levels hybridizing with this clone were not regulated by TSH, being the same in FRTL-5 cells grown with or without the hormone in the medium. In addition, no differences were found in Northern blot analysis using either AE2 from thyroid or AE2 cDNA from rat stomach (RSAE2-1 provided by G. Shull).

For the second approach an antibody reported to react with AE2 was used. The major immunoreactive band in FRTL-5 cells after Western blotting had a molecular weight of ~ 130 KD. Although the immunoreactivity was less in cells grown with TSH, the difference could be related to differences in glycosylation; in fact a different antibody showed that the amount of immunoreactive protein in cells treated with or without TSH was the same. Membranes from rat uterus, reported to have high levels of AE2, showed a major immunoreactive band with a MW of ~ 150 KD. Thyroid membranes from Fisher rats also had a similar high MW form of AE2 suggesting that FRTL-5 might express a truncated form of AE2.

In summary, cloning studies and immunostaining analysis identified AE2 in rat thyroid cells; transfection studies will show if an AE2 encoded protein can account for the DIDS- enhanced iodide accumulation.

200. P54 AMMONIUM PERSULFATE: AN ALTERNATIVE OXIDIZING REAGENT FOR MEASURING URINARY IODINE.

S. Pino, S-L Fang, and L.E. Braverman, Division of Endocrinology, University of Massachusetts Medical School, Worcester, MA.

The most accurate and reproducible method for measuring iodide employs chloric acid as the oxidizing agent to remove interfering substances. Unfortunately, chloric acid is a potential hazard requiring an explosion proof hood among other precautions. We have developed a simple, convenient, and economic method for measuring urinary iodide using 1M ammonium persulfate as the oxidizing reagent for the removal of interfering substances. Ammonium persulfate is a non-explosive, non-hazardous chemical compared to chloric acid. The oxidation procedure can be completed in 30 minutes at a temperature of 90-95C. The iodide in the urine is then measured by a modification of the traditional colorimetric method of Sandell and Kolthoff which is based on the catalytic effect of iodide on the reduction of ceric sulfate by arsenite utilizing a Technicon Autoanalyzer. 110 urine samples collected from a mixed population of healthy males and females, ranging in age from 6 to 79 years and living in the United States, were analyzed for urine iodide content by two methods: the proposed ammonium persulfate method and our standard chloric acid method of Bennotti et al. (Clin. Chem. 11:932, 1965). The ammonium persulfate method has an intra assay CV of 9.1% at 5.4±0.5 ug I/dl (mean±SD), 7.8% at 18.5±1.4 ug I/dl, and 4.0% at 44.9±1.8 ug I/dl. The inter assay CV is 10.2% at 5.9±0.6 and 7.9% at 41.5±3.3. Recovery of iodide added to urine in vitro was 107%, 94%, and 97% for 5.3 ug I/dl, 9.8 ug I/dl and 46.2 ug I/dl, respectively. Addition of potassium thiocyanate prepared in iodine free water to a final concentration of 1.25-20 mg/L did not affect the assay. The standard curve was linear up to 0.15 ug I. The lower limit of detectability was 0.0034 ug I. Values for iodide in 110 urines measured by the reference chloric acid method ranged from 0.5 to 102 ug I/dl. The persulfate method (Y) correlated extremely closely with the reference chloric acid method (x) by the method of Pearson. Thus, $Y=0.862x + 0.9224$ug I/dl and $r=0.975$. Conclusion: A new, safe, and simple method for measuring urinary iodide is described which uses ammonium persulfate rather than chloric acid as the oxidizing agent for removal of interfering substances. This method can more easily be adapted to laboratories which do not have the appropriate equipment to use chloric acid and eliminates the hazard of chloric acid.

201. P55 RESORCINOL EFFECT ON IODIDE UPTAKE IN FRTL-5 THYROID CELLS. E. Gaitan, R.C. Cooksey, J. Legan, Division of Endocrinology, University of Mississippi Medical School and VA Medical Center, Jackson, Mississippi, U.S.A.

Resorcinol, a coal-derived antithyroid/goitrogenic pollutant and degradation by-product of humic substances in nature, increases thyroid I^- uptake **in vivo** in the Gunn rat ($p<0.002$), while concomitantly increasing thyroid weight and decreasing thyroid hormone synthesis (Excerpta Med. ICS652:823,1984) by inhibition of thyroid peroxidase (TPO) (J.Toxicol.Environ.Health 37:467, 1992). Therefore, the effect(s) of resorcinol on I^- uptake were investigated **in vitro** using the functional rat thyroid cell line, FRTL-5. Results of tests with 24-well determinations per sample were analyzed by Student-Newman-Keuls test at 5% level. Resorcinol (0.5,1,2,5,10, 20,50,100μM) in the presence of TSH (100μU) (6H-medium), produced higher ^{125}I uptake (pmol I^-/μg DNA) at all concentrations (α0.05) than TSH alone (7.2±0.6SD), reaching the highest I^-uptake at 2μM (10.1±0.7SD). Higher ^{125}I uptake (α0.05) was also produced by resorcinol in TSH-free(5H)medium but only at 20,50,100μM. Ouabain (1,10mM), an inhibitor of Na^+-K^+-ATPase, suppressed (α0.05) in a dose-dependent manner ^{125}I uptake reaching same values at both 1 and 10mM concentrations in cells exposed to TSH alone (6H-medium) and TSH plus resorcinol (2μM)(NS), as well as in cells in TSH-free(5H) medium alone and TSH-free(5H)medium plus resorcinol (50μM)(NS). Potassium perchlorate (KClO4) (1,10μM), a competitive monovalent anion inhibitor, also suppressed in a dose-dependent manner (α0.05) ^{125}I accumulation by FRTL-5 cells, but in contrast with Ouabain suppression, ^{125}I values were still higher (α0.05) in cells exposed to resorcinol at the lowest KClO4 concentration (1μM), both in the presence and absence of TSH. Lower concentrations of resorcinol (0.5,1,2μM) in the presence of TSH (6H medium) increased [^{3}H]-thymidine incorporation into DNA (cpm/μg DNA) (α0.05) but higher concentrations (5,10,20,50,100μM) inhibited it in a dose-dependent manner (α0.05). [^{3}H]Tdr-incorporation into DNA was also increased by resorcinol in TSH-free(5H)medium, but only at higher concentrations (50,100μM)(α0.05). cAMp-production (pmoles/μg DNA) was increased by resorcinol (α0.05) both in the presence (6H) and absence (5H) of TSH. Results demonstrate that resorcinol stimulates I^- uptake by thyroid cells and that this stimulatory effect is mainly mediated by Na^+-K^+-ATPase in someway resembling the action of TSH. This action of resorcinol is independent of its inhibitory effect on TPO and hormone synthesis.

202. P56 THYROID HORMONOGENESIS IS DIRECTLY MODULATED BY N-GLYCANS AT THE N-TERMINAL DOMAIN OF HUMAN THYROGLOBULIN.

P.J. Lejeune, B. Mallet, N. Baudry, P. Niccoli & P. Carayon. Unité 38 INSERM, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 5, France.

Thyroglobulin (Tg), the major glycoprotein of the thyroid gland, is the substrate of thyroid hormones (T4 and T3) biosynthesis which occurs at the apical membrane and involves iodination of Tyr residues into iodotyrosines and coupling of few of them into hormones. It is known that Tg N-glycans play a role in recycling poorly iodinated Tg through a GlcNAc receptor. Therefore, poorly iodinated Tg is not degraded after endocytosis but, rather, recycled via Golgi apparatus to the colloid in order to complete its iodination and, ultimately, proceed to hormone formation. In the present study, we show that the presence and the structure of N-glycans are not only involved in Tg traffic but are also directly implicated in hormone synthesis.

The N-terminal domain of human Tg (NTD, Asn1-Met171, N-glycosylated at Asn57 and Asn91) contains the preferential T4-forming site at Tyr5 and is also able to form T4 in vitro. NTDs were purified after CNBr treatment from Tg preparations with low and mild iodine content in vivo and from poorly iodinated Tg after in vitro iodination and coupling. Using affinity chromatography on ConA- and RCA120-Sepharose columns, the NTDs were separated in five isoforms according to the type and the degree of N-glycosylation and were tested for T4 content. Our results showed that 1/ in vivo as well as in vitro, unglycosylated isoforms did not form hormone, while fully or partially (at Asn91) glycosylated ones did; 2/ the presence of high mannose type structures enhanced the hormone content; 3/ desialylation did not affect in vitro hormone synthesis.

In conclusion, direct involvement of N-glycans in thyroid hormonogenesis open the possibility that 1/ thyrotropin, which modulates the number and the nature of the oligosaccharide side chains born by Tg could also modulate the formation of T4 residues through the N-glycosylation process; 2/ defect in hormonosynthesis may derive from abnormal glycosylation of the NTD. Furthermore, domain rather than whole molecule, should be considered when ascribing a role to N-glycans in the function of proteins.

203. P57 THYROGLOBULIN ENDOCYTOSIS IS SELECTIVE. BUT DOES IT INVOLVE A SPECIFIC RECEPTOR ?

A. Giraud, J. Lanet and J.L. Franc, INSERM U 38, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cédex 5, France.

In thyroid follicles, the iodinated thyroglobulin (Tg) present in the lumen is taken back into thyroid cells by ill-defined mechanism(s) of endocytosis. While some previous experiments lead to the conclusion that apical endocytosis does not exhibit selectivity for Tg (1), other experiments are in agreement with a coated-pit mediated process (2) and with a Tg uptake mediated by low affinity binding sites (3). These apparently conflicting results prompted us to study the endocytosis of Tg with an experimental system deviced to prevent artifacts as far as possible.

Endocytosis experiments of ^{125}I-labeled porcine Tg or bovine gamma globulin (γG) were performed in siliconized flasks with suspension culture of porcine thyroid cells forming inside-out follicles (4). The cells were treated with 10^{-4}M dibutyryl cyclic AMP for 24h before each experiment. In some cases the coated-pit pathway was inhibited either by hyperosmolarity or by cytoplasm acidification.

The results obtained show that :

- there is a preferential binding and subsequent endocytosis of Tg compared to γG.
- Tg endocytosis is at least partly mediated by coated pits.
- Tg endocytosis occurs through numerous binding sites (more than 10^6 sites per cell) of low affinity (Kd between 10^{-5} and 10^{-6}M).
- the amount of Tg recovered in cells is strongly enhanced by the presence in the medium of high concentrations of ovomucoid, a glycoprotein bearing terminal GlcNAc. This may reflect the saturation of the recycling receptor described by Miquelis et al. (5) (a GlcNAc receptor present in thyroid cells).

In conclusion Tg endocytosis is selective. However the high number of binding sites associated to a very low affinity suggests that Tg could adhere to a range of non specific sites on the cell surface.

Whether this selective Tg binding and endocytosis is tissue specific is currently under investigation. Further studies will be necessary to determine the physico-chemical basis of the preferential Tg binding (hydrophobicity, surface charge of the molecule...).

References

(1) Kostrouch,Z., Bernier-Valentin,F., Munari-Silem,Y., Rajas,F., Rabilloud,R. and Rousset,B. (1993) Endocrinology, 132,2645-2653.
(2) Bernier-Valentin,F., Rostrouch,Z., Rabilloud,R. and Rousset,B. (1991) Endocrinology, 129,2194-2201.
(3) Lemansky,P. and Herzog,V. (1992) Eur. J. Biochem., 209,111-119.
(4) Espanet,H., Alquier,C. and Mauchamp,J. (1992) Exp. Cell Res.,200,473-480.
(5) Miquelis,R., Courageot,J., Jacq,A., Blanck,O., Perrin,C. and Bastiani,P. (1993) J. Cell Biol., 123,1695-1706.

204. P58 NEW SPECIFIC INHIBITORS OF Na^+/I^- SYMPORTER, TETRAHYDROPYRIDINE DERIVATIVES, IN CULTURED RAT THYROID (FRTL-5) CELLS.

T.Saito, Third Department of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi 409-38, JAPAN

Although iodide transport mechnism is important in thyroid physiology, molecular structure of Na^+/I^- symporter is not known. To search for this problem, we have developed six new inhibitors of Na^+/I^- symporter from tetrahydropyridine derivatives. IC50s of these six compounds (A1-6) were 15, 8, 30, 40, >100, and >100 μM, respectively, using cultured rat thyroid FRTL-5 cells. These defferences of the inhibitory effect on $^{125}I^-$ uptake seems to depend on the varieties of their residues. As A2 had the strongest inhibitory effect, we have then synthesized another compound (A2N) whose structure is similar to A2 but contains an amino residue. IC50 of A2N was 20 μM and $^{125}I^-$ uptake was inhibited up to 87% at the concentration of 100 μM. In order to characterize the inhibitory effect of the compound, iodide uptake assay was performed at five concentrations of extracellular Na^+ in the presence of various concentrations of A2N. A2N decreased $^{125}I^-$ uptake in a dose dependent manner and a higher concentration of extracellular Na^+ recruited $^{125}I^-$ uptake of the cells. The kinetic analysis revealed that Ki of A2N, as determined from a Dixon plot, was approximately 20 μM, suggesting that it had a higher affinity with the symporter than other inhibitors such as TRP-P-2 and chloride channel blockers. Furthermore, these inhibitors did not affect cAMP accumulations stimulated by thyrotropin (TSH). As A2N seems to be a specific inhibitor of Na^+/I^- symporter, these findings suggest that A2N may be a convinient tool for the identification of Na^+/I^- symporter.

205. P59 ROLE OF GUANYLATE CYCLASE-cGMP IN THE REGULATION OF IODIDE UPTAKE BY CALF THYROID PRIMARY CULTURES.LV Bocanera,L Krawiec,D Silberschmidt,O Pignataro,GJ Juvenal,LB Pregliasco,MA Pisarev
Div.Bioquim.Nuclear,CNEA;IBYME and CONICET,Buenos Aires,Argentina

The regulation and role of guanylate cyclase (GC) and cyclic GMP (cGMP) was studied in calf thyroid cells in primary culture. Soluble GC was stimulated by sodium nitroprusside (SNP).The lowest dose was 10 μM (9.37$\pm$0.55 pmol cGMP/min/mg prot. vs control 4.25$\pm$0.06 $p<0.01$, as mean$\pm$SEM),and stimulation was observed after 5 min incubation (122% $p<0.01$). SNP increased cGMP and cAMP. For cGMP the increase occured after 5 min (SNP 2.35$\pm$0.34 pmol/mg prot vs control 1.18$\pm$0.12 $p<0.01$), and remained elevated up to 60 min (SNP 2.71$\pm$0.30 $p<0.01$). TSH did not alter cGMP levels increased by SNP. For cAMP the increase occur after 15 min (SNP 9.54$\pm$1.14 pmol/mg prot.vs control 4.94$\pm$0.37 $p<0.01$) and returned to basal values at 60 min. TSH also increased cAMP (TSH 7.06$\pm$0.72 pmol/mg prot. $p<0.01$). At 5 min both compounds were additive and this effect disappeared afterwards. TSH-stimulated ^{125}I uptake (0.5 mU/ml 72 hs) was inhibited by SNP (TSH 4006$\pm$176 cpm/well vs SNP+TSH 1171$\pm$23 $p<0.01$), due to decreased influx,without change in the efflux. This effect was mimicked by 10 μM 8Br-cGMP, supporting that this nucleotide mediates SNP action (TSH 2858$\pm$446 cpm/well vs 8Br-cGMP+TSH 1907$\pm$101 $p<0.05$). SNP also decreased the ^{125}I uptake stimulated by forskolin (FK) 10 μM (FK 4509$\pm$355 cpm/well vs SNP+FK 3208$\pm$264 $p<0.005$), while 50 μM A-23187 blocked the effect of TSH on the same parameter. TSH increased Na^+K^+ATPase and addition of SNP caused a significant inhibition (control 29616$\pm$1503 dpm ^{32}P/mg prot.; TSH 75753$\pm$10006 $p<0.01$ vs control; SNP+TSH 14107$\pm$9028 $p<0.01$ vs TSH). This would suggest that the blockade of ^{125}I uptake and the inhibition of ATPase caused by SNP are related. The present results show that the soluble GC is stimulated by SNP. The GC-cGMP system would play an inhibitory role in the TSH regulation of iodide uptake.

206. P60 DISSOCIATION AND REASSOCIATION OF NON-COVALENTLY LINKED DIMERS OF HUMAN 19 S THYROGLOBULIN TREATED WITH UREA. F. Gentile*, B. M. Veneziani# and C. Sellitto#, *Centro di Endocrinologia e Oncologia Sperimentale del Consiglio Nazionale delle Ricerche and #Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università "Federico II", Naples, Italy.

The effect of increasing concentrations of urea on the association of non-covalently linked dimers of poorly-iodinated human 19 S thyroglobulin (Tg) was analyzed by native polyacrylamide gel electrophoresis in urea transverse gradients. Complete dissociation of native 19 S Tg dimers was observed in 2.0 M urea both at pH 7.4 and at pH 9.0. At urea concentrations between 2.5 M and 5.5 M Tg monomers partially reassociated into dimers which in native electrophoresis migrated slower than native dimers. These anomalous dimers redissociated at urea concentrations above 5.5 M. When 19 S Tg denatured in urea was renatured by dialysis and/or native electrophoresis in the absence of urea, the recovery of dimers with the same migration as native dimers was dependent on the concentration of urea to which 19 S Tg had been previously exposed. Native dimers were formed after renaturation of samples already exposed to urea concentrations not higher than 2.5 M, whereas anomalously slow dimers were formed with different yields upon renaturation of Tg samples exposed to higher urea concentrations. When Tg denatured in urea was diluted to a concentration of 0.01 mg Tg/ml prior to removal of urea by dialysis and then concentrated again, native dimers were formed in the samples exposed to urea concentrations not higher than 2.5 M, while no dimers were formed in those exposed to higher urea concentrations. In conclusion, complete dissociation of non-covalent 19 S Tg dimers occurs in 2.0 M urea and is fully reversible. Incubation of Tg at higher urea concentrations results in the exposure of alternative contact surfaces and the formation of dimers that seem to have a less compact structure. It appears that the intermonomeric contact sites of native dimers are stably exposed in the native state and in urea up to 2.5 M, but they are lost at higher urea concentrations. Instead, the contact sites involved in the formation of "slow" dimers appear to be exposed as the result of local unfolding events occurring over a definite range of urea concentrations, and to be stabilized by the reciprocal interaction. The possibility of dissociating 19 S Tg dimers reversibly is being exploited in studies of the relationship between dissociation and proteolytic accessibility of Tg which may shed light on the location of the intermonomeric contact sites of 19 S Tg.

207. P61 mRNA'S OF SEVERAL LENGTHS ENCODE THYROGLOBULIN'S C TERMINUS. M. E. Mason, B. Struyk, J. T. Dunn, University of Virginia, Charlottesville, VA.

Thyroglobulin (Tg) contains specific sites for thyroid hormone formation. Abnormalities in Tg are associated with inadequate hormone production and thyroid disease in humans and animals. Previous work indicates heterogeneity in the region of the important hormonogenic sites near Tg's C terminus in certain familial goiters, in contrast to the invariant N terminus. In the present work, we examined mRNA from this C-terminal region in 10 human thyroids from subjects with thyroid conditions and disease that included sporadic multinodular goiter, familial goiter, Pendred's syndrome, Graves' disease, Hashimoto's thyroiditis and thyroid cancer. We extracted total RNA and used the RNAse protection assay as follows: ^{32}P-labeled RNA probes encompassing all or part of the region of Tg cDNA nucleotides 7808-8260, were gel- purified, hybridized with human thyroid RNA samples, and after digestion of the unhybridized RNA probe with ribonucleases, were separated by PAGE and examined by autoradiography. The major species detected corresponded in size to full length Tg mRNA, but smaller species were also noted. For example, when using a probe corresponding to Tg nucleotides 7889-8086, we observed the expected full length species of 197 nucleotides and an additional prominent fragment of 170 size, as well as 3 less abundant bands of approximately 130, 124, and 120 nucleotides in length. These smaller species varied in quantity in different diseased thyroids studied, but did not appear to be specific for a particular thyroid disease. Every one of the thyroids examined so far has shown some degree of heterogeneity by this technique. We conclude that the mRNA encoding Tg's C-terminus has widespread heterogeneity and suggest that this may affect thyroid hormone formation and contribute to the development of goiter.

208. P62 THE TRANSIENT EXPRESSION OF LARGE HUMAN THYROGLOBULIN FRAGMENTS IN MAMMALIAN CELLS. Simone A.R. van de Graaf, Erwin Pauws, Carrie Ris-Stalpers, Jan J.M. de Vijlder. Academic Medical Center, Experimental Pediatric Endocrinology, University of Amsterdam, The Netherlands.

In our continuing study to unravel the role of thyroglobulin (TG) in the thyroid hormone synthesis we have subcloned human TG cDNA in four overlapping fragments using the polymerase chain reaction. The cDNA fragments approximately 2.3 kb each encoding the amino acids minus 19 - 684 (TgI), 648 - 1392 (TgII), 1283 - 1981 (TgIII) and 1908 - 2746 (TgIV), respectively (1) were subcloned downstream of a CMV promoter. TgI contains the wild type (WT) 5' upstream sequence with the signal peptide and translation starts from the original ATG. In the other three constructs an artificial consensus Kozak sequence is incorporated upstream of an internal ATG to ensure initiation of translation. The constructs were transiently transfected by electroporation in human thyroid cells (HTori-3) and in monkey kidney cells (COS-1). Protein expression was determined in cell-lysates by Western blotting and immuno-chemiluminescence. In whole cells the expression of TG was visualized by using a polyclonal TG antibody coupled to a FITC-labelled second antibody. Preliminary results indicate that i) using SDS PAGE, the expressed TG proteins are of the calculated MW: 76, 82, 77 and 93 kDa for TgI, II, III and IV, respectively; ii) all four TG fragments remain intracellular in both HTori-3 and COS-1 cells and are not secreted in measurable amounts into the medium; iii) the protein TgI (containing the WT signal peptide) is mainly localized in the rough endoplasmatic reticulum whereas the other three large TG fragments (TgII, III, IV) show a more diffuse pattern. To further investigate the influence of the signal peptide on the localization of the TG constructs we are now subcloning the WT signal peptide sequence upstream of TgII, III and IV cDNA constructs. We are also investigating the T4 forming potential of each TG construct after expression in HTori-3 and COS-1 cells.

(1) Y.Malthiery & S.Lissitzky, Eur.J.Biochem. 165,491-498 (1987)

209. P63 **URSODEOXYCHOLIC ACID (UDCA) INHIBITS IODINE METABOLISM IN PORCINE THYROID CELLS IN PRIMARY CULTURE: IS UDCA A BENEFICIAL ANTITHYROID DRUG?** Y. Takiyama, R. Kanri and I. Makino, The Second Department of Internal Medicine, Asahikawa Medical College, Asahikawa 078, JAPAN

Ursodeoxycholic acid (UDCA), derivative of bile acid has been used to treat cholesterol gallstones. We have shown that bile acid decreases iodide uptake in porcine thyroid cells. This suggests that UDCA may also be used as an antithyroid drug. In the present study we examined whether UDCA inhibits iodine metabolism in porcine thyroid cells.

Porcine thyroid cells obtained from a slaughter house, were cultured in the presence of 2 % fetal calf serum (FCS) for 24 hrs. These cells were exposed to UDCA in F12 medium without FCS and phenol red, followed by incubating with Na[^{125}I] for 1 hr. Organification of iodide was measured by counting TCA-insoluble fraction from the culture medium after 3 days incubation with Na[^{125}I]. TSH-induced iodide uptake was inhibited by only 24 hour incubation with UDCA. After 72 hours, 250 μM UDCA inhibited TSH-induced iodide uptake 41.4 ± 18.6 % of control cells untreated with UDCA. These effects were also seen in a dose-dependent manner within pharmacological concentration (25 to 250 μM). The release of organified iodide into medium completely suppressed by the treatment of UDCA. Baseline iodide uptake and organified iodide release were not changed by UDCA. Cytotoxicity assay using dimethylthiazol-2-yl-diphenyl tetrasolium bromide showed negative results. These results suggest that the effects of UDCA are not due to cytotoxicity. Both TSH-induced iodide uptake and iodine organification depend on cAMP signal. UDCA inhibited not only cAMP accumulation at steady state in the cells treated with TSH for 1 or 3 days but also inhibited both 8BrcAMP-stimulated iodide uptake and organified iodine release. These data suggest that UDCA decreases iodine metabolism, inhibiting cAMP production and post-cAMP pathway.

In summary, UDCA inhibits thyroid cell iodine metabolism under pharmacological concentrations. These data suggest that thyroid functions need to be monitored when UDCA are used to treat patients. UDCA might be a useful antithyroid drug, when the thionamides are not tolerated because of their side effects.

210. P64 IODOAMINO ACID DETERMINATION IN THYROGLOBULIN: A RAPID AND SENSITIVE MICROMETHOD.

N. Baudry, R. Koukiekolo, L. Vinet, P.J. Lejeune, J.L. Franc & B. Mallet . Unité 38 INSERM, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 5, France.

Among chemical methods used for estimation of iodoamino acid residues, very few are sensitive enough. It is, thus, necessary to turn to radiolabeling. In this study we developed a new method involving rapid high-performance liquid chromatography to separate monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T3) and thyroxine (T4), followed by a sensitive spectrophotometric detection using a microassay system based on the catalytic Sandell-Kolthoff reaction.

Separation of MIT, DIT, T3 and T4 was carried out onto a Lichrosorb RP-8 chromatographic column and elution was performed with a linear gradient of acetonitrile-water (10:90 to 60:40) with a flow rate of 0.8 ml/min during 45 min. Fractions were collected and aliquots were put into wells of microtiter plates containing PBS buffer, pH 9.0 and arsenious acid, then ceric ammonium sulfate was added. The decrease in O.D. in the presence of iodide and/or organic iodine was monitored, using a microtiterplate reader, at 414 nm after 10 min incubation. Whatever the iodoamino acid residue, quantitative recovery was about 80%, coefficients of variation ranged from 1.1 to 5.8% and limit of detection was under 0.6 pmol. Standard curves were linear up to 25 pmol after an incubation time of 10 min. However, shorter incubation time can be used for higher iodoamino acid concentrations.

Enzymatic hydrolysates from thyroglobulins more or less iodinated in vivo or in vitro were analyzed. The results were fully comparable with those obtained by the common method using ion-exchange chromatography followed by simultaneous detection with cerium-arsenic reaction.

In conclusion, this new microassay is particularly convenient for its rapidity (less than 2 h) and its sensitivity (<1pmol). Moreover, it can test many samples simultaneously and does not require labeled iodine. Many potential applications can be foreseen for detecting iodine-containing compounds in various situations.

211. P65 MATERNAL "COMPOUND W", A POSSIBLE INDICATOR OF FETAL THYROID FUNCTION DURING MATERNAL HYPERTHYROIDISM ON PTU THERAPY

L. VanMiddlesworth[1], S. M. Guerra[1], B. M. Mercer[2], S. R. Rose[3], S. Burstein[3], and S.Y. Wu[4]. Dept. Medicine[1], Dept. Obstetrics & Gynecology[2], Dept. Pediatrics[3], Univ. Of Tenn., Memphis, TN; Depts. Of Nuclear Medicine and Medicine[4], VA-Univ. of Calif., Irvine Medical Center, Long Beach, CA.

Recently Wu et al. (JCE&M 1994, 78:1505) suggested that the human fetus may be responsible for a maternal blood-borne compound which cross-reacts immunologically with 3,3'-diiodothyronine sulfate (T_2S), designated "Comp W". It is hypothesized that this compound is produced in the fetus from thyroid hormone and equilibrated with maternal blood. A pregnant hyperthyroid patient at 31 weeks gestation had Total T_4 = 23.7 μg/dl, FT_4 = 2.9 (normal 0.7 - 1.8) and TSI index = 8.3 (normal 1.3). She was maintained on 300 mg propylthiouracil (PTU) per day but she was considered clinically hyperthyroid at 34 weeks gestation with subnormal FT_4 = 0.4 ng/dl but T_3 = 350 ng/dl and TSH = 0.14 μU/ml. Her "Comp W" was 76 ng T_2S-equivalent/dl which was less than that in 22 patients with normal pregnancies from 34 to 40 weeks gestation (217 ± 108 ng/dl, mean ± 1 S.D.). PTU was reduced to 150 mg/d, and T_4, 0.1 mg/d, was added to her treatment. Four weeks later she delivered a baby with normal thyroid newborn-screening tests. Upon delivery at 38 weeks, the mother had normal Total T_4 of 6.9 μg/dl, but TSH = <0.05 μU/ml. At birth "Comp W" of maternal and cord blood were normal at 196 and 195 ng/dl. After 4 days, the baby was considered euthyroid with normal Total T_4 = 11.4 μg/dl but "Comp W" was 129 ng/dl, which was greater than normal maternal values on the third and fifth days post-partum. The baby developed transient hyperthyroidism and was euthyroid after 2 months without specific therapy. It is considered that the maternal "Comp W" may have indicated PTU therapy was excessive for the fetus at 34 weeks gestation and reduction of PTU with added T_4 may have helped to assure normal thyroid newborn-screening of the infant.

212. P66 GRAVES'DISEASE - HIGH DOSED METHIMAZOLE THERAPY AND LEVOTHYROXINE ADMINISTRATION DO NOT LEAD TO BETTER LONG TIME RESULTS.

W.Meng, Th.Niemeyer, G.Kirsch, S.Krabbe and A.Schindler, Medizinische Universitaetsklinik, Greifswald, Germany

In a prospective randomized study we examined the effect of a highly dosed methimazole(MMI) treatment (n=73, 40 mg/day, 1 year, T4 substitutive) in comparison with a low dosed therapy (n=76, 10 mg/day, T4 sporadic). 2. A retrospective study was conducted to test the hypothesis that the remission rate might be increased by TSH suppressive treatment with Levothyroxine (T4 during and 1 year after MMI treatment: n=72, TSH <0,5 mU/l, without T4 n=106, TSH >0,5 mU/l). The patients were followed-up for 2 years.

No differences in the relapse rates were observed. The relapse rate was during the first two years in the high dosed group 49%, in the low dosed group 47%, in cases with T4 45% and in patients without T4 administration 49%. The rate of side effects was 17% in the 40 mg group and 10.5% in the 10 mg group.

group	n	rate of persistences	rate of recurrences after 1 year	2 years
40 mg MMI	73	11%	36%	49%
10 mg MMI	76	17%	38%	47%
T4 during/after	72	13%	34%	45%
without T4	106	17%	40%	49%

Conclusion: High doses of MMI or T4 administration do not improve the long term results in Graves'disease. Our data correspond with the results of a european multicentre study (2) and are the first arguments against the findings of Hashizume et al.(1).

1. Hashizume,K. et al.: N.Engl.J.Med.324 (1991) 947-953.
2. Reinwein,D. et al.: JCEM 76 (1993) 1516-1521

213. P67 ROLE OF THYROID PEROXIDASE IN MINOCYCLINE-INDUCED BLACK PIGMENTATION OF THE THYROID: ANTITHYROID EFFECTS OF MINOCYCLINE. Martha L. Dorris and Alvin Taurog. University of Texas Southwestern Medical Center, Dallas, Texas

Minocycline (MN) is a member of the tetracycline family of antibiotics. Two recent double-blind,.placebo-controlled studies reported favorable results with MN in the long-term treatment of rheumatoid arthritis (Arthritis Rheum. 37:629, 1994; Ann. Int. Med. 132:81, 1995). Earlier reports, however, indicate that MN has antithyroid effects in laboratory animals. Administration of MN to rats, dogs, and monkeys caused a black discoloration of the thyroid (Toxicol. Appl. Pharmacol. 11:150; 1967). MN-induced black thyroids have also been observed in humans (Am. J. Path. 117:98, 1984). Goitrogenic effects and inhibition of thyroid hormone formation were reported in rats treated with large doses of MN (Endocrin. 90: 1192, 1972). The aim of the present study was twofold: 1) To test the hypothesis (J. Pharmacol. Exp. Therap. 266:1164, 1993) that thyroid peroxidase (TPO) is involved in the generation of the MN-induced black pigment, and 2) To test MN for inhibition of TPO-catalyzed iodination and coupling. Formation of the black pigment was studied at pH7.0 with varying concentrations of MN, TPO, and glucose-glucose oxidase. When 0.25 mg/ml MN was incubated at 37° with the H_2O_2 generating system, glucose (1 mg/ml) - glucose oxidase (200mU/ml), no color change was observed after 1.5h. However, when the incubation system included 50nM TPO, a distinct black oxidation product was observed. This result provides direct evidence that TPO is involved in the production of the MN-induced black thyroid. In an iodination system containing TPO (25nM), goiter Tg (1.0μM), $^{125}I^-$(100μM), glucose (1mg/ml), and glucose oxidase (50mU/ml), at pH7.0, 31% of the $^{125}I^-$ was organically bound after 20 min at 37°C. This value was reduced to 4.7% by 25μM MN, 7.6% by 40μM PTU, and 6.8% by 20μM MMI. In a coupling system at pH7.0, 37°C, containing 1.5μM preiodinated ^{125}I-Tg (30 atoms I/molecule Tg) 24nM TPO, 1μM free diiodotyrosine, 1mg/ml glucose, and 20mU/ml glucose oxidase, 1.22 residues T_4 were formed per molecule Tg in 10 min. Formation of T_4 was completely inhibited by 25μM MN, but only 50% inhibited by 25μM PTU, and 30% inhibited by 25μM MMI. Thus, MN is at least as potent as conventional antithyroid drugs in inhibiting both TPO-catalyzed iodination and coupling. It seems advisable, therefore, to monitor thyroid function in patients receiving long-term MN therapy.
Supported by NIDDK 03612.

214. P68 EFFECT OF THE PRESENCE OF ANTITHYROID PEROXIDASE (TPO) ANTIBODY ON THE DEVELOPMENT OF POSTPARTUM THYROID DISEASE (PPT) IN A SUBSEQUENT PREGNANCY. F. Ammari,[1] C.J. Richards, Department of Medicine[1], University of Wales College of Medicine, Cardiff and Dept. Obstetrics, Caerphilly Miners District Hospital, Cardiff, U.K.

PPT, characterised by hyper or hypothyroidism (or both) occurs in 50% of anti TPO Ab +ve women as detected early in gestation. In order to evaluate the effect of the presence of anti TPO antibody in pregnancy on the subsequent development of PPT following a further pregnancy we have studied women who have had two pregnancies observed over 2 or 3 separate detailed studies of postpartum thyroid disease in our area during the past 10 years. Thyroid function (FT4, FT3 and TSH) and anti TPO Ab were measured (by ELISA) from 6 wks postpartum and monthly for 6 months. Thirteen anti TPO negative women who were also studied during two post-partum periods were used as a control group.

A total of 50 women had two observed pregnancies and this included 2 women with 3 deliveries. In 17 anti TPO Ab -ve women no change in antibody status or thyroid function was observed in the second pregnancy. Of the 33 anti TPO Ab +ve women 21 did not have PPT in pregnancy one but 5 (14%) developed PPT in pregnancy 2; 13(62%) remained euthyroid and 3 seroconverted to anti TPO Ab -ve in pregnancy 2. Of 12 PPT +ve women in pregnancy one, 10 (83%) developed recurrent PPT while 2 did not after a mean of 39.8 months (range 12-65 months). In two anti TPO Ab +ve patients with 3 pregnancies both patients remained euthyroid each time, one had PPT the first time but remained euthyroid after.

The mean time interval between pregnancies in those women who developed PPT in both pregnancies (40.3 months ± 14SD) compared to those who were anti TPO Ab +ve only in both pregnancies (35.4 ± 14.4) was not significantly different. Those who were anti TPO Ab +ve in pregnancy 1 and PPT in pregnancy 2 (n=5) had a similar interpregnancy time interval.

This study has indicated a high risk of developing PPT following a subsequent pregnancy if it had occurred previously. However, there is also a significant chance (24%) of PPT occurrence when women had been euthyroid after a first pregnancy. These data emphasize the value of screening for anti TPO Ab early in gestation, especially if there is a previous history of PPT or anti TPO Ab positivity in a previous pregnancy.

215. P69 **METHIMAZOLE TREATMENT OF MATERNAL HYPERTHYROIDISM DURING LACTATION.**

F. Azizi. Endocrine Research Center, Shaheed Beheshti University of Medical Sciences, Eveen, Tehran, I.R. Iran.

For many years, breast-feeding was forbidden if antithyroid drugs were being used. Recently mothers with Graves' disease under PTU treatment have been advised of safety of breast-feeding for their infants. The issue with methimazole (MMI) is not clear. Only one paper has recommended the saftey of carbimazole up to 15 mg daily (equal to 10 mg MMI). To study the effect of MMI therapy in lactating mothers on the thyroid function of their breast-fed infants, we studied 15 newborns of women with thyrotoxicosis who had received MMI during pregnancy and permitted to breast-fed exclusively after delivery while taking 5 mg MMI daily (part one), and 20 infants whose mothers developed thyrotoxicosis 2 to 11 months after delivery and received 10-20 mg MMI daily for one month and allowed to breast-feed (part two). In part one, no clinical evidence of thyroid dysfunction was detected in newborns one month after breast-feeding, and T4, T3 and TSH in infants: 12.4 ± 1.2 μg/dl, 198 ± 14 ng/dl and 1.2 ± 0.9 μU/ml respectively, were all within normal range. In part two one month following MMI 10 mg/d in 14 mothers, four were still clinically hyperthyroid and one hypothyroid; all infants were euthyroid. In mothers, FT4I decreased from 19.8 ± 3.8 to 12.1 ± 4.2 ($P<0.001$), and FT3I decreased from 451 ± 97 to 194 ± 44 ($P<0.001$).In infants, there was no statistical difference in hormone concentrations before and after initiation of MMI therapy, T4: 12.0 ± 2.3 vs 12.3 ± 1.2 μg/dl, T3: 201 ± 20 vs 192 ± 15 ng/dl and TSH: 1.5 ± 0.8 vs 1.3 ± 0.9 μU/ml, respectively. In 6 lactating thyrotoxic women one month after MMI 20 mg daily, all infants were euthyroid: T4 = 11.1 ± 1.3 μg/dl, T3 = 207 ± 18 ng/dl and TSH = 1.8 ± 1.3 μU/ml. Two mothers were hypothyroid one month after therapy (TSH 24& 102 μU/ml). Both infants were euthyroid with TSH values of 2.2 and 2.6 μU/ml respectively.

We conclude that treatment of hyperthyroid mothers with up to 20 mg/d MMI is safe to their breast-fed infants.

216. P70 ANTITHYROID TREATMENT OF MATERNAL HYPERTHYROIDISM DURING LACTATION.

Y. Abe[1], H.Sato[1], H. Sakai[1], and N. Ooyama[2], 1) Department of Internal Medicine, School of Medicine, Tokai University, Isehara 259-11 and 2) Department of Pediatrics, School of Medicine, Kitasato University, Sagamihara 228, Japan

Most prescribing information states that thyrotoxic mothers treated with 1-methyl-2-mercaptoimidazole (MMI) should no breast-feed. Therefore if the patients want to breast-feed her baby, the antithyroid drug should be changed from MMI to propylthiouracil (PTU) during the pregnancy. However there are some reports indicating that at very small dosage, MMI is probably a safe drug for the nursing mother. The purpose of this study was to obtain basic pharmacokinetic data on MMI for the treatment of Graves' disease in nursing women and to find a safety treatment of nursing women with MMI. This project was approved by the hospital ethical committee. Methimazole in serum, milk and thyroid homogenate were determined by HPCL ; the level of sensitivity was 0.01μg/ml. *Kinetics of plasma and milk MMI after a single oral dose* ; Six Graves' patients (lactating women) were given a single oral 15-mg dose of MMI at 6 pm after giving informed consent. Blood samples and breast milks were obtained for MMI measurement at 1, 2, 4, 6 and 12 h. Throughout the 12h collection period, mean serum and milk MMI levels were nearly identical. The mean peak serum MMI level was 0.31±0.09 (SEM) μg/ml after 2 h , while the peak milk MMI concentrations was 0.32±0.10 μg/ml and also occurred at 2 h. The half-life of MMI calculate from the elimination rate constant was 5.4±2.1 hr. in plasma and 4.2±0.8 hr. in milk. The mean MMI levels after 12 h were 0.04±0.02μg/ml in serum and 0.03±0.01μg/ml in milk. There was good correlation between serum MMI concentrations and milk MMI concentrations ($r=0.998$, $p<0.0001$). *Intrathyroidal concentration* ; 43 patients with Graves' disease who were underwent thyroidectomy were studied. Small pieces of the thyroid gland were kept frozen for MMI assay. Time after the last dose to removal of the thyroid tissue ranged from 5 to 24hr. Intrathyroidal concentration of MMI at 8hr and 24hr after 15mg MMI oral dose was 1.48±1.06 μg/g and 1.30±0.97 μg/g respectively. There was no correlation between the intrathyroidal MMI concentrations and the serum MMI concentrations. Methimazole thus accumulate in the thyroid gland and remain partly unmetabolized for long periods. *Infant thyroid functions*; Based upon these studies it was decided that lactation should be permitted in the daytime if patient was treated with 5 to15 mg of MMI as a single daily doses at evening. In the night the infant was nursed with breast-milk which was collected in the daytime or bottle feeding. After giving informed concent five lactating women who developed an allergy to propylthiouracil were treated with 5 to 15mg MMI, administered at 6pm and permitted breast-feeding in the daytime. Clinical signs and symptoms of hypothyroidism and hyperthyroidism were not observed in any of the children during the three months of study. At 4, 30 and 90 d after the concentrations of TSH, free T4 and freeT3 in all infants were within the normal range for newborn infants. MMI in serum of infants were not detected. These results revealed that MMIwas concentrated in the thyroid gland and that the intrathyoidal concentration were maintained for 24 h in spite of short half-life in serum. On the other hand, milk MMI concentration at 12h after the administration of MMIwas very low. It suggested that a single daily dose of MMI is adequate for the treatment of Graves' disease in nursing women. We conclude that breast-feeding can be permitted if the treatment was performed with a small single daily dose of methimazole.

217. P71 EXCESSIVE THYROIDAL STIMULATION IN GEMELLAR PREGNANCY. J.P. Grün, S. Meuris, P. De Nayer*, D. Glinoer. Univ. Hospital St-Pierre (ULB) and UCL*, Brussels, Belgium.

Aim of present work was to assess in detail the role of human chorionic gonadotropin (hCG) to stimulate the maternal thyroid gland in the early months of gestation. For this purpose, we investigated prospectively 30 healthy women in whom pregnancy had been successfully achieved by in vitro fertilization techniques, which allowed for the precise determination of gestational age. Women were divided in 2 groups based on the number of foetuses : singleton (SP; n = 17) and gemellar (GP; n = 13) pregnancies. At 6, 8, 9, 10, 11, 15 and 19 wks, following serum parameters were measured : intact heterodimeric hCG, free β-hCG subunit, TSH and free T_4 concentrations.
In GP compared to SP, peak hCG levels (9-11 wks) were significantly higher (171,000 ± 12,500 *vs* 65,500 ± 7,600 U/L; mean ± SE; $P < 0.001$), and also much more prolonged. HCG values above 75,000 U/L lasted for less than 1 wk in SP, while up to 6 wks in GP. Free β-hCG subunit values paralleled that of intact hCG in both groups; the ratios of free β-/hCG were similar in SP and GP, and did not vary with gestation time. Concerning thyroid function, GP was associated with a more profound and frequent lowering in TSH, compared with SP : TSH values were ≤ 0.20 mU/L in 21 % in SP, compared with 60 % in GP (among the latter, TSH was transiently undetectable in 80 % of the cases). Furthermore, while FT_4 remained normal in SP (9-20 ng/L), it was supranormal (up to 40 ng/L) in 4 instances among GP.
In summary, in GP the placenta produces large amounts of hCG during prolonged periods of time. It is both the amplitude and duration of hCG production which are responsible for excessive thyroidal stimulation, often leading to increased FT_4 and reduced TSH levels, i.e. the syndrome of gestational transient thyrotoxicosis. Present results confirm our earlier reports on the role of hCG to stimulate maternal thyroid function in the first trimester of pregnancy. Even though the production of a variant hCG molecule with potent thyrotropic activity cannot be excluded, this hypothesis is not required to explain our data. Clinicians should be aware of the frequent occurrence of significant thyroidal disturbances associated with hCG stimulation, particularly in gemellar pregnancy.

218. P72 A RANDOMIZED TRIAL OF ANTITHYROID DRUG TREATMENT IN GRAVES DISEASE: FOLLOW-UP REPORT FOR UP TO 7 YEARS. D.Reinwein, A.Rawert, G.Benker and the European Multicenter Study Group. University of Essen, D - 45122 Essen, Germany

A randomized trial of two fixed doses of methimazole (MMI) in Graves' disease has been conducted in 7 European countries, and the results in 309 patients reported previously after one year without thyroid medication did not show any difference in relapse rate (1). We have conducted an extended follow-up of 199 patients who were in remission by the end of the trial, looking at possible differences in relapse rates and laboratory findings.

Results. The median of follow-up was 1125 days (range, 90-2795 days) in the 10 mg group, and1490 days (range, 180 - 2980) in the 40 mg group. Follow-up examination discovered 51 additional relapses in the 199 patients. 10 patients had undergone surgery for cosmetic reasons, and 5 patients had developed spontaneous hypothyroidism. In summary, including the patients who had already relapsed during the first year of follow-up, 158 patients had recurrencies of Graves' disease, 75/155 in the low-dose group, and 83/149 in the high-dose group (p=0.2). Using Cox's proportional hazard model, no difference in the probability of recurrency could be shown between the two dose groups (p=0.52). The time interval until relapse was not different between the two groups (mean, 514 vs. 387 days). TSH, MAK, TAK, and TBIAb did not differ in the patients remaining euthyroid. No MMI dose effect was seen on thyroid volume or on the clinical course of endocrine ophthalmopathy.

Conclusion. There is no detectable difference in the clinical and immunological course of Graves' disease when treated for one year with either 10 or 40 mg of methimazole.

1. Reinwein,D., Benker, G., Lazarus, J.H., Alexander, W.D.: A prospective randomized trial of antithyroid drug dose in Graves' disease therapy. J Clin Endocrinol Metab 76 (1993) 1516-21

219. P73 DETERMINANTS OF THE RESPONSE TO METHIMAZOLE (MMI) IN GRAVES' DISEASE. A STUDY IN 509 PATIENTS. G. Benker, D. Reinwein, H. Hirche and the European Multicenter Study Group. University of Essen, D-45122 Essen, Germany.

To investigate the factors which determine the initial response to methimazole (defined as duration until euthyroidism is achieved), 509 patients with Graves' disease from different European countries with low-normal and subnormal iodine supply were followed during the initial course of treatment. Patients were randomized to treatment with either 10 or 40 mg of methimazole per day for one year, with levothyroxine supplementation as required to maintain euthyroidism. Investigations were carried out before treatment and at 3 and 6 weeks, 3, 6, 9, and 12 months.

Results: 40.2% of patients responded to 10 mg of methimazole within 3 weeks, and 77.5% within 6 weeks. The corresponding figures for 40 mg of methimazole were 64.6 and 92.6%, respectively. Significant associations between duration of hyperthyroidism and the following variables vere found: Goitre size, urinary iodide excretion, MMI dose, presence of TSH receptor antibodies (TBIAb), index of disease severity (Crooks), and pretreatment thyroid hormone levels. Response to MMI was delayed in patients with large goitres, iodine excretion of >100 µg/g creatinine, high pretreatment thyroid hormone levels, elevated levels of TBIAb, and treatment with only 10 mg of MMI daily. In the 10 mg group, 46% of patients were euthyroid within 3 weeks when urinary iodide was <50 µg, and only 27%, when iodide was >100 µg. By stepwise logistic regression, the main factors for the response to MMI were daily dose, pretreatment T3 levels, and goitre size.

Conclusion: Daily dose, pretreatment serum T3 levels, and goitre size are the main determinants of the therapeutic response to MMI in Graves' disease, at least in areas comprising low, subnormal, and low-normal iodine supply.

220. P74 ANTITHYROID DRUG TREATMENT OF HYPERTHYROIDISM FOR 10 TO 28 YEARS D.W.Slingerland and B.A.Burrows, DVA Medical Center, Boston, MA

There are 3 reasons for considering the use of antithyroid medication (ATD) as an open-ended treatment for hyperthyroidism:(1)the alternatives usually result in hypothyroidism, (2) the future holds promise of a more primary therapy, and (3) ATD is a simple non-destructive treatment. Eighteen of over 100 patients have been treated long term (10-28 years) with ATD (av14.8, mean 12yrs). Of this group, 14 have been euthyroid off treatment for 1 to 27 years (av10, mean8), one received radioiodine for an huge goiter, one died of unrelated cause while still on therapy, one is still on ATD, and one became hypothyroid. There were no serious untoward effects in these patients. Suppression tests (1 week of triiodothyronine) have been used to decide when to stop therapy. Of the 15 patients so tested, 10 were suppressed (uptake =20% of 131-I at 24 hrs.) and remained euthyroid.However,there were 7 instances in 6 of these patients of recurrence following cessation of therapy after an earlier suppression. In addition, 3 patients remained euthyroid after non-suppressed, but relatively low, uptakes of 22, 30, and 35%. Of 16 instances of suppression, 7 recurred off ATD all of whom later suppressed without recurrence off ATD. There were 29 instances in which continued disease activity was apparent following either suppressed or unsuppressed uptakes. There were 4 recurrences in the one patient, who is still on therapy, 5-24 months after unsuccessful suppressions.Only one patient was carried through therapy with no evidence of continuing disease other than failure to suppress.Evidence of continuing disease included elevated thyroxine while on ATD due to non-compliance or inadequate dosage, immediate post-suppression evidence of continuing activity , and recrudescence/recurrence 3 months to 2½ years after stopping ATD.In sum, ATD continued indefinitely, and despite poor suppression predictions, is simple, non-destructive and resulted in this group in a 93% "cure" rate with an average follow up of 10 years.

221. P75 **RESPONSE TO METHIMAZOLE IN THYROTOXIC PATIENTS WITH LOW AND OPTIUM IODINE INTAKE.** **F.Azizi. Endocrine Research Center, Shaheed Beheshti University of Medical Sciences, Eveen, Tehran, I.R. Iran.**

We have previously reported that the response to thionomides differs in thyrotoxic patients residing in areas with low and high iodine intake. To study the influence of consumption of optium amounts of iodine on the response to methimazole (MMI) in an area of iodine deficiency, we studied the effect of MMI 20 mg daily for one month and 10 mg daily for an additional month in 36 patients with diffuse toxic goiter in Tehran. Urinary iodine excretion was between 10.2 to 35.0 μg/dl in 21 (Optimum Iodine=OI) and 2.3 to 9.5 μg/dl in 15 patients (Low Iodine=LI). Thyroid hormone and TSH concentrations were evaluated before and after one and two months of MMI treatment. OI patients had higher levels of serum T4 and FT4I. After four weeks of treatment with MMI, thyroid hormone parameters decreased significantly in both groups. FT4I was lower (6.9±4.1 vs 9.6±3.4; P<0.05) and serum TSH was higher (1.5±1.6 vs 0.6±0.7 μU/ml; P<0.05) in LI as compared to OI, in LI, FT4I was subnormal in 6 (40%). At the end of two months of treatment, further decrease in thyroid parameters was evident. Serum T4 (4.1±1.6 vs 6.1±1.7 μg/dl, P<0.001), FT4I (3.6±1.7 vs 5.9±2.0, P<0.001) and FT3I (105±26 vs 125±32, P<0.05) were lower and serum TSH (14.6±20.2 vs 2.7±2.4 μU/ml, P<0.005) and T3/T4 ratio (37.0±24.0 vs 22.1±6.9, P<0.01) were higher in LI as compared to OI. FT4I in 10 (67%) and FT3I in 2 (13%) patients in LI were subnormal, and 9 (60%) had increased TSH. There was subnormal FT4I in 6 (29%) and subnormal FT3I in 2 (10%) patients in OI group, but none had abnormal TSH.

Conclusion: Compared to patients with iodine depiciency, optimum consumption of iodide mildly attenuates the response to MMI in patients with diffuse toxic goiter. However, it does not cause refractoriness of response to thionamides seen in patients residing in areas of "iodine excess".

222. P76 Post-abortion thyroid dysfunction: Results of a prospective study. Alex Stagnaro-Green and Albert G. Thomas, Mount Sinai School of Medicine, New York, N.Y.

Recent studies have shown that post-partum thyroid dysfunction is a common disorder, occurring in up to 9% of women in the New York metropolitan area. In 1993 we reported the first case of post-abortion thyroid dysfunction. The present study attempts to document hormonal and immune changes post-abortion and the incidence of post-abortion thyroiditis. Thirty-three women had a TSH and thyroid autoantibodies (thyroid peroxidase and antithyroglobulin) performed at the time of abortion (defined as either an elective or spontaneous termination of pregnancy). All women were seen at 3, 6, and 9 months post-abortion for repeat TSH and thyroid autoantibody determination. Post-abortion thyroid dysfunction was defined as a TSH ‹ 0.2 or › 5.0 post abortion, with a normal TSH at the time of abortion. Four women were dropped from the study due to either hyperthyroidism discovered at initial screening (n=2) or repeat pregnancy (n=2) prior to the sixth month follow-up. Seventeen percent (5/29) of the remaining patients were lost to follow-up. The mean age of the remaining 24 women was 29 years old (range 17-42), and 58% of the women were black, 38% hispanic, and 4% white. Pregnancy history revealed an average of 3.5 prior pregnancies (range 0-11). The average gestational age at the time of abortion was 11 weeks (range 5-22). Eight percent (2/24) of the women exhibited thyroid autoantibodies at the time of abortion and another 2 women (8%) developed thyroid antibodies post-abortion. None of the 24 women however, developed post-abortion thyroiditis. We tentatively conclude that post-abortion thyroid dysfunction is a rare event.

223. P77 FOLLOW-UP OF PATIENTS WITH GRAVES' DISEASE AFTER METHIMAZOLE TREATMENT.

T. Rago, C. Mammoli, S. Pallini, E. Fiore, R. Rocchi, F. Latrofa, P. Agretti, P. Vitti.
Istituto di Endocrinologia, University of Pisa, Pisa Italy.

.Discrepant data are still reported on the relapse rate of hyperthyroidism after methimazole (MMI) treatment for Graves' disease.

Three hundred and four consecutive patients with hyperthyroid Graves' disease (243 females, 61 males, age 32.4±12 years) treated with MMI in the period 1980-1992 were included in the study. Several patients were excluded because were addressed to radioiodine or surgery for severe ophthalmopathy or huge goiter.

MMI was administered for 21±12 mo and patients tapered to keep euthyroid with no addition of L-thyroxine. The mean follow-up period after MMI withdrawal was 20.2±18 mo (minimum 6 mo).

192 (145 females, 47 males)/304 (63.1%) patients had a relapse of hyperthyroidism 1-192 mo after MMI withdrawal (mean 10.1±21). Relapse was observed in 9/12 (75%) patients treated for < 12 mo and in 183/292 (62.7) patients treated for > 12 mo (with no diffrence between 12, 18 or > 18 mo). The relapse rate was dependent on the age (< 30 yr: 103/151, 68.2%; 30-40 yr: 51/77, 66,2%; >40 yr: 38/76, 50%) and size of goiter (> 70 ml: 30/32, 94%, 40-70 ml: 139/228, 61%, <40 ml: 18/39, 46%). TSH receptor antibody (TRAb) levels at the end of treatment were reduced in all patients, and were undetectable in 58%. Relapse was observed in 33% of patients with undetactable TRAb and in 94% of those with detectable TRAb. 1 patient with elevated TRAb levels who developed spontaneous hypothyroidism had a blocking rather than stimulating antibody (TSAb) as shown by bioassay on thyroid cells in vitro. TSAb results were not more predictive than TRAb results.

In conclusion: 1) the relapse rate of hyperthyroidism after MMI treatment for Graves'disease was higher than that reported in previous studies; 2) TRAb or TSAb was not predictive of relapse in more than 30% of patients; 3) the most reliable clinical parameters for predicting relapse of hyperthyroidism were the age (< 40 yr) and size of goiter (> 40 ml) .

224. P78 PERI-PARTUM THYROID DEFICIENCY. E.A. Laryea [1] and J.H. Dusseault [2]

[1] McGill University, Montreal, Quebec, Canada [2] Universite de Laval, Montreal, Quebec, Canada

Because of the adverse effect of hypothyroidism on fertility it is rare for hypothyroidism to be present in pregnant women. Most of such cases have been discovered before term and had been treated. During the early periods of gestation, normal fetal brain development needs normal and maternal thyroid function. This makes early diagnosis of hypothyroidism in pregnant women very important.

During the early part of 1987 and late 1988 we performed thyroid function tests on each pregnant woman 24 hours after delivery of a live baby.

Out of a total of 2000 cases, 24 had differing grades of thyroid deficiency. In all of these cases the TSH is elevated, however the Free T_4 was either low or normal. Three out of twenty four had at least laboratory criteria consistent with hypothyroidism and are all on thyroid replacement therapy now.

We conclude that the incidence of hypothyroidism in pregnancy in this series is at least equal to that of neonatal hypothyroidism. Screening, therefore, for maternal hypothyroidism during the first weeks of pregnancy may be useful.

225. P79 PAINFUL GRAVES' DISEASE: CLINICAL PICTURES AND ITS UNIQUE OUTCOME

S. Fukata, K. Kuma, M. Sugawara*. Kuma Hospital, Kobe, Japan, *West Los Angeles, VA Medical Center, and UCLA School of Medicine, Los Angeles, California, USA

We previously reported a new type of Graves' disease - progressive thyroid failure after painful episodes (Arch Intern Med. 1993; 153: 2147). We accumulated total 12 patients with this disorder. We describe clinical pictures of painful Graves' disease and its unique outcome based on our observation of the 12 patients. There were 9 women and 3 men with the mean age of 58.9 years (28 to 64 years-old). All of them had newly diagnosed Graves' disease with elevated serum thyroid hormone levels, increased 24h radioiodine uptake, and positive thyrotropin binding inhibitory immunoglobulin (TBII). Nine patients were treated with methimazole and the other 3 patients were untreated because progressive thyroid failure was expected after painful episode. All patients experienced 1 to 5 episodes of severe pain and tenderness over the thyroid gland with inflammatory signs such as fever, an increased erythrocyte sedimentation rate, and positive C reacting protein. Cytological findings did not show any evidence of subacute thyroiditis. Four patients developed hypothyroidism rapidly after single episode of pain in the thyroid gland. Five patients, after experiencing several painful episodes in the thyroid gland, progressed to permanent hypothyroidism over 2 years without antithyroid medication. The rest of the 3 patients did not develop hypothyroidism up to 2 years of observation, and these patients required antithyroid drug therapy for Graves' disease. All 9 patients who developed hypothyroidism had strongly positive antithyroglobulin and antimicrosomal antibodies. Interestingly, 2 of the 3 patients who did not develop hypothyroidism had negative antithyroglobulin and antimicrosomal antibodies. Glucocorticoid therapy (prednisolone 10 mg po tid) was highly effective in relieving thyroid pain in 11 of the 12 patients. However, one patient had persistent painful episodes despite glucocorcicoid therapy and required surgical removal of the thyroid gland.

Conclusions 1) Painful Graves' disease needs to be recognized because progressive thyroid failure is a common outcome. 2) The cause of painful Graves' disease is unknown, but it may be related to the immunological event based on strongly positive thyroid antibodies. 3) Glucocorcicoid therapy is effective for relieving pain although it does not affect the outcome.

226. P80 FLUCTUATION OF PANCREATIC BETA-CELL FUNCTION AND PERIODIC PARALYSIS IN PATIENTS WITH GRAVES' DISEASE.

K. Notsu, M. Imaoka, Y. Ito, J. Saito*, S. Ohguni** and Y. Kato**. Department of Medicine and of Neurology*, Shimane Prefectural Central Hospital and First Division*, Department of Medicine, Shimane Medical University, Izumo, JAPAN

The onset of periodic paralysis (PP) in some patients is before or simultaneous with that of Graves' disease (Gr) and others have the attack of PP after treatment with antithyroid drugs (ATD). Including this phenomenon, the pathogenesis of PP still remains unclear. It is suspected that excessive insulin secretion and supressed serum levels of potassium play an important role in PP. Pancreatic beta-cell function (BCF) was examined in 72 patients with untreated Gr. Intravenous glucose tolerance tests (ivG) were performed and plasma glucose and C-peptide (CPR) levels were measured. The initial phase of CPR secretion (sum of serum CPR at 1 and 3 min after iv injection of 10g glucose: ICPR) was calculated and utilized as a marker of BCF. The sex ratio was 18:54 (m:f) and the mean (+SD) age was 45.1 (+14.5) yr. Fifteen of 72 patients had glucose intolerance and the mean ICPR was 1555.7 (+728.2) pmol/L (Group A) . That in the other 57 with normal glucose tolerance was 3806.5 (+1489.5) pmol/L (Group B), which was significantly higher than in Group A and in normal controls (2747.3+993.0 pmol/L). There was a significantly negative correlation between age and ICPR value ($r=-0.61, n=72, p<0.001$). Twenty-one of 57 patients received ivG at three weeks after an initial therapy with ATD. Although thyroid function in these patients gradually improved, ICPR values in 12 of 21 increased after the therapy (Group B1). Those in the other 9 decreased (Group B2). The mean ICPR in groups B1 and B2 was 3210.7 (+993) pmol/L and was 4865.7 (+1588.8) pmol/L, respectively. The difference was significant ($p=0.0087$). The mean ICPR value at three weeks after the therapy in these two groups was not different. The mean age in Group B1 (53.0+11.2 yr) was significantly higher than in Group B2 (38.7+8.7 yr) ($p=0.015$). Three male patients, whose age was 24, 34 and 34, had PP before the diagnosis of Gr and ICPR values before treatment were 7977.1 pmol/L, 6884.8 pmol/L and 7282 pmol/L, respectively. They belonged to Group B2. A 53-yr-old man who belonged to Group B1 had PP after the therapy with ATD. His ICPR level before treatment was 3508.6 pmol/L and at the attack of PP was 6289 pmol/L. These four patients had significantly increased ICPR levels at the attack of PP. These results may show that (1) PP is not observed in diabetic patients with impaired BCF, especially in aged, (2) The young men with increased BCF have PP before (or same) the diagnosis of Gr, (3) BCF in relatively aged patients increases after an initial treatment with ATD and (4) those patients have PP after manifestation of typical clinical findings of Gr.

227. P81 PHOSPHOLIPASE C AND CA^{2+} RESPONSES INDUCED BY UNTREATED GRAVES' BUT NOT REMISSION GRAVES' IGGS AND THEIR POTENTIATION BY AN ADENOSINE DERIVATIVE. A. Kuwabara, F. Okajima, K. Sho, I. Kobayashi and Y. Kondo. Institute for Mol. Cell. Regulation and Dept. Laboratory Medicine, Gunma University, Maebashi, Japan

Most of immunoglobulin (IgG) fractions obtained from Graves' disease patients stimulate adenylyl cyclase, which is defined as TSAb. Although a few cAMP-independent actions of Graves' IgGs including Ca^{2+} mobilization have been reported, the Ca^{2+} signalling induced by Graves' IgGs is still not very clear, especially in quantitative aspects and the relationship to remission of the disease. Aim of the present study is to characterize Graves' IgG-induced signalling pathways leading to the Ca^{2+} mobilization in FRTL-5 thyroid cells and examine importance of the response as a clinical measure. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) increase by a Graves' IgG preparation was demonstrated by the digital video-imaging of fura2-loaded cells grown on coverslips, which showed a transient and rather synchronized $[Ca^{2+}]_i$ increase with a peak at around 30 sec in more than 60 % of the cells. Fluolometry of suspensions of fura2-loaded cells showed that untreated Graves' IgGs induced a transient Ca^{2+} response in a dose dependent manner. This response was enhanced by the addition of an adenosine derivative, N^6-(L-2-phenylisopropyl)adenosine (PIA), which itself has no or much less Ca^{2+} mobilizing activity. Because this is a typical feature of receptor-mediated activations of the phospholipase C (PLC)-Ca^{2+} system, the above-mentioned results suggest that the $[Ca^{2+}]_i$ increase by Graves' IgGs is due to the activation of the PLC-Ca^{2+} pathway. Although some variation of the Ca^{2+} mobilizing activity among 12 untreated Graves' IgGs was observed, the mean value was several times higher than that of 8 normal subjects, the difference being statistically significant. After the challenge by 12 remission Graves' IgGs, however, $[Ca^{2+}]_i$ remained at the normal level. In a separate experiment, we also observed PLC activation by Graves' IgGs in the presence of PIA. There was no statistical correlation between the cAMP response and either PLC or Ca^{2+} responses to Graves' IgGs; some IgG preparations showed the higher Ca^{2+} response but the lower TSAb and *vise versa*.

Summarizing the results, we suggest that Graves' IgGs are composed of a variety of IgGs, one with a capacity to superiorly activate the adenylyl cyclase-cAMP pathway and the other with an activity to preferably stimulate the PLC-Ca^{2+} pathway, probably both through a single species of TSH receptor in FRTL-5 thyroid cells. In relation to this, it is noted that in some cases, there is discrepancy between TSAb values and symptom. Thus, the PLC or Ca^{2+} response of cultured thyroid cells to Graves' IgG fractions would provide a new clinical measure for Graves' disease, which may reflect other aspects of the disease than those reflected on TSAb values.

228. P82 CLINICAL APPLICATION OF COLOR DOPPLER IMAGING TO THE DIFFERENTIAL DIAGNOSIS OF THYROTOXICOSIS. M. Kitaoka and H. Ishido, Division of Endocrinology and Metabolism, Showa General Hospital, Kodaira, Tokyo.

The differential diagnosis of thyrotoxicosis is of great clinical importance in the assessment of patients with diffuse thyroid disease. Both destructive thyroiditis and Graves' disease(diffuse toxic goiter) may result in thyrotoxicosis, but the therapeutic approaches to theses two pathological processes are completely different. Color Doppler imaging is thought to be effective in distinguishing between destructive thyroiditis and Graves' disease, and its clinical usefulness was evaluated in this study.

Forty five patients with Graves' disease, 20 patients with destructive Thyroiditis(11 cases were sub-acute thyroiditis and 9 cases were painless thyroiditis) were examined using color Doppler ultrasonography with 7.5MHz scanner.

The 45 patients with Graves' disease were classified into the following three groups: 26 patients with remarkable color flow images (so-called "thyroid inferno") with homogeneous internal echoes, 9 patients with heterogeneous internal echoes and remarkable dolor flow images in the hypoechoic area, and 10 patients with remarkable color flow images in the normoechoic area and no color flow in the hypoechoic area.

Fourteen patients with destructive thyroiditis in the thyrotoxic phase showed no color flow images in the hypoechoic area. Six patients with destructive thyroiditis in the recovery phase showed a few color flow images in the hypoechoic area.

The above findings indicate that color Doppler imaging is useful in the differential diagnosis of thyrotoxicosis and also suggest that this method may prove valuable in helping to clarify the pathophysiology of Graves' disease.

229. P83 **SUCCESSFUL THERAPY RESTORES TO NORMAL THE INCREASED LEVELS OF ENDOTHELIN-1 IN ADULT HYPERTHYROID PATIENTS** C Letizia, M Centanni, R Cesareo, A De Ciocchis, S Cerci, A Di Gregorio, D Scavo. I Patologia Medica & Endocrine Section, Dept of Experimental Medicine, University "La Sapienza", Roma, Italy

In a previous study we have shown that endothelin-1 (ET-1) concentrations are almost doubled in hyperthyroid patients when compared to age-matched controls, whereas normal levels have been found in the hypothyroids. However, the effect on human ET-1 levels of therapeutic correction of hyper- or hypothyroidism is unknown. The aim of this study has been to investigate the plasma levels of ET-1 in adult subjects with a different thyroid homeostasis before and after a successful treatment. Plasma levels of ET-1 have been measured in 42 subjects (19 M, 23W, aged from 27 to 58) divided as follows: a) 20 healthy subjects used as controls; b) 14 patients with definite hyperthyroidism; c) 8 hypothyroid patients. Blood samples were obtained under no treatment, 1 month and 6 months after the beginning of an appropriate therapy. Extracted plasma ET-1 has been determined by a specific radioimmunoassay using rabbit anti-endothelin antisera (Peninsula Lab, Belmont, Ca). Data have been expressed as the mean ± SD and evaluated by the analysis of the variance. The mean plasma levels of ET-1 in the control group were 10.9 ± 2.2 pg/ml. In hyperthyroid subjects, as expected, the ET-1 concentrations were significantly higher (19.6 ± 5.1 pg/ml; +80%; $p<0.0001$) than in the control group. 13 out of 14 hyperthyroid patients (93%) showed ET-1 levels above 15 pg/ml, the upper limit of the normal range. The use of antithyroid drugs reduced the ET-1 levels to 13.4± 8.2 pg/ml (-32%; $p<0.001$) after 1 month of treatment and to control levels (10.6 ± 4.5 pg/ml; -46%; $p<0.0001$) after 6 months. In contrast, hypothyroid subjects showed no differences as compared to controls either before the treatment (10.9 ± 2.0 pg/ml p=ns) or following a 1 month or 6 months of replacement therapy (11.0 ± 2.9 pg/ml and 11.4 ± 3.9 pg/ml p= ns for both). The main finding of this study is that in hyperthyroid patients the increase of circulating ET-1 levels is progressively abolished by successful antithyroid therapy.

230. P84 SERUM THYROGLOBULIN CONCENTRATION AS AN INDICATOR OF REMISSION IN ANTI-THYROID DRUG TREATMENT FOR GRAVES' DISEASE.

Y. Murakami, S. Morita, T. Yokozawa. First Department of Internal Medicine, Osaka Medical College, Takatsuki 569, The Kuma Hospital, Kobe 690, Japan.

We previously reported serum thyroglobulin (Tg) concentration in hyperthyroid patients with Graves' disease were high (210 ± 181 ng/ml Mean ± SD), and were decreased to normal (33.8 ± 31.5 ng/ml) in remitted patients after anti-thyroid drug (ATD) treatment. In another study utilizing a Tg IRMA kit which can exclude the affection of serum anti-Tg autoantibody, we also reported that 76 out of 83 patients (94 %) remitted with ATD treatment had normal serum Tg concentration. In this study, we have examined the possibility if serum Tg level during ATD therapy is a reliable indicator to decide the discontinuation of the medication.

Forty-five patients with Graves' disease under conditions as follows were selected for this study; 1) minimal ATD dosage (MMI 5 mg a day or PTU 50 mg a day), 2) normal concentration of serum TSH, 3) 1) and 2) conditions persisted more than 3 months. The ATD treatment was stopped, and the subjects were divided into 2 groups according to serum Tg concentration at the time of ATD cessation; 35 patients with normal serum Tg lower than 45 ng/ ml and 10 patients with high serum Tg. The patients were followed up for at least one year.

The remission was obtained in 29 out of 35 patients (83 %) in the normal serum Tg group. The remission was obtained in three out of 10 patients (30 %) in the high serum Tg group: the incidence of remission was significantly greater in the normal serum Tg group than in the high serum Tg group ($p < 0.01$). The thyroid volume at the time of ATD cessation was not significantly different between the normal serum Tg group and the high serum Tg (31 ± 14 ml vs. 41 ± 25 ml). The duration of ATD treatment was not also significantly different between the 2 groups (the normal serum Tg group of 45 ± 35 months vs. the high serum Tg group of 30 ± 12 months).

Conclusion: In ATD treatment for patients with Graves' disease, normalized level of serum Tg indicate a greater possibility of remission of hyperthyroidism even if the thyroid is still enlarged.

231. P85 PHENOTYPE OF FAMILIAL NON AUTOIMMUNE DIFFUSE THYROTOXICOSIS. J.L. Leclère[1], C. Schvartz[2], J. Parma[3], J. Van Sande[3], M. Decoulx[4], J. Orgiazzi[5]. [1]Clinique d'Endocrinologie, Hopital de Brabois, CHU de Nancy, France. [2]Service de Médecine Nucléaire, Institut J. Godinot, CHU de Reims, France. [3]Institut de Recherche Interdisciplinaire, Université de Bruxelles, Belgium. [4]Clinique Médicale, CHU de Lille, France. [5]Service de Médecine Interne, CHU de Lyon Sud, Lyon, France.

Familial non autoimmune diffuse thyrotoxicosis has been first described in 1982. The pathology and the behavior after transplantation on nude mouse of thyroid tissue being similar to that of toxic nodule, the term of "toxic hyperplasia" was proposed to this new form of thyrotoxicosis. This concept has been gradually confirmed by the recognition of two other families, and finally admitted since the identification of its pathophysiology: a germline mutation in the thyrotropin receptor (TSHR) gene. A case of non familial toxic hyperplasia due to a somatic mutation has been recently described. Then, it is of great interest to identify similar cases in the future, but a systematic screening by molecular analysis is not yet realistic.

The aim of this study is to determine the phenotype of toxic hyperplasia from the two proven mutated families (the numbers correspond to the number of affected patients / examined patients):

1- high incidence of cases of thyrotoxicosis (28/72)
2- frequent precocity of the onset of disease (8/8)
3- moderate or minimal goiter in young patients (8/8)
4- constant absence of ophthalmopathy (0/28)
5- total absence of thyroglobulin, thyroperoxydase and TSHR antibodies (0/28)
6- absence of linkage with the usual HLA phenotype encountered in Graves'disease (0/12)
7- systematic recurrence after antithyroid drug therapy (19/19)
8- absence of histological Graves' aspects of thyroid tissue (lymphoid infiltration, presence of plasma cells, aberrant expression of HLA II by thyroid cells,...)
9- recurrence of goiter and thyrotoxicosis on residual thyroid tissue after incomplete destruction of thyroid tissue by ^{131}I or surgery

Among these characteristics, the most suggestive features are 1, 2, 7, 8 and 9.

232. P86 GRAVES' DISEASE AND AUTOIMMUNE FACTOR VIII DEFICIENCY. R. Sievert and M.I. Surks, Department of Medicine, Montefiore Medical Center and the Albert Einstein College of Medicine, Bronx, New York.

Spontaneously acquired factor VIII deficiency is an uncommon disorder in which auto-antibodies inhibit factor VIII activity leading to a hemorrhagic diathesis that is fatal in 20% of patients. We now describe the first case in which hyperthyroidism due to Graves' disease and factor VIII deficiency occurred simultaneously. A 61 year old woman with Hashimoto's thyroiditis had been treated with l-thyroxine (0.05 ug/day) for 10 years. L-thyroxine was then stopped because of decreased serum TSH. Two months later, she developed hematomas and ecchymoses in both forearms and right thigh. Activated partial thromboplastin time (APTT) was 77 sec (normal: 23-33); factor VIII activity was decreased to 17% (normal: 50-150%)] in association with a factor VIII inhibitor, 37 Bethesda units; (normal: 0). Symptoms and signs of hyperthyroidism were absent but she had a diffusely enlarged thyroid gland and was biochemically hyperthyroid: free T4 = 4.3 ng/dl, serum TSH = <0.01 uU/ml; and thyroid stimulating immunoglobulin (TSI) = 221% (normal: <130%). The 24 hour thyroid [^{123}I] uptake was 55% and a scan showed diffuse uptake in an enlarged gland. She was treated with iv gamma globulin, prednisone and cytoxan. No antithyroid treatment was given. There were no further hemorrhagic events. After 2 months, factor VIII activity had increased to 51%, APTT had decreased to 36 sec and factor VIII inhibitor was undetectable. A decrease in serum free T4 (1.3 ng/dl) and TSI (151 %) also occurred. The probability that these autoimmune disorders occurred by chance alone is minute. Therefore, an underlying derangement in immune regulation may have resulted in the simultaneous production of TSI and anti-factor VIII antibodies. Anti-factor VIII antibodies may occur more frequently in Graves' disease but at insufficient titer to produce a hemorrhagic disorder.

233. P87 THE CLINICAL DILEMMAS PRESENTED BY PATIENTS WITH DOWN'S SYNDROME (DS) AND GRAVES' DISEASE (GD). J. M. Hughes, Mary Imogene Bassett Hospital, Cooperstown, New York, USA.

Adult patients with DS (trisomy 21) have an increased incidence of thyroid dysfunction, usually hypothyroidism and rarely GD. Moreover, the GD may manifest atypically and therefore be missed. 4 females and 1 male with a mean age of 28 y (range 9 to 51) with DS and GD are discussed. There were only 4 consistent presenting symptoms, of a possible 15. All had weight loss (mean 6.0 kg). In 2, behavioral changes and hyperdefecation were noted. 1 had menstrual irregularity. Physical findings were also subtle. Mean pulse rate was 96 bpm (range 70 to 120). Only one had an obvious goiter, 50 gms. The mean thyroid weight was 25 gms (range not palpable to 50). 2 were hyperactive on exam. None had tremor, ophthalmopathy or dermopathy. Skin and hair changes were not present. On laboratory, free T_4 and total T_3 levels were markedly elevated: mean FT_4 - 5.5 ng/dl (normal 0.8 - 1.6) and TT_3 - 310 ng/dl (normal 80 - 200). TSH levels were suppressed. In 4, anti-microsomal antibody levels were obtained and were present. In 3, thyroid scans were possible and diagnostic of GD. With therapy, 3 are euthyroid after PTU and 2 after I^{131}. In summary, the paucity of clinical findings, other than weight loss, makes the recognition of possible GD difficult in DS patients. However, once suspected, the laboratory diagnosis is classic. Furthermore, patients respond well to traditional therapy. Finally, thyroid scans and I^{131} therapy may at times be difficult to obtain, depending on patient cooperation.

234. P88 RELATIONSHIPS BETWEEN PROGNOSIS OF GRAVES' DISEASE AND THYROID RELATED ANTIBODIES. T. Morita, S. Kubota, S. Murakami, I. Hayaki, K. Tamagawa, A. Oshima, Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

We followed 61 patients with Graves' disease under treatment with antithyroid drugs (ATD) for 4 to 6 years. Twenty-one patients had positive TGHA (by hemagglutination method) and positive MCHA (by hemagglutination method) (group A), twenty-one patients had negative TGHA and positive MCHA (group B), and nineteen patients had negative TGHA and negative MCHA (group C) at their initial visit. We evaluated TSH receptor antibodies (TRAb), Tg antibody (TgAb) and TPO antibody (TPOAb) (by direct method) at 0, 3, 6, and 12 months after treatment. TRAb and TPOAb gradually decreased during treatment ($P<0.0001$ and $P<0.005$, respectively), however TgAb did not decrease significantly even in group A. After 4 to 6 years, thirty-three patients (11 in group A, 12 in group B and 10 in group C) remitted (remission group), and the other 28 patients needed continuous therapy (non-remission group). TRAb values at initial visit in the remission group were significantly lower than those in the non-remission group, however the time course of TRAb showed no significant difference between the two groups (by two-way ANOVA). TPOAb values and TgAb values at initial visit were similar in the remission and non-remission groups. The time course of these antibodies showed no significant difference between the remission and non-remission groups. In conclusion, TRAb and TPOAb during the first year of ATD treatment decreased significantly, however their time course was not related to the prognosis of Graves' disease. Only TRAb values at initial visit related to the prognosis.

235. P89 A CASE REPORT OF GRAVES' DISEASE TREATED BY ULTRASOUND GUIDED PER-CUTANEOUS ETHANOL INJECTION. A. Kawauchi, Y. Ban, M. Taniyama, M. Kaname, H. Nagakura, M.Kaihara, K. Kushima ,Y.Hashimoto, T.Sawada,M. Kusano. Depts. of Surgery, 3rd Internal Medicine and Clinical Pathology, Showa Univ., Sch. of Med., Tokyo, JAPAN

Recently, ultrasound guided per-cutaneous ethanol injection (PEI) therapy was already apllied widely not only for hepatocelluler carcinomas but also for primary parathyroid adenomas , benign thyroid functioning adenomas, reccurent thyroid carcinomas and the other organ tumors. On the other hand, there is no report of PEI therapy in Graves'disease. This time, we had a oppotunity to treat a Graves' disease female case with US guided PEI therapy, became euthyroid state without any other therapies.

A 45 year old woman with palpitation, body weightloss,exophthalmus and diffuse goiter which was found on a routine physical examination, visited the outpatient clinic in our hospital 3 years before. Because of remarkable increasing serum T3 , T4, F-T3 and F-T4 levels and suppressing serum TSH level, she had diagnosed as a hyperthyroid Graves' disease and started to take anti-thyroid drugs(ATD). But, she had stoped taking ATD 3 times for 3 years and refused both the RI and surgical therapy. Therefore,with the informed consent in both talking and writing, PEI therapy under US observation had been performed 4 times for 3 weeks in admission, using 25.7 ml ethanol totally. The serum thyroid hormone levels increased from latent hyperthyroid state to hyperhtyroid state immediately after PEI therapy. Especially,the serum T4, F-T3 andF-T4 values changed remarkably ,but the serum T-3 value still remained at upper limmited point during the therapy. Two weeks after PEI therapy, the serum hormone levels decreased to normal range and the complaines disappeared without ATD. Ultrasound color Doppler flow mapping and RI findings showed the decreasing thyroid blood flow obviously comparing with the condition before PEI therapy. Now, the patient is still under following up in the outpatient clinic carefully, but major side effects of PEI therapy didn't be seen yet. Through this experience, as our impression, PEI therapy will be established as a current therapy of Graves' disease in the near future for the patient who couldn't be indicated or accepted the other therapies.

236. P90 CHRONIC STRESS ASSOCIATED WITH PANIC DISORDER DOES NOT PRECIPITATE GRAVES' HYPERTHYROIDISM

E. Fiore, *G. Perugi, P. Lapi, L. Montanelli, *G.B. Cassano, C. Mammoli
Institute of Endocrinology and *Institute of Psychiatry, University di Pisa

A possible role of stress in developing Graves' disease has been suggested, but this evidence is still controversial. This is mainly due to the difficulty to define stress and to distinguish stress symptoms from hyperthyroid symptoms. The present study was carried out to evaluate the occurence of Graves' disease in a typical stress condition such as panic disorder. To this purpose 41 patients (M=6, F=35; age=22-56) with panic disorder were collected. Patients had been suffering from panic attacks since 1 to 30 years, with a mean disease period of 9.6 years. The control group included 458 age and sex matched normal subjects, without past or present history of psychiatric disorders. Patients and controls were submitted to a full thyroid evaluation, including interview, physical examination, assays for free thyroid hormones, ultra-sensitive thyrotropin (TSH), anti-thyroglobulin (TgAb), anti-thyroperoxidase (TPOAb) and anti-TSH receptor antibodies (TRAb). In selected cases thyroid ultrasonography was also performed.

None of patients had a history of hyperthyroidism. On physical examination all patients were clinically euthyroid without any evidence of endocrine exophthalmos. Serum free thyroid hormones and TSH were within the normal range in all patients but one. She was a 55 years old woman with undetectable TSH and normal free thyroid hormones. In the subsequent 1.5 year of follow up TSH returned within the normal range and she did not develop overt hyperthyroidism. Circulating TPOAb were found positive in 7/41 (17%) (range 54-1000 U/ml) patients with panic disorder. Six out of 41 (14.7%) patients (all TPOAb positive) had also circulating TgAb (range 75-540 U/ml). The prevalence of circulating TPOAb and TgAb was significantly higher in patients with panic disorder than in controls (TPOAb: χ^2=8.45, p<0.01; TgAb: χ^2=12.04, p<0.001). TRAb were not detected in any patient of the study group. None of patients with positive thyroid antibodies showed a thyroid hypoechogenic pattern, typical of autoimmune thyroid disease.

In conclusion, patients with panic disorder show a greater prevalence of humoral thyroid autoimmunity with respect to the general population. The question of whether this phenomenon results from a genetic link between panic disorder and thyroid autoimmunity, or is the consequence of an immune alteration due to the psychiatric condition, is a matter of speculation. Despite this autoimmune derangement, we did not find any clinical or serological evidence of hyperthyroidism. Therefore our study shows that stress due to panic disorder does not precipitate Graves' disease even in patients with subclinical thyroid autoimmunity.

237. P91 **ALTERATIONS IN CORTICOSTEROIDS WITH LOW ALDOSTERONE LEVELS IN GRAVES' DISEASE**

B.A.K. Khalid, M.L. Ng, M.S.L. Lo, H. Kamarulzaman,
Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur 50300, Malaysia.

Asian patients with Graves' Disease are prone to hypokalaemic periodic paralysis which may occur during, before or after the thyrotoxic phase. Various theories have been postulated for its etiology. Clinically the hypokalaemia may be reversed by spironolactone, an aldosterone antagonist, suggesting excess mineralocorticoid activity. As cross-sectional study was done amongst Malaysians with Graves' thyrotoxicosis to determine alterations in corticosteroid levels. Age and sex matched. Normals were used as controls.

In 169 normal controls, the serum aldosterone after 4 hours upright posture and 3 days normal salt diet was 221 ± 18 pg/ml (mean ± SEM) which was significantly higher than in hyperthyroid (102 ± 11 pg/ml, $P<0.001$, n=90), euthyroid patients on antithyroid drugs (87 ± 9 pg/ml, $P<0.001$, n=94) and overtreated patients who became hypothyroid (73 ±11 pg/ml, $P<0.001$, n=26). In 24 patients with non thyroidal ilness, the serum aldosterone levels were not significantly different. There were no significant differences in the peripheral renin levels, which remained within normal levels in all groups. The plasma cortisol levels were significantly higher at both the morning and evening with absence of diurnal variations. The low aldosterone levels correlated negatively to the raised cortisol levels implying secondary suppression. Whether periodic paralysis is due to excessive cortisol levels in the kidneys is yet to be proven.

238. P92 LONG PROGNOSIS OF THYROTOXICOSIS TREATED WITH GLUCOCORTICOID WITH OR WITHOUT THIONAMIDE IN GRAVES' DISEASE.

T.Yagura, K.Sugiyama, T.Yamamoto, T.Yamada and H.Ishii, Tenri Hospital, Tenri, Nara, Japan.

We investigated long prognosis of thyroid function in 14 patients (5 males and 9 females) of Graves' disease who were treated with glucocorticoid with or without thionamide. Two patients were given glucocorticoid for SLE and 12 for infiltrative ophthalmopathy. Their age ranged from 41 to 77 (mean 56 ± SD 10) years-old. Duration of thionamide therapy before glucocorticoid was less than 1 month in 8 patients and more than 4 months in 6. All patients had positive TSH binding inhibitory immunoglobulin (TBII). Two patietns were started with steroid pulse therapy and remaining 12 did with continuous steroid of 58 ± 19 mg/day equivalent to prednisolone. Patients who were treated with radioisotope or subtotal thyroidectomy were not selected for subjects. Except one patient who did not need, all were administered thionamide in conjunction with steroid.

Five patient did not need thionamide and kept euthyroidism within 12 months after initiation of steroid (group A) and most of them occurred within 5 months. TBII also became negative within 8 (3.6 ± 3.2) months. Group A was followed 8 to 58 (36 ± 32) months and they all kept euthyroidism without thionamide at the end of observation. Among remaining 9 patients (group B), eight were taking thionamide and one was in mild hyperthyroidism without medication because of allergy after 12 months. Group B was followed for 55 to 108 (72 ± 19) months. TBII became negative in five (group B1) at 12 months and 2 of them acquired remission later but 3 did not. Remaining 4 patients of group B still had positeve TBII (group B2) at 12 months and none of them reached remission at the end of further observation.

When rapid disappearance of TBII is observed in Graves' disease treated with pulse or continuous large dose steroid therapy, early discontinuation of thionamide and remission will be expected.

239. P93 **THE TYPE III 5-DEIODINASE GENE IN *RANA CATESBEIANA* (RC) RESPONDS TO THYROID HORMONE (TH) IN BOTH THE TADPOLE AND THE ADULT FROG.**

Kathryn B. Becker and Valerie Anne Galton, Dartmouth Medical School, Lebanon, NH, USA.

The type III 5-deiodinase (5D) catalyzes the conversion of T_4 and T_3 to their respective inactive derivatives, rT_3 and 3,3'-T_2. We have recently reported that the *X.laevis* 5D is a selenoprotein encoded by the TH-responsive gene, XL-15 (St.Germain *et al.* PNAS, USA 91: 7767,1994), and have isolated from an RC cDNA library a partial cDNA (RCD10), which is the RC homologue of XL-15 (Galton *et al.*Thyroid 4: suppl 1,S96,1994). RCD10 has now been extended to yield RC5D, a 1534 bp cDNA which contains the entire coding region of the RC 5D gene, including a conserved TGA encoding selenocysteine, and sequences in the 3' untranslated region which dictate the incorporation of selenocysteine. Capped mRNA transcripts of RC5D induce 5D activity when injected into *X. laevis* oocytes. Studies have revealed that the marked response of the RC5D gene to TH is transient. When tadpoles were exposed to 10 nM T_3 (in bath water), levels of RC5D mRNA and 5D activity in tail, liver, eye, leg and kidney were greatly increased after 3 days but had decreased to pre-exposure levels after 10 days. A more extensive time/response study (0, 0.25, 0.67, 1, 2, 3, 5, 12 days) indicated that RC5D mRNA levels and 5D activity in both liver and tail were maximal at 1-2 and 5 days respectively. In the adult frog, which is generally considered to be resistant to TH action, 5D expression was also markedly enhanced by TH, although other genes that are TH-responsive in the tadpole (TRα, TRβ,and carbamyl phosphate synthetase) were unaffected. The effect on 5D expression may be the only unequivocal example of a TH action in the adult frog demonstrated to date. Moreover, in view of the response of the 5D gene, the resistance of other genes to TH cannot be attributed to failure of the hormone to access their transcriptional regulatory elements.

RC5D expression was localized within tissues utilizing reverse transcription (RT) *in situ* PCR. Sections of tail and liver from control and T_3-treated tadpoles were examined. The PCR products were visualized with alkaline phosphatase-conjugated anti-digoxigenin antibody. In tail, staining was observed in nuclei of muscle cells, the capillary endothelial cells and skin. In liver, more than 95% of the hepatocytes were positive. No staining was observed when the RT was omitted and all cells were positive in sections not receiving DNase treatment. This pattern was seen in tissues from both control (low copy number) and T_3-treated tadpoles indicating 1) the extreme sensitivity of the technique and 2) that T_3 does not enhance 5D activity by increasing either the number or type of cells expressing the gene.

These findings support the view that the 5D plays a major role in regulating intracellular TH levels in both developing and adult amphibia.

240. P94 EFFECT OF CERAMIDE AND PROTEIN KINASE C ON THE REGULATION OF TYPE I 5'-DEIODINASE.

K. Mori, S. Stone, and W.J. DeVito, Div. of End, UMass. Med. Sch, Worcester, MA 01655.

Tumor necrosis factor-α (TNF-α) has numerous effects on a number of cell types, including thyrocytes. Two TNF receptors, TR55 and TR75 have been identified in a number of cell lines. TNF binding to the TR55 receptor may trigger specific signal transduction cascades including protein kinase C (PKC) and the hydrolysis of sphingomyelin to ceramide. We have recently shown that TNF-α and interferon-γ (IFN-γ) act synergistically to inhibit the expression of 5'-deiodinase (5'D-I). In the present studies, we have used FRTL5 cells to determine if PKC or ceramide inhibits 5'D-I activity and gene expression in a manner similar to TNF-α. FRTL-5 cells were incubated in media containing 5% calf serum, in the absence of TSH, for 7 days prior to use. Incubation of FRTL-5 cells with sphingomyelinase (Spg) (1 U/ml) or 12-O-tetradecanoyl-phorbol 13-acetate (TPA) (100 nM) for 3 days had no effect on cell viability. Addition of Spg to TSH-stimulated cells resulted in a dose dependent decrease in 5' D-I activity (71$\pm$ 5; 0.01 U/ml, 42 $\pm$ 7; 0.1 U/ml, and 40 $\pm$ 5; 1.0 U/ml, percent of TSH stimulated cells), and 5'D-I mRNA levels (82; 0.01 U/ml, 83; 0.1 U/ml, and 20; 1.0 U/ml, percent). Similarly, addition of TPA to TSH-stimulated cells resulted in a dose dependent decrease in 5' D-I activity (56 $\pm$ 7; 1 nM/ml, 33 $\pm$ 3; 10 nM, and 24 $\pm$ 1; 100 nM, percent of control), and in 5'D-I mRNA levels (114; 1 nM/ml, 76; 10 nM, and 24; 100 nM, percent). When the same blots were analyzed for thyroglobulin mRNA levels, no effect of TPA was found. When cells were co-incubated with INFγ (12.5 u/ml) and either Spg (0.1-1.0 U/ml) or TPA (1-100 nM) alone, or with TPA and Spg in combination, however, there was no synergistic effect on 5'D-I activity or mRNA levels. In conclusion, we found that activation of ceramide or PKC alone is sufficient to block the TSH-induced increase in 5' D-I activity and mRNA levels. In the presence of INF-γ, however, activation of either pathway alone is insufficient to result in a synergistic inhibition of 5'D-I activity or gene expression. These data suggest that TNF-induced activation of additional pathways, are required for the synergistic inhibition of thyroid function by TNF-α and IFN-γ.

241. P95 **CHARACTERIZATION OF IODOTHYRONINE SULFOTRANSFERASE ACTIVITY IN RAT LIVER.**

T J Visser and E Kaptein. Dept Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands.

Sulfation accelerates the degradation of different iodothyronines, including T4, T3 and 3,3'T2 (T2), by the type I iodothyronine deiodinase in both human and rat liver. However, little is known about the enzyme(s) calatyzing the sulfation of iodothyronines. We have now characterized the iodothyronine sulfotransferase (IST) activity of rat liver cytosol. Based on earlier findings a convenient IST assay was developed using [^{125}]T2 as the preferred substrate and unlabeled PAPS as the cofactor. Usually, rat liver cytosol was incubated at 37 C with 1 μM T2 and 50 μM PAPS in phosphate buffer. T2 sulfate was separated from T2 on Sephadex LH-20 minicolumns. T2 ST activity was optimal at pH 8.0, but all further studies were done at pH 7.2. T2 ST activity was increased slightly by addition of 2 mM EDTA but was hardly affected by Ca^{2+}, Mg^{2+} or DTT. Under the selected reaction conditions (25 μg/ml; 15 min) T2 sulfation was linear with cytosolic protein concentration and reaction time. Hepatic T2 ST activity was >2-fold higher in male than in female rats, which was associated with a lower K_m value for T2 (1.9 vs 3.8 μM) and a slightly higher V_{max} value (2.0 vs 1.2 nmol/min/mg protein) in males vs females; the K_m value for PAPS was similar in males and females (6.1 vs 4.5 μM). Iodothyronine analogs dose-dependently inhibited T2 sulfation, with potencies decreasing in the order (IC_{50} values in μM): 3,3'T2 (1.3) > 3'T1 (1.8) > 3',5'T2 (5.3) > rT3 (13) > T4 (22) > T3 (45) > T0 (52) > 3T1 (100) > 3,5T2 (»100). T2 ST activity was also inhibited dose-dependently by a variety of phenol (P) derivatives (IC_{50} values in μM): P (13), 2,6-Cl_2P (0.25), 2,6-Br_2P (0.3), 2,4,6-Br_3P (0.06), 2,4,6-I_3P (0.3), Cl_5P (0.005), 4-NO_2P (0.7), 2,6-Cl_2-4-NO_2P (0.2) and 2,6-I_2-4-NO_2P (0.9). Relative to T2, sulfation of T3 by male rat liver cytosol was very slow, characterized by a K_m value of 48 μM and V_{max} of 0.22 nmol/min/mg protein. T3 ST activity was inhibited dose-dependently by T2 and Triac, with IC_{50} values of 4.8 and 15 μM, respectively. Preliminary studies demonstrated a wide range of T2 ST activity in human liver cytosol up to 137 pmol/min/mg protein. These results indicate that T2 is a convenient substrate for rat liver cytosol IST activity. The greater IST activity in male than in female rats as well as the profile of inhibition by phenols suggest that arylsulfotransferase type IV (AST IV) is a major enzyme for thyroid hormone sulfation in rat liver.

242. P96 SELECTIVE MACROPHAGE DEPLETION IN THE LIVER DOES NOT PREVENT THE DEVELOPMENT OF THE LOW T_3 SYNDROME IN THE MOUSE.

A. Boelen, MC Platvoet-ter Schiphorst and WM Wiersinga, Dept. of Endocrinology, Academic Medical Center, University of Amsterdam, The Netherlands.

The pathogenesis of the sick euthyroid syndrome is still incompletely understood. Cytokines have been implicated to play a causative role in the decrease of serum T_3 during illness. We have previously shown that administration of bacterial endotoxin (LPS) to mice results in a decrease of serum T_3 and T_4 and of liver 5'-deiodinase mRNA. Liver macrophages (Kupffer cells) are considered as the main source of cytokine production in the liver. The aim of the present study was to investigate the role of Kupffer cells in the low T_3 syndrome in mouse (induced by LPS or by food deprivation), using selective in vivo macrophage depletion. Methods: Selective macrophage depletion of liver and spleen macrophages was accomplished by intravenous injection with liposomes encapsulating dichloromethylene diphosphonate (CL_2MDP); depletion was checked by histological examination of spleen sections stained with acid phosphatase. Two types of experiments were performed: A) Administration of LPS, or vehicle in pair-fed controls (PFC), with or without i.v. injection of liposomes 48h previously. B) Food deprivation for 24h or food ad lib, with or without liposomes 48h previously. Results: Liposomes effectively depleted macrophages (Mø) in liver and spleen as evident from disappearance of acid phosphatase staining. Furthermore, LPS-induced serum TNFα and IL-6 levels were markedly reduced in liposome treated animals. LPS and fasting decreased serum T_3, T_4 and liver 5'-deiodinase mRNA relative to controls at 24h. The decrease was similar in Mø+ve and Mø-ve groups (see Table; values in nmol/l as mean ± SD, 6-8 mice per group)

	PFC, Mø+ve	LPS, Mø+ve	PFC, Mø-ve	LPS, Mø-ve
serum T_3	1.47 ± 0.29	1.08 ± 0.26	1.31 ± 0.19	1.00 ± 0.22
serum T_4	32 ± 6	17 ± 5	26 ± 7	22 ± 9
	C, Mø+ve	fasting, Mø+ve	C, Mø-ve	fasting, Mø-ve
serum T_3	1.81 ± 0.34	1.26 ± 0.13	1.78 ± 0.21	1.29 ± 0.12
serum T_4	27 ± 6	18 ± 6	23 ± 5	18 ± 2

Conclusion: Selective in vivo macrophage depletion of liver and spleen does not prevent the LPS- or starvation-induced sick euthyroid syndrome in mice. We conclude that cytokines generated in the Kupffercel do not play a substantial role in the pathogenesis of the low T_3 syndrome in this experimental model.

243. P97 **EFFECT OF T3 ADMINISTRATION ON THE EXPRESSION OF TYPE I 5'-DEIODINASE MESSENGER RNA OF THE LIVER IN STREPTOZOTOSIN INDUCED DIABETIC RATS.** S.Tabata, T.Yonemoto, and M.Horimoto, 2nd Dept. of Internal Medicine, Kansai Medical University, Moriguchi, Osaka 570, Japan.

In poorly controlled diabetics, serum T3 levels are frequently decreased. This decrease is considered to be due to à decrease in type I 5'-deiodinase (5'-D) activity from T4 to T3, but the detailed mechanism is still unclear. In the present study, we measured 5'-D activity and its mRNA in rat liver and observed the relationship of 5'-D and serum T4 and T3 levels after giving T3 to streptozotosin (STZ) -induced diabetic rats, in order to study the mechanism of the decrease in 5'-D activity. Methods : 100mg/kg BW of STZ was intraperitoneally administered to 20 male Wistar rats weighing 150-200g. After blood glucose levels significantly increased, the rats were divided into four groups as follows : (1) STZ alone, (2) STZ plus T3, (3) STZ plus insulin, (4) STZ, T3, and insulin. Intermediate-acting insulin and T3 were administered daily at 4 units/100g BW and 5 μ g/ 100g BW, respectively. The rats were decapitated together with normal control rats ten days later. Serum levels of T4, T3, TSH, and glucose were measured, followed by the determination of 5'-D activity and 5'-D mRNA in the liver. 5'-D mRNA was determined using the Northern blot analysis, while 5'-D activity was measured by I release from ^{125}I-rT3 as reported previously. Results : Blood glucose levels were markedly higher in group (1) than in the control group, while the level was almost normalized in group (3) and (4) . T3 level was markedly reduced in group (1) , but in groups (2) and (3) it was similar to the level in the control group. In group (4) , T3 level was rather elevated. T4 levels were reduced in all the groups. TSH level in groups (1) and (3) was as high as in the control group, while in groups (2) and (4) it was suppressed. 5'-D activity in group (1) was apparently reduced as compared with the control group. On the other hand, in groups (2) and (3) it was almost the same level in the control group, and it was rather raised in group (4) . The band of 5'-D mRNA on Northern blotting was located at about 2.1kbp, with no change between the groups. 5'-D mRNA expression in the liver of each group was compared to that in the control group,with the control level being set at 100%. The levels in group (1) , (2) , (3) , and (4) were 20 ± 4%, 74 ± 23%, 87 ± 8%, and 166 ± 26%, respectively. Compared to the control group, only group (1) showed a significant decrease. In group (2) , 5'-D mRNA tended to increase, and in group (3) , it was increased significantly compared with group (1) . Moreover , compared to groups (2) and (3) , it was significantly increased in group (4) . The T3 level, 5'-D mRNA level, and 5'-D activity correlated well with each other. Conclusion : Decreased hepatic 5'-D mRNA in diabetics are suggested to be associated with decrease in serum T3 level caused by the insufficient insulin action.

244. P98 **INHIBITION OF IODIDE TRANSPORT AND ORGANIFICATION BY SODIUM NITROPRUSSIDE IN CULTURED BOVINE THYROID CELLS.** **M.E. Costamagna, A.M. Masini-Repiso, A.M. Cabanillas, C.G. Pellizas, M. Di Fulvio and A.H. Coleoni. Department of Clinical Biochemistry, Faculty of Chemical Sciences, National University of Córdoba, Córdoba, Argentina.**

Sodium nitroprusside (SNP) spontaneously breaks down in solution to produce nitric oxide (NO). In many cell types NO stimulates cytosolic guanylate cyclase activity. A recent report suggests that NO may regulate secretion from endocrine organs. It has been demonstrated that SNP used as a source of NO increases cGMP levels in thyroid cells. A reduction in iodide uptake and no change in hormone secretion were observed in bovine and human thyroid cells respectively. The aim of this work was to further study the effect of SNP on the uptake, transport and organification of iodide in TSH-stimulated bovine thyroid cells in primary culture. The morphology of the cells was also observed. Iodide uptake and organification were measured after 60 min of cell incubation at 37 C with 1 µCi Na^{131}I (carrier free). Uptake (organified plus non-organified iodide) was determined by measuring total intracellular radiactivity incorporated into cells and iodide organification by protein-bound ^{131}I after precipitation with trichloroacetic acid. Values were expressed as c.p.m./µg DNA. Iodide transport was measured as the iodide concentrating activity of cells in the described conditions plus 0.1 µmol/L KI and 1 mmol/L methylmercaptoimidazole (MMI). Iodide diffusion was measured by adding 1 mmol/L $KClO_4$. Values were expressed as the ratio of c.p.m.per mg DNA of cells/ c.p.m. per µL medium. As variable iodination values among cultures were obtained, results were expressed as percent of TSH value. The table below indicates results after 24 h incubation with SNP of one from three similar experiments.

Treatment (24h) (n=6)	Uptake (%)	Organification (%)	Transport (%)
TSH 1mU/mL	100 ± 29	100 ± 27	100 ± 20
+ SNP 10µmol/L	55 ± 11 ^{b}	69 ± 23^{a}	47 ± 5^{c}
+ SNP 50µmol/L	19 ± 5 ^{c}	23 ± 7^{c}	33 ± 5^{c}
+ SNP 100µmol/L	10 ± 6 ^{c}	15 ± 6^{c}	24 ± 6^{c}
+ SNP 500µmol/L	3 ± 1 ^{c}	7 ± 3^{c}	20 ± 3^{c}

Values as MEAN ± SD. a=P<0.05; b=P<0.01; c=P<0.001 vs. TSH (ANOVA)

A strong dose-dependent inhibition of the three parameters assayed was observed. At longer periods a sustained inhibition on the progressive increase in iodide uptake induced by TSH was also obtained. Uptake values with 1 mU/mL TSH (cpm/ µgDNA; MEAN ± SD) were: 24h: 39±4; 48h: 28±8; 72h: 80±36; 96h:185±48. Values with SNP (100 µmol/L) were (% of TSH value): 16±10*; 5±2*; 5±1* and 3±3* respectively (* = P < 0.001; n=6). The reduction of iodide uptake appears to be due to an impairment of both iodide transport and organification. The inhibition of iodide organification could not be totally attributable to the reduction in the iodide transport since this reached lower inhibition levels. Morphological changes compatible with cell dedifferentiation were induced by SNP. In a dose-dependent fashion, SNP produced spreading out and loss of follicular formations with the appearance of monolayers of flattened cells. Trypan blue exclusion staining of SNP treated cells showed that cell viability was similar to that in untreated cells. We conclude that SNP exerts some dedifferentiating effects on primary bovine thyroid cells mainly evidenced by a reduced iodide organification.

245. P99 **ALTERED THYROID HORMONE METABOLISM IN EUTHYROID PATIENTS WITH COMPLEX VENTRICULAR ARRHYTHMIAS AND ITS NORMALIZATION AFTER CHRONIC AMIODARONE TREATMENT.** G. Iervasi, A. Clerico, A. Pilo, S. Berti, R. Bonini, S. Turchi, C. Manfredi, A. Biagini, R. Bianchi, and L. Donato, CNR Institute of Clinical Physiology, Pisa, Italy.

One of the most important causes of sudden cardiac death is ventricular fibrillation, often preceded by complex ventricular arrhythmias (CVA). Since there is growing evidence that thyroid hormones may affect cardiac excitability, we tested the hypothesis that an altered peripheral metbolism of thyroid hormones might play a role in the pathogenesis of CVA and that anti-arrhythmic action of amiodarone (A) may be mediated by its effect on thyroid hormone metabolism. Peripheral thyroid hormone metabolism was investigated by using a double-tracer kinetic method in 10 patients with CVA before and during chronic (6 months) A treatment, as well as in 10 well-matched controls. The main parameters of thyroid hormones kinetics were determined from plasma disappearance curves of ^{125}I-T4 and ^{131}I-T3 and appearance curve of ^{125}I-T3 from ^{125}I-T4 after ^{125}I-T4 and ^{131}I-T3 iv bolus injection, using a six-pool model. Long-term A treatment was able to significantly CVA in nine patients as evaluated by 48-h Holter monitoring. The kinetic results (mean±SD) found in the 10 patients and in the 10 controls are reported in the Table:

Subjects	Serum T4 (μg/dl)	T4 PR (μg/day/m^2)	% conv	Serum T3 (ng/dl)	T3 PR (μg/day/m^2)	T3 SR (μg/day/m^2)
Patients						
Basal condition	7.7±1.2	41±14	45±20	112±22	18±3.1	3.3±2.5
Treatment	9.9±1.6	59±23	25±18	104±22	14±3.0	4.0±2.3
P (paired *t* test)	0.0071	0.0161	0.0018	n.s.	n.s.	n.s.
Controls	7.9±1.0	56±9.0	27±4.0	125±12	16±2.5	4.0±1.9
P* vs *basal condition*	n.s.	0.012	0.0102	n.s.	n.s.	n.s.
P* vs *treatment*	0.01	n.s.	n.s.	n.s.	n.s.	n.s.

PR= production rate; SR= thyroidal secretion rate; % conv= T4 to T3 conversion ratio. * unpaired *t* test

The unresponsive patient did not show any change in the peripheral thyroid metabolism before and after treatment. Our data indicate that: 1) patients with CVA and normal levels of thyroid hormones may have a peculiar alteration of metabolism of thyroid hormones, characterized by increased T4 to T3 conversion and reduced T4 production; 2) the long-term therapy with A able to suppress CVA normalizes the metabolism of thyroid hormones. Our results suggest that an altered thyroid metabolism may have a role in the pathogenesis of CVA.

246. P100 **2-Iodohexadecanal, the putative XI, derives from plasmalogens, is present in the human thyroid and binds covalently to thyroid proteins:** V. Panneels, H. Van Den Bergen, P. Niccoli, J. Van Sande, J.C. Braekman and J.M. Boeynaems. Institute of Interdisciplinary Research, School of Medicine, Université Libre de Bruxelles, Brussels, Belgium.

- Iodide, at pharmacological dose, inhibits various metabolic pathways of the thyroid gland, making it an autoregulatory component of the system. Most of the inhibitory effects of iodide, like the Wolff-Chaikoff effect and the inhibition of adenylyl cyclase require the trapping and the organification of iodide, suggesting that they are mediated by iodinated derivatives (called XI). The major iodolipid of the thyroid gland, 2-iodohexadecanal (IHDA), has been identified in horse *(in vitro)* and rat *(in vivo)* thyroids. IHDA reproduced all the characteristics of iodide inhibitory effects on H_2O_2 production, responsible for the Wolff-Chaikoff effect, and on adenylyl cyclase, suggesting that IHDA might be the mediator of those actions. In this study, we have checked the presence of IHDA in human thyroid and further investigated the mechanisms of IHDA biosynthesis and action.

- **Plasmalogens are the precursor of IHDA:** plasmalogens are glycerophospholipids containing a vinyl ether group (-CH=CH-O-) highly reactive with oxidant molecules. It has been postulated that reactive forms of iodide can attack this vinyl ether group of plasmalogens, followed by a cleavage of this group and generation of IHDA. This hypothesis was supported by the fact that incubation of bovine brain plasmalogens with lactoperoxidase, iodide and H_2O_2 generated IHDA. Using TLC and RP-HPLC, we have now demonstrated the incorporation of [3H]-hexadecanol in the plasmalogens of dog thyrocytes in culture and the subsequent formation of [3H]-IHDA in response to iodide addition. This production of [3H]-IHDA was inhibited by methimazole (500μM). [3H]-palmitate was used as control: it was incorporated into several classes of lipids, but not in plasmalogens, and no labelled IHDA could be detected in response to iodide.

- **IHDA is produced *in vivo* by human thyroid gland:** lipids were extracted from human thyroid tissue (7g) of a patient suffering from Basedow Grave's disease and treated with lugol. IHDA was identified on the basis of coelution with standard IHDA in gas chromatography and of mass spectrometry.

- In order to characterize the **mechanism of IHDA action** in the thyroid, its covalent binding to proteins was investigated. [3H]-IHDA labelled a large number of proteins following addition to thyroid membranes, but the rapid time course of this binding was not consistent with the slow onset of the IHDA effects. A smaller number of proteins were labelled following iodide addition to [3H]-hexadecanol-labelled dog thyrocytes and might represent the physiological targets of IHDA.

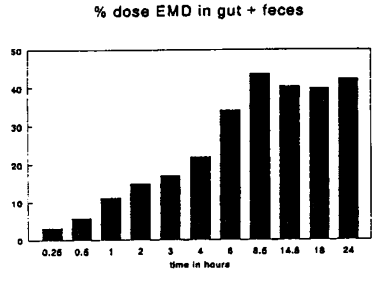
In conclusion, we have demonstrated that the IHDA formed upon exposure of the thyroid to iodide derives from plasmalogens. We also have shown that the biosynthesis of IHDA is largely increased in response to a concentration of iodide which induces the Wolff-Chaikoff effect. We have detected IHDA in human thyroid treated by lugol.

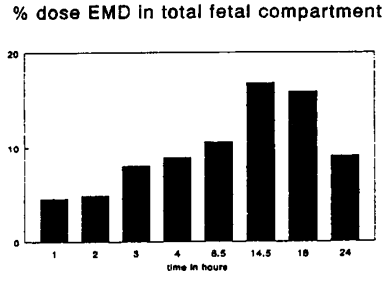
247. P101 **ONTOGENY OF THE DIFFERENCES IN THYROID HORMONE LEVELS AMONG OUTBRED RAT STOCKS AND BETWEEN GENDERS.** A. G. Amador, D. Pittman and A. Caruso, Division of Research, Department of Obstetrics & Gynecology, SIU School of Medicine, Springfield, Illinois, USA.

Differences in thyroid hormone levels have been reported among normal adults from various rat stocks (Pittman *et al*, 1993), and from several species (Amador & Hilgers, 1994). In those studies, differences were also observed in the sexual dimorphism of thyroid hormone levels. The present study was designed to determine at what point during the ontogeny of thyroid hormone metabolism the afore mentioned differences appear. Thus, thyroids and livers from 21, 28, 35 and 42 day (d) old male (m) and female (f) Long-Evans (LE), Wistar (W) and Sprague-Dawley (SD) rats were obtained, weighed, and homogenized in d_2H_2O. Thyroid thyroxine (T_4) and triiodothyronine (T_3), as well as hepatic T_3 were then determined using solid-phase radioimmunoassays. Thyroid weight was higher in mLE than in fLE only at 42d, and in fSD is was higher than in mSD only at 42d. No differences in thyroid weight were observed in W rats. The concentration of T_4 was higher in fLE at 42d than in mLE. It was higher in fW than in mW except at 42d. Also, in mSD it was higher than in fSD at 21d, and again at 42d. Male SD rats tended to have the highest T_4 concentrations and fW the lowest. The thyroid concentration of T_3 was higher in fW than in mW at 28d. No differences in thyroid T_3 levels were observed between genders in LE and SD rats. Liver weight tended to be highest in mLE and lowest in fSD rats. Liver weight was higher in mLE than in fLE at all ages, and in mSD than in fSD except at 21d. No differences in liver weight were observed in W rats. The concentration of liver T_3 was higher in fLE than in mLE at 21 and 28d. It was higher in mW than in fW only at 21d. No differences in the concentration of liver T_3 were measured in SD rats. Thus, sexual dimorphism occurs during the ontogeny of thyroid hormone metabolism, but its characteristics tend to be stock-specific. The present results indicate that genomic-dependent intra-species and inter-gender differences in the thyroid and liver levels of thyroid hormones, observed in adult animals, are present during development, and are independent of gonadal maturation. (Support was provided by the SIU-ADRAF Student Research Fund, the SIU-OB/GYN Departmental Research Fund, and the ReproGen Research Fund.)

248. P102 **^{125}I-FLAVONOID CROSSES THE PLACENTA AND ACCUMULATES IN FETAL COMPARTMENT IN THE RAT.** J.P.Schroder-van der Elst*, D. van der Heide, P.M. Versloot, T. Chen** and H. Rokos**. Human and Animal Physiology, Agricultural University, Wageningen,* and Endocrinolgy, University Hospital, Leiden, The Netherlands;** Henning Berlin GmbH, Germany.

EMD 21388, a synthetic flavonoid is a potent inhibitor of the in vitro binding of T_4 to transthyretin (TTR). It is unknown if this flavonoid crosses the placenta and if it interferes with T_4 binding in the fetal compartment. In 8 pregnant rats (20 ds) we studied the distribution of the iodinated homologue of EMD 21388, EMD 49209, in maternal tissues, intestinal contents and fetal tissues. After a bolus injection of EMD 49209, the rats were bled and perfused at different time points up to 24 hours. Whole fetal units (fetus + maternal + fetal placenta in amnion sac), fetal organs (plasma, liver, brain, thyroid), maternal plasma, several tissues and intestinal contents were counted, homogenized, extracted and submitted to C18 column chromatography and/or HPLC. After 24 hrs only 10% dose was metabolized to free ^{125}I. Only small amounts of radioactive metabolites could be detected. The % dose in all maternal tissues and plasma decreased fast with time to levels of less than 0.01 % dose/gram. In maternal brain no ^{125}I-EMD was found.EMD 49209 was excreted very fast into the gut. This increased to 40% dose after 8 hrs (fg.right). In all fetal units together the % dose EMD increases with time to 15 hrs to 18%. After 24 hrs still 10% dose is present in the total fetal units (fig left). As 50-60% dose is already excreted (urine + feces + gut), this means that 25-30% EMD still present in the animal is found in the fetal compartment. In comparison with the maternal brain in which no EMD was detected, the fetal brain con-tained signigicant amounts of ^{125}I EMD. As it has been shown that the fetal brain contains high concentrations of TTR, our results indicate a preferential pathway for this flavonoid to the fetal brain. This suggests an essential interference of this flavonoid with T_4 binding to TTR in the fetal rat.





249. P103 **GENDER-SPECIFIC CHANGES IN HEPATIC THYROID HORMONE METABOLISM DURING SHORT-TERM FASTING AND LONG-TERM FOOD RESTRICTION IN RATS.**

GAC van Haasteren, E Linkels, E Kaptein, WJ de Greef and TJ Visser. Depts Endocrinology and Reproduction, and Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands.

Short-term fasting induces marked reductions in serum TSH, T4 and T3, and hepatic type I iodothyronine deiodinase (ID1) activity in rats, but little is known of the effects of short- or long-term food restriction (FR) on thyroid hormone conjugation in the liver. In this study groups of male and female Wistar rats were subjected to complete fasting for 3 days (3DF) or to FR to 1/3 of normal intake (FR33) for 3 weeks. Liver microsomal ID1 activity was determined with 1 μM rT3 as substrate and 5 mM DTT as cofactor, microsomal UDP-glucuronyl-transferase (UGT) acivity with 1 μM T4 or T3 and 5 mM UDPGA as cofactor, and cytosolic sulfotransferase (ST) activity with 1 μM 3,3'-T2 (T2) as substrate and 10 μM PAPS as cofactor. The results of FR33 vs fed controls (ctrl) are presented in the table (mean ± SD pmol/min/mg; n=5-8; ¶, $p<0.01$ vs ctrl; #, $p<0.01$ vs. ctrl males).

Group	ID1	T4 UGT	T3 UGT	T2 ST
Male ctrl	733 ± 112	1.39 ± 0.14	0.93 ± 0.05	278 ± 20
Male FR33	346 ± 66¶	2.29 ± 0.14¶	1.05 ± 0.06¶	138 ± 54¶
Female ctrl	266 ± 37#	1.56 ± 0.10	1.00 ± 0.15	130 ± 13#
Female FR33	114 ± 23¶	2.52 ± 0.25¶	0.66 ± 0.18¶	144 ± 21

ID1 activity was lower in female than in male ctrl rats and was strongly decreased by FR33 and 3DF in both sexes. T4 UGT activity was not different between ctrl males and females and was significantly increased by FR33 and 3DF in both sexes. Similar results were obtained with rT3 and bilirubin as the substrates, supporting previous data that bilirubin UGT is a major enzyme for T4 and rT3 glucuronidation. T3 UGT activity was not different between ctrl males and females. FR33, but not 3DF, strongly reduced T3 UGT activity in females but not in males. The same results were obtained with androsterone as the substrate, supporting previous data that T3 is largely glucuronidated by androsterone UGT. T2 ST activity was ≈2-fold higher in male than in female ctrl rats. FR33, but not 3DF, decreased T2 ST activity in males to the level in ctrl females but did not affect the latter. Similar results were obtained with T3 as the substrate. These results indicate important gender- and substrate-specific changes in hepatic iodothyronine conjugation during short- and long-term food restriction which contribute to the complex alterations in thyroid hormone metabolism in these conditions.

250. P104 **T_2 AND TRIAC ARE SULFATED IN HEPG2 CELLS, WHEREAS T_3 SULFATION IS ABSENT.**

M de Jong, EJ Rolleman, HF Bernard. Departments of Internal Medicine III and Nuclear Medicine, Erasmus University Hospital, Rotterdam, The Netherlands.

We showed that the human hepatoma cell line HepG2 is a good model for normal human hepatocytes with regard to thyroid hormone metabolism (1). Both cell types show little T_3 deiodination due to deficient sulfation (2) (V_{max} of T_3S deiodination is 30-fold higher compared to T_3). To investigate if sulfation of other iodothyronines is also absent in HepG2 cells, we report here studies with T_2 and Triac. Methods: ^{125}I-3,3'-T_2 and ^{125}I-Triac were incubated with HepG2 cells in culture for 24h in DMEM-F12 with 0.5% BSA at 37C. Medium was analyzed by LH-20 chromatography for produced iodide, conjugates and remaining iodothyronines. To investigate the nature of conjugates, 0.5 ml medium was treated for 1h at 80C with 0.5 ml 1 M HCl, under which condition sulfates are hydrolyzed, while ether glucuronides are stable (3), or for 1h at 37 C with 50 µl 1 M NaOH, under these conditions ester glucuronides of Triac (produced in human liver) are hydrolyzed (4)). Results: see Table, mean ± SD, n≥3, @:$p<0.001$. Discussion: T_2 results indicate that the T_2-conjugates are predominantly T_2 sulfate, whereas we did not find T_3 sulfation in HepG2 cells (2). This could be explained by a difference in K_m of T_2 and T_3 sulfation (as found in rat liver; 2 versus 48 µM). For Triac the most important metabolite is normally Triac glucuronide, but the results obtained here show that Triac is sulfated in HepG2 cells and not glucuronidated, probably due to a deficiency of ester glucuronidation in this cell line. HepG2 cells are thus not always a good model for human hepatocytes to study thyroid hormone metabolism.

Table:	% Iodide	% Conjugates
T_2	47.3 ± 3.8	48.2 ± 2.7
T_2 (HCl)	47.3 ± 2.8	3.53 ± 0.2@
T_2 (NaOH)	47.2 ± 3.0	48.0 ± 2.8
Triac	10.5 ± 1.2	31.4 ± 2.6
Triac (HCl)	12.9 ± 1.2	0.94 ± 0.4@
Triac (NaOH)	11.7 ± 1.0	30.9 ± 3.2

(1) J.Endocrinol.Invest 16:73,1993; (2) BBA 1157:114-118,1993; (3) Endocrinology 117:1-7,1985; (4) Endocrinology 135:1004-1009,1994.

251. P105 THYROXINE BINDING GLOBULIN (TBG) INHIBITS TRANSTHYRETIN (TTR) BINDING BY MONOCYTES. G.C.Schussler and M.G.Yap, State U. of NY Health Science Center, Brooklyn.

TTR, a transport protein for T_4 and retinol binding protein, has an antiinflammatory effect on monocytes (Inflam 16:471). Like previously studied cells (JBC 265:1425), monocytes (THP1) bind TTR and the TTR-T_4 complex at high affinity (Kd 10^{-9} M), limited capacity sites. During investigation of TTR mediated T_4 uptake by monocytes, it was unexpectedly found that purified TBG, added in order to reduce the free T_4 concentration, decreased TTR as well as T_4 binding to the monocytes. The inhibition of ^{125}I TTR uptake was concentration dependent between 4 x 10^{-9} M and 4 x 10^{-12} M TBG in serum free solution. In the presence of 1% human serum which contains about 4 x 10^{-9} M TBG, the addition of 4 x 10^{-9} M TBG further decreased ^{125}I TTR binding but lower concentrations were ineffective. To eliminate the possibility that cellular TTR binding was inhibited by a contaminant rather than TBG itself, TBG was preincubated with varying concentrations of a polyvalent antiTBG antibody (IgG fraction). The antibody alone did not affect TTR or T_4 binding to monocytes but, depending on the concentration at which it was used, TBG antibody diminished or abolished the TBG inhibition of TTR binding. The inhibition of cellular T_4 binding was proportionately affected supporting the interpretation that the antibody inactivated TBG. Concentrations of TBG that inhibited monocyte TTR binding had no effect on TTR uptake by hepatocytes (HepG2), although high affinity TTR binding was readily demonstrable. Inhibition of TTR binding to its binding site by protein interaction with TBG is unlikely because this should be demonstrable regardless of the cell used and because the effect of TBG on TTR uptake occurs even with a large molar excess of TTR. Thus far we have not been able to find saturable TBG binding and competition for the TTR binding site by TBG seems unlikely The mechanism by which TBG inhibits TTR binding to monocytes and whether this inhibition of binding diminishes the antiinflammatory effect of TTR remain to be determined.

252. P106 SERUM IODTHYRONINE CONCENTRATIONS IN INTESTINALLY DECONTAMINATED RATS TREATED WITH TYPE I T4 5'-DEIODINASE INHIBITOR. I.E. Veronikis, S. Alex, S.L. Fang, G.E. Wright, and C.H. Emerson, University of Massachusetts Medical School, Worcester, Massachusetts.

Anaerobic intestinal bacteria produce enzymes that hydrolyze T4 and T3 conjugates and therefore promote the enteric reabsorption of thyroid hormones. This could be more important in rats whose type 1 5'-deiodinase activity (5'D-I) is inhibited since their biliary T3 sulfate (T3S) levels are elevated. Studies were performed to test the importance of enteric bacteria in thyroid economy and determine if reduction of intestinal bacterial arylsulfatase (AStase) activity augments the relatively weak ability of 5'D-I inhibitors to lower serum T3 levels. Adult male rats received enteric antibiotics or no antibiotics for 12 days and then, in half of the rats in each group, treatment with 6-anilino-2-thiouracil (ATU), a 5'D-I inhibitor that does not affect thyroid hormone synthesis, was added for 10 days. Intestinal arylsulfatase activity was strikingly reduced by approximately 87 % in the antibiotic groups and liver 5'D-I activity was decreased by 65 % in ATU treated rats. The data are shown as mean ± SE.

Treatment	T4, ug/dl	T3, ng/dl	rT3, pg/ml	T4S, ng/dl	T3S, ng/dl
None	5.2 ± 0.3	107 ± 4	110 ± 7	2.8 ± 0.4	6 ± 1.3
Antibiotics	5.7 ± 0.2	130 ± 7	105 ± 13	1.8 ± 0.2	3.6 ± 1
ATU	9 ± 0.3	97 ± 6	159 ± 7	6.1 ± 1.4	10.6 ± 2
Antibiotics + ATU	7 ± 0.3	107 ± 7	197 ± 14	6.5 ± 0.9	9.7 ± 2
Two Way ANOVA	$p < 0.01$*	$p < 0.02$**	$p < 0.01$*	$p < 0.01$*	$p < 0.01$*

ATU treatment was associated with a significant increase in serum T4*, rT3*, T4S*, and T3S* and a marginal decrease in serum T3** concentrations. Antibiotic treatment did not lower serum thyroid hormone concentrations or increase serum TSH or thyroid weight. In fact it was associated with a modest increase in serum T3**. These results suggest that anaerobic enteric bacteria do not have an important role in recycling thyroid hormones.

253. P107 RELATIONSHIP BETWEEN SERUM T_3 AND SERUM INTERLEUKIN-8, INTERLEUKIN-10 OR INTERFERONγ IN PATIENTS WITH NONTHYROIDAL ILLNESS.

MC Platvoet-ter Schiphorst and A Boelen. -Dept. of Endocrinology, Academic Medical Center, University of Amsterdam, The Netherlands.

Cytokines have been implicated in the pathogenesis of the low T_3 syndrome during illness. In particular, a strong negative relationship has been observed between serum T_3 and serum IL-6 and a variety of soluble cytokine receptors in patients with nonthyroidal illness. The low T_3 syndrome seems to be an useful mechanism to counteract excessive catabolism during illness and would fit in the broader concept of the acute phase response as a defense mechanism of the body regulated by proinflammatory and antiinflammatory cytokines. The aim of the present study was to evaluate any association in NTI-patients between serum T_3 and interleukin-8 (IL-8), interleukin-10 (IL-10) or interferonγ (IFNγ). Patients. Inclusion criteria: consecutive hospitalized patients of the Department of Internal Medicine (n=140). Exclusion criteria: thyroid or pituitary/hypothalamic disease, drugs known to affect thyroid hormone metabolism (like steroids, antiepileptics) or to induce cytokines (n=40). The remaining patients were divided in three groups: gr A ($T_3 \geq 1.30$nmol/l, $T_4 \geq 75$nmol/l), gr. B ($T_3 < 1.30$nmol/l, $T_4 \geq 75$nmol/l), and gr. C ($T_3 < 1.30$nmol/l, $T_4 < 75$nmol/l). Methods. ELISA's were used for measurement of IL-8 (Central Laboratory of the Red Cross), IL-10 (Schering-Plough) and IFNγ (Medgenix). Differences between groups were analyzed by the Kruskal-Wallis test and correlations by Spearman rank test. Undetectable cytokine concentrations were taken as 50% of the detectionlimit in the calculations. Results. The median values (range between parentheses) are given in the table. No differences between the groups were observed.

	group A (n=41)	group B (n=46)	group C (n=13)
T_3 (nmol/l)	1.55 (1.3-2.35)	0.98 (0.43-1.29)	0.80 (0.15-1.02)
IFNγ (IU/ml)	<0.2 (<0.2-0.66)	<0.2 (<0.2-1.95)	<0.2 (<0.2-0.22)
IL-8 (pg/ml)	<2 (<2-951)	<2 (<2-232)	<2 (<2-671)
IL-10 (pg/ml)	<100 (<100-1387)	<100 (<100-10986)	<100 (<100-8371)

IFNγ was undetectable (<0.2 IU/ml) in 80%, IL-8 (<2 pg/ml) in 72% and IL-10 (<100 pg/ml) in 67% of the patients. Taken together all 100 patients, a small negative correlation was found between serum T_3 and serum IL-8 ($r=-0.25$, $p<0.05$) but not between serum T_3 and serum IL-10 or IFNγ. Conclusion. The low T_3 syndrome during illness is slightly associated with serum IL-8 but not with serum IL-10 or IFNγ. These results do not imply substantial involvement of these cytokines in the pathogenesis of the sick euthyroid syndrome.

9:00 A.M. 254. TSH RECEPTOR MUTATIONS IN THYROID DIFFERENTIATED CARCINOMAS WITH CONSTITUTIVE ACTIVATION OF ADENYLATE CYCLASE. D. Russo[1,2], F. Arturi[1], M. Schlumberger[4], J.A. DuVillard[3], B. Caillou[4], R. Monier[4], H.G. Suarez[3] and S. Filetti[1], [1]Cattedra di Endocrinologia, Dipartimento di Medicina Sperimentale e Clinica; [2]Cattedra di Farmacologia, Facoltà di Farmacia, Università di Reggio Calabria, Catanzaro, Italy; [3]Institut de Recherches Scientifiques sur le Cancer, CNRS, Villejuif, and [4]Institut Gustave-Roussy, Villejuif, France.

Constitutive activation of the cAMP transducing cascade may act as an oncogenic mechanism in thyroid tumorigenesis. Indeed, activating mutations of the Gs alpha protein (gsp) and the TSH Receptor (TSHR) genes, have been found in thyroid hyperfunctioning adenomas. In the present study we assessed the possibility of the presence of critical mutations in the TSHR gene, in a series of six differentiated carcinomas selected on the basis of: a) high basal adenyl cyclase activity and a poor response to TSH stimulation, assessed in tumor membrane preparations; b) absence of gsp activation. In these tumors, the intracytoplasmic domains of the TSHR involved in signal transduction, all encoded by the exon 10 of the TSHR gene, were analyzed. DNA fragments of the TSHR C-terminal part (exon 10) was obtained from the amplification by PCR of genomic DNA extracted by the tumors and the adjacent normal tissue. Following PCR, the samples were sequenced by the method of Sanger for detection of mutations in the TSHR gene and blotted in nitrocellular filters and hybridized with a wild-type or mutated oligonucleotide encompassing the areas investigated. In three out of the six tumors examined, we found the presence of a base substitution at residue 623 (GCC --> TCC), together with the wild-type sequence. The mutation, somatic and heterozygotic, determines the substitution of a Serine for an Alanine in the third intracellular loop of the receptor, in a region critical for signal transduction. This substitution is able to constitutively activate the TSHR in terms of cAMP generation, after transfection of CHO cells with a mutant TSHR cDNA bearing a Serine at residue 623. One tumor bearing a TSHR mutation presented also a N-ras alteration (GGT --> GCT) in codon 13. Analysis of the lung metastatic tissue of this sample revealed also the presence of the same mutations in TSHR and N-ras genes. Our data represent the first report of alterations in the TSHR gene detected in thyroid malignant neoplasia. TSHR mutations may therefore participate, as well as the Gs alpha protein, in the oncogenesis of some differentiated thyroid carcinomas presenting increased basal levels of cAMP and a poor response to TSH.

This study was supported in part by a grant from AIRC.

9:15 A.M. 255. INTERFERRON-γ(IFNγ) SUPPRESSES THYROTROPIN RECEPTOR(TSHR) PROMOTER ACTIVITY BY REDUCING THE DNA BINDING OF THYROID TRANSCRIPTION FACTOR-1(TTF-1) TO ITS RECOGNITION SITE. K.Ohe, S.Ikuyama and H.Nawata, Third Department of Internal Medicine, Kyushu University Faculty of Medicine, Fukuoka 812-82, Japan.

IFNγ, which is known to play an important role in developping autoimmune thyroid diseases, decreases the expression of thyroid-specific genes, such as TSHR, thyroid peroxidase and thyroglubulin. In the present study, we determined the mechanisms of IFNγ-induced suppression of TSHR gene expression. In the previous studies, we cloned 1.7kb of 5'-flanking region of the rat TSHR gene, and identified several important *cis*-acting and *trans*-acting elements in its promoter region (Numbering system of the nucleotides; see Mol Endo 6:793, 1992). The reporter plasmids containing the 1.7kb promoter region, and 5'-deletions thereof, ligated to bacterial chloramphenicol acetyltransferase(CAT) gene were transiently transfected into FRTL-5 rat thyroid cells using DEAE-dextran. The cells were incubated in the 6H medium with or without 100U/ml rat IFNγ for 36hr, then, CAT activities were measured to assess the promoter activity. Significantly lower CAT activities were expressed when the cells were treated with IFNγ. Among several 5'-deletions of the promoter region, 5'-199, which contains "the minimal promoter region", showed the strongest suppression by IFNγ (<20%), while 5'-177, which is devoid of the downstream TTF-1 binding site, exerted less IFNγ-induced suppression, as compared to the 5'-199 construct. IFNγ suppressed the 5'-199 promoter activity in a dose-dependent manner from 3.12 to 50U/ml, and the suppressive effect was rat IFNγ-specific since human IFNγ, IFNα and IFNβ showed negligible effect on the promoter activity in FRTL-5 cells. When the sequence of the downstream TTF-1 site was mutated to the one to which TTF-1 could not bind, the overall promoter activity decreased in conjunction with marked reduction of IFNγ-induced supressibility. In addition, IFNγ did not suppress the TSHR promoter activity in FRT rat thyroid cells which do not express TTF-1. These functional data suggested that alteration of the TTF-1 binding could be involved in the IFNγ-induced suppression of the TSHR promoter activity. To test this, we performed gel mobility shift analyses using the oligonucleotide containing the downstream TTF-1 binding site(-189 - -175bp) as a probe. Although both nuclear extracts prepared from IFNγ-treated and non-treated FRTL-5 cells formed specific TTF-1-DNA complexes, the complex formation was less evident when the extract from IFNγ-treated cells was used. Northern blot analyses showed that TTF-1 RNA level in the IFNγ-treated cells was apparently comparable with that in the non-treated cells. From these results, we concluded that IFNγ suppresses the TSHR promoter activity by reducing the binding of TTF-1 to its recognition site in the minimal promoter region.

9:30 A.M. 256. CLONING OF THE cDNA FOR THE THYROID TRANSCRIPTION FACTOR-2.
M. Zannini, K. Sato, M. I. Arnone and R. Di Lauro, Stazione Zoologica "Anton Dohrn", Naples, Italy.

The cDNA for the Thyroid Transcription Factor-2 (TTF-2) has recently been cloned. The clone has been identified as TTF-2 on the basis of the following criteria: it is able to bind to the sequences on the thyroglobulin and thyroperoxidase promoters that had been previously described as binding site for a thyroid-specific factor named TTF-2; it is tightly regulated at the transcriptional level by insulin as it has been described for the TTF-2 binding activity. In situ hybridization experiments have shown that TTF-2 is expressed very early during mouse development suggesting that it could play a role in the commitment of the thyroid cell precursors of the embryo. Moreover its expression is restricted to the thyroid and to the anterior pituitary both during development and in adult tissues.
At variance from other thyroid transcription factors that are differently affected upon transformation with a variety of oncogenes, TTF-2 is very sensitive to the action of oncogenes, being absent in all transformed thyroid cell lines tested.
The full length cloning of the cDNA will allow to investigate its role in the thyroid specific expression of the thyroglobulin and thyroperoxidase promoters and hence in the establishment of the differentiated thyroid phenotype.

9:45 A.M. 257. **ISOLATION OF LYMPHOCYTES PRODUCING HUMAN IgG CLASS MONOCLONAL TSAb AND ANALYSIS OF VARIABLE REGIONS OF THEIR Ig GENES** T. Akamizu, H. Li, J. Okuda, H. Sugawa and F. Matsuda# Department of Laboratory Medicine, Faculty of Medicine, and #the Center for Molecular Biology and Genetics, Kyoto University, Kyoto, Japan

We have previously prepared Epstein-Barr virus (EBV)-transformed human B cell clones producing IgM class monoclonal anti-thyrotropin receptor antibodies (TRAb) from patients with Graves' disease. We then isolated and characterized Ig H chain genes of several B cell clones with TSH binding inhibitor immunoglobulin (TBII) or thyroid-stimulating antibody (TSAb) activities [J Immunol,**152**:1485,1994]. In autoimmune diseases, however, IgG class autoantibodies are generally considered to be more pathogenic than IgM class ones. In order to increase the chance of acquisition of cells producing IgG class TRAb, we depleted IgM^+ cells before EBV-transformation with a Magnetic Cell Separator. As the results, we obtained 4 independent B cell clones producing IgG class monoclonal TSAb from three patients with Graves' disease. None of these clones showed any TBII activity.

Next, 2 of 4 TSAb clones, B6B7 and F3F3, were selected for molecular genetic analysis. Their heavy and light chain cDNAs were isolated using the reverse transcriptase, immunoglobulin gene specific primers and PCR, and were sequenced. V_H genes of both clones belonged to $V_{H\text{-}IV}$ gene family, while V_κ genes belonged to different V_κ gene families. Interestingly, the most homologous V_H segment of B6B7 clone is V4-59 which was also used in a IgM class TSAb, 82-1 [ibid.], suggesting the restricted usage of V_H segments for TSAb. A number of somatic mutations were observed in variable regions of both chains of these clones. Although several replacement (R) and silent (S) mutations were found in both CDR and framework region, the R/S ratios of CDR were considerably higher than those of the framework region, suggesting the importance of somatic mutations for TSAb activity.

In conclusion, human IgG class monoclonal TSAb were isolated and their primary structures were clarifed at the molecular level.

10:30 A.M. 258. A RANDOMISED OPEN TRIAL OF CARBIMAZOLE ALONE VERSUS CARBIMAZOLE AND THYROXINE IN THE TREATMENT OF GRAVES' DISEASE. B. McIver, P.H. Rae, G. Beckett, A.E. Gold and A.D. Toft, University Department of Medicine and University Department of Clinical Biochemistry, Royal Infirmary, Edinburgh, Scotland, EH3 9YW.

The treatment of Graves' disease with a combination of antithyroid drugs and thyroxine for 18 months followed by thyroxine alone for up to a further 3 years has previously been reported to result in a 20-fold reduction in the risk of relapse, compared with antithyroid drugs alone (1). We have performed an open, prospective, randomised trial of carbimazole alone (Group A) versus carbimazole plus thyroxine (Group B) in one hundred and eleven patients with Graves' disease (89 female, 22 male; mean age 34 years, range: 18-72 years). Patients in both groups received carbimazole 40 mg/day for one month. In Group A the dose of carbimazole thereafter was adjusted to maintain the TSH within the normal range. In Group B, the dose of carbimazole remained unchanged, and thyroxine 0.1 mg/day was added, with the dose adjusted to maintain a suppressed TSH (<0.05 mU/l). Carbimazole was continued for 18 months in both groups. In Group B, thyroxine was continued for a further 18 months. Twenty patients (18%) have withdrawn from the study or been lost to follow up. Of the remaining 91 patients, 50 have completed 18 months of antithyroid drug treatment, and have been followed for a further 14 ± 7.5 months. TSH receptor antibody levels (TRAb) fell in both groups over the 18 months of carbimazole treatment, from 23.6 ± 4.2 to 3.2 ± 1.1 U/l in Group A and from 30.6 ± 4.6 to 5.3 ± 1.6 U/l in Group B. There was no difference in the rate of fall of TRAb between the two groups. Eight patients in Group A and 11 patients in Group B have relapsed following withdrawal of carbimazole, after an average of 5.8 ± 1.7 months (Group A) and 5.8 ± 1.3 months (Group B). Life-table analysis shows no difference between the rates of relapse in the first two years after carbimazole withdrawal ($p = 0.8$). In conclusion, combination therapy with antithyroid drugs and thyroxine does not affect either the rate of fall of TRAb levels, or the risk of relapse in our patients following carbimazole withdrawal.

(1) Hashizume K., et al. Administration of Thyroxine in treated Graves' disease. (1991) New England Journal of Medicine, 324: 947-53.

10:45 A.M. 259. Identification of a T3 Responsive Element in the Upstream Regulatory Region of the Human Type I 5'-Deiodinase Gene. T.Jakobs, C.Schmutzler and J.Köhrle, Klinische Forschergruppe, Medizinische Poliklinik, University of Würzburg, Würzburg, Germany

The selenoenzyme iodothyronine-5'deiodinase type I (5'DI) activates the prohormone thyroxine to the thyroid hormone 3,3',5-tri-iodothyronine (T3). Thereby 5'DI is one of the key enzymes in the T3-mediated control of growth, differentiation and basal metabolism. We report the identification of a genomic 5'DI clone approximately 25 kb in length by screening of a human DNA library with a cDNA fragment of the 5'DI coding region. This genomic clone contains 1500 bp of the gene's upstream regulatory sequences. At nucleotides -696 to -681 (with respect to the initiator codon) a T3 responsive element (TRE) consisting of an ideal direct repeat of two AGGTCA halfsites with a spacing of four nucleotides (DR+4) and a third putative AGTTCA halfsite with a spacing of another two nucleotides was found. This TRE was functionally characterized by electrophoretic mobility shift assays. Nuclear extracts prepared from HepG2 cells produce a specific retardation of a radiolabeled oligonucleotide comprising the DR+4+2 element. Antibodies against T3-receptor α and β produced the expected supershift of the complex. In addition, a putative NFκB-binding site followed by an AP-1 site was found at nucleotides -797 to -782. The intron/exon structure of the 5'DI coding region was determined by sequencing of the exon-intron boundaries. The transcription startpoint of the 5'DI was mapped to nucleotides -23 and -24 by PCR amplification of a cDNA complementary to the 5' end of the mRNA. Neither a TATA nor a CAAT-box was detected within the usual distance to the transcription initiaton site but a GC-box typical of the promotor of house-keeping genes was found at nucleotides -68 to -62. The identification of these response elements can provide the molecular basis of the observed induction of the 5'DI gene by thyroid hormone and the modulation by cytokines and factors associated with the low T3 syndrome. - Supported by DFG and EC grants.

11:00 A.M. 260. EXPRESSION OF THE MYELIN BASIC PROTEIN GENE IN DIFFERENTIATING PRIMARY OLIGODENDROCYTES: THE T3RE SEQUENCE (-186 TO -163) IS NECESSARY FOR BOTH T3-DEPENDENT AND T3-INDEPENDENT REGULATION. K.A.. Strait and J.H. Oppenheimer. Dept of Medicine and Dept. Cell Biol. U of Minnesota, USA

The rise in myelin basic protein (MBP) and other T3 target gene expression during rat brain development exhibits both a T3-dependent and a T3-independent phases (Mol. Endorinol. 1992 6 : 1847). T3 accelerates the expression of MBP and other target genes, thus resulting in an earlier attainment of the same maximal value ultimately reached in the absence of hormone. To elucidate the molecular basis of these mechanisms, we have studied the in vitro differentiation of a primary culture system of progenitor cells (0-2A) into oligodendrocytes. Northern analysis indicated a rise in TRβ1 mRNA preceded the rise in MBP mRNA, with maximal MBP expression attained between days 6 and 8. We transfected oligodendrocytes with 1300 bp of the upstream regulatory region of the MBP promoter which includes the TRE sequence reported by Farsetti et al (JBC 1991 266: 23226), fused to luciferase. Compared to baseline expression of this construct when transfected prior to differentiation (day 0), cells transfected on the third day of culture showed a 4-fold induction in the presence of T3 but only 1.5-fold in its absence. Transfection of cells on day 6 resulted in a 5-fold increase in the presence of T3 and an unexpected 5-fold increase in the absence of T3. Thus, during oligodendrocyte differentiation in culture, the MBP gene undergoes a T3-sensitive phase (day 3), whereas expression in mature cells (day 6) is elevated independently of T3. To examine the role of the MBP T3RE sequence we created a GG to TT mutation in each half-site. Control transfections of Neuro-2A cells with the mutant MBP construct (mMBP-luc) confirmed the loss of T3 inducibility (0.9-fold) compared to wild type MBP-luc (6.5-fold). Transfection of mMBP-luc into oligodendrocytes resulted in the expected loss of T3 induction on day 3 (1.1-fold mMBP-luc vs. 4-fold wt MBP-luc). Unexpectedly, however, the absence of a T3RE in mMBP-luc also blocked T3-independent expression in mature oligodendrocytes on day 6 (1.6-fold mMBP-luc vs. 5-fold wtMBP-luc). Conclusion: The T3RE sequence (-186 to -163) in the MBP gene is necessary not only for the T3-induced rise of MBP but also for T3-independent gene expression in mature oligodendrocytes. We hypothesize that the MBP T3RE sequence in mature oligodendrocytes facilitates T3-independent regulation of MBP either by supporting ligand-independent actions of the TR or by binding transcription factors other than TR to one or both of the T3RE half-sites .

11:15 A.M. 261. **CROSS-TALK BY MUTANT $T_3ß_1$ RECEPTORS INTERFERES WITH DUAL HORMONAL SYNERGY:A NEW MOLECULAR MECHANISM OF HORMONE RESISTANCE ?** P.G. Walfish*, Y-F Yang*, L.A. Chang*, T. Yoganathan*, E. Pisano* and T.R. Butt+. Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada* and SmithKline Beecham, King of Prussia, PA 19403, U.S.A.+

To determine whether rat $T_3ß_1$ ligand binding domain mutants (TRm) interfere not only with cognate ligand (T3) effects on $T_3ß_1$ wild-type (TRw) but also with 9 cis retinoic acid (9c-RA) induction of retinoid X receptors (RXRs) and dual (T3 + 9c-RA) hormonal transactivational effects on TR/RXR heterodimers, we used yeast (S.cerevisiae) which is devoid of endogenous TRs and RXRs. The expression plasmids contained the following receptors: TRw; or one of four TRm consisting of either a E1 subdomain D300A or Δ286-305 (having a ≈ 60 and 95% respective reduction in heterodimerization with RXRs); or a E3 subdomain T419 (having a 42 a.a. C terminus truncation with disruption of the 9th heptad); or a F subdomain T455 (having 6 a.a. C terminus truncation with retained RXR heterodimerization but absent T3 transactivation); or RXRα and RXRγ. Various combinations of these receptors were co-expressed in triple or quadruple transformations in the presence of ß-gal reporters containing one of the following hormone response elements (HREs): rat growth hormone inverted palindrome (rGH) x 3, chicken lysozyme silencer everted palindrome (F2) x 1 or variously spaced consensus AGGTCA repeats x 1 of DR1,3,4,5, or natural genes with imperfect hexameric direct repeats to include apolipoprotein A1 (ApoA1) x 2, acyl CoA oxidase (AOx) x 2, or mutated chicken lysozyme F2 3bp spaced everted palindrome (F2M) x 1. Compared with TRw, all TRms had varying degrees of defective constitutive and T3 induced transactivation. Interference with TRw by TRms in the absence of RXRs was only evident in the presence of rGH and F2. When co-expresssed as TR/RXR heterodimers, substitution for TRw of TRms defective in E1 or E3 subdomains inhibited the anticipated increase in transactivation resulting from dual (T3 + 9c-RA) hormonal synergy mediated by RXRγ but not RXRα as well as specific consensus and natural direct repeat HREs. Co-expression of both TRw and TRm in the presence of RXRγ and specific HREs significantly reduced 9c-RA and dual (T3 + 9c-RA) hormonal synergy responses for TRms with defective dimerization domains (ie. Δ286-305 and T419). However, TRms with functional heterodimerization domains (ie. T455 and D300A) also had a significant reduction (30-50%) in T3 and dual (T3 + 9c-RA) hormonal induction (T455 > D300A) in the presence of either DR4, DR5, AOx or ApoA1 but not rGH and F2 HREs, whereas they maintained 9c-RA dependent up-regulation to varying degrees. **Conclusions: 1)** In the presence of specific natural and consensus direct repeat HREs, TRw induced dual (T3 + 9c-RA) hormonal synergy through specific cross-talk with RXRγ. **2)** In the presence of RXRγ, substitution of TRm for TRw or co-expression of TRm with TRw had varying degress of inhibiton on these ligand-induced signalling pathways. **3)** Since several of the rat $TRß_1$ mutants resemble the human $TRß_1$ dominant negatives mutants of resistance to thyroid hormone, these observations could represent a new and clinically relevant molecular mechanism of hormone resistance.

11:30 A.M.
262. THYROID HORMONE RECEPTOR AND CREB ASSOCIATE BY PROTEIN-PROTEIN INTERACTION. A. Lin, B. Stevenin and S.L. Lee. Division of Endocrinology, Metabolism, and Molecular Medicine, New England Medical Center Hospital, Tufts University School of Medicine, Boston, MA 02111.

CREB is a major positive transcriptional activator while thyroid hormone (T3) is a repressor of TRH gene expression. The major basal enhancer of the rat TRH gene is a unique overlapping binding site for CREB and thyroid hormone receptor (TR). T3 inhibition of TRH gene expression may occur due to variation in CREB or phosphorylated (activated) CREB (phosphoCREB) levels, TR (alone or as a heterodimers with RXR) competition with CREB for a DNA binding site or TR interference of CREB activity via protein-protein interactions. We are currently examining these hypotheses for T3 inhibition of TRH promoter activity in our laboratory. Gel shift analyses of the multifunctional basal enhancer of the TRH gene revealed specific binding with bacterially expressed CREB and *in vitro* translated TRβ1. TRβ1 bound primarily as a monomer under conditions in which TRβ1 binds to the chick lysozyme F2 TRE as a dimer. T3 did not alter the binding pattern of TRβ1 with the TRH probe. Gel shift assays performed with TRβ1 and *in vitro* synthesized RXRβ demonstrated heterodimer formation. Gel shift competition studies were performed under conditions of protein excess and limiting probe. Small quantities of CREB easily competed off TRβ1 binding while excess TRβ1 could not compete off the CREB complexes from the TRH element. It was observed during some sets of competitions that the migration of the CREB band was retarded and was consistent with a protein-protein interaction between CREB and TRβ1. To determine if there was a specific interaction between CREB and TRβ1, we performed immunoprecipitation assays using bacterially expressed CREB and *in vitro* translated TRβ1-FLAG (template provided by Dr. Fred Schaufele, UCSF). Using unlabeled TRβ1-FLAG, ^{32}P-labeled phosphoCREB and a monoclonal FLAG antibody, CREB was recovered after immunoprecipitation. The amount of TR-CREB complex was not significantly altered by 50nM T3, dephosphorylation of CREB, or phosphorylation of TRβ1-FLAG. Immunoprecipitation assays using truncated TRβ1-FLAG proteins indicate that the site of interaction with CREB occurs at the TRβ1 c-terminus. This data suggests that thyroid hormone may alter transcription without direct binding to a TRE and thus provide a possible explanation for the difficulty in past studies to define a consensus negative TRE.

9:00 A.M. 263. **PAPILLARY THYROID CANCER IN CHILDREN EXPOSED TO CHERNOBYL NUCLEAR ACCIDENT: MOLECULAR ANALYSIS OF TUMOR SPECIMENS.** L. Fugazzola, I. Bongarzone, S. Pilotti, T.V. Vorontsova#, P. Collini, L. De Gregorio, S. Rao, L. Astakhova#, E.P. Demidchik#, M. Prat°, F. Pacini*, A. Pinchera*, M.A. Pierotti - Istituto Nazionale Tumori, Milano; °Dipartimento di Scienze Biomediche e Oncologia, Torino; *Istituto di Endocrinologia, Pisa, Italy; #Institute of Endocrinology, Minsk, Byelorussia.

Molecular analysis of thyroid tumors from children exposed to Chernobyl nuclear accident could provide answers to the many questions risen following the disaster about its linkage with the dramatically increased incidence of papillary thyroid cancer. To this purpose we have analyzed 12 samples of primary papillary thyroid cancer from children living in Belarus at the time of the accident. Southern blot analysis was accomplished on 6 of them and we found RET rearrangements in 4 cases; 3 of them were found to belong to the oncogene RET/ptc3 (the product of the fusion between ele1 gene and the TK domain of RET) and 1 to the oncogene RET/ptc2 (RIα gene and TK domain of RET). RET oncogenic activation was biologically confirmed by DNA transfection assay on NIH-3T3 cells and the presence of chimeric transcripts was assessed by RT-PCR. Moreover, to characterize the fusion point of RET TK domain with the activating gene, we performed nucleotide sequencing in all rearranged samples. K ras oncogenic activation, reported by some authors to be induced by ionizing radiations, was not found in any case. On the other hand all cases examined (n = 12) showed overexpression of the met oncogene, the receptor for HGF/SF, whereas the inactivation of the tumor suppressor gene p53 was not observed in any case. In conclusion, our study reports the first complete molecular analysis of fresh samples of papillary thyroid cancer from children exposed to Chernobyl nuclear accident. In particular, the activation of RET oncogene was found with a frequency higher than that reported in all the studies up to now published (66.6%). This result, together with previous observations of RET rearrangements in a patient exposed to radiation and in cell lines irradiated "in vitro", seem to indicate a possible relation between radiation, RET oncogenic activation and papillary thyroid cancer.

This work was supported by CNR "ACRO Project" and Associazione Italiana Ricerca Cancro.

9:15 A.M. 264. **PROGNOSTIC VALUE OF c-MET EXPRESSION IN PAPILLARY THYROID CARCINOMAS.** A. Belfiore, P. *Gangemi, M.G. Santonocito, G.L. La Rosa, A. Costantino, A. *Fiumara, R. Vigneri. - Cattedra di Endocrinologia, University of Catania and *Servizio di Anatomia Patologica, V. Emanuele Hospital, Catania, Italy.

The HGF/SF receptor, c-Met, is a transmembrane tyrosine kinase receptor which mediates both mitogenic and motogenic effects; c-Met is overexpressed in a proportion of papillary thyroid carcinomas. In the present study we evaluated c-Met expression in 59 archival formalin-fixed paraffin-embedded papillary thyroid carcinomas by immunohistochemistry and correlated it with the clinical outcome. Both the proportion (0-5) and the intensity (0-3) of positive stained cells were scored. A total score, combining the proportion and the intensity score was calculated, ranging from 0 to 8.

Results: c-Met expression was observed in 49 of the 59 papillary carcinomas (83%) but was undetectable in the peritumoral tissue. The 59 carcinomas examined were subdivided in 2 groups: one with a absent/moderate c-Met expression (n=34; total score ≤5) and one with high c-Met expression (n=25; total score >5). At tumor presentation, the two groups were similar for patient age (36.6±16.4 and 33.9±16.2 years, respectively), tumor size (2.4±1.5 vs. 2.5±1.8 cm in diameter), presence of multiple cancer foci, extrathyroidal and lymph-node involvement. However, angioinvasivity was more frequent in carcinomas with absent/moderate c-Met in respect to carcinomas with high c-Met expression (4/34=11.8% vs. 0/25%). Distant metastases were diagnosed at the first total body scan in 2/34 (5.9%) cases with absent/moderate c-Met expression and in none of the group with high c-Met. During the follow-up period, which was similar in the two groups (58.6±28.9 and. 63.2±29 months, respectively), 4 patients were diagnosed lung metastases and 2 both lung and bone metastases. All 6 patients were in the group with a absent/moderate c-Met expression.

Conclusions: a) c-Met is undetectable in normal thyroid tissue but it is expressed at variable levels in malignant thyroid papillary carcinoma cells; b) c-Met expression appears to be a strong independent prognostic factor in papillary thyroid cancer since 8/34 (23.5%) tumors with absent/moderate c-Met developped distant metastases as compared to 0/24 tumors with high c-Met expression.

Supported in part by AIRC (Associazione Italiana per la Ricerca sul Cancro)

9:30 A.M. 265. **RELATIONSHIP OF SPECIFIC MUTATIONS OF THE RET PROTO-ONCOGENE TO DISEASE PHENOTYPE IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 2A.**

H.M. Heshmati[1], H. Gharib[1], H. Abu-Lebdeh[1], S. Khosla[1], N.M. Lindor[2], and S.N. Thibodeau[3], Divisions of Endocrinology/Metabolism[1], Medical Genetics[2], and Laboratory Genetics[3], Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905.

Multiple endocrine neoplasia type 2A (MEN 2A) is a rare autosomal dominant cancer syndrome with incomplete clinical penetrance. It is characterized by medullary thyroid carcinoma (MTC) in more than 95 % of cases, pheochromocytoma (Pheo) in 50 % of cases, and hyperparathyroidism (HPT) in 20 % of cases. RET proto-oncogene germline mutations in exons 10 and 11 on chromosome 10 are present in 95 % of MEN 2A families. The present study evaluates the relationship of specific mutations of the RET proto-oncogene to disease phenotype (presence of MTC, Pheo and HPT) in patients with MEN 2A. We studied 82 patients (43 males, 39 females) from 19 MEN 2A kindreds known to have positive RET mutations. DNA sequence analysis was performed on exons 10 and 11 of the RET proto-oncogene. Biochemical tests included measurement of basal and/or pentagastrin-stimulated plasma calcitonin, urinary free catecholamines and/or metanephrines, and serum calcium and/or parathormone. Statistical evaluation was performed with the chi-square test. RET mutations involved codons 618 (four kindreds, 29 patients) and 634 (15 kindreds, 53 patients). TGC to CGC (cysteine to arginine) mutation on codon 634 was present in nine kindreds (30 patients). Elevated plasma calcitonin levels and/or histologically proved MTC were present in 79 patients (96 %). Pheo was present in 32 patients (39 %) and HPT in 22 patients (27 %). Patients with codon 634 mutations had a higher frequency of Pheo than those with codon 618 mutations (51 % versus 17 %, $P < 0.005$). However, the frequency of HPT was not statistically different between patients with codon 634 and 618 mutations (30 % versus 21 %). With codon 634 mutations, subjects who had the TGC to CGC mutation were more prone to develop Pheo than those with other codon 634 mutations (63 % versus 35 %, $P < 0.05$). In conclusion, specific mutations of the RET proto-oncogene strongly predispose to the development of Pheo in MEN 2A. Patients with codon 634 mutations, and especially the subset TGC to CGC mutation, require careful follow-up to detect Pheo at early stage.

9:45 A.M. 266. MEDULLARY CARCINOMA OF THE THYROID. A STUDY ON PROGNOSTIC FACTORS IN 117 CONSECUTIVE PATIENTS [1] L. Scopsi, G. Sampietro, P. Boracchi [2], M. Gullo, F. Cusumano [3], S. Pilotti, Endocrinology Unit, Division of Pathological Anatomy and [3] Division of Surgical Oncology C, Istituto Nazionale Tumori, Milan, Italy, [2] Istituto di Statistica Medica e Biometria, University of Milan, Milan, Italy

Background. 1) To document the long-term outcome in medullary thyroid carcinoma (MTC) patients and 2) to evaluate the diagnostic and prognostic utility of a number of clinicopathologic features in the management of MTC. **Methods**. A retrospective review was carried out of 117 patients with MTC diagnosed and treated at the Istituto Nazionale Tumori, Milan, Italy, within a 30-year period up to February 1994. The case series included 62 male and 55 female patients, with a median follow-up of 4.5 years (one fourth of the patients had a follow-up >135 months). Of these, eight were of familial type, viz., five MEN2A, one FMTC, and two MEN2B. Statistical univariate analysis of relapse-free survival (RFS) and overall survival (OS) was carried out for 18 clinicopathologic variables, including sex, age at diagnosis, blood group, phenotype, time from first sign to hospitalization, TNM staging, completeness of surgical excision, postoperative calcitoninemia, histologic subtype, tumor size, tumor focality, thyroid capsule, amyloid, C cell hyperplasia, calcitonin (CT), katacalcin (KT) and CT gene-related peptide immunostaining patterns of the primary tumor, and pattern of regional lymph node metastases. Chi-square statistics, Fisher's exact test, and multiple correspondence analysis (MCA) were applied to the set of all possible two-way contingency tables obtained by cross-classification of the sample under study according to the 18 variables mentioned above. **Results**. Risk factors of poor survival (RFS and/or OS) were male sex, age over 60 years, mixed cell subtype, tumor size >4 cm, spread of primary tumor beyond thyroid capsule, lack of amyloid, N1 (particularly N1B) stage, M1 stage, and heterogenous CT and KT immunostaining pattern. In multivariate analysis, only capsule status, M stage, and age at diagnosis were retained in the final model for OS. In that for RFS, capsule status was retained together with gender and amyloid status. After MCA, the variables shown to be predictors of survival were associated with the first two axes. In keeping with the results of multivariate analysis for OS and RFS, patients' characteristics of favorable or unfavorable prognosis were grouped in two different profiles for both axes. **Conclusions**. Patients over 60, with primary tumor extending into neck soft tissues and/or with distant metastases at presentation were at high risk to die precociously of their disease, while male sex and absence of amyloid marked those patients at high risk for recurrences. Extrathyroidal tumor invasion appeared to be the worst prognostic factor and might allow breakdown of patients in two categories, amenable to different therapeutical regimens. In fact, reoperation for neck residual disease seems a useless exercise in pT4 patients, especially if over 60 and males. For T0-T3/M0 patients, especially if young, an aggressive surgical regimen is instead warranted.

[1] Data obtained through the *Gruppo di Studio sul Carcinoma Midollare della Tiroide* of the *Societa` Italiana di Cancerologia*. Financial support has been given by the *Associazione Italiana per la Ricerca sul Cancro*.

9:00 A.M. 267. THE TRANSCRIPTION FACTOR GHF-1, BUT NOT THE SPLICE VARIANT GHF-2, COOPERATES WITH THYROID HORMONE AND RETINOIC ACID RECEPTORS IN RAT GROWTH HORMONE GENE EXPRESSION. P. Peña, A.Sanchez-Pacheco, T. Palomino and A. Aranda. Instituto de Investigaciones Biomédicas. CSIC. Madrid. Spain.

The pituitary-specific transcription factor GHF-1 (or Pit-1), a member of the homeobox POU family of DNA-binding proteins, plays an important role in growth hormone (GH) gene expression. Differential splicing of the GHF-1 primary transcript gives rise to a functionally distinct isoform called GHF-2 in which an additional 26 aminoacids are inserted into the activation domain of the protein. Other factors such as thyroid hormone (T3) and retinoic acid (RA) also regulate GH gene transcription. A hormone response element (TRE) located close to two GHF-1 binding sites in the GH promoter mediates the response to both ligands. The T3 receptor (TR) and the RA receptor (RAR) heterodimerize with the retinoid X receptor (RXR) and bind to the same element.

We have compared the ability of GHF-1 and GHF-2 to stimulate the rat GH promoter and to cooperate with T3 and RA receptors in non-pituitary (Cos-7) and pituitary (GH4C1) cell lines. In transient transfection assays a construct containing the rat GH promoter (-530GH-CAT) was essentially silent in Cos-7 cells but its activity increased significantly after co-tranfection with a vector expressing GHF-1. Expression of TR/RXR or RAR/RXR heterodimers also activated the promoter, even in the absence of GHF-1, and the combination of the transcription factor and the receptors produced a further increase. The receptors enhanced promoter expression in a ligand-independent manner, but a maximal promoter activation was found in the presence of GHF-1 and the ligand-occupied receptors. However, cotransfection with a vector expressing the GHF-2 isoform had little, if any, stimulatory effect on the GH promoter and did not show cooperation with T3 or RA receptors even in the presence of ligands. In pituitary GH4C1 cells, T3 and RA increased GH gene expression, and over-expression of GHF-1 enhanced the response to both ligands. In contrast, transfection with GHF-2 markedly blocked the response to T3 and RA, antagonizing the effect of GHF-1. GHF-1 inhibits binding of receptor heterodimers as well as RXR homodimers to DNA suggesting direct protein to protein interaction between the receptors and the transcription factor. As compared with GHF-1, GHF-2 showed a decreased ability to bind the cognate site in the rat GH promoter but retained the capacity to displace the receptor dimers from the response element.

These results show that alternative splicing of the GHF-1 gene gives rise to two isoforms that differ in their transactivating properties and in their ability to synergize with the nuclear thyroid hormone and retinoic acid receptors on GH gene expression.

9:15 A.M. 268. **DIRECT PROTEIN INTERACTIONS BETWEEN THYROID HORMONE RECEPTOR ISOFORMS AND GENERAL TRANSCRIPTION FACTORS.** Kevin J. Petty, University of Texas Southwestern Medical Center, Dallas, TX, U.S.A.

Thyroid hormone receptors (TR's) influence gene expression through effects on transcription that require the general transcription factors (GTF's). The general transcription factor IIB (TFIIB) binds directly to both TRα1 and TRß. It has been reported that another GTF (TATA binding protein or TBP) did not bind directly to TRα1 but did bind to TRß suggesting that these TR isoforms differ in their mechanisms of GTF interaction and transcriptional regulation. To examine this possibility, direct interactions of the human TRα1, TRß, or c-erbAα2 proteins with the human TFIIB, TBP, or retinoid X receptor α (RXRα) proteins were quantitated. The TR's were synthesized in reticulocyte lysates and labeled with 35S. The TFIIB, TBP, and RXRα were expressed in bacteria with histidine tags and purified. Equimolar amounts of each purified protein were immobilized on a nickel affinity resin and mixed with a constant amount of one of the 35S-labeled TR isoforms in the absence or presence of 0.1 μM T3. The proteins bound to the resin were washed extensively, eluted with SDS, and quantitated by scintillation counting and were also evaluated by SDS-PAGE + autoradiography. The total 35S protein bound to the histidine-tagged protein (in dpm) was divided by the dpm of background binding in a reaction without histidine tagged protein (see table below). A separate reaction included purified, histidine-tagged ß-galactosidase as a negative control. The numbers represent relative stability of binding between the two proteins used in each reaction.

	none	ß-gal	RXRα		TFIIB		TBP	
			-T3	+T3	-T3	+T3	-T3	+T3
TRα1	1.0	0.9	90.9	103.6	4.6	2.5	22.2	15.1
TRß	1.0	1.1	97.3	119.8	11.9	8.0	37.6	22.6
c-erbAα2	1.0	1.2	1.5	1.4	4.3	4.4	21.7	24.1

The relative binding strength of the TR's (α1 and ß) were RXRα>>TBP>TFIIB, and for c-erbAα2 were TBP>TFIIB (no binding to RXRα). TRß bound more stably to TFIIB and TBP than did the other TR isoforms. T3 produced 15-20% increases in the binding of RXRα to both TRα1 and TRß, and decreases of 30-40% in the binding of TRα1 or TRß to either TFIIB or TBP. T3 had no effect on c-erbAα2 interactions. These results indicate that all of the TR isoforms are capable of interacting directly with TFIIB and TBP although TRß binds more readily to these GTF's. The direct binding of c-erbAα2 to these general transcription factors supports a model in which c-erbAα2 could inhibit TR function by competing with TR's for binding to GTF's without binding to DNA.

9:30 A.M. 269. INTERACTION OF NATURAL AND ARTIFICIAL THYROID HORMONE RECEPTOR MUTANTS WITH A POTENTIAL TRANSCRIPTIONAL CO-ACTIVATOR T.N.Collingwood, V.Cavaillès*, O.Rajanayagam, M.Adams, C.Matthews, M.G.Parker * and V.K.K.Chatterjee Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Rd, Cambridge CB2 2QQ, U.K and * Molecular Endocrinology Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.

The ligand-dependent activation function of the thyroid hormone β receptor (TRβ) localises to its hormone-binding domain. Our analyses of natural mutant receptors in thyroid hormone resistance syndrome (RTH), have shown that codon 453 mutations (P453A, P453H, P453S, P453T) impair this function. This residue precedes a putative amphipathic α-helical sequence of nine amino acids at the receptor carboxyterminus. We have now identified a novel mutation (L454V), involving a hydrophobic residue within this motif in an RTH kindred. Three affected individuals showed characteristic biochemical abnormalities (mean FT4 59pmol/L, FT3 16pmol/L, TSH 1.1mU/L) with an absence of symptoms (proband, sister) or thyrotoxic features (daughter). The mutant protein bound ligand comparably to wild type receptor (WT Ka = $1.8x10^{10}M^{-1}$, L454V= $1.3x10^{10}M^{-1}$). However, hormone-dependent transactivation by the mutant receptor was markedly impaired on direct repeat, palindromic or everted repeat thyroid response elements (TREs), although it formed homo and heterodimers on all three TREs. Systematic mutational analyses of this region generated additional artificial mutants of this hydrophobic (L454A) or negatively charged residues (E457A, E457D) with similar properties. Both the natural and artificial mutants were powerful dominant negative inhibitors of wild type TRβ action. Studies with the mouse estrogen receptor (mER), have identified three proteins of 160, 140 and 80kDa (RIP160, RIP140 and RIP80) in mammalian cells, which interact with ER in a hormone-dependent manner. We therefore examined the interaction of RIP140 with wild type and mutant TRs. Immobilised fusion proteins of wild type or mutant receptor hormone binding domains linked to glutathione-S-transferase (GST-TR) were incubated with ^{35S}methionine-labelled RIP140 in the absence or presence of ligand. Wild type GST-TR exhibited negligible specific interaction in the absence of hormone but a marked increase in binding with 1μM T3, comparable to estradiol-dependent binding of RIP 140 to GST-ER. In comparison, the mutants receptors showed negligible (L454A,L454V) or very slight (E457D, E457A) increases in hormone-dependent binding to RIP140. Our data indicate the importance of hydrophobic as well as charged residues within the amphipathic helix for both hormone-dependent transactivation and protein interactions. The potent dominant negative activity of these mutants also suggests that they are unlikely to inhibit wild type receptor action by titrating this putative co-activator.

9:45 A.M. 270. STUDIES OF THE FUNCTIONS OF DIFFERENT MUTANT T3 RECEPTORS WITH TRUNCATION OF THE EXTREME C-TERMINAL ACTIVATION DOMAIN. H.Nakamura, Y.Miyoshi*, K.Nishiyama, K.Komatsu, K.Nakao*. Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; Kyoto University School of Medicine*, Sakyoku, Kyoto, Japan.

Recently the extreme C-terminal activation domain, Tau4 or AP2, has been elucidated in T3 receptor(TR). We studied the functions of three mutant(m) TRβ1s with different truncation in this domain found in patients with generalized resistance to thyroid hormone. In a 6-y.o. Japanese girl, two consecutive base substitutions in her TRβ1 gene changed the 451st codon(TTC) to stop codon(TAA), resulting in 11-amino acid(aa) deletion(F451X). This girl had severe mental retardation, delayed speech development, attention deficit hyperactivity disorder (ADHD) and SITSH. Another mTR with 13-aa deletion (E449X) was found in a Japanese 16-y.o. boy who showed SITSH and low TBG, but no any clinical signs and symptoms except for goiter. The third truncated TR, C446X with 16-aa deletion, was found by Dr.Dorin in a 31-y.o. American male (Mol Cell Endocrinol 99:81,1994), who shows severe mental retardation, ADHD, deafness, and short stature.

The functions of these three truncated TRs expressed in CV1 cells were studied. In every mTR, T3 binding activity was very low and transcriptional activity on $TREpal_2$- and DR4-CAT reporter genes was negligible even with 1μM T3. Each mTR exhibited not only very strong dominant negative activity against wild TRβ1, but also remarkable silencing activity. Interestingly, the dominant negative and silencing activity were the strongest in F451X. The mTRs suppressed the retinoic acid receptor and vitamin D3 receptor functions, and F451X again exhibited the strongest inhibition. A gel shift study revealed no apparent difference among mutant and wild TRs. We also constructed a mutant TRα1(αF397X) which has the same C-terminal deletion as F451X. When compared with F451X, αF397X showed remarkable, but less prominent, dominant negative activity. In summary, among three mTRs with deletion in the Tau4 domain, F451X with 11-aa deletion showed the strongest dominant negative and silencing activity. The inhibitory activity was more prominent in TRβ1 than in TRα1, which have the same truncation. Although the functional properties of E449X and C447X were indistinguishable, clinical features of each patient was quite different. The CNS involvement might not necessarily correlate with severity of mTR's dominant negative activity.

10:30 A.M.
271.

A LONGITUDINAL STUDY OF THYROID DISORDERS IN THE COMMUNITY. M.P.J.Vanderpump, W.M.G.Tunbridge, J.M.French, D.Appleton, F.Clark, J.Grimley Evans and E.T.Young. Department of Medicine, Newcastle General Hospital, Newcastle upon Tyne, UK.

The original Whickham survey documented the prevalence of thyroid disorders in a randomly selected sample of 2779 adults which matched the population of Great Britain in age, sex and social class. In a twenty-year follow-up study outcomes in terms of morbidity and mortality were determined for over 97% of the original sample in order to determine the incidence and natural history of thyroid disease in this cohort. Of the 1877 known survivors, 96% participated in the follow-up study. The mean incidence (with 95% confidence intervals) of spontaneous hypothyroidism in women was 3.5/1000 survivors/year (2.8 - 4.5) rising to 4.1/1000 survivors/year (3.3 - 5.0) for all causes of hypothyroidism and in men was 0.6/1000 survivors/year (0.3 - 1.2). The mean incidence of hyperthyroidism in women was 0.8/1000 survivors/year (0.5 - 1.4) and was negligible in men. Similar incidence rates were calculated for the deceased subjects. The hazard rate of hypothyroidism and hyperthyroidism showed an increase with age in hypothyroidism but no age relationship in hyperthyroidism. The risk of having developed hypothyroidism at follow-up was examined with respect to risk factors identified at first survey. A positive family history of any form of thyroid disease, the presence of a goitre at either survey, fasting cholesterol and triglyceride levels at first survey, or parity in women at first survey were not associated with an increased risk of hypothyroidism. The odds ratios (with 95% confidence intervals) of developing hypothyroidism with a) raised serum thyrotropin (TSH) alone were 8 (3 - 20) for women and 44 (19 - 104) for men; b) positive thyroid antibodies alone were 8 (5 - 15) for women and 25 (10 - 63) for men; c) both raised serum TSH and thyroid antibodies were 38 (22 - 65) for women and 173 (81 - 370) for men. A logit model indicated that increasing values of serum TSH above 2mU/L at first survey increased the probability of developing hypothyroidism which was further increased in the presence of thyroid antibodies. This historical cohort study has provided incidence data for thyroid disorders over a twenty-year period for a representative cross-sectional sample of the population, and has allowed the determination of the importance for thyroid disease of prognostic risk factors identified twenty years earlier.

10:45 A.M.
272.

IODINE SUPPLEMENTATION MUST BE MONITORED AT THE POPULATION LEVEL IN IODINE DEFICIENT AREAS. A.M. Ermans[1], D. Gullo[2], S.G. Mugisho[3], M. Tshibangu[3], R. Tonglet[4], P. Iurato[2], M.W. Mukalay[3], E. Mahangaiko[3], P. Bourdoux[1], ULB-Cemubac Brussels[1], University of Catania[2], Kirotshe Zaire[3], UCL Woluwe[4].

ICCIDD, UNICEF and WHO have promoted the use of iodized salt as the universal prophylaxis intervention for IDD. To achieve this goal they recommend at least a 100 ppm iodination level in tropical countries. Salt with such iodination level was sold for about two years in a goitrous district of Kivu (Zaire) where recent data indicate a subsequent increase in daily iodine intake ranging from 200 to 500 µg iodine/day.

The aim of our study was to evaluate the effects of this high iodine intake in goitrous subjects living in that area. Adult subjects (n=190) with visible multinodular goiter were investigated for the size of their goiter (echography), the level of circulating thyroid hormones and TSH and the concentration of urinary iodine.

Median urinary iodine concentration in our study population was 24 µg/dL. Serum undetectable TSH, the classical marker of hyperthyroidism, was noted in 14.2 % of the 190 subjects studied. Fourteen subjects (7.4 %) had also clear-cut elevated serum T4 (mean 24.1 µg/dL) and T3 (mean 472 ng/dL) typical of severe thyrotoxicosis. Their urinary iodine concentration was not different from the one of the whole population. TSI was negative in all of them. Their mean age was 35 years (15 to 50) with a male to female ratio of 0.22 and all of them had a goiter larger than 60 mL. Most of them complained of fatigue, palpitation, diarrhoea, had tachycardia and weight loss but none had exophthalmos. During a one year follow-up, markedly elevated values of serum T4 and T3 failed to return within the normal range. Because of the exacerbation of clinical symptoms two patients had to be treated with antithyroid drugs.

The induction of thyrotoxicosis in about 10 % of the sample population we have studied and the observation that the disease is not self limiting in the presence of a high iodine supply are mandatory reasons for reducing the iodination level of salt in Kivu. Contrary to the common opinion, our data demonstrate that iodine induced thyrotoxicosis is not rare, not transient and not restricted to old subjects. It is also concluded that monitoring of iodine supplementation in iodine deficient areas has to be conducted at the population level and not on the basis of worldwide criteria.

11:00 A.M. 273. HIGH FREQUENCY OF GOITRE AND SUBCLINICAL HYPERTHYROIDISM IN A LOW IODINE INTAKE AREA VERSUS HIGH FREQUENCY OF SUBCLINICAL HYPOTHYROIDISM IN A HIGH IODINE INTAKE AREA. A COMPARATIVE EPIDEMIOLOGICAL STUDY IN ELDERLY SUBJECTS.

P. Laurberg, A.B. Hreidarsson, K.M. Pedersen, N. Sigfusson, E. Iversen. Dept. of Endocrinology, Aalborg, Denmark, Dept. of Medicine Landspitalinn and The Heart Preventive Clinic, Reykjavik, Iceland.

Subclinical hypo- end hyperthyroidism are common among elderly subjects. The importance of the iodine intake level of the area for the prevalence of these abnormalities in elderly subjects was evaluated. Population samples of 68 years old subjects were examined in East Jutland (EaJu) Denmark (N=423, M/F 185/238) and Iceland (Ice) (N=100, M/F 46/54) where previous studies have demonstrated long-standing relatively low (~ 50 μg/day) and high (~ 300 μg/day) iodine intake. Despite the absence of significant goitre in young subjects in the low iodine intake area, goitre was common in elderly subjects, especially in elderly females (F/M:12.2/2.7% had been operated for goitre or had easily visible goitre). This was much less common in the high iodine intake area (F/M: 1.9/2.2%), ($P<0.05$ in females, NS in males). In the low iodine intake area many subjects had sTSH below the reference range (sTSH <0.4 mU/l: F/M: 10.9/5.9%, sTSH <0.1 mU/l: F/M: 3.8/1.6%, with elevated sT_4 and/or T_3 (marginal in majority) in F/M: 3.8/1.1%. (excluding subjects receiving T_4)). No cases of low sTSH were observed in the high iodine intake area ($P<0.001$). In contrast elevated sTSH was much more common in the high iodine intake area (sTSH >5.0 mU/l in F/M: 20.4/6.5%, versus F/M 3.4/1.1% in the low iodine intake area ($P<0.001$), sTSH >10 mU/l F/M: 5.6/2.2 versus 1.3/0.5%). None of the subjects with elevated sTSH had subnormal sT_4. The majority of subjects with elevated sTSH had detectable TPO-Ab and/or TG-Ab. However despite the much higher frequency of high sTSH in Iceland, the prevalence of circulating thyroid Ab using very sensitive methods was higher in East Jutland (EaJu: Tg-Ab >100 U/l F/M 41.3/17.8%, >10,000 U/l 17.5/6.9%, Ice: Tg-Ab >100 U/l F/M 14.8/8.7%, Tg-Ab >10,000 U/l 5.5/2.2%). Similar differences were found in the prevalence of TPO-Ab: e.g. in EaJu TPO-Ab >10 U/l were found in 18% of the population, but only in 9% in Ice ($P<0.05$).

Conclusion: In an area with relatively low iodine intake goitre and subclinical or borderline hyperthyroidism was very common in elderly subjects. This was not found in a high iodine intake area. However in this area subclinical hypothyroidism was much more common. Many subjects with elevated sTSH had thyroid antibodies in serum, but the overall prevalence of thyroid antibodies was higher in the low iodine intake area. This discrepant pattern could be secondary to iodine deficiency induced thyroid abnormalities. The importance of the observed aberations for public health remains to be evaluated.

11:15 A.M. 274. RARITY OF ONCOGENIC MUTATIONS IN THE TSH RECEPTOR OF AUTONOMOUSLY FUNCTIONING THYROID NODULES IN JAPAN

Y.Nagayama, A.Takeshita, N.Ishikawa,T.Yamashita and T.Obara, Nagasaki University School of Medicine, Nagasaski; Ito Hospital, Tokyo; Tokyo Women's Medical College, Tokyo, Japan

Recenty, somatic mutations have been identified in the transmembrane regions and cytoplasmic loops of the TSH receptor (TSHR) in hyperfunctioning thyroid adenomas and familial hyperthyroidism. These mutant receptors constitutively stimulate adenylyl cyclase and cAMP accumulation, leading to thyroid tumor formation and hyperthyroidism. In the present study, we evaluated the frequency of constitutively activating mutations of the TSHR in a large series of autonomously functioning thyroid nodules (AFTNs) in Japan. Forty five AFTNs (38 solitary hyperfunctioning thyroid adenomas and 7 toxic multinodular goiters) were analyzed. Genomic DNA was extracted from paraffin-embedded tissue sections, from which DNA fragments encoding the "hot spot" for the constitutively activating TSHR mutations (the third cytoplasmic loop and sixth transmembrane segment) were amplified by PCR and analyzed by SSCP. Only one sample from a hyperfunctioning adenoma displayed a migration abnormality. Sequence analysis of this DNA fragment demonstrated an unusual mutation of alternate three base deletions at nucleotides 1953 to 1957 (AAA GAT ACC to AAG TCC), resulting in one amino acid deletion (Asp at 619) and one conservative amino acid substitution (Thr to Ser at 620). Normal nucleotide sequence of this region in a non-tumor tissue was confirmed in genomic DNA of peripheral leukocytes in this case, suggesting these nucleotide alterations are a somatic mutation. Functional property of this mutation (TSHR △619) was then evaluated with in vitro mutagenesis and transfection studies. Unexpectedly, the functional characteristics of TSHR △619 were essentially identical to those of the wt-TSHR; there was no differences in TSH binding affinities and basal and TSH-stimulated levels of cAMP and IP3 between TSHR △619 and the wt-TSHR. Finally, normal nucleotide sequence in this region was confirmed in randomly selected 10 samples with a normal migrating pattern in SSCP.

In conclusion, previously identified constitutively activating oncogenic mutations in the TSHR do not appear to be present in AFTNs at high frequency in Japan. The oncogenic potential of a novel somatic mutation TSHR△619 is at present uncertain because of its normal function.

11:30 A.M.
275.

MULTIPLE CHANGES IN THYROID STATUS IN PATIENTS TREATED WITH RECOMBINANT INTERFERON-α (rIFN-α). R. Minelli, T. Giuberti, C. Schianchi, S. Marchelli, E. Gardini, M. Salvi, L. Braverman, and E. Roti. Thyroid and Infectious Disease Units, Univ. Parma, Parma, Italy and the Endocrine Division, Univ. Mass. Med. Sch., Worcester, MA.

Treatment with interferons has been reported to induce both hypo- and hyperthyroidism. We have prospectively evaluated 32 patients (pt) with HCV chronic active hepatitis treated with rIFN-α. Mean age was 39.1±2.1 (mean±SE) yr (26 men, 6 women). Before treatment, pt were evaluated clinically; serum FT4, FT3, TSH, TPOAb, and TRAb were measured; and an I-C104 discharge test was carried out. After baseline studies were completed, rIFN-α therapy (14.3±1.0 MU/wk) was administered for 6.5±0.4 mo. Clinical evaluation and thyroid function tests were carried out every mo during rIFN-α therapy. At the 6th mo of treatment or sooner if rIFN-α was discontinued, the I-C104 test was repeated. Before treatment, all pt had normal serum TSH (0.7±0.1 m U/L), FT4 (14.7±0.5 pmol/L) and FT3 (4.6±0.1 pmol/L) concentrations;all had negative I-C104 tests and serum TRAb levels; and 3 pt had positive TPOAb. Eleven pt developed changes in thyroid function and/or developed positive thyroid Ab during rIFN-α therapy and 21 pt did not. TPOAb values markedly increased in the 3 pt with positive TPOAb before rIFN-α therapy and 2 other patients developed TPOAb during therapy. Two different pt became thyrotoxic with low thyroid RAIU and negative TRAb, suggesting thyroiditis; 2 pt developed subclinical hyperthyroidism (suppressed TSH and normal FT4 and FT3) with an elevated thyroid RAIU and negative TRAb; and 1 pt became hypothyroid with positive TPOAb. During rIFN-α therapy, seven of the 11 pt developed a positive I-C104 discharge test, indicating impaired thyroidal organification of iodide. Two of these pt were retested after rIFN-α was discontinued and the I-C104 test reverted to normal. Three additional pt treated with rIFN-α were evaluated only when thyroid dysfunction appeared. All three were euthyroid and one had positive TPOAb before treatment. Two pt were thyrotoxic with a low RAIU and one was hypothyroid at the time of our evaluation during rIFN-α therapy. Conclusion: These results suggest that rIFN-α treatment may induce various thyroid disorders including hypothyroidism, hyperthyroidism, and thyrotoxicosis probably secondary to destructive thyroiditis. Positive TPOAb did not predict the development of thyroid dysfunction during rINF-α therapy. Finally, we have observed for the first time that rINF-α treatment may induce a positive I-C104 test *in vivo* indicating impaired intrathyroid organification of iodide, consistent with recent *in vitro* studies demonstrating that rIFN-α impairs iodide organification in human thyroid follicles (Yamazoki et al. JCEM 77:1439, 1993).

11:45 A.M.
276.

THYROXINE ADMINISTRATION TO INFANTS OF LESS THAN 30 WEEKS GESTATIONAL AGE DECREASES PLASMA TRIIODOTHYRONINE CONCENTRATIONS; RESULTS OF A RANDOMIZED TRIAL. AG van Wassenaer, JH Kok, T Vulsma. Academic Medical Center, Amsterdam, The Netherlands.

Introduction Transient hypothyroxinemia is commonly found in the first 4-8 postnatal weeks in (very) preterm infants. Low T4 levels found in preterm neonates have been related to impaired developmental outcome at 2 years. However, until now it is not clear whether thyroxine supplementation is required. We therefore carried out a randomized, placebo-controlled trial of T4 administration in infants of less than 30 weeks gestational age, aiming to study the influence of T4 administration on the developmental outcome at 2 years. In an earlier trial report, no effect of T4 administration was found on mortality and morbidity. The effects of T4 administration on thyroid hormone metabolism is here presented.

Patients and Methods 200 infants entered the study: 100 in the T4 treatment group (T-group) and 100 in the placebo group (P-group). T4 (8 μg/kg birthweight) or placebo were administered daily during the first 6 postnatal weeks, starting between 12 and 24 hours after birth. On days 1 and 3, weekly during the treatment period, and 2 weeks after stopping T4/placebo, 1 ml blood was drawn. T4, FT4, T3, rT3, TSH and TBG were measured.

Results Plasma T4 and FT4 were significantly increased in the T-group during the whole treatment period; after stopping trial medication, T4 and FT4 levels became similar in both study groups. TSH secretion was depressed in the T-group. T3 levels were decreased in the T-group from day 14 on, until the end of the treatment period. rT3 levels were significantly higher in the T-group.

Discussion This is the first randomized study, examining the effect of T4 administration on thyroid hormone metabolism in very preterm infants with transient hypothyroxinemia. T4 administration carried out as in this trial is effective in treating hypothyroxinemia. It has no permanent effect on thyroid function. It decreases plasma T3 levels, without influencing infant mortality and morbidity. The T3 decrease can be explained by decreased T3 release from the thyroid caused by the depressed TSH. Also, the increased rT3 levels could have decreased type 2 deiodinase. This study confirms the limited role hepatic type 1 deiodinase plays in sustaining plasma T3 levels in these very preterm infants.

10:30 A.M. 277. HETERODIMERIZATION OF THYROID HORMONE RECEPTOR α1 AND ITS VARIANT ERBAα2 WITH RETINOID X RECEPTOR. Y.-Z. Yang and R.J. Koenig, Division of Endocrinology, University of Michigan Medical Center, Ann Arbor, MI 48109-0678.

The gene *ErbAα* encodes two proteins, thyroid hormone receptor α1 (TRα1) and erbAα2, that are identical for their first 370 amino acids but then diverge due to alternative splicing. While TRα1 is a bona fide TR, erbAα2 is unable to bind T3 and functions as a repressor of T3 action. TRα1 forms heterodimers with retinoid X receptors (RXRs) on various T3 response elements (TREs). Since the TR heterodimerization domain known as the 9th heptad spans the alternative splice site, it is partially missing in erbAα2. Therefore it is not surprising that erbAα2 fails to heterodimerize with RXR on TREs arranged as palindromes (Pal) or inverted Pals (IP). However, the observation that erbAα2-RXR heterodimers form on direct repeat TREs (DR) (Nagaya & Jameson, JBC 268:24278) was unexpected and raised questions about the role of the 9th heptad in heterodimerization. We made 2 mutant erbAα2 proteins to address this question. The full 9th heptad was restored in erbAα2 by inserting the missing portion (TRα1 amino acids 371-6) after amino acid 370, creating erbAα2+9H. As a control, six alanines were inserted at the same position of erbAα2 to make erbAα2+ala. A mutant TRα1 also was made, TRα1-9H, in which the distal portion of the 9th heptad (aa 371-6) was replaced by aa 371-6 of erbAα2. Proteins were translated in reticulocyte lysate. Electrophoretic mobility shift assays were used to test the heterodimerization of each of these proteins with RXR on DR, Pal and IP TREs. The ability to heterodimerize is summarized as follows

	TRα1	TRα1-9H	erbAα2	erbAα2+9H	erbAα2+ala
DR	++++	++++	+++	+++	+++
Pal	++++	+	0	+++	1/2+
IP	++++	+++	0	+++	+++

Conclusions: 1) The full 9th heptad is unnecessary for heterodimerization on a DR TRE; 2) The specific 9th heptad amino acid sequence is critical for heterodimerization on Pal; 3) the 9th heptad is important for heterodimerization on IP, but it primarily serves as a non-specific spacer to prevent C-terminal sequences in erbAα2 from impairing heterodimerization. Therefore, the dimerization interface seems to be different on each orientation of TRE (DR, Pal, IP). The inhibitory domain that is relieved in erbAα2+ala is currently under study, and could be the phosphorylation site identified by Katz & Lazar (Endo Soc '94). Also under study are the effects of these mutations on gene expression.

10:45 A.M. 278. **AMINO ACID CHARGE OF THE NINTH HEPTAD REPEAT OF THE THYROID HORMONE RECEPTOR DICTATES HOMO AND HETERODIMERIZATION ON A NUMBER OF THYROID HORMONE RESPONSE ELEMENTS.** T. Monden, J.D. Safer, A.N. Hollenberg, and F.E. Wondisford. Thyroid Unit, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston MA.

Artificial or natural mutations of the C-terminal end of the ninth heptad, amino acids 428 (L428R) and 429 (R429Q) of the human thyroid hormone receptor (TR) β-1, respectively, selectively disrupts hetero- or homodimerizationon on a number of thyroid hormone response elements. To explore further whether charge near the ninth heptad of TR determines the nature of TR complexes bound to DNA, we mutated individually or collectively codons 428 and 429 of TR β-1 and tested the DNA-binding of these mutants, in the absence or presence of retinoid X receptor (RXR), to a direct repeat with 4 bp spacing (DR+4), a palindromic element (Pal) or a negative thyroid hormone response element from the human TRH gene. We have previously reported that the double mutant, LR428-429RQ regains its ability to homodimerization versus the single mutant R429Q, suggesting that a negative charge at codon 429 and not the amino acid change itself is responsible for this defect. In contrast, when codon 428 is changed to Q as a single mutant (L428Q) or double mutant with R429Q (LR428-429QQ), homodimerization is still impaired but heterodimerization was not affected or enhanced on DR+4 and Pal DNA probes. Unlike the L428R mutation, the L428Q mutant had no defect in heterodimerization on the Pal element. Again this illustrates the importance of a specific amino acid change, and not the potential disruption of the heptad, as determining the heterodimerization defect. Interestingly, any of these mutations caused heterodimerization to be lost on a negative thyroid hormone response element present in the proximal human TRH promoter. These data indicate that a net negative charge at the C-terminal end of the ninth heptad impairs homodimerization of TR and favors heterodimerization with RXR. Selective properties of TR on certain thyroid hormone response elements may be reduced or enhanced by modifying the charge near the ninth heptad of the receptor.

11:00 A.M. 279. **THE FUNCTION OF RETINOID X RECEPTOR (RXR) ON A NEGATIVE T3 RESPONSE ELEMENT (nTRE).**

T.Takeda and T.Nagasawa Thyroid Study Unit, Department of Medicine, The University of Chicago, Chicago, IL 60637 U.S.A.

Retinoid X receptors (RXR) form heterodimers with thyroid hormone receptor (TR). Heterodimerization plays an important role in TR-mediated transcription on positive T3 response elements (pTRE). However the function of RXR on a negative TRE (nTRE) is unclear. We analyzed the role of RXR in TR-mediated transcriptional inhibition of a nTRE. Transient cotransfection studies were performed in JEG3 cells using the rat TSHα promoter gene (containing nTRE) fused to luciferase gene as a reporter. JEG3 cells were grown in 24-well plates and transfected using 250 ng of reporter gene, 500 ng of β-galactosidase gene, and different amounts of TRβ1 wild type (WT) expression vector. We examined TRβ1WT expression vector dose-dependent inhibition of luciferase activity, at 1 nM T3 to determine the ideal condition for the experiments. The maximal and half-maximal inhibition were found at 6 ng/well (168 ng/ 10 cm dish) and 0.4 ng/well (11.2 ng/ 10 cm dish) of TRβ1WT vector, respectively. T3 dose dependent inhibition was studied using 0.4, 0.8, and 3.2 ng TRβ1WT vector per well. Appropriate conditions to study TRβ1-T3 inhibition of TSHα transactivation were found using 0.8 ng TRβ1 and 0.1 nM T3, which is in a linear portion of the dose-response curve. Different amounts of RXRγ expression vectors were transfected with or without TR in the presence or absence of 0.1 nM T3 and/or 100 nM 9-cis retinoic acid (9cisRA). As the amount of transfected RXRγ vector was increased, T3 inhibition was blocked in the presence of 9cisRA. Transfection studies were also performed using mutant TRs, Mf (G345R) and GH (R316H). Mf has a non-detectable T3 binding and a strong dominant negative effect, while GH has a weak dominant negative effect although it also has non-detectable T3 binding. The T3 inhibition of TSHα expression was blocked more by 9cisRA-activated-RXRγ using Mf and GH than by TRβ1WT. These results suggest that 9cisRA-activated-RXR contributes to transcriptional regulation of a nTRE and may also influence the transcriptional response to mutant receptors.

11:15 A.M. 280. ROLE OF RXRα ON THE STIMULATION OF THE RAT UNCOUPLING PROTEIN GENE BY T_3. R. Rabelo, C. Reyes, A. Schifman, J. E. Silva. Division of Endocrinology, Jewish General Hospital, Lady Davis Institute, McGill University, Montreal, Quebec, Canada.

We have previously localized two T_3-response elements (TREs) 27 bp apart from each other within a critical enhancer high upstream in the rat uncoupling protein (UCP) gene. The upstream TRE (upTRE) is an everted repeat (ACCCCTactgAGGCAA) and the downstream TRE (dnTRE) a DR-3 (AGGGCAgcaAGGTCA). Both TREs are necessary for full T_3 responsiveness and contributed equally to the T_3 effect in transfection assays. In electrophoretic mobility shift assays (EMSA), beta1 T_3 receptors (β_1T_3R) homodimers bind to both TREs but the addition of T_3 to the assay virtually abolishes this binding. These findings suggest that the transactivation by T_3 depends on the binding of heterodimers to the UCP TREs, most likely T_3R-RXR. The goal of these studies was to define the role of RXR heterodimers in the transactivation of UCP gene by T_3. Studies included transient transfection assays, EMSA and footprinting (methylation protection). In the hibernoma cell line HIB-1B (BAT-derived) transiently co-transfected with β_1T_3R, RXRα and a reporter gene containing both TREs, 9-cis-retinoic acid (9-cis-RA) stimulated reporter expression albeit to a less extent than T_3 and the effect of both ligands together was at least additive. The binding to both TREs of β_1T_3R-RXRα heterodimers was stronger than that of β_1T_3R homodimers, and the addition of T_3 to the binding mixture increased the intensity of the heterodimeric bands. Footprinting analysis demonstrated that β_1T_3R-RXRα heterodimers protected both TREs from methylation as previously shown for β_1T_3R homodimers but with some distinct differences. For the upTRE, the first guanine (G) of the 5' half-site was not protected by β_1T_3R homodimeric binding while the fourth G of the same motif was not protected by the binding of β_1T_3R-RXRα heterodimers. For the dnTRE the Gs protected by both types of dimers were the same, but a G located 7 bp upstream of the 5' half-site and the third G of this motif became hypersensitive to methylation, suggesting a conformational change induced by the heterodimer. Mutation and/or deletion of the 3' half-sites of both up- and dnTRE abolished the binding of β_1T_3R homodimers and T_3 transactivation but seemed not to affect the binding of β_1T_3R-RXRα heterodimer. Furthermore, the 5' half-sites of these mutated TREs showed the similar footprinting pattern as that induced by the heterodimer on the intact TRE. In conclusion: 1) β_1T_3R forms heterodimers with RXRα on both UCP TREs; 2) the formation of heterodimers is enhanced by T_3; 3) both 9-cis-RA and T_3 stimulate transactivation mediated by heterodimers; 4) mutation of lower half site of the TREs abolishes responsiveness to T_3 and the formation of homodimers, but not of heterodimers; 5) heterodimers appear to bind with polarity to the TREs, RXRα firmly anchoring the dimer to the 5' half-site but the contact of β_1T_3R with the 3' half-site is essential for transactivation.

11:30 A.M. 281. MOLECULAR BASIS OF DIFFERENTIAL INTERACTIONS OF HUMAN THYROID HORMONE RECEPTOR ISOFORMS WITH HORMONE RESPONSE ELEMENTS. X-G Zhu, P. McPhie+, S-y Cheng, Laboratory of Molcular Biology, NCI and +Laboratory of Biochemical Pharmacology, NIDDK, National Institutes of Health, Bethesda, MD, U.S.A. 20892-4255

Recent studies have indicated that most of the thyroid hormone effects are mediated by the interaction of thyroid hormone nuclear receptors (TRs) with the specific DNA sequences, known as thyroid hormone response elements (TREs), in the promoter region of the target genes. Four functional domains, A/B, C, D and E can be assigned to each of the two TR isoforms, α1 and β1. These two TR isoforms are highly homologous except in the amino terminal A/B domain. It is known that they have very similar binding affinity to the thyroid hormone, 3, 3',5-triiodo-L-thyronine (T3) and have similar specificity for T3 analogs. However, it is less well understood whether they interact with differential activity with the three types of TREs in which the half-site binding motifs are arranged in an everted repeat (F2), inverted repeat (Pal), or direct repeat separated by 4 gaps (DR4). In the present study, we analyzed the binding of human TRα1 (h-TRα1) and human TRβ1 (h-TRβ1) to the three TREs by electrophoretic gel mobility shift assay. We found that h-TRβ1 bound to the three TREs as a homodimer with apparent Kd of 0.9, 4.1 and 5.7 nM for F2, DR4 and Pal, respectively. In the presence of 10 nM T3, h-TRβ1 bound to all three TREs as a monomer with a similar apparent Kd of ~1 μM. In contrast, h-TRα1 bound to the three TREs both as a homodimer and a monomer. Apparent Kds for dimeric binding are 1.2, 4.8 and 7.2 nM, for F2, DR4 and Pal, respectively, and for monomeric binding is ~3 nM for the three TREs. These results indicate that h-TRα1 and h-TRβ1 bound to TREs with differential activity. Furthermore, these data also show the extent of positive cooperativity in dimeric binding to TREs was h-TRβ1>>h-TRα1. Thus, the stronger positive cooperativity in the dimeric binding of unliganded h-TRβ1 precluded its binding to TREs as a monomer. F2-bound h-TRβ1 and h-TRα1 were dissociated by T3 in a concentration-dependent manner with a similar ED_{50} of 4 -5 nM, suggesting that the different extents of positive cooperativity in the homodimeric binding in the unliganded state was independent of the hormone binding domain. Furthermore, the different TRE binding activity of the two isoforms was not due to the differences in their A/B domain as deletion of A/B domain from h-TRβ1 molecule did not affect its binding to three TREs. This TRE-dependent differential activity of the TR isoforms may serve as one of the important regulatory mechanisms for achieving diveristy and specificty of pleitropic T3 effects.

11:45 A.M. 282. VITAMIN D RECEPTORS (VDRs) HAVE DOMINANT NEGATIVE ACTIVITY ON T3-RECEPTOR (TR) TRANSCRIPTIONAL ACTIVITY. P.M. Yen, Y. Liu, W.W. Chin, Div. of Genetics, Dept. of Med., Brigham & Women's Hosp; Howard Hughes Med. Inst. & Harvard Medical School; Boston, MA.

Vitamin D receptors transactivate via hormone response elements (HREs) containing half-sites arranged as direct repeats separated by three nucleotides. Recently, Schrader et al. (Nature 370:382-386 (1994)) demonstrated that VDR and TR can form dimers on the calbindin HRE, and that both vitamin D and T_3 can co-regulate transcription via this element. In order to understand further potential VDR and TR interactions, we have examined VDR, TR, and retinoid X receptor (RXR) binding to two thyroid hormone response elements (TREs), DR4 and F2, and a retinoic acid response element, DR5. VDR/RXR bound well to DR4 and DR5 (with half-sites arranged as direct repeats separated by four and five nucleotides, respectively) using the electrophoretic mobility shift assay. Surprisingly, VDR/RXR also bound well to F2, the chick lysozyme TRE (with half sites arranged as an inverted palindrome). Addition of vitamin D did not alter VDR/RXR heterodimer binding to these elements. Moreover, no VDR homodimers or VDR/TR dimers were observed on these HREs. In co-transfection experiments using CV-1 cells, we observed that VDR did not repress basal transcription in the absence of ligand or stimulate transcription in the presence of 10^{-6}M vitamin D when using reporter plasmids containing these HREs. Interestingly, VDR had dominant negative activity as it blocked T_3-mediated transcriptional activity by both TRα and TRβ on these elements. These results demonstrate that VDR/RXR heterodimers can bind promiscuously to a wide range of HREs, including inverted palindromes. Additionally, they suggest a novel repressor function by VDR on T_3-mediated transcription which may be significant in tissues where VDR and TR are co-expressed (e.g, bone).

283. P1 ALPHA-INTERFERON(IFN) THERAPY AND AUTOIMMUNE THYROID DISEASE

K. Tsuboi, H. Oshima, R. Yuasa, T. Nagayama, F. Ihara, S. Otsuka and Y. Miyachi
Toho University School of Medicine, Tokyo

The mechanism of alpha-interferon(αIFN) induced thyroid dysfunction has not been fully elucidated. In our experiment, the pattern of thyroid abnormality which developed after αINF therapy for chronic active hepatitis type C was similar to that of silent thyroiditis; thyrotoxicosis followed by transient hypothyroidism, positive thyroid autoantibodies. To confirm this, we screened thyroid state before αIFN therapy and followed during and after treatment.

METHOD: 101 patients(50 males) with chronic hepatitis type C who admitted for the liver biopsy and who have not been diagnosed any thyroid disease before, were administered. Patients were examined the thyroid with palpation and ultrasonography(US). Thyroid function was estimated with serum TSH(sensitive assay), freeT4 and freeT3. The autoantibodies(Ab) to thyroid microsomal antigen(TPO) and Ab to thyroglobulin(Tg) were evaluated with haemagglutination method(MCHA and TGHA) and with direct RIA method(TPOAb and TgAb). All patients are living in iodine sufficient area and having normal iodine diet. When patients had histologically comfirmed and eligible for the therapy, we used n-IFNα, r-IFNα-2a or r-IFNα-2b for 24 weeks.

RESULTS: 38 males (76.0 %) and 40 females (78.4 %) had palpable goiter, while normal Japanese did not. There was no correlation between age and incidence of goiter. Sixty-five % of goiter was small but elastic hard. Seven patients (2 males) were hypothyroidism (TSH>5μU/ml) before treatment. 6.8% of patients had TGHA, 19.4% had MCHA, 57.9% had TgAb, 52.6% had TPOAb. 71.9% of patients has TPOAb and/or TgAb. On follow-up study, 22 patients developed transient thyroid dysfunction (prevalence was 30% with n-IFNα , 58% with r-IFNα-2a, 35% with r-IFNα-2b) at the end of the treatment or later. Of the patients who developed transient thyroid dysfunction, only three were negative both TPOAb and TgAb. The parameter for liver function and liver biopsy performed 6 month after completion of IFN therapy, revealed that patients who have favorable result of hepatitis have developed transient thyroid dysfunction, while patients who have no change or worse did not.

CONCLUSION: Prevalence of goiter and thyroid autoantibodies were extreamly high in patients with chronic hepatitis type C. We have found concealed Hashimoto disease in patients with chronic hepatitis type C. Thyroid dysfunction which developed during or after αIFN was transient. These results support that patients who develope the thyroid dysfunction during or after αIFN therapy must be having autoimmune thyroid disease before therapy. Direct RIA method for thyroid autoantibodies is sensitive to detect and to predict autoimmune thyroid disease.

284. P2 GENETICS OF GRAVES' DISEASE: LINKAGE TO HLA AND IMMUNOGLOBULIN (Gm and Km) ALLOTYPES IN PATIENTS AND THEIR FAMILIES.

S Ratanachaiyavong[1], D C Shields[2] and A M McGregor[3], Department of Medicine, Songkla University, Songkla, Thailand[1]; Department of Genetics, Trinity College, Dublin, Ireland[2]; Department of Medicine, King's College School of Medicine, London, UK[3]

Graves' disease (GD) is likely to be multifactorial and polygenic in its aetiology. Whilst associations between genes in the major histocompatibility complex (HLA genes) and GD are well recognised, controversy surrounds the possible association of these genes within the families of patients with GD. On the one hand classical linkage analysis fails to show an association (Roman S H et al, 1992 JCEM 74; 496) whereas combined segregation and linkage analysis does (Ratanachaiyavong S et al, 1994 Amer. J. Hum. Genet. 55; 540). In extensive studies using combinations of HLA polymorphic markers spanning 3,800 kb (from HLA-A to HLA-DPB1) of the human MHC region we have investigated unrelated patients with GD (n=100), normal controls (n=100) and 217 members of 21 families of patients with GD which included 51 members affected with GD and 45 unaffected members who were thyroid autoantibody positive. These studies demonstrate that HLA-DR17 (DR3) contributes the strongest genetic susceptibility not only to GD but also to thyroid autoantibody production. Positive lod scores of 3.89 and 6.64 were obtained under the single and two locus models respectively. However, HLA-DR17 is unlikely to be the only disease susceptibility allele. Investigation of immunoglobulin (Gm and Km) allotypes (by a haemagglutination inhibition assay) in these families did not show any significant difference in the frequencies of the Gm and Km1 markers between family members. However, when both HLA and either Gm or Km1 were considered together, significant interactions were observed. The interaction between HLA and heavy chain (Gm) allotypes, after correction, achieved significance in the antibody positive family members for both the Gm (fnb) and Gm (axzg) haplotypes alone or together ($p<0.005$, $p<0.02$, $p<0.004$ respectively), as compared with the antibody negative family members. Similar results were obtained with the light chain (Km1) marker in its interaction with HLA in the antibody positive family members ($p<0.004$). Further analysis also indicates the strong influence of these genetic markers on the development of GD and thyroid autoantibodies in both males and the younger members (<35 years) of the families. These studies further extend the genetic linkage within families of patients with GD.

285. P3 **EFFECTS OF EXOGENOUS HUMAN INTERFERON (IFN) - γ OR IFN-NEUTRALIZING MONOCLONAL ANTIBODY ON XENOGRAFTED HUMAN GRAVES' THYROID TISSUE IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE.**

N.Yoshikawa, S. Ikehara[1], H.Kumazawa[2], T.Yamashita[2], M.Inada. The Second Dept. of Internal Medicine, Dept. of Pathology[1] and Otorhinolaryngology[2], Kansai Medical University, Moriguchi, Osaka 570, Japan

We studied the effects of exogenous human IFN- γ or a neutralizing monoclonal antibody (mAb) to IFN- γ on xenografted human Graves' thyroid in SCID mice to investigate a possible role of IFN- γ in the pathogenesis of human Graves' disease. Human thyroid tissues from four patients with Graves' disease were xenografted into SCID mice. Two weeks after xenografting, human immunoglobulin (Ig) -G was detectable in all mice with Graves' thyroid grafts. Mice were divided into three groups with human IgG levels similar to each other. Mice in the first group were treated with IFN- γ daily for 6 weeks ; mice in the second (similar) group were treated with a monoclonal antibody (mAb) to IFN- γ ; mice in the third group were given mice IgG only (control group) . Blood samples were taken every two weeks for human IgG and thyroid-specific autoantibody (Tg-antibody (Ab) , TPO-Ab, and thyroid stimulating antibody (TSAb)) . After 6 weeks' treatment, mice were killed ; thyroid xenograft was examined for thyrocyte HLA-DR expression. Human IgGs were equally produced in all three groups ; mice treated with IFN- γ showed significantly lower amounts of thyroid autoantibodies than those seen for control group. Treatment of a mAb to IFN- γ , in contrast, caused significantly higher amounts of thyroid autoantibodies than those in control group. Thyrocyte HLA-DR expression was markedly increased in xenografts from mice with IFN- γ administration. In conclusion, 1) IFN- γ might down-regulate the production of thyroid-specific autoantibodies but not human IgG, at least under these circumstances ; there thus may be the specific inhibitory effects of IFN- γ to thyroid-specific autoantibody production of intrathyroidal B lymphocytes, and 2) this animal model may help to elucidate the possible role of cytokine (s) in the pathogenesis of Graves' disease.

286. P4 **THYROID HORMONE AUTOANTIBODIES AFTER FINE NEEDLE BIOPSY OF THE THYROID.** S.Benvenga, L.Bartolone,S.Squadrito,S.Battiato,F.Trimarchi. Cattedra di Endocrinologia, University of Messina, 98125 Messina, Italy.

Circulating thyroid hormone antibodies (THAb) are the rarest thyroid Ab and only in ~60% of cases are associated with thyroglobulin Ab (TgAb). Their prevalence (~0.03%) is relatively greater in autoimmune thyroid diseases (ATD) and T3Ab prevail over T4Ab. Although conclusive evidence is lacking, Tg is believed to be the corresponding antigen. However, since circulating Tg - but not Tg stored in the thyroid gland (tTg) - is virtually devoid of T3 and T4, only tTg can mount an immune response. Thus far a limited number of TgAb -ve patients were evaluated for Tg increase in serum 3 to 180 min after thyroid fine needle biopsy (FNAB): this increase occurred in 11/25 (=44%) and 11/15 (=73%) patients (JCEM 56:26, 1983; Horm Metab Res 17: 49, 1984). The latter study also evaluated TgAb appearance within 2 months after FNAB and found no positivization. With these premises we ascertained if leakage of tTg following diagnostic FNAB could represent sufficient stimulus to elicit the appearance of THAb within 1 yr from FNAB. Preliminarly, we verified (courtesy of Dr. A.Schneider, Chicago) that FNAB causes the release into the bloodstream of heterologous (and potentially immunogenic) molecules of Tg. METHODS. 213 patients with "cold" nodules (73.3% were TgAb-ve) presented regularly at the scheduled intervals (1-3 h, 3-5 d, 15 d, 1, 3, 6, 12 mos) after FNAB. FNAB diagnoses were: colloid goiter (C.G.), 71.5%; adenomatous g., 12.4%; cancer, 2.9%; lymphocytic or subacute thyroiditis, 4.3%; inadequate, 8.9%. These parameters were measured pre- and post-FNAB: Ab to T3 and T4 of both the IgM and IgG class (radioimmunoprecipitation technique, JEI 6: 203, 1983), TgAb (IRMA by CIS) and, in AbTg-ve patients, Tg (IRMA by CIS). RESULTS. 1) Serum Tg increased by 15 to 2500% (peak at 1-3 h almost always) in 74% of patients. 2) Over the follow-up period, 93% of the patients TgAb-ve remained so and 7% converted to +ve. Within the TgAb+ve group, TgAb levels increased only in 11.4%. 3) In the pre-FNAB sampling, no patient was THAb+ve. After FNAB, there were a total of 8 (3.8%) THAb+ve cases: 5 of the type IgM-T3 (in 1/5 followed by the appearance of IgG-T3), 2 of IgM-T4 (in 1/5 coexisting with IgG-T4), 2 of IgG-T3 and 1 of IgG-T4. IgM-T3 occurred always at 15 d, IgM-T4 always at 1 mo, IgG-T3 at 1 and 3 mo in o1 and 6-12 mos in the other patient, IgG-T4 at 1 mo. These 8 THAb+ve patients were TgAb-ve (n=5) and TgAb+ve (n=3; 82 to 387 U/ml; n.v.<50); following FNAB, TgAb status did not change. The following patterns were seen: IgM-T3 only (n=4), IgM-T3 first, then IgG-T3 (n=1); IgG-T3 only (n=1); IgM-T4 only (n=1); IgM-T4 coexisting with IgG-T4 (n=1). Diagnoses were: CG (n=6), CG with Hurtle cells (n=1), Hurtle cell carcinoma (n=1). CONCLUSIONS: 1) THAb (IgM > IgG) of restricted specificity (against T3 only or T4 only and T3Ab more frequent than T4Ab) can be elicited by tTg release following FNAB even in patients with no ATD who are (and remain) TgAb-ve. Our data, thus, provide compelling evidence for tTg being the antigen of THAb. 2) Since the 2.5% prevalence of THAb (IgG) reported in the literature for non toxic (or colloid) goiter is the same as that (3/152= 2.0%) in our study, we postulate that a similar, but not artifactually induced, leakage of tTg is the pathogenethic mechanism for THAb in this (and probably other) non ATD. 3) THAb prevalence after FNAB is 126-fold higher than in unselected thyroid patients (3.8 vs 0.03%). Spontaneously occurring THAb (IgG) are rare because, at least in non ATD, a primary immune response (IgM) is rarely followed by a secondary one (IgG), probably due to the requirement of a continuous leakage of tTg.

287. P5 LACK OF B7-1/BB1 AND B7-2/B70 EXPRESSION ON THYROCYTES FROM PATIENTS WITH GRAVES' DISEASE: delivery of the costimulatory signals from bystander professional antigen presenting cells. N. Matsuoka, A. Kawakami, M. Tsuboi, H. Kimura, M. Kita, K. Eguchi. The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki city, Nagasaki, Japan.

Objectives Previously we reported that thyrocytes from patients with Graves' disease are capable of inducing proliferation of autologous peripheral blood T cells in response to soluble antigen and the synergistic augmentation of T cell response by adding suboptimal numbers of monocytes. In the present study, we analyzed the responsibility of costimulatory molecules expressed on the surface of thyrocytes and intrathyroidal mononuclear cells for Ag-specific T cell proliferation.

Methods 1) Graves' thyroids were mechanically minced, enzymatically digested and cultured with/without cytokines. 2) costimulatory molecules expressed on the surface of thyrocytes and mononuclear cells were stained by specific monoclonal antibodies (mAbs) and analyzed by flowcytometor. 3) autologous T cells were stimulated by thyrocytes and /or monocytes in the presence of purified protein derivative (PPD). 4) mAbs specific for costimulatory molecules were studied their blocking capacity for T cell proliferation in response to PPD.

Results On the surface of Graves' thyrocytes, ICAM-1 and LFA-3 were constitutively expressed, however, non of ICAM-2, VCAM-1, B7-1 nor B7-2 were detected and induced by cytokines. The most potent costimulator of T cells, B7-1 was expressed on the intrathyroidal monocytes alone and B7-2 was present on intrathyroidal lymphocytes and both peripheral blood (PBM) and intrathyroidal monocytes (ITM), however, density of B7-2 was higher on ITM than on PBM. The intensity of CD28 expression on intrathyroidal $CD8^{bright+}$ cells were less than that on peripheral blood $CD8^{bright+}$ cells. Ag-specific T cell response induced by thyrocytes was greatly blocked by anti-ICAM-1mAb and partially blocked by anti-LFA-3mAb. Furthermore, synergistic augmentation of T cell response by adding suboptimal numbers of monocytes was lowered by either anti-B7-1mAb or anti-B7-2mAb down to the same level as thyrocytes alone used as antigen presentation cells (APC).

Conclusions Our study suggests that the specific fashion in antigen presentation which is characterized by co-operation of certain kind of epithelia expressed MHC molecules and infiltrating bystander professional APC providing costimulatory signals at the inflammatory site of autoimmune diseases.

288. P6 REGULATION OF FAS ANTIGEN-MEDIATED APOPTOSIS ON HUMAN THYROCYTES BY THYROID-STIMULATING HORMONE(TSH), INTERLEUKIN-1(IL-1), AND INTERFERON-γ (IFN-γ). A. Kawakami, K. Eguchi, N. Matsuoka, M. Tsuboi, M. Kita, H. Kimura, S. Nagataki. The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki 852, Japan.

Objective. Apoptosis, a physiological cell death, has been shown to be involved in tissue homeostasis as well as in tissue regression. In this study, we tried to determine whether Fas antigen-mediated apoptosis occurs in human thyrocytes.

Methods. Thyroid tissues were obtained by subtotal thyroidectomy from patients with Graves' disease who had received antithyroidal drug therapy for several weeks and were euthyroid. The normal thyrocytes were obtained from the tissues adjacent to thyroid carcinoma. To isolate thyrocytes, thyroid tissues obtained were minced and digested with collagenase. Thyrocytes were cultured in RPMI1640 containg 2% bovine serum albumin(BSA) with various kinds of reagents(bovineTSH; bTSH, 8-bromo-cAMP, IL-1β, IFN-γ) for indicated times. Fas antigen and bcl-2 expression on thyrocytes were determined by the flowcytometer and Fas antigen-mediated apoptosis of the cells were determined using flowcytometric analysis to detect hypodiploid DNA.

Results. 1) Fas antigen expression on thyrocytes was inhibited by TSH as low as 0.1mM and maximal suppression was seen around 5mM. 8-bromo-cAMP could also inhibit Fas antigen expression. 2) In contrast, IL-1β and IFN-γ treatment markedly increased Fas antigen expression on thyrocytes. 3) Fas antigen-mediated apoptosis of thyrocytes could only be found on thyrocytes treated with IL-1β and IFN-γ. Unstimulated thyrocytes and TSH-treated thyrocytes were resistant against Fas antigen-mediated apoptosis. 4) Bcl-2 was expressed around 90% of thyrocytes and did not change after stimulation with TSH, IL-1β, and IFN-γ.

Conclusion. Fas antigen-mediated apoptosis can be occured on human thyrocytes and this mechanism appeared to be dependent on the degree of Fas antigen expression on thyrocytes. TSH may suppress the apoptotic process of thyrocytes due to the down regulation of the Fas antigen expression and be involved in the development of goiter.

289. P7 ASSOCIATION OF HLA-A ALLELES WITH GRAVES' DISEASE AND HASHIMOTO'S THYROIDITIS : IMPLICATIONS OF NATURALLY PROCESSED MHC-BOUND PEPTIDES.

T. Sudo, H. Tamai, CJ. Savoie, T. Morita and T. Sasazuki, Department of Psychosomatic Medicine (T. S., H. T., T. M.), Faculty of Medicine, and Department of Genetics, Medical Institute of Bioregulation (T. S., CJ. S.), Kyushu University, Fukuoka 812, Japan.

We have previously reported that both Graves' disease and Hashimoto's thyroiditis are associated with HLA-A2 in the Japanese population. To further examine the susceptible genes to these diseases, the HLA-A allele was defined in 87 patients with Graves' disease, 100 patients with Hashimoto's thyroiditis and 250 healthy controls by DNA typing using the PCR-sequence specific oligonucleotide probes (SSOPs) method. In our PCR-SSOPs method, eighty-four types of SSOPs were designed for all polymorphic regions in exon 2 and exon 3 of HLA-A gene to allow for identification of all known alleles. The frequency of HLA-A*0206 was increased in Graves' disease (patients versus controls, 32% vs 15%, R.R. = 2.73, $P < o.oool$) and that of -A*0207 was increased in Hashimoto's thyroiditis (17% vs 10%, R.R. = 1.94, $P < o.o5$). In contrast, the frequency of -A*0201 was similar to each other. To investigate the molecular basis of this association, the natural peptides bound to the HLA-A2 subtypes (-A*0201, -A*0206 and -A*0207) were compared. Acid extracted peptides from immuono-affinity purified HLA-A2 molecules were analyzed by microsequencing and by tandem mass spectrometry. Although the HLA-A*0206 and -A*0207 molecules differ by a single amino acid from -A*0201, the allele-specific peptide-motifs including the dominant anchor amino acid residues were considerably different among these three HLA-A2 subtypes, indicating that the majority of bound peptides differ from each other.

Our results support an important role for the MHC class I molecule in the pathogenesis of autoimmune thyroid diseases, and also may explain why the associations between HLA-A2 and either disease has been reported in the Caucasoid population in which HLA-A*0201 is the predominantly expressed A2 subtype. Moreover, the allele specific peptide-motifs defined in this study should contribute to the identification of pathogenic epitopes.

290. P8 INDUCTION OF THYROID AUTOANTIBODIES IN NATIVE MICE BY IDIOTYPIC MANIPULATION

Y. Tomer[1], B. Gilburd[2], J. Sack[2], A. Meshorer[3], C.L. Burek[4], N.R. Rose[4], Y. Shoenfeld[2], [1]Division of Endocrinology, Mount Sinai School of Medicine, N.Y, N.Y.; [2]Sheba Medical Center, [3]Weizmann Institute of Science, Israel; [4]Department of Immunology, The Johns Hopkins University, Baltimore, MD.

Background: We have shown that idiotypic manipulation can induce, in naive mice, systemic autoimmune diseases (e.g. SLE) mediated mainly by autoantibodies. Objective: To examine whether idiotypic manipulation can induce organ specific autoantibodies and disease mediated by cellular mechanisms, namely, experimental autoimmune thyroiditis. Methods: 15 BALB/c mice were immunized with a monoclonal mouse anti-human thyroglobulin (hTg) antibody (Ab); 15 controls were immunized with normal mouse IgG. Mice were tested for production of anti-Tg Ab's, and for the development of hypothyroidism and histological manifestations of thyroiditis. Results: Mice immunized with anti-hTg Ab developed, 6 weeks after immunization, Ab's to hTg, but not to dsDNA, cardiolipin, or myeloperoxidase. The specificity of binding to hTg was confirmed by immunoblot analysis which demonstrated binding of the anti-hTg monoclonal Ab (MAb) used for immunizations and of the mouse sera to a 300 kD band corresponding to hTg. To confirm that the anti-hTg Ab's detected in the mice were different from the injected MAb, we determined the IgG subclass of the anti-Tg MAb injected and of those produced in the mice. Whereas the IgG subclass of the anti-hTg MAb used for immunization was IgG1-kappa, the anti-hTg Ab's of the immunized mice demonstrated polyclonal IgG subclasses, thus ruling out the possibility that we were detecting the same Ab that was injected. The presence of anti-Tg Ab's was associated with low production of thyroid hormones (T_4 levels were 3.7 ± 0.75 ug/dl and 5.0 ± 0.76 ug/dl in mice injected with anti-hTg and mouse IgM, respectively, $p= 0.0012$). During a follow-up of 20 weeks the mice did not develop histological signs of thyroiditis. Conclusion: Idiotypic manipulation can induce anti-thyroglobulin autoantibodies, and low thyroid hormone levels in naive mice.

291. P9 UNIQUE HLA CLASS II ASSOCIATION IN JUVENILE GRAVES.

L. Lavard, H. Perrild, B. B. Jacobsen, H. O. Madsen, A. Svejgaard.
Depts. of Ped., Int.Med. and Clin. Immun., Glostrup, Bispebjerg , Odense University Hospitals and Rigshospitalet, Denmark.

The association between HLA-DR and HLA-DQ and juvenile Graves's disease has been investigated in 85 Danish caucasian patients with onset of juvenile Graves' between the age of 0-18 years and a control group of healthy Danish blood donors.
DNA-typing by PCR-SSO(Polymerase chain reaction with sequence specific oligo hydridization). The types for HLA-DRB1, HLA-DRB3, HLA-DQA1 and HLA-DQB1 loci were determined. For the latter only presence of DQB1*0201 was determined.
HLA-DR: DRB1*0301 was very frequent(64%) compared to healthy controls(23%) ($p = 10(-11)$) and compared to adult onset Graves'(50%). DRB1*0701 was totally absent, compared to 11% in the control group($p = 10(-8)$). Concerning the DRB3 locus, there was no significant difference between the patients and controls.
HLA-DQ: 64% of the patients had DQA1*0501,(controls 40%), DQB1*0201 was present in all DRB1*0301 positive patients, and absent in all DRB1*0301 negative patients. Stratification DRB1*0301 positive or negative revealed no association with DQ within the strata indicating that the DQ-associations were due to linkage disequilibrium.
No relations between specific DR-, DQ-types and sex, age of diagnosis, thyroid ophthalmopathy, relapse rate nor presence of anti-TPO antibodies were found.
In conclusion: juvenile Graves' disease in caucasians is closely associated with DRB1*0301, and the associations with DQA1*0501 and DQB1*0201 are due to linkage disequilibrium.
DRB1*0701 have a protective effect.
There was no association between HLA-DR/DQ-types and the clinical course or presence of anti-TPO antibodies.

292. P10 IMMUNOGENETIC MARKERS IN MEXICAN PATIENTS WITH GRAVES DISEASE.-V. Gómez, O. González, J. Granados. Hospital Adolfo López Mateos, ISSSTE, Instituto Nacional de la Nutrición Salvador Zubirán, México D.F., México.

Introduction.- The origin of autoimmune thyroid disease is multifactorial, and a genetic susceptibility for its development is well recognized. Class II alleles from the major histocompatibility complex (MHC) genes have been found to be associated with Graves Disease, the relevant allele however, varies among the different populations so far studied. Objectives.- To identify the relevant MHC alleles in the genetic susceptibility for the development of Graves Disease in Mexicans. Methods.- Class I and Class II MHC genes were studied in 30 Mexican Mestizo patients with GD by using standard serological techniques, class II Alleles were also identified by PCR amplification of DNA from DRB1, DRB3, DRB4, DRB5, DQB1 genes; results were compared to those present in a group of 100 ethnically matched normal controls. Statistical analysis were performed with the X2 and Fisher's Exact tests by using the EPISTAT statistical program. Results.- As compared to normal controls, GD patients showed significantly increased frequencies of HLA-DR3 ($p<0.005$), DR5 ($p<0.005$) and DQB1*0302 ($p<0.001$). None of the patients were homozygous DR3 or DR5 and neither of them were heterozygous DR3/DR5. Gene frequencies of the remaining class I and class II alleles were similar between patients and normal controls. Conclusions.- These data identify a primary role of class II MHC genes in the genetic susceptibility to GD in Mexicans and further suggest the existance of at least two point putations within the 6p chromosome, one of them related to the DR5 (DRB1*1101),DQB1*0302 haplotype and the other one related to DR3 (DRB1*0301) haplotypes.

293. P11 IMMUNE FUNCTION IN THYROID DISORDERS. P.D. Kallio and E.D. Murphy, Evanston Hospital, Evanston, Illinois, U.S.A.

This study sought to determine the immune response in various thyroid disorders. Analysis of lymphocyte subsets by flow cytometry and testing of sera for soluble CD antigens (CD8, CD23, CD25) was performed. Assay results for the CD antigens have been reported previously and were most useful for defining treatment outcome in Graves hyperthyroidism. Flow cytometry was done with whole blood samples from patients with untreated Graves (7), PTU treated--on PTU (4) and after PTU (5), euthyroid after radioactive iodine treatment (7) {RAI Rx}, euthyroid with ophthalmopathy (3), chronic thyroiditis (23), hyperthyroid phase of subacute thyroiditis (5), euthyroid after subacute thyroiditis (9), and normal controls (21). Graves hyperthyroid patients had more B cells ($p<0.001$) and an increased number of activated B cells (CD5+, CD23+, CD38+, CD5+CD23+, CD5+CD38+, all $p<0.001$). Euthyroid PTU patients retained increased B cells ($p<0.001$) and B cell activation along with activated CD8 suppressor cells (CD28-CD11b-, $p<0.05$). All lymphocyte subsets returned to normal for patients euthyroid after PTU. RAI Rx patients had more B cells ($p<0.001$) and more activated B cells (CD5+,CD23, CD5+CD23+, $p<0.01$, $p<0.001$, $p<0.01$). Patients with euthyroid ophthalmopathy showed increased activation of CD4 cells (DR+, $p<0.02$), CD8 cells (DR+, $p<0.005$), and of CD3 cells (DR+CD25-$p<0.01$). Chronic thyroiditis patients showed decreased CD25 as compared to controls on CD4+DR- cells ($p<0.01$) and on CD8 cells ($p<0.02$). Hyperthyroid subacute thyroiditis patients had normal lymphocyte levels but CD8+DR+ and CD3+DR+CD25- levels increased after becoming euthyroid ($p<0.02$, $p<0.005$). These disorders may thus show varying immune responses. Graves hyperthyroidism is related to B cell activity and ophthalmopathy to CD8 and CD4 cells. Chronic thyroiditis, a gradual process, showed less CD4 and CD8 activity. Hyperthyroid subacute thyroiditis had no immediate immune response but paradoxically had increased activated CD8+ cells in its recovery phase.

294. P12 HLA CLASS II GENOTYPE ANALYSIS IN THYROPEROXIDASE (TPO) ANTIBODY POSITIVE PATIENTS EXAMINED THE IMMUNE-DOMINANT LESIONS OF TPO BY RECOMBINANT ANTIGEN-BINDING FRAGMENTS [F(ab)]. T. Nishikawa, Y. Shirahige, T. Tominaga, K.Ashizawa, T. Kiriyama, N. Yokoyama. The First Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan.

[Objective]. Major histocompatibility complex (MHC) class II molecules are pivotal to antigen recognition by T-lymphocytes and play a major role in immune reactions. The purpose of the study is to investigate the relationship between HLA class II antigens and the TPO immune-dominant domain in autoimmune thyroid disease.

[Methods] Recombinant F(ab)s are now available to investigate the immune dominant lesions in TPO antibody, which is divided into two different domains (domain Aand B). In this study, we analyzed 50 TPO antibody positive patients by 4 different recombinant F(ab)s and by HLA class II antigens. To examine the immune-dominant domain of TPO, binding competition studies were performed by incubating patients' sera with ^{125}I-TPO in the presence or absence of maximum concentrations (10^{-8} mol/l) of TPO specific F(ab). Antigen-IgG complexes were precipitated with protein A. HLA class II genotypes (HLADQB1, DRB1 and DPB1) were tested by the PCR-RFLP method.

[Results]

(1) There was no correlation between the immune-dominant domains of TPO and specific HLA class II genotypes in these 50 patients. However,

(2) The frequencies of HLA DQB1 0303 and 0401 increased in the study group. (25.0% vs. Japanese control 14.8%, $Pc<0.01$; 23.0% vs. Japanese control 13.0%, $Pc<0.01$, respectively)

(3) The frequencies of HLA DRB10405 and 0901 also increased in the same study group (23.0% vs. Japanese control 13.2%, $Pc<0.01$; 24.0% vs. Japanese control 14.1%, $Pc<0.01$, respectively.)

[Conclusion] Some HLA class II antigens may confer susceptibility to TPO antibody expression, although the immune-dominant lesion was not related to any HLA class II genotype.

295. P13 IMMUNOHISTOCHEMICAL DETECTION OF APOPTOSIS IN AUTOIMMUNE THYROID DISORDER. M. Koga, M. Sato, M. Migita, K. Nonaka, Department of Medicine, Kurume University School of Medicine, Kurume, Japan

To evaluate a possible involvement of apoptosis in autoimmune thyroid disorders (AITD), we investigated the expression of Fas antigen, which mediated apoptosis, and bcl-2, which suppressed Fas-mediated apoptosis, in thyroid tissue from patients with Graves' disease (GD) and from patients with Hashimoto disease (HT), and in extraocular muscle (EM) and orbital fat tissue (FAT) from patients with thyroid-associated ophthalomopathy by immunohistochemical staining with mouse monoclonal antibodies against Fas antigen and bcl-2. Apoptosis was detected by in situ end-labeling of fragmented DNA using Apop tag kit. Both Fas antigen and bcl-2 were detected in mainly thyroid follicular cells from all of 3 GD patients and 3 HT patients. The expression of bcl-2 was slightly stronger in GD patients than in HT patients. Apop tag-positive cells were detected all of 6 GD patients and 5 of 6 HT patients. In the orbit, Fas antigen was expressed in extraocular muscle cells from 11 of 19 TAO patients. Apop tag-positive cells were found in interstitium and some muscle fibers from 8 of 9 TAO patients. Weak expression of Fas antigen and bcl-2 was detected in FAT from 3 of 20 patients and 15 of 20 patients with TAO, respectively. These data suggest that Fas-mediated apoptosis may be present in thyroid tissue and in orbital tissue in AITD and TAO. Further investigations are indicated to clarify the role of Fas-mediated apoptosis in the development of AITD and TAO.

296. P14 CD31, CD 62P, AND CD62E IDENTIFY A SPECIFIC PATTERN OF ENDOTHELIAL ACTIVATION IN GRAVES'DISEASE (GD).V. D. Aubert, M.C. Bene, J. Leclère, G.C. Faure. Clinique Medicale & Endocrinologique and Laboratoire d'Immunologie, Faculté de Médecine & CHU Nancy, France.

Using immunohistological techniques, we reported a comparative investigation of the endothelial expression of adhesion molecules CD62P or PADGEM (Platelet Activation Dependant Granule External Membran Protein), CD62E or ELAM-1 (Endothelial Leukocyte Adhesion molecule), CD31 or PECAM (Platelet Endothelial Cell Adhesion Molecule) in human thyroid samples from patients with and without auto-immune diseases. 19 thyroid glands were studied, 13 with auto-immune thyroiditis (12 GD, 2 Hashimoto's diseases) and 6 as control non auto-immune tissues (3 benign adenomas, 3 multinodular goiters). All samples were examined in indirect immunofluorescence with monoclonal antibodies CD62P, CD62E, CD31, performed on frozen-cut sections of the thyroid samples. Small capillaries more numerous and of smaller size could be seen in all GD thyroid glands with CD31. All proliferation capillaries, venules and high endothelial venules (HEV) were brightly stained. Their location was correlated with the characteristic"festooned" pattern of the epithelial basement membrane in GD. Vascular structures displaying the morphology of HEV were clairly identified with CD62P in Hashimoto's and in Graves' diseases. In control samples, the endothelial layer of large blood vessels only were positive and mostly located in the fibroconnective trabeculae separating vesicles'foci with CD31 whereas CD62P expression was not detected. CD62E was expressed on the apical pole of endothelial cells in 8/13 cases of auto-immune thyroiditis and only in 2 controls. Thyroid samples from patients with GD displayed a characteristic pattern of capillary proliferation with CD62P, CD62E and CD31 expression on endothelial cells.

In conclusion adhesion molecules seem to be likely actively involved in auto-immune thyroiditis with an important role in the migration of leukocytes through the intact vessel wall.

297. P15 NO DOSE RELATED EFFECT OF METHIMAZOLE ON THE INTENSITY OF THE INTRATHYROIDAL AUTOIMMUNE PROCESS IN RELAPSING GRAVES' DISEASE

R. Paschke, M. Vogg Service de Génétique Médicale, Université Libre de Bruxelles, Belgium

Decreased lymphocytic infiltration and declining thyroid autoantibodies after and during treatment of patients with Graves' disease with methimazole suggest immunosuppressive actions of antithyroid drugs. However, the recent report of an equal efficacy of low and high dose carbimazole treatment of Graves' disease seems to contradict the immunosuppression thesis.

We therefore determined the intrathyroidal methimazole (M) concentrations with a HPLC method and the cumulative preoperative methimazole doses in 17 patients undergoing subtotal thyroid resection for relapsing Graves' disease. The intensity of the intrathyroidal infiltration by immunoglobulin G-producing plasma cells (P), activated T cells (T), antigen presenting cells (A) and the total number of lymphocytes (L) were identified immunohistologically with monoclonal antibodies for immunoglobulin light chains kappa and lambda, UCHL1 and the S100 antibody respectively followed by morphometry. The intrathyroidal M concentration did not correlate with the intensity of the intrathyroidal infiltration by P ($r = -0.18$), T ($r = -0.1$), A ($r = -0.19$) and L ($r = -0.04$). This was also true for the cumulative preoperative methimazole doses. Comparison of groups with significantly different intrathyroidal methimazole concentrations (134 ng / g, $n = 8$ versus 993 ng / g, $n = 7$) showed no significant differences for any of the intrathyroidal immunocompetent cells.

These findings suggest that there is no dose related effect of M on the intensity of the intrathyroidal autoimmune process of patients with relapsing Graves' disease. They provide an explanation why it does not seem to be justified to recommend higher M doses than required for the control of hyperthyroidism with the goal of immunosuppression.

298. P16 DOES THYROXINE ADMINISTRATION POSTPARTUM (PP) REDUCE ANTI TPO ANTIBODY ASSOCIATED DEPRESSION?

JH Lazarus, B Harris[1], AB Parkes, CJ Richards[2] and RG Newcombe[3]. Depts. Medicine, Psychological Medicine[1], Obstetrics[2], and Medical Computing and Statistics[3], University of Wales College of Medicine, Cardiff, Wales, U.K.

An increase in depressive symptomatology and in research diagnostic criteria (RDC) positive depression has been noted in anti TPO Ab +ve (ascertained 16 weeks antenatally) women PP compared to anti TPO -ve PP women irrespective of thyroid function (Br Med J 305:152;1992). The mean time PP of maximum incidence of depression is 5 months. To assess the effect of thyroxine 0.1mg administration PP we have performed a randomised double-blind placebo-controlled trial in 372 PP anti TPO Ab +ve (>30KIU/L) women of whom 187 received T4 0.1mg/day from 6-24 wks PP compared to 185 PP women receiving placebo. Women were assessed at 6,12,16,20 and 24 wks PP by a variety of psychiatric instruments as well as measurement of thyroid function (FT4,FT3,TSH). As 400 women are required for statistical analysis these data are preliminary.

Results, analysed by covariance for the 20 week time point only, comparing active treatment to placebo and adjusting for the baseline visit are shown (Table).

Test	Point Estimate	95% Confidence Interval	p value
Montgomery-Asberg	-.12	-1.68 to +1.44	0.88
Edinburgh	-.86	-1.89 to +0.16	0.099
Kellner-Sheffield (KS)	-1.71	-3.67 to +0.24	0.086
General Health Questionnaire	-1.53	-3.02 to -0.04	0.045

These preliminary data suggest a significant benefit of T4 in terms of the GHQ and approaching significance for the Edinburgh and KS scales at 5 months PP. A full analysis at all time points is required to establish the therapeutic efficacy of T4 in reducing depression in the PP period.

299. P17 THYROID HYPOFUNCTION WITH Ab-TPO MAY BE A FURTHER RISK FACTOR OF PROGRESSION IN HIV INFECTION.

A. Olivieri*, E. Medda*, M. Sorcini*, P. Narciso°, , M. D'Archivio*, S. Baccarini*, M.E. Grandolfo* and S. Carta*.
*Istituto Superiore di Sanità, Roma. ° Ospedale L. Spallanzani,Roma-Italy

In HIV infection thyroid function was reported to be altered even early in the course of the infection (J. Clin. Endocrinol. Metab. 70:566, 1990). In a previous study (J. Endocrinol. Invest. 16: 407,1993) we found a thyroid hypofunction with Ab-TPO occurence, more pronounced in advanced stages of HIV infection.

In order to investigate whether the hypothyroid condition may be a further risk factor of infection advance, 68 HIV infected patients at different stages of infection were clinically examined 22 months after the thyroid function assessment. At enrolling time the patients were classified as follows: 26 asymptomatic seropositive (ASYM), 31 with AIDS-related complex (ARC) and 11 with AIDS.

At enrollment, a decreasing trend was observed throughout the stages of infection in the 68 HIV patients for mean values of total and free thyroid hormones, with exception ot total T3; in fact it was normal in all patients. An increasing trend throughout the stages was observed for TSH,TBG and Ab-TPO. Ab-TPO positivity was present in 20.5% (14/68) of HIV patients: 11.5% of ASYM, 23% of ARC and 36.4% of AIDS patients. All the patients were divided into 2 groups according to FT4 levels at enrolling time: Group A (n=48) with FT4 $\geq$ 0.8 ng/dl (mean $\pm$ S.D.: 0.9$\pm$0.1) and Group B (n=20) with FT4 < 0.8 ng/dl (mean $\pm$ S.D.: 0.6 $\pm$ 0.07). Clinical progression of HIV disease in the patients was defined as clinical transition throughout the stages of the infection according to CDC classification. Clinical evolution with a stage advance was observed in 47% (32/68) of the patients. At the end of 22 months, the Group A showed a lower percentage of patients (40%) with clinical evolution than Group B (65%). Moreover, 71% (10/14) of patients with Ab-TPO showed a clinical advance.

Cox's proportional hazard model was used to analyze the association between HIV progression and enrolling FT4 values and Ab-TPO, while simoultaneously controlling for age, stage of infection and CD4 counts at enrollment.

As expected, Cox's regression analysis showed that patients with very low CD4 counts at enrollment (<200/mm^3) had a high relative risk (RR) of clinical progression (RR=2.2). Nevertheless a trend of increasing risk of progression was observed with decreasing FT4 levels (FT4= 0.8-0.9 ng/dl, RR=1.20; FT4<0.8 ng/dl, RR=1.36). This risk increases when patients had low FT4 and Ab-TPO (RR=1.86). These results suggest that thyroid hypofunction may be considered a further risk factor of progression in HIV infection.

300. P18 **Autoimmune prone BB rats show a high intrinsic thyrocyte metabolism and are capable to cope with mild iodine deficiency**

P.J. Simons, P. Mooij, H.A. Drexhage, Dept. of Immunology, Erasmus University, Rotterdam, The Netherlands

Objective: We earlier showed[1] that autoimmune-prone BB rats are capable to compensate for mild iodine deficiency, this in contrast to Wistar rats that become gradually hypothyroid. When female autoimmune-prone BB rats and female Wistar rats were kept on a low iodine diet (LID) for a period of 18 weeks (from 3 weeks of age onwards, the LID consisted of 1% $KCLO_4$ in the drinkingwater for 2 days, followed by distilled water and iodine deficient pellets for the remaining period) the BB rats developed only a transient subclinical hypothyroidism from 3 till 6 weeks of LID (NID vs LID values after 6 weeks of diet: T_4 = 44.8 $\pm$ 8.8 (mean $\pm$ SD) vs 18.1 $\pm$ 2.4 nmol/l (mean $\pm$ SD), $p<0.05$; TSH = 1.59 $\pm$ 0.9 (mean $\pm$ SD) vs 10.7 $\pm$ 3.6 ng/ml (mean $\pm$ SD), $p<0.05$). Euthyroidism was fully restored in BB rats after 9 weeks of the low iodine diet and NID vs LID values were: T_4 = 56.5 $\pm$ 8.6 (mean $\pm$ SD) vs 43.8 $\pm$ 6.5 nmol/l (mean $\pm$ SD) and TSH = 1.35 $\pm$ 0.4 (mean $\pm$ SD) vs 3.0 $\pm$ 1.1 ng/ml (mean $\pm$ SD). Wistar rats however became gradually hypothyroid in the period of LID.

A hypothetical explanation for the compensating mechanisms in the BB rat is an autonomous high intrinsic metabolism of BB-thyrocytes such as has been described in another thyroid autoimmune animal model, the Obese Strain (OS) chicken. This was studied.

Design: We isolated thyroid follicles from BB rats and Wistar rats at various ages (week 3-22), cultured these for 24 hrs in vitro in Ham's F12, and thereafter studied their production of T_4 and T_3 into the culture fluid under basal conditions and during TSH stimulation (various dosages).

Results: BB thyrocytes differed from Wistar thyrocytes, in that they produced more thyroid hormones both under basal conditions as well as after TSH stimulation, particularly when thyroid follicles had been isolated from animals of 8 weeks of age and older (60% more under basal conditions, 120% more during TSH stimulation, both $p<0.05$).

Conclusion: BB thyrocytes have a raised intrinsic capacity to produce thyroid hormones as compared to Wistar rats, particularly at the time just before thyroid autoimmune development. This high intrinsic metabolism of BB thyrocytes starting in puberty (1) explains the capability of pubertal BB rats to cope with mild iodine deficient diets and (2) is likely connected to the development of thyroid autoimmunity in the animals around that time.

[1] P. Mooij, Acad. Thesis, Rotterdam, 1993.

301. P19 THYROID AUTOIMMUNITY ASSOCIATED WITH LIPOPROTEIN(a) INCREASE

Helga Lotz and Giovanni B. Salabè - Istituto di Medicina Sperimentale, Consiglio Nazionale delle Ricerche, Viale Marx 15-43, 00137 Roma, Italy.

Elevated serum lipoprotein(a) has been shown an independent risk factor for cardiovascular heart disease (CHD). Recently in autoimmune female hypothyroids on l-T4 therapy elevated (Lpa) has been documented (Klausen et al.1992). It is therefore of interest if thyroid autoimmunity in its asymptomatic expression in general population shows elevated Lp(a). 30 male and 29 female thyroid antibody positive (T-Abs) euthyroid **normolipidemic** blood donors, screened out from 428 male and 162 female donors compared with their respective T-Abs negative controls resulted in highly significant difference in Lp(a) distribution (Friedman two way analysis of variance: p=0.0000), which could be attributed to normolipidemic euthyroid male donors with T-Abs compared to donors without T-Abs. (Lp(a) mean 19.7±15.9 mg/dl vs 12.7±17.5 mg/dl; median 17.0 mg/dl vs 4.0 mg/dl: Mann Whitney: p=0.0000). IRMA TSH (detection limit 0.02mU/L) mean 1.6±1.1 mU/L vs 1.2±0.6 mU/L. In 29 premenopausal females with T-Abs compared to 72 females without T-Abs no increase of Lp(a) could be evidenced (Lp(a) mean 13.2±16.1 mg/dl vs 12.3±13.9 mg/dl, median 5.2 mg/dl vs 8.7 mg/dl: Mann Whitney p=0.9776).TSH mean 2.1±1.0mU/L vs 1.3±0.7mU/L. Comparing 20 postmenopausal autoimmune hypothyroid patients on l-T4 therapy, 23 female congenital adult hypothyroids (5 dyshormonogenetic and 18 ectopic) on l-T4 together with the 2 groups of female donors resulted in significantly different distribution of Lp(a) (Kruskal Wallis one way analysis of variance: p=0.0179), due to female autoimmune hypothyroids on l-T4 compared to congenital hypothyroids on l-T4 (mean Lp(a) 21.1±18.1 mg/dl vs 14.0±17.7 mg/dl; median 21.0 mg/dl vs 9.0 mg/dl: Mann Whitney p=0.0005).TSH 3.0±1.5 mU/L vs 3.1±1.8mU/L.
In conclusion: Thyroid antibody positive euthyroid normolipidemic male blood donors show increased serum Lp(a), premenopausal females do not, supporting an Lp(a) lowering effect of female hormones shown in an increasing number of studies. Association of euthyroid autoimmunity with increased Lp(a) serum levels might be the link with increased prevalence of thyroid autoimmunity in CHD shown in the past. Preliminary results in 66 male myocardial infarct survivors (MI) show increased median Lp(a) in T-Abs positive MI vs T-Abs negative MI: 28.0 mg/dl vs 12.0 mg/dl.

302. P20 THYROID-STIMULATING HORMONE-LIKE ACTIVITY OF THE ANTI-α GALACTOSYL ANTIBODY (ANTI-GAL) IN ENDEMIC GOITER PATIENTS.

G Medeiros-Neto, ES Umezawa, MJF Martins, MLC Correa, VCC Guimarães, M Knobel, D Gianella-Neto. Inst Tropical Med and Thyroid Laboratory, University of Sao Paulo Medical School, 01065-970 Sao Paulo, Brazil.

Anti-GAL is a human natural polyclonal antibody that constitutes approximately 1% of the circulating IgG and that interacts specifically with the mammalian carbohydrate epitope α-galactosyl. This epitope is abundant on thyroid follicular cells. Assays performed with porcine thyrocytes have indicated that anti-GAL can mimic *in vitro* TSH effects in stimulation for cAMP synthesis. We hypothesized that anti-GAL titers might be elevated in endemic goiter subjects. Serum was obtained from 36 patients with very large multinodular goiters (grade III and IV) and constituted Group I. Patients with grade I or II smaller goiters constituted group II (n = 20) and 20 normal controls formed group III. All three groups had approximately the same iodide intake through iodized salt and there was no significant difference in urinary iodide excretion (171 ± 72 μgI/dL urine). Thyroid function tests indicated that group II had a significantly higher serum levels of T_3 (3.02 ± 2.39 nmol/L), serum total T_4 (128.7 ± 57.4 nmol/L), serum free T_4 (21.4 ± 17.1 pmol/L) as compared with group I. Also serum TSH was significantly lower in group II (0.74 ± 0.9 mU/L) as compared with group I (1.31 ± 1.4 mU/L). As expected the group I with larger goiters had marked elevation of serum Tg (221 ± 207 μg/L) as compared with group II (12.4 ± 10.8 μg/L). Serum anti-GAL antibodies were elevated in both goitrous groups (1.182 ± 0.150 U) as compared with normal subjects (0.45 ± 0.44 U). Anti-GAL antibodies were isolated by affinity chromatography on melibiose-Sepharose and tested *in vitro* in CHO cells transfected with human TSH receptor. As these cells lack the α-galactosyl epitopes there was no significant change in cAMP accumulation. In contrast incubation of anti-GAL with the mouse 3T3 cells transfected with hTSH-r (expressing α-GAL epitopes) resulted in an increase in cAMP synthesis. This stimulation is likely to be the result of antibody binding to the hTSH-r molecule. The present study suggests that anti-GAL antibodies may potentially interact with α-GAL epitopes and may exert a TSH-mimicking effect that could be related to growth of thyroid cells.

303. P21 AMIODARONE INDUCES A DIFFERENT PATTERN OF ULTRASTRUCTURAL CHANGES TO IODINE EXCESS ALONE IN BB/W RAT THYROID.

Vicki Pitsiavas, Mu Li, Peter Smerdely [§], Steven C Boyages. Departments of Clinical and Laboratory Endocrinology, Westmead Hospital, Westmead NSW 2145.[§] Department of Aged Care, St George Hospital, Kogarah NSW 2217. AUSTRALIA

Amiodarone (AMD) is frequently the cause of thyroid function abnormalities which have been attributed in part to its high iodine content. Emerging evidence however suggests that the iodine content is not the sole cause of AMD thyroid dysfunction. Previous studies on the effects of high concentrations of iodine on thyroid ultrastructure have shown marked changes. These effects have been extrapolated to AMD. From functional studies this may not be true. Therefore the aim of the study was to determine the effects of AMD and iodine on thyroid ultrastructure and to determine any differences.

BB/W rats were weaned at 4 weeks and divided into three groups of three rats. Group 1 was given sterile drinking water, Group 2 was given AMD (30mg/Kg) drinking water, Group 3 was given NaI (10mg/Kg) drinking water. The doses of AMD and iodine were equivalent in iodide content. Rats were sacrificed at 15 weeks of age, thyroids were removed and processed for electron microscopy.

Results showed distinct differences between the three groups.

Group 1 (Controls): Thyroid follicles appeared to be morphologically intact under EM. Thyrocytes appeared to have normal subcellular structures and the colloid space appeared homogenous.

Group 2 (AMD): Thyroid follicular structure appeared distorted. Many thyrocytes had irregular shaped nucleus with dilated RER. Mitochondria were mostly oval shaped. Lysosomes were present in increased numbers compared with the control group. Secondary lysosomes containing lipofuscin bodies were also evident. Many cells appeared to be undergoing apoptotic changes. Inclusion bodies were also present

Group 3 (NaI): Thyroid follicles appeared morphologically intact. Thyrocytes had a regular nucleus. Lysosomes were present as well as secondary lysosomes but lipofuscin bodies were absent.

We conclude that AMD induces ultrastructural changes in the thyroid which differ markedly from those caused by iodine in iodide-equivalent concentrations. Thyroid follicles appeared more distorted, nuclear chromatin appeared aggregated, and there were increased lipofuscin bodies present in AMD treated rats compared with iodine treated rats. Further, AMD caused apoptotic cell death at this concentration whilst iodine did not. We recommend further studies into the mechanism of these observed differences between amiodarone and iodine.

304. P22 TISSUE EOSINOPHILIA AND EOSINOPHIL DEGRANULATION IN RIEDEL'S INVASIVE FIBROUS THYROIDITIS.

A. E. Heufelder[1], J. R. Goellner[2], G. J. Gleich[3], and I. D. Hay[4]. Medizinische Klinik[1], Klinikum Innenstadt, Ludwig-Maximilians-Universität, München, Germany, and Departments of Pathology[2], Immunology[3], and Internal Medicine[4], Mayo Clinic/Foundation, Rochester, MN 55905.

The etiology of Riedel's invasive fibrous thyroiditis (IFT), an entity distinct from the more common fibrous variant of Hashimoto's thyroiditis, has remained obscure. The typical histological features, in particular the presence of an invasive fibrosclerotic process in conjunction with a prominent inflammatory infiltrate, suggest that the release of fibrogenic cytokines and other factors from these cellular infiltrates may play an important role in the pathogenesis of IFT. Our observation, in routinely processed tissue sections obtained from patients with documented IFT, of a striking degree of tissue eosinophilia led us to hypothesize that eosinophils and their release products may play an active role in the evolution of this disease. Immunofluorescence staining with a highly specific, affinity-purified polyclonal rabbit antibody directed against human eosinophile granule major basic protein (MBP) revealed the presence of marked tissue eosinophilia and abundant extracellular deposition of MBP in all specimens derived from 16 patients with IFT. Eosinophil degranulation was most prominent in perivascular areas, in the inflamed loose connective tissue, and in the interstitial connective tissue between striated muscle fibers. By contrast, only occasional eosinophils and no extracellular deposits of major basic protein were detected in thyroid tissues obtained from 18 normal control individuals or patients with multinodular goiter, Graves' disease, or Hashimoto's thyroiditis. The presence of marked eosinophil infiltration and extracellular deposition of MBP in tissues affected by IFT and other associated fibrosclerotic manifestations suggests an as yet unrecognized role for eosinophils and their fibrogenic products in propagating fibrogenesis in IFT.

Supported by Deutsche Forschungsgemeinschaft (He 1485/5-1).

305. P23 **GLUCOSE METABOLISM IN PERIPHERAL LYMPHOCYTES IN GRAVES' DISEASE: RELATIONSHIP WITH SERUM THYROID AUTOANTIBODIES.** Marisa C. Werner, João H. Romaldini, Luis F. B. Costa Rosa, Rui Curi. Department of Endocrinology, HSPE-IAMSPE and Institute of Biomedical Sciences, University of São Paulo, Brazil.

Glucose metabolism, as reflected by the maximum activities of hexokinase(HK) and citrate sintase(CS) is increased in peripheral lymphocytes in untreated Graves'disease patients. The HK and CS activities decrease after methimazole(MMI) Graves'disease treatment(ATA, 1994, Abstract 79). To evaluate if the HK and CS activities in lymphocytes from Graves'disease patients are related to serum concentrations of anti-TSH receptor (TRAb), antithyroid peroxidase (TPOAb) and antithyroglobulin (TgAb) antibodies we studied 3 groups of patients: untreated hyperthyroid (UH, n=12), MMI treated hyperthyroid (TH, n=11) and MMI treated euthyroid (TEU, n=10) patients. Serum TRAb values were determined by RRA, serum TPOAb and TgAb by an IRMA method. Serum TSH, T_3 and T_4 concentrations were measured by chemiluminescent method. The maximum enzyme activities of HK and CS in blood lymphocytes were measured by spectrophotometry.

Group	HK	CS	TRAb	TPOAb	TgAb	T3	T4	TSH
	(nmol/min/mg/protein)		(%)	(UI/mL)		(ng/dL)	(μg/dL)	(μU/mL)
UH	263.2±40.7	73.1±12.7	21.6±20	1118±1189	247±289	3.1±0.5	14.9±2.6	<0.03
TH	231.5±54.5**	63.7±20.7**	50.6±41#	862±1245	370±607	3.2±0.8**	13.8±3.1**	<0.03
TEU	174.2±46.6*	36.3±19.5*	16.2±9.7**	773±1191	309±443	1.3±0.4*	7.9±1.3*	0.17±0.3

* = p<0.001, UH vs TEU ** = p<0.05, TH vs TEU # = p<0.05, UH vs TH (t test) (means ± SD)

In the UH group a negative correlation was found between HK and CS activities and TPOAb concentration (r = -0.77, p<0.01 and r = -0.71, p<0.01, respectively) as well as with CS activities and TRAb values (r = -0.66, p<0.02). After MMI administration no correlation could be found even in the TH patients whose enzyme activities and thyroid hormone levels did not differ from those of UH group. In conclusion, 1) the higher HK and CS enzyme activities found in the UH and TH groups but not in the TEU patients seem to indicate that these alterations are related to the elevated T3 and T4 values. 2) The high T3 and T4 levels in the UH group were related to a reduced thyroid antibodies production and might exert a protective role on immune response to thyroid antigens mediated throughout their action on glucose metabolism. 3) The lack of correlation between HK and CS enzyme activities and thyroid autoantibodies in the TH and TEU patients may suggest a direct action of MMI on peripheral lymphocytes of Graves'disease.

306. P24 FREE RADICALS AND ANTIOXIDANT DEFENSES IN GRAVE'S DISEASE. M. Abalovich*,D. Bequelman*,C. Reides#,M. Repetto#, S. Llesuy#, S. Gutierrez*, A. Guitelman*.* Endocrinology division, Durand Hospital.# General and Inorganic Chemistry division, School of Pharmacy and Biochemistry, University of Buenos Aires. Buenos Aires, Argentina.

It has been described a decrease of some antioxidant compounds in patients suffering hypertyroidism. The aim of this work was to evaluate the participation of free radicals in active Grave's disease evaluating the antioxidant/prooxidant balance. We studied 12 hyperthyroid patients (9 female and 3 male) of $\bar{x}$= 34 years old, T_3= 446 ± 59 ng%; T_4= 19 ± 1 μg%; anti TSH receptor antibodies= 32 ± 6 %; Intake of I_{131}= 59 ± 6 %; coursing $\bar{x}$= 8.3 months of evolution. The parameters evaluated were: 1) Tert-Butyl hydroperoxide intiated chemiluminescense (Ql), 2) superoxide dismutase (SOD) and catalase (CAT), 3) glutathione (GSH) and total plasma antioxidant capacity (TRAP). 10 normal adults were taken as controls (C). Results: Levels of Ql were higher (p<0.005) in Grave's disease= 11236 ± 379 cpm/mg of haemoglobin than controls= 4082 ± 695 cpm/mg of haemoglobin; levels of SOD and GSH were lower in Grave's disease than in controls (SOD= 0.46 ± 0.01 and 0.74 ± 0.02 U/mg de haemoglobin p< 0.005; GSH= 2.34 ± 0.06 and 3.52 ± 0.13 μM/ml of erythrocytes p<0.05); CAT and TRAP did not show significant differences. The high levels of Ql and the low ones of SOD and GSH in erythrocytes suggest the participation of free radicals in hypertyroidism caused by Grave's disease. From these results we could speculate on the benefits of the administration of an antioxidant therapy together with the habitual treatment of hypertyroydism. (Values are expressed as $\bar{X}$ ± ESM).

307. P25 Effect of methimazole (MMI) on T cell recognition of human thyroglobulin (hTg).

H. Sarui, S. Sakata, H. Takuno, I. Matsui, K. Yasuda. The Third Department of Internal Medicine, Gifu University School of Medicine, Gifu 500, Japan.

[Objective] The in vitro effect of methimazole (MMI) on T-cell recognition against human thyroglobulin (hTg) was studied using lymphocytes obtained from different strains of mice immunized with hTg. [Method] Mice with H-2k haplotype (C3H/He, B10BR), H-2d haplotype (Balb/C, B10D2), and H-2b haplotype (C57BL/6, C57BL/10) were immunized with hTg emulsified with FCA. One week after the immunization, regional lymph nodes were obtained. Lymphocytes from the lymph nodes were cultured for 72 hour hTg or Con A in the presence or absence of different concentrations of MMI. Concentration of MMI tested were 10^{-3}M, 10^{-4}M, and 10^{-5}M. Blastogenesis of lymphocytes was evaluated by measurements of the cellular uptake of [^{3}H] thymidine. [Results and discussion] Addition of MMI in the culture medium enhanced ConA-induced blastogenesis of lymphocytes in a dose dependent manner in all the strains of mice. On the other hand, addition of different concentrations of MMI on hTg-induced blastogenesis showed different effects. For example, addition of 10^{-4}M MMI enhanced the blastogenesis in 4 strains of mice (Balb/C, B10D2, C3H/He, B10BR) whereas no significant enhancement was obtained in the rest of two strains of mice. Addition of 10^{-3}M MMI suppressed hTg induced blastogenesis in every 6 strains of mice. addition of 10^{-5}M MMI showed no such effect. It was concluded that MMI has both immunosuppressive and immunoenhancement effects on lymphocytes depending on its concentration.

308. P26 **HODGKIN'S DISEASE IS ASSOCIATED WITH INCREASED ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY AGAINST HUMAN ORBITAL MUSCLE CELLS (ADCC)**

M.D. Ringel, T. Taylor, C.E. Freter, L. Diehl, R. Howard, and K.D. Burman. The Washington Hospital Center, Georgetown University Medical Center, and Walter Reed Army Medical Center, Washington DC.

Patients with Hodgkin's disease (HD) have been reported to develop thyroid function abnormalities and orbitopathy. We studied the incidence of autoantibodies against thyroid antigens and orbital muscle cells in patients with HD (n=20) and controls (n=10). The following serological tests were evaluated: antibodies against TG and TPO, TSI, TBII, TSH, FT4, FT3, western blots for the orbital 64 Kd antigen, and an ADCC LDH release assay using human orbital muscle cells (J. Wall and A. Barsouk). HD patients were subdivided into those treated either with external beam radiation therapy (XRT, n=15) or chemotherapy (MOPP/ABVD, n=5) alone. The ADCC assay was significantly elevated in patients with HD (35% v. 10%, p=0.04) compared with controls. Also, HD patients treated with XRT were more likely to have a positive ADCC than those receiving chemotherapy alone (42% v. 0%, p=0.11). In comparison with controls, patients with HD tended to have elevated anti-TPO antibodies (20% v. 0%, p=0.34) and TSI (10% v. 0%, p=0.15). The incidence of elevated anti-TG antibody (10%) and TBII (0%) were identical in the HD and control patients. There was no difference between HD patients treated with XRT versus chemotherapy for the anti-TG, anti-TPO, TSI and TBII assays. The 64 Kd antigen was found in one HD patient (5%). TSH and free T4 were not significantly different between the control and HD groups; however, the free T3 was lower in the HD group (2.13 pg/ml v. 2.64 pg/ml p=0.013). In summary, these data suggest: (1) ADCC against human orbital muscle cells is increased in patients with HD with a trend suggesting a relationship with thyroidal XRT and (2) autoantibodies against the thyroid gland tended to be elevated in patients with HD, unrelated to treatment modality. Further studies assessing the immunologic abnormalities in Hodgkin's disease are warranted.

309. P27 IMMUNOHISTOCHEMICAL STUDY OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THYROID DISORDER. A. Inagaki, K. Iwase, T. Tsujimura, S. Jimbo and K. Miura. Division of Endocrine Surgery, Fujita Health University School of Medicine, Toyoake, Aichi470-11, Japan.

Vascular endothelial growth factor (VEGF) is a specific mitogen for endothelial cells in vitro and an angiogenic factor in vivo. Its role in other cell types is not yet clear. There is a close relationship between the angiogenesis and the development and spreading of various tumors. VEGF is one of the factors related to angiogenesis of tumors, and its expression is considered to be stronger in malignant tumors. Since VEGF is a widely distributed protein, this property may have relevance to a variety of physiological and pathological proliferative processes. We studied the localization of VEGF in human thyroid tissues with various thyroid disorders to explore its possible involvement in proliferative processes.

Immunohistochemistry was performed on formalin-fixed paraffin-embedded thyroid tissue specimens. Thyroid tissues were obtained by surgical resection from patients with papillary carcinoma, 12; follicular carcinoma, 2; medullary carcinoma, 2; poorly differentiated carcinoma, 1; anaplastic carcinoma, 3; malignant lymphoma, 2; follicular adenoma, 7; adenomatous goiter, 4; Graves' disease, 4. A purified rabbit polyclonal anti-human VEGF antibody and a labelled streptavidin biotin peroxidase complex detection system were used.

VEGF was not identified in normal thyroid follicular cells. Some but not all thyroid tumor cells expressed VEGF in the cytoplasm (papillary carcinoma, 8/12; follicular carcinoma, 1/2; medullary carcinoma, 2/2; poorly differentiated carcinoma, 1/1; follicular adenoma, 3/7; adenomatous goiter, 2/4). In benign follicular adenoma and adenomatous goiter the expression of VEGF was found weakly in small parts of the tumor tissues, whereas in malignant tumors it was found strongly in many cells. However, VEGF was not expressed in anaplastic carcinoma and malignant lymphoma. In Graves' disease the expression of VEGF was not detected either.

Inefficient vascular supply and the resultant reduction in tissue oxygen lead to angiogenesis in order to satisfy the needs of the tissue. VEGF probably functions as a hypoxia-inducible angiogenic factor, and the expression of VEGF is stronger in malignant tumors which need more oxygen supply to proliferate. In thyroid tissues well differentiated carcinomas expressed VEGF stronger than benign follicular tumors, although VEGF was not expressed in anaplastic cartinoma which is an extremely undifferentiated tumor. In Graves' disease composed of nontransformed cells the expression of VEGF was not detected. In conclusion, the expression of VEGF is thought to be affected by the transformation and differentiation of tumors.

310. P28 **IN SITU HYBRIDISATION AND IMMUNOHISTOCHEMICAL STUDY OF THYROPEROXIDASE EXPRESSION IN THYROID TUMORS:** C. de Micco, M. Grino, F. Kopp, S. Garcia. Pathology Institute, Faculty of Medicine, INSERM U38, INSERM U297, Marseille, France.

Changes in thyroid peroxidase (TPO) expression, leading to a decrease in anti-TPO monoclonal antibody MAb 47 fixation by immunohistochemistry (IHC), have been found in malignant thyroid tumors but not in normal and benign thyroid tissues. Our purpose is to study by in situ hybridisation (ISH) in normal and tumor thyroid tissues the expression of TPO m-RNA sequences corresponding to various areas of the TPO molecule and especially to the epitope recognized by MAB 47. MRNA expression was compared with level of TPO synthesis disclosed by IHC. The study was performed on frozen sections of 8 normal thyroids, 20 papillary carcinoma, 5 colloid nodules, 5 follicular adenoma and one normal pancreas. Four synthetic strands of 36 nucleotides labelled by tailing with S35 using 3' terminal transferase were used as antisense probes for ISH: P1 is complementary to sequence 93-129 in exon 2, P2 to sequence 2635-2671 in exon 15, P3 to sequence 2224-2260 which codes for TPO MAb 47 epitope, in exon 12 , and P4 to sequence 2848-2884 in exon 17. Sense probes complementary to P1 to P4 were used as negative controls. IHC was performed with one polyclonal anti TPO antibody (PAb) and with MAb 47 anti TPO monoclonal antibody. Intensity of ISH and IHC reactions was quoted on a semi quantitative scale from 0 to ++. A low level labelling, considered as background and quoted 0, was observed on normal pancreatic tissue with antisense probes and on normal thyroid tissues with sense probes. On normal thyroid a good level of fixation quoted ++ was got in all samples with P1, P2, P4, PAb, MAb 47 and in 3 samples with P3 ; the five other samples reacted only slightly with P3. All colloid nodules and follicular adenoma reacted strongly with the four probes and both antibodies. Results in papillary cancers are summarized in the following table:

Reaction	P1	P2	P3	P4	PAb	MAb47
0	2*	3	9	5	0	5
+	8	9	5	4	6	11
++	10	8	6	11	11	1
Nb. Cas.	20	20	20	20	17	17

*Number of cases

TPO was always found by IHC with PAb, but usually the level of staining was lower than in normal and benign thyroid tissues. With MAb 47 staining appeared null or feeble in 16/17 cases.The level of mRNA found by ISH with P1, P2, and P4 was generally correlated with the amount of TPO disclosed by PAb. The TPO mRNA sequence complementary to P3, which codes for TPO epitope recognized by MAb 47, as the epitope itself, was not or weakly expressed in most of the cases. Thus disappearence of TPO MAb47 epitope in thyroid cancers coincided with reduced expression of the corresponding mRNA sequence. This could be due to a modification in the regulation of TPO synthesis with predominant production of a molecular form lacking the segment recognized by MAbb47, or to another abnormality in TPO mRNA maturation secondary to malignant transformation. Although TPO mRNA with deleted sequences in exon 12 has not been described yet our present results fits better with the first hypothesis.

311. P29 **GROWTH ACTIVITY IN HYPERPLASTIC AND NEOPLASTIC HUMAN THYROIDS AS ESTIMATED BY IMMUNOHISTOCHEMISTY USING ANTIBODY MIB-1.** R. Katoh, C.E. Bray, K. Suzuki, A. Komiyama, A.Hemmi and A. Kawaoi. Department of Pathology,Yamanashi Medical University,Tamaho, Japan

Estimation of growth activity has emerged as a major approach for the determination of the agressiveness, and metastatic potential of a variety of human neoplasms. Up to the present time, however, there have been few studies of the utility of this approach in endocrine tumors. In this study, growth activity (GA) was examined by immunohistochemistry using the proliferation-associated antibody MIB-1 in 232 thyroid lesions. The GA in adenomatous goiter (MIB-1-positive cell rate: 0.73%) and Graves' disease (1.68%) tended to be higher than that in normal tissue (0.19%). The mean value for differentiated thyroid carcinomas (2.00%) was much lower than those of other organ carcinomas (44.67%). Of the thyroid carcinomas, the highest GA value was observed in undifferentiated carcinoma (32.67%). Follicular carcinoma (3.18%) showed a higher GA than papillary carcinoma (1.83%). There was no significant difference between GA of follicular carcinoma and hypercellular adenoma (solid/trabecular or tubular subtype: 3.03%). Widely invasive follicular carcinomas showed a higher GA than minimally invasive cases. No significant correlations between the GA and patient's age, sex, tumor diameter, metastasis or histological features were observed in papillary carcinomas. Familial medullary carcinomas showed a higher GA than sporadic cases. Latent small papillary carcinomas had very low GA. In conclusion, immunohistochemical investigation using the antibody MIB-1 provides useful information concerning the growth of thyroid lesions, and it could lead to increases in our understanding of the basic biological mechanisms of thyroid diseases. Although immunohistochemistry using MIB-1 may be useful in identifying subsets of tumors that will have an agressive course, it seems, in practice, to be of limited value for prediction of whether a tumor is benign or malignant and/or to evaluate the prognosis of patients with thyroid carcinoma.

312. P30 **The natural Protein Kinase Cα Mutant Is present in Human Thyroid Neoplasms.** Prévostel, V. Alvaro, F. de Boisvilliers, A. Martin, C. Jaffiol and D. Joubert. INSERM U401 et Hôpital Lapeyronie, 34094 Montpellier Cedex 05, France.

An altered protein expression of Ca^{2+}-dependent protein kinase C (PKC) isoforms and a point mutation in the PKCα cDNA (position 908 of the nucleotide sequence, position 294 of the amino acid sequence, substitution of an aspartic acid by a glycine) have been previously described in a subpopulation of human pituitary tumors. In this work, we screened 16 thyroid tissue samples (4 follicular adenomas, 5 colloid adenomas, 3 papillary carcinomas, 1 follicular carcinoma and 3 normal tissues adjacent to the tumors) for the presence of the PKCα point mutation and for PKC α, β1, β2, ε and δ protein expression.

Screening for the presence of the PKCα mutant was performed by a subcloning technic. The polymerase chain reaction products were generated using reverse-transcribed cDNAs, subcloned and sequenced (10 clones were routinely sequenced). The PKCα point mutation at position 908 of the cDNA sequence was found in 4 out of the 9 adenomas and in the follicular carcinoma. It was neither detected in the papillary carcinomas nor in the adjacent normal tissues (one was the adjacent normal tissue of the follicular carcinoma; in this sample, genomic DNA and cDNA were used to look for the presence of the mutant), demonstrating the somatic nature of this mutant. Western blot analysis of PKC isoforms showed that the expression of all isoforms was higher in the thyroid neoplasms as compared with their adjacent normal tissue (n=3). It was also higher in the samples containing the PKC mutant (2 follicular adenomas, 2 colloid adenomas and the follicular carcinoma) as compared with the tumors where it was not detected (3 papillary carcinomas and 5 adenomas). Samples could be ordered according to their increasing PKC expression as follows : normal adjacent tissue < follicular adenomas without PKCα mutant ≤ papillary carcinoma < follicular adenomas with PKC mutant ≤ follicular carcinoma with PKC mutant.

In conclusion, the discovery of the PKCα mutant in thyroid neoplasms demonstrates that this mutant is not particular to human pituitary tumors. It is a somatic mutation and its presence is concomitant with high levels of all of the PKC isoforms analyzed.The presence of the PKC mutant in thyroid neoplasms raises the question of its importance in thyroid tumorigenesis.

313. P31 IMMUNOHISTOCHEMICAL STUDY OF THROMBOSPONDIN AND ITS RECEPTORS IN NORMAL THYROID AND IN BENIGN AND MALIGNANT THYROID TUMORS

M. PATEY*, B. DELEMER***, D. CLAISSE**, G. BELLON**, M. PLUOT*, B. HAYE**

* Department of Pathology. C.H.U. Robert Debré, REIMS, FRANCE.
** Department of Biochemistry. Université Reims Champagne Ardenne. REIMS, FRANCE.
*** Department of Endocrinology. C.H.U. Maison Blanche. REIMS, FRANCE

Thrombospondin (TSP) is an extracellular matrix glycoprotein which possesses multiple cell adhesive domains among them a cysteine-serine-valine-threonine-cysteine-glycine (CSVTCG) sequence and a Arg-Gly-Asp (RGDA) domain interacting with the CD36 receptor and the α_v ß3 integrin receptor respectively. TSP has been previously reported to be synthetized and secreted by culture thyroid cells (Prabakaran *et al* 1993, Bellon *et al* 1994).
The aim of this study was to localize TSP and its receptors in normal, benign and neoplasic thyroid tissues since TSP has been reported to play a role in tumor progression.

Material and methods

Fresh tissue samples from 28 histologically confirmed cases of thyroid carcinoma including 6 papillary, 3 follicular, 1 anaplastic carcinoma, 6 cases of normal thyroid tissue, 6 cases of adenoma and 6 cases of nodular goiters were investigated. Frozen sections were examined by an immunohistochemical method using Labeling Streptavidin Biotin immunoperoxidase staining procedure with the following antibodies : monoclonal (Mabs) antihuman TSP (Sigma), monoclonal antihuman CD36 (Immunotech) and polyclonal antihuman ß3 integrin (Euromedex) with positive and negative controls.

Results

Mabs anti-TSP reacts strongly with the desmoplastic stromal area of papillary carcinoma and with the rare fibrous richly vascularized stroma of follicular and anaplastic carcinoma. In constrast TSP is poorly expressed reduced to the vessel walls in normal tissue and benign adenoma. In nodular goiters, TSP is only found in areas of dense fibrous scars.
The CD 36 receptor is restricted to the endothelial cells of capillaries.
On the other hand ß3 integrin receptor is expressed by thyrocytes and the intensity of staining seems to correlate with that of TSP.

Conclusion

The TSP and its receptor ß3 integrin expression increase in neoplasic thyroid tissues. These findings suggest that the TSP may play a important role in tumor progression and in tumor neovascularisation.

314. P32 ALTERED METABOLISM OF SUPEROXIDE RADICALS IN THYROID CANCER

DJ Conway, K. Sheahan, J. Jacob, NJ O'Higgins and PPA Smyth. Endocrine Laboratory, Departments of Medicine & Surgery, University College Dublin & St Vincent's Hospital, Dublin.

Cytochemical measurement of Glucose-6-Phosphate Dehydrogenase (G6PD) activity is routinely performed under an atmosphere of N_2 to prevent H^+ reacting preferentially with O_2 and thus being unavailable to reduce a neotetrazolium (NT) H^+ acceptor. G6PD activity was minimal when benign tissues were reacted under an atmosphere of O_2. An unexplained finding was that in many malignant tissues a significant amount of G6PD activity is retained under O_2. In this study G6PD measured in frozen sections from benign thyroid tissues (N=17) gave a mean of 36.4±1.4 U under O_2. In contrast 6 malignant thyroid tissues (3 follicular, 2 papillary and 1 lymphoma) demonstrated O_2 insensitivity in that significant G6PD activity was retained under O_2 (N_2 38.6±2.0 U; O_2 35.5±2.0U; 95% G6PD activity retained). O_2 insensitivity in malignant tissues has been attributed to the inability of such tissues to remove superoxide radicals. In order to test the ability of thyroid tissue to metabolise superoxide radicals, frozen sections were incubated with the enzyme superoxide dismutase (SOD; 42-420IU/ml) prior to G6PD measurement. Addition of SOD abolished the O_2 insensitivity of G6PD in 3 malignant thyroids, 2 follicular and 1 lymphoma, rendering their behaviour similar to benign tissues. G6PD activity in N2 was unaffected by SOD addition.

The findings provide a theoretical basis for the O_2 insensitivity phenomenon based on failure of malignant tissues to metabolise O_2 radicals.They demonstrate that this investigation is a true metabolic index differentiating between benign and malignant thyroid tissues with the potential of providing a new metabolic test to complement existing histological or cytological diagnosis of thyroid carcinoma.

315. P33 TYROSINE PHOSPHORYLATION PLAYS AN IMPORTANT ROLE IN IFNγ INDUCED MHC CLASS I AND CLASS II EXPRESSION IN THYROID CANCER. Y. Suzuki, Y. Tanaka, H. Nakamura, K. Kobayashi and T. Mori, 2nd Department of Surgery, Tottori University, Yonago

Major histocompatibility complex(MHC) antigens on cancer cells are involved in a variety of immune functions relating to tumor immunity. MHC class I molecules have been shown to act as restriction elements in the lysis of target cells by cytotoxic T lymphocytes(CTL). MHC class II molecules are known to present antigens to helper T cells, and to induce or regulate autologous T helper cell activation. Cancer cells, however, reduce their molecules and escape the host's immune reactions. It is well known that IFNγ(interferon gamma) upregulates the expression of MHC molecules and restores immunogenesity of cancer cells. Nevertheless the mechanisms of IFNγ controlling MHC expression on cancer cells still have not been elucidated, especially in MHC class I. Recent biochemical studies indicate that tyrosine phosphorylation by JAK tyrosin kinase acts as an important step for the mechanisms of a signal transduction system activated by IFNγ. In this report, therefore, we examined the role of tyrosine phosphorylation in the signal transduction of IFNγ induced MHC expression in a thyroid cancer cell line using tyrosine kinase inhibitors herbimycin A. Herbimycin A inhibits both IFNγ inducible MHC class I and class II expressions (a decreases in mean fluorescence up to 30%), which were analyzed by flow cytometer. In relation to this phenomenon, laser microscopic detection with an FITC labeled anti-phosphotyrosine mAb showed that IFNγ induces an increase in protein tyrosine phosphorylation (an increase in mean fluorescence up to 300%) within 5 min. Herbimycin A also inhibited the IFNγ induced enhancement of tyrosine phosphorylation. In conclusion, tyrosine phosphorylation plays an important role in both IFNγ induced MHC class I and class II expression. A JAK-STAT (signal transducers and activators of transcription) signal transduction system was strongly suspected the association of IFNγ induced MHC expression, and particularly MHC class I, because previous efforts failed to associate IFNγ induced MHC class I expression with any other signal transduction systems.

316. P34 **SEQUENTIAL CHANGES IN SERUM TPO FOLLOWING RADIOIODINE THERAPY OF PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA.** M. Ozata , *H. Bayhan , **N. Bingöl , *S. Dündar , *S. Ilgın , İ. Kurt , Z. Beyhan, A. Çorakcı and M.A. Gündoğan. Departments of Endocrinology and Metabolism and *Nuclear Medicine, Gülhane School of Medicine 06018 Etlik-Ankara and **Bayındır Medical Center, Ankara, Turkey.

To determine the detectability and the time course of serum thyroid peroxidase (TPO) levels before and 1,2,4 and 6 months after ^{131}I administration, we evaluated TPO in 13 selected patients (mean age 40.2 ± 13.2 yr) with differentiated thyroid carcinoma (DTC) whose sera did not contain antimicrosomal or antithyroglobulin antibodies. All patients received ^{131}I therapy 6 or 8 weeks after thyroid surgery for ablation of the postsurgical thyroid remnant. Serum samples were also collected from 10 normal subjects. Measurement of TPO was carried out by a new commercially available immunoluminometric assay (LUMItest TPO, B.R.A.H.M.S. Diagnostica GmbH, Berlin, Germany) with a sensitivity of 30 pg/mL. Serum thyroglobulin (Tg) was measured by RIA with a sensitvity of 2.6 ng/mL before and 6 months after ^{131}I administration. In all patients a standard-total body scan was obtained before and 6 months after ^{131}I administration. TPO was undetectable in all sera from normal subjects. However, serum TPO became detectable in all patients with DTC during the study while rescans are either negative or positive and appeared not to be related to the radioiodine dose given, histology of the thyroid tumor, residual thyroid volume, TSH levels, or age of patients. However, a significant negative correlation was present between TPO levels before ^{131}I administration and the time from surgery ($r = -0.82$, $P < 0.001$). Six of 13 patients had increased TPO levels 1 month after ^{131}I administration. Serum TPO levels tended to decrease during follow-up in most patients (7 of 10) with a negative rescan, although individual variability occurred. However, in 3 patients with positive rescans, TPO levels increased during follow up. We found no correlation between Tg and TPO levels measured before and six months after ^{131}I administration, and Tg levels also decreased in most patients with a negative rescan. Our results suggest that 1-TPO is undetectable in normal subjects. 2-TPO is detectable in all patients with DTC either before or after ^{131}I administration and appears to be related only to the time from surgery but not to age, histology of tumor, residual thyroid volume or radioiodine dose given. 3-It is not clear why TPO levels are not correlated with Tg in patients with a negative rescan. Perhaps, the amounts of Tg and TPO released are different after thyroid surgery or ^{131}I administration. 4-Decreased TPO levels during follow-up might be a marker for the success of radioiodine therapy. However, long-term studies in a large number of patients are needed to clarify this finding.

317. P35 IMMUNOHISTOCHEMICAL STUDY OF SMALL HEAT SHOCK PROTEIN, HSP28 IN VARIOUS THYROID DISORDERS. K. Iwase, K. Kato*, T. Tsujimura, A. Inagaki, S. Jimbo and K. Miura. Division of Endocrine Surgery, Fujita Health Univ. School of Medicine, Toyoake, Aichi 470-11, *Institute for Developmental Res., Aichi Prefecture Colony, Aichi 480-03, Japan.

Heat shock and other environmental and pathophysiologic stresses stimulate synthesis of heat shock proteins (HSPs). These proteins may active in development of resistance to stressful conditions and agents such as oxygen free radicals or cytotoxic drugs. HSP28 is an important small stress protein found in human cells, both cancer and normal cells. Recently described immunological functions and role in cell differentiation for heat shock proteins prompted us to determine the localization of HSP28 in human thyroid tissues with various thyroid disorders.

Immunohistochemistry was performed on formalin-fixed paraffin-embedded thyroid tissue specimens. Thyroid tissues were obtained by surgical resection from patients with various thyroid disorders (papillary carcinoma, 17; follicular carcinoma, 1; medullary carcinoma, 3; anaplastic carcinoma, 3; malignant lymphoma, 2; follicular adenoma, 5; adenomatous goiter, 3; Graves' disease, 10). A purified rabbit polyclonal anti-human HSP28 antibody and a labelled streptavidin biotin peroxidase complex detection system were used for these studies.

HSP28 was identified in cytoplasm of normal follicular cell, but not in the follicular lumen. Some but not all thyroid tumor or hyperplastic follicular cells also expressed this protein in cytoplasm. Many papillary carcinomas expressed HSP28, predominantly in the tumor cells which showed papillary growth or existed at the edge of the tumor. Other malignant tumors expressed HSP28 similarly at the edge of the tumor, but all 3 anaplastic carcinomas did not express it anywhere. Follicular adenoma and adenomatous goiter expressed it in the cells which grew papillary or composed micro-follicles. HSP28 was not expressed in the tumor or hyperplastic tissues with fibrous or cystic degeneration. Many Graves' disease strongly expressed HSP28 in the well proliferated or papillary growing follicular cells, but not in large follicles which may be affected by a long term anti-thyroidal drug therapy.

HSP28, besides its role in various stressful conditions, may function as a molecular chaperone and in signal transcription pathways of different cell regulators. The correlation of the presence of HSP28 with hormone receptors in breast cancers, and that with the degree of tumor differentiation in some tumors have been observed. In conclusion, HSP28 seems to relate with cell proliferation and differentiation in the thyroid since it exists predominantly in well proliferative tumor cells and hyperplastic follicular cells, especially in papillary growing cells.

318. P36 IODINE THERAPY REDUCES INDUCED CARCINOGENESIS IN MAMMARY GLANDS OF NORMAL AND IODINE DEFICIENT RATS. B.A. Eskin, C.E. Grotkowski and C. P. Connolly, Medical College of Pennsylvania and Hahnemann University, Philadelphia, Pennsylvania, U.S.A.

Iodine deficiency (ID) produces fibrocystic disease (FCD) in mammary glands of rats. Furthermore, iodine (I_2) has been seen to be more efficacious than iodide in clearing the FCD. Employing this specificity and dosage, this study was done to determine whether carcinoma induction is enhanced in ID and how I_2 affects induced cancers. Mammary gland cancers in both ID and normal rats were compared and evaluated after I_2 treatment. Two groups of one hundred sixty (160) Sprague-Dawley rats were divided and maintained on either a normal or ID diet. ID was induced and maintained by Remington Diet and doubly distilled drinking water (DDW). Each subdivision was given a 15 mg DMBA bolus or a sham gavage. Half of each group received 20 μg/d of diatomic iodine (I_2) formulation in drinking water, started one week before the carcinogen. Tumors produced were measured bi-weekly with a special caliper through weeks 14-22 following the bolus. The mean tumor volume (MTV) was calculated using the formula: $4/3\pi([L+W]/2)$, with the greatest diameter the length (L), and corresponding orthogonal diameter its width (W). Histopathology was determined by random biopsies and at necropsy. Animals were cared for by the NIH Guide standards. The results show that ID rats produce statistically higher ($p<0.01$) MTV after DMBA than normal iodine rats. Treatment with iodine early effectively reduces MTV in both ID and euiodine rats (<0.01, <0.01); however, the effect appears slightly greater in ID animals (42.8 vs 31.6%). Mammary gland histopathology was studied randomly and shows that the level of malignancy appeared to be reduced in rats given iodine. From this data, it appears that ID causes an increase in the MTV of rat mammary gland tumors induced by DMBA. Further, iodine (I_2) in low doses reduces the tumor size and histologic malignancy of DMBA carcinogenesis in both ID and euiodine rats. Further research evaluating dosages and histopathology are in progress.

319. P37 **EFFICACY OF VITAMIN D3 AND ITS ANALOG,22-OXA-1,25-DIHYDROXYVITAMIN D3(OCT) ON TSH DEPENDENCY,AND INHIBITION OF PROMOTION IN THE THYROID ANAPALASTIC CARCINOMA CELL LINE,TTA-1.**H.FURUKAWA,S.SUZUKI,K.KIMAN, A.TSUCHIYA, R.ABE, The Department of surgery II, Fukushima Medical College,Fukushima,Japan

Anapalastic carcinoma of thyroid is uncommon and is associated with a poor prognosis. Therefore, there is not found an effecive treatment for this carcinoma. We have examined the TSH dependency observed in differentiated thyroid carcinoma and the inhibition of promotion by 1 α ,25(OH)2D3(VD3) and its analog,22-oxa-1,25-Dihydroxyvitamin D3(OCT) without hypercalcemia in the thyroid anapalstic carcinoma cell line,TTA-1 .

TTA-1, established by Dr.A.Yoshida ,and TAC-1(thyroid anapalastic carcinoma cell) which has been cultured from primarily resected material in IInd Dept. Surgery of Fukusima Medical College were used for experiment. The control were primary cultured cells ,TPC-1,2,3,4, which were all papillary carcinoma of thyroid obtained in the IInd Dept. Surgery. VD3 and OCT were given by Chugai Pharm.Co.. Cells were cultured in Dalbecco Modified Eagle medium supplemented with 10% fetal bovine serum at 37C in a humidified atmosphere of 5% CO2. Viability tumor cells were comfirmed by succinate dehydrogenase inhibition(SDI) method.

Growth of TPC-1,2,3,4,and TAC-1 were promoted depend on dose of TSH, but TTA-1 was proliferated only at the high dose of TSH. Cell growthes in VD3 additive culture were indicated all diphasic pattern at the point of 10-9M/ml. OTC was more effective inhibition in cell growth of anaplastic carcinoma cell lines than in that of papillary carcinoma cell lines.

Conclusively, high dose TSH , low dose VD3 and OTC might be useful for the inhibition of growth of anapastic thyroid cancer.

320. P38 **BOTH THYROID-SPECIFIC AND UBIQUITOUS FACTORS ARE NECESSARY TO INDUCE HORMONAL REGULATION OF RAT THYROPEROXIDASE GENE PROMOTER.** **L. Ortiz, P. Aza-Blanc and P. Santisteban. Instituto de Investigaciones Biomédicas (CSIC). Arturo Duperier 4. 28029 Madrid. (Spain).**

The mechanism for hormonal regulation of rat thyroperoxidase (TPO) gene transcription is well known. Its promoter has been characterized and different transcription factors bind to this promoter. Some of them are ubiquitous (UFB) and some thyroid-specific (TTF-1, TTF-2 and Pax-8). We have recently demonstrated that the TPO promoter activity is hormonally regulated by TSH/cAMP and insulin/IGF-I mainly through the *cis*-regulatory element where the transcription factor TTF-2 binds. This *cis* element acts as a hormone response element since heterologous promoter constructs containing four, eight or twelve tandem repeats of an oligonucleotide that includes the TTF-2 binding site increase their activity in response to TSH/cAMP and insulin/IGF-I. The fact that a high copy number of the TTF-2 binding site is needed to work efficiently as a hormone response element suggest a role for the other transcription factors either TTF-1, Pax-8 and/or UFB. ***The aim*** of the present work was to demonstrate a role for such transcription factor binding sites in the TPO promoter context and if these sites affect the hormonal response of the TTF-2 binding site.

Different constructs of the TPO promoter, fused to the luciferase reporter gene, were made : (1) Containing only the TTF-1/Pax-8 binding site. (2) The previous one plus the TTF-2 binding site, maintaining or not the endogenous distance between both sites. (3) The previous ones plus the UFB/TTF-1 binding site. These constructs were made either in sense or antisense orientation of the different elements. Then were transfected to FRTL-5 cells and their response to TSH/cAMP and to insulin/IGF-I were determined. ***The results*** obtained have shown that a unique binding site of any transcription factor is not sufficient to obtain a complete hormonal response of the TPO promoter. To obtain an efficient hormonal induction of the TPO transcription with only one TTF-2 binding site, is absolutely necessary the precise position of the UFB binding site. Moreover, TTF-1/Pax-8 binding site acts amplifying both basal and hormonaly regulated transcription of the TPO. In the absence of the TTF-2 binding site no hormonal response was obtained, observation that confirm the importance of this factor in the regulation of the TPO promoter activity by hormones. The fact that a precise distance between UFB and TTF-2 binding sites must be maintained, together with previous data of Francis-Lang et al. (Mol. Cell. Biol. 12, 576-588, 1992), showing that mutations that inhibiting the UFB binding also affect the TTF-2 binding, suggest a possible interaction between this two factors.

Looking the UFB binding sequence we found a very high similarity with half of the CTF-1/NF-1 binding sequence. In order to demonstrate the previous observed identity, we made electrophoretic mobility shift assays with nuclear extracts from FRTL-5 thyroid and from He-La cells (that contain high amount of CTF-1/NF-1 factor). As synthetic oligonucleotide were used the UFB binding site and the canonical CTF-1/NF-1 binding sequence. By competition and super-shift assays using α-CTF-1/NF-1 antibodies, our preliminary results suggest that UFB could be the constitutive CTF-1/NF-1 transcription factor or a protein very close related. Demonstrate this homology is important for future studies of how the thyroid-specific transcription factors contact with the basic transcriptional machinery in order to transcribe the TPO gene.

Supported by grants BIO CT-93.0454 (CE) and DGICYT PM-92-0012 (Spain)

321. P39 RELATIONSHIP BETWEEN THE EXPRESSION OF THYROID-SPECIFIC GENES AND THEIR TRANSCRIPTION FACTORS IN HUMAN THYROID TUMOR CELL LINES. P. Ros [1], D. Rossi [1], C. Domínguez [1] and P. Santisteban [1]. (1) Instituto de Investigaciones Biomedicas. CSIC. (2) Servicio de Endocrinología Pediátrica, Hospital Ramón y Cajal. Madrid (Spain)

TTF-1 and Pax-8 are thyroid transcription factors responsible for the tissue-specific Thyroglobulin (Tg) and Thyroperoxidase (TPO) gene expression. Both of them bind to Tg and TPO gene promoter. Furthermore, it has been recently reported that TTF-1 also binds to TSH receptor (TSH-R) gene promoter. These factors have been cloned disclosing that they contain homeo and paired boxes respectively. The importance of this kind of genes is their implication in cell differentiation. In the case of TTF-1 and Pax-8 is accepted that both are determinants of thyroid cell phenotype since oncogene transformed thyroid cells and thyroid neoplasias loss or have altered their expression. The availability of three human thyroid cell lines from different carcinomas (follicular (FRO), papillary (NPA) and anaplastic (ARO)), give us the opportunity to study the correlation between Tg, TPO, and TSH-R gene expression and their thyroid transcription factors TTF-1 and Pax-8. The cells were a gift from Dr. J. Fagin, (Los Angeles, CA).

Total RNA were isolated from thyroid and non thyroid cells. As thyroid cells we have used the above cells, FRTL-5 cells and normal human thyroid tissue. As a non thyroid cells we have used He-La, COS-7 and Hep-G2 cells. After northern-blot analysis, RT-PCR and hybridization with the different cDNA probes, no Tg, TPO nor TSH-R gene expression was found in all the carcinoma thyroid cells line. Since the expression of these genes is controlled by the thyroid transcription factors TTF-1 and Pax-8, we analyzed the expression of these factors by northern-blot, RT-PCR and western-blot analysis. The results obtained have indicated the virtual absence of both thyroid transcription factors in the FRO, NPA and ARO human cells. All the genes analyzed were positive in the control human thyroid tissue and in the FRTL-5 cells. Therefore, this data confirm that the absent of Tg, TPO and TSH-R gene expression is associated with that of thyroid-specific transcription factors also in human thyroid cells line. We study later the activity of the Tg, TPO and TSH-R promoters in all the cells line. The different promoters fused to reporter genes were transfected to the different cells type. The results shown very low activity of Tg , TPO and TSH-R promoter in NPA, FRO and ARO human thyroid cells as well in non thyroid cells. Co-transfection of each promoter, together with an expression vector for TTF-1 increase the activity of Tg and TPO promoter. Although this factor binds to the TSH-R promoter the increases found, after co-transfection, are lower that those found with Tg and TPO promoter. This finding suggests that TTF-1 is a crucial factor for Tg and TPO gene expression. However, may be another factors different of TTF-1 could be more important for the control of TSH-R gene expression .

(Supported by Grants : DGICYT PM92-0012 (Spain) and BIO-CT93.0454 (CE)

322. P40 THE REASON WHY THE THYROID SECRETES MAINLY THYROXINE. J.J.M. de Vijlder, M.T. den Hartog. Academic Medical Center, University of Amsterdam, Pediatric Endocrinology, Emma Children's Hospital AMC, The Netherlands.

Thyroid hormone is secreted by the thyroid gland mainly as thyroxine (T_4) whereas only a minor amount is secreted as 3,5,3' triodothyronine (T_3). This phenomenon is not yet clearly understood. Biosynthesis of thyroid hormone is an oxidative coupling reaction between two iodotyrosine-residues within a thyroglobulin (Tg) molecule. This reaction is catalyzed by thyroid peroxidase (TPO). In accordance with thyroid hormone secretion T_4 formation in Tg is greatly favored over that of T_3. Besides T_4 and T_3 we also found 3,3',5' triiodothyronine (rT_3) and traces of 3,3' diiodothyronine (T_2) in purified human (h) highly iodinated Tg.

In order to study the mechanism of iodothyronine formation and to understand why mainly T_4 is formed, iodine-free hTg was non-enzymatically iodinated. After removal of non-incorporated iodide this iodothyronine-deficient Tg containing 20 mol I/mol Tg, was incubated with lactoperoxidase (LPO) and a hydrogen peroxide generating system at a pH ranging between 4 and 9. The content of T_4, T_3, rT_3 and T_2 in Tg was measured by radioimmunoassays after hydrolysis of Tg. T_4 formation markedly increased between pH 4 and pH 8 following a curve similar to the ionization curve of the phenolic hydroxyl group of di-iodotyrosine (DIT) with a pKa value of 6.5. T_2 formation was only observed at high pH, starting to increase from pH 6, following the phenolic hydroxyl ionization curve of mono-iodotyrosine with a pKa of 8.7. T_3 and rT_3 showed patterns that could be interpreted as a combination of both ionization curves. These results demonstrate that ionization is a requirement for optimal thyroid hormonogenesis and since ionization facilitates the removal of an electron under oxidative conditions the pH dependence agrees with the involvement of phenoxy radicals in thyroid hormonogenesis. Since the phenolic hydroxyl group of DIT, and not of MIT, is largely ionized under physiological conditions, it is understandable that mainly T_4 is formed and secreted by the thyroid.

323. P41 MENADIONE (VITAMIN K3) IS THE MOST POTENT STIMULATOR OF SUPEROXIDE AND HYDROGEN PEROXIDE IN THE THYROID CELL. M. Sugawara, K. Wen, West Los Angeles VA Medical Center and University of California School of Medicine, Los Angeles, California

Hydrogen peroxide (H_2O_2) is needed for thyroid hormone formation. It has been believed that H_2O_2 in the thyroid cell is produced without producing superoxide (O_2^-), a precursor of H_2O_2. We describe menadione mediated O_2^- and H_2O_2 generation in the thyroid cell. Methods: FRTL-5 rat thyroid cells cultured in the presence of 2 mU/ml TSH or absence of TSH were exposed to 0 to 50 μM menadione for 1 h at 37 C. The amount of O_2^- and H_2O_2 produced during 1 h incubation was measured by the cytochrome-c method and the homovanillic acid-horseraddish peroxidase method, repectively. In some experiments, cells were preincubated with 25 μM menadione for 30 min, followed by washing to remove menadione; then, H_2O_2 formation was measured. The amount of H_2O_2 generated by phorbol myristate acetate (PMA) and ionomycin (calcium ionophore), known H_2O_2 stimulators in the thyroid cell, was compared with that by menadione. Results: The amount of H_2O_2 generated by menadione was always 2 to 3-fold greater than that produced by PMA or ionomycin. Menadione produced O_2^- in FRTL-5 cells; PMA or ionomycin did not produce O_2^-. Washed cells after menadione treatment for 30 min did not stimulate H_2O_2 generation, suggesting that menadione in the extracellular space stimulated H_2O_2 generation. Addition of SOD to the medium increased menadione mediated H_2O_2 production by 20%, indicating conversion of O_2^- to H_2O_2 in the extracellular space. Menadione-mediated H_2O_2 generation was dependent on the presence of extracellular calcium and increased in the presence of TSH and not by addition of PMA. Superoxide formation by menadione was not dependent on calcium or TSH. Cell free experiments showed that both the soluble fraction and the membrane fraction produced O_2^- and H_2O_2 in the presence of menadione and 100 μM NADPH. However, the amount of O_2^- and H_2O_2 generated by the membrane fraction was much greater than that by the soluble fraction. Nitric oxide synthase, cytoplasmic enzyme, produced O_2^- and H_2O_2 in the presence of NADPH and menadione. Conclusions: 1. Menadion was the most potent stimulator of O_2^- and H_2O_2 in the thyroid cell. 2. The mechanism of H_2O_2 generated by menadione appears to involve calcium independent O_2^- formation and calcium dependent H_2O_2 formation through the interaction between NADPH oxidation and menadione reduction. 3. Nitric oxide synthase can also be the source of H_2O_2 as long as NADPH and menadione are available.

324. P42 THYROTROPIN MODULATES THE NADPH OXIDASE ACTIVITY IN PRIMARY CULTURE OF PORCINE THYROID CELLS. [1]D.P. Carvalho, [2]B. Haye, [1]C. Dupuy, [1]J. Pommier and [1]A. Virion, [1]Unité de Recherche sur la Glande Thyroïde, INSERM U-96, Bicêtre and [2]Université de Reims Champagne Ardenne, UFR Sciences, ERS-CNRS F0017, Reims, France.

Thyrotropin (TSH) promotes the expression of thyroid-specific proteins, such as thyroglobulin and thyroperoxidase, in primary culture of thyroid cells. H_2O_2 generation is a limiting step on thyroid hormone biosynthesis, and its production is also believed to be under TSH control. Thus, in the present study we evaluated the role of TSH on the NADPH oxidase and cytochrome c reductase activities, which are the putative enzymes involved on H_2O_2 generation in the thyroid gland. The NADPH oxidase H_2O_2 generating activity was measured using the horseradish peroxidase-scopoletin method. Porcine thyroid cells in primary culture were treated with TSH (0.1 mU/ml) for 4 days in the presence or in the absence of 12-*O* -tetradecanoylphorbol 13-acetate (TPA; 10^{-7} M) or cycloheximide (10^{-4} M). NADPH oxidase activity in the cellular particulate fractions (3,000 x g, 20 min) was significantly increased by TSH (0.1 mU/ml TSH: 35.9 ± 4.7 nmol H_2O_2 / h / mg protein ; control: 1.8 ± 0.8 nmol H_2O_2 / h / mg protein; $p< 0.001$) in a dose dependent manner, with a maximal effect at 1 mU/ml TSH. The increase in NADPH oxidase activity produced by TSH was partially blocked by TPA (TSH+TPA: 11.6 ± 2.3 nmol H_2O_2 / h / mg; $p< 0.005$) or cycloheximide (TSH+cycloheximide: 14.2 ± 2.9 nmol H_2O_2 / h / mg; $p< 0.01$). Furthermore, a 4-day forskolin (10^{-5}M) treatment mimicked TSH action on the NADPH oxidase activity (forskolin: 54.3 ± 10.2 nmol H_2O_2 / h / mg). On the other hand, the cytochrome c reductase activity was neither changed by TSH (0.1 mU/ml TSH: 143 ± 12.5 nmol NADPH oxidized / h / mg; control: 197.9 ± 39,4 nmol NADPH oxidized / h / mg) nor by forskolin (158.9 ± 19.4 nmol NADPH oxidized / h / mg) treatments, showing that this enzyme is constitutive, and might play no major role on the thyroid H_2O_2 generating system. Our results reinforce the idea that NADPH oxidase is the enzyme responsible for H_2O_2 generation in the thyroid gland, since its activity is strongly regulated by TSH, possibly through the cyclic AMP cascade. In conclusion, the NADPH oxidase seems to be another marker of thyroid differentiation as well as thyroperoxidase and thyroglobulin, and therefore could play a key role in thyroid hormone production.

* D.P. Carvalho is recipient of a fellowship from the CNPq, Brazil.

325. P43 NADPH OXIDASE FROM THYROID PLASMA MEMBRANE : IRREVERSIBLE ATP ACTIVATION AND ATP DEPENDENT Ca^{2+} DESENSITIZATION. Y. Gorin, C. Dupuy, J. Pommier and A. Virion, Unité de Recherche sur la Glande Thyroïde, INSERM U-96, Bicêtre, France.

The thyroid plasma membrane contains a Ca^{2+}-dependent NADPH oxidase which provides H_2O_2 for thyroid peroxidase-catalyzed biosynthesis of thyroid hormone. The Ca^{2+} effect was reversible, *in vitro*, with particulate fraction or plasma membrane and did not require ATP-Mg^{2+}. This could be mediated by an inhibitory protein component. However, fully active enzyme could be obtained in the absence of Ca^{2+} by limited proteolysis with α-chymotrypsin or by treatment with $ZnCl_2$. Incubation of porcine thyroid homogenate with ATP-Mg^{2+} before preparing the particulate or plasma membrane fraction induced : i) a 2-3 fold irreversible increase in NADPH oxidase activity ii) Ca^{2+}-desensitization of the enzyme (30% -100%) together with enhanced activity. These ATP effects were Ca^{2+}-dependent. The ATP effects were reproduced by pyrophosphate and the nonhydrolyzable ATP analog, AMP-PNP. Otherwise, incubation of the homogenate with a serine protease and cysteine protease inhibitor cocktail inhibited the ATP activating effect, but not the ATP-dependent desensitization. Our results suggested that the ATP effects do not involve protein kinase or ATPase activities. Moreover, the ATP activating effect and ATP-dependent Ca^{2+} desensitization were shown to be independent. ATP activation involved a proteolytic activity and could be due to an irreversible change in the enzyme itself or its environment via ATP binding to a site yet to be determined. The irreversible ATP-dependent desensitization occured only with the membrane fraction incubated with a 3,000xg supernatant obtained from the native homogenate. This effect was not observed with the 105,000xg supernatant of the same homogenate, indicating that factors implicated in the desensitization process are associated with the light particulate fraction. This Ca^{2+} independent state of NADPH oxidase and activation of the enzyme could be physiological and occur at the apical membrane during exocytosis or endocytosis, allowing enzymatic activity to continue after the calcium level has returned to the pre-trigger state. Ca^{2+} and ATP could thus provoke *in vivo* a cascade of events involving associated proteases or other factors and thus bring about changes in the enzyme activity.

326. P44 **ENZYMATIC ACTIVITY OF HUMAN THYROID PEROXIDASE PRODUCED IN INSECT CELLS IN THE PRESENCE OF HEMATIN.** Ji-Lao Fan and G.S. Seetharamaiah, Department of Microbiology/Immunology, University of Texas Medical Branch, Galveston, TX 77555.

Thyroid peroxidase (TPO) is an essential enzyme for thyroid hormone biosynthesis and is an autoantigen against which antibodies are found in a number of autoimmune thyroid disorders. Large quantities of pure TPO are essential for understanding its structure and role in normal thyroid function and thyroid diseases. Therefore, we produced large quantities of human TPO (hTPO) using a baculovirus expression system and purified the protein to homogeneity using high-performance liquid chromatography. The purified protein was identified as hTPO by enzyme-linked immunosorbant assay, western blot analysis, and amino acid sequence analyses. Carbohydrate analysis of the recombinant hTPO showed that the protein is glycosylated and mannose is the major oligosaccharide. We have extended the carbohydrate analysis by establishing the occurrence of N-acetyl galactosamine which gave strong indication that hTPO contained O-glycosyl moietes. Purified hTPO reacted specifically with sera from patients with Hashimoto's thyroiditis. Crude as well as purified hTPO did not show any enzymatic activity when produced in sf9 insect cells grown in serum free medium. Whereas, hTPO produced in sf9 insect cells grown in the presence of 10% fetal bovine serum containing 1ug/ml of hematin was enzymatically active. However, specific enzymatic activity of the recombinant hTPO was found to be much lower than that often found with hTPO purified from thyroid tissue. This difference in enzymatic activity might reflect improper processing of the protein in insect cells. Nevertheless, availability of purified hTPO in relatively large quantities should allow further structural and immunological studies.

327. P45 **CONSTITUTIVE cAMP STIMULATION DECREASES MYC/MAX AND AP-1 SITE BINDING PROTEIN EXPRESSION IN FRTL-5 THYROID CELLS.** M.A. Zeiger, P. Catureguli, M.A. Levine and M. Saji. Departments of Surgery, Pathology and Medicine, Johns Hopkins University, Baltimore MD 21205

We have created a permanently transfected FRTL-5 cell line (TGCT8) in which the thyroglobulin (TG) promoter directs expression of the cholera toxin (CT) A1 subunit. These cells recapitulate the activating mutations of Gsα that are present in a subset of thyroid adenomas and well-differentiated thyroid carcinomas and demonstrate constitutively elevated cAMP levels. More importantly, these cells grow in nude mice and result in poorly differentiated tumors that are metastatic to lung. We know that both c-myc and c-fos play a critical role in the control of cell proliferation and that up to 60 % of thyroid carcinomas exhibit increased mRNA expression of these oncogenes. In addition, we know that both TSH and insulin/IGF-1 increase c-myc and c-fos mRNA expression in FRTL-5 cells. We therefore examined TGCT8 cells for the expression of myc/Max and AP-1 site binding proteins.

TGCT8 cells were cultured with or without 1 μg/ml insulin (a strong stimulator of TG promoter) for 2 days; wild type FRTL-5 cells were cultured with insulin and/or 100 ng/ml CT as control. In order to measure myc/Max and AP-1 site binding proteins, oligonucleotides representing consensus sequences of these binding sites were labelled with [^{32}P]ATP, respectively. Electrophoretic mobility shift assays (EMSA) were performed utilizing the [^{32}P]labelled oligonucleotides and nuclear protein isolated from TGCT8 and wild type FRTL-5 cells. Each oligonucleotide formed one specific complex with nuclear protein from these cells. Specific antibodies against fos family member showed that the AP-1 DNA/protein complex contained c-fos, fra2 and junB. Basal levels of myc/Max and AP-1 site binding protein were equal in TGCT8 and wild type cells. Both CT and insulin decreased these protein levels in wild type cells. Insulin treatment of TGCT8 cells decreased both myc/Max and AP-1 DNA/protein complex formation. Northern analyses, however, revealed that both CT and insulin increased c-myc and c-fos mRNA expression in wild type cells within 4 hours after treatment. In conclusion, TGCT8 cells expressing constitutively elevated cAMP levels have decreased myc/Max and AP-1 protein/DNA complex formation. This phenomenon is mimicked by CT and insulin treatment of wild type FRTL-5 cells. These data suggest that protein and not mRNA levels of these DNA binding proteins may be important to our further understanding of thyroid tumoigenesis.

328. P46 THYROID PEROXIDASE ACTIVITY IS INHIBITED BY SOME AMINOACIDS. D. Rosenthal, D.P. Carvalho, S.M. Coelho and M.A.S. Camacho; Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Normal thyroid peroxidase (TPO) iodide-oxidation and protein-iodination activities can be inhibited "in vitro" by several hydrolyzed proteins such as human TPO preparations, bovine serum albumin and casein. The aim of the present study was to determine whether this inhibitory effect on TPO activity could be produced by some aminoacid(s). Among the aminoacids tested, cysteine, methionine and tryptophan were found to act as competitive inhibitors of the TPO iodide-oxidation reaction, and at 50μM completely inhibited the "in vitro" iodide-oxidation activity and reduced the protein-iodination activity by more than 60%. Tyrosine, phenylalanine, and histidine also inhibited the iodide-oxidation activity, but to a lesser extent, and all other aminoacids tested (cystine, proline, arginine, leucine and isoleucine, threonine, asparagine, lysine and valine) produced no significant effect on TPO activity. Cysteine also produced a transient inhibition of the TPO guaiacol-oxidation reaction, which is not significantly affected by any of the other aminoacids. The iodide-oxidation inhibitory activity produced by cysteine, methionine and tryptophan can be overcome by increasing the iodide concentration, but is unaffected when the cofactor (H_2O_2) concentration is increased. Thus, it seems that the inhibitory aminoacids could interfere with TPO iodide-oxidation reaction by interacting with the oxidized form of iodine and/or the iodide-binding site of TPO. It remains to be seen if these inhibitory aminoacids, as such or as major constituents of a small peptide, might be the TPO inhibitor found in some human dyshormonogenetic goiters with organification defects.

Supported by FINEP, CNPq and CEPEG/UFRJ.

329. P47 MUC1 MUCIN EXPRESSION IN PAPILLARY THYROID CARCINOMA : COMPARISON OF MICRO CARCINOMA, MACROCARCINOMA AND CASES AFTER THE CHERNOBYL ACCIDENT.

C. Julié*, T. Delbot**, L. Leenhardt**, A.M. Bergemer*, B.Helal***, B. Franc*
*Department of Pathology, Ambroise Paré Hospital, 92100, Boulogne-Billancourt., **Department of Nuclear Medicine, Pitié Hospital, Paris, 75013,*** Department of Nuclear Medicine, Kremlin-Bicêtre Hospital, Le Kremlin-Bicêtre, 94275, France.

The Apomucin MUC1 has been described in a variety of carcinoma tissues. It has been suggest that MUC1 mucin expression may be related to tumor progression to advanced stages. We have previously described MUC1 mucin expression in papillary thyroid carcinoma. The aim of this study was to determine if there was any variation in MUC1 mucin expression according to tumor behaviour. Therefore 36 cases have been reviewed. 13 micro papillary thyroid carcinoma (<1cm), 13 macropapillary carcinoma (1,5 - 7cm), 10 macropapillary carcinoma (1,5 - 7 cm) in children of the city of Kiev exposed to radiation effect, as a result of Chernobyl accident, have been compared. A known follow-up was available in 30 cases : 19 had regional lymph node metastasis and/or a large extent out of the thyroid gland. MUC1 immunoreactivity was assessed using a MUC1 mucin-specific monoclonal antibody, on formalin-fixed paraffin-embedded tissue sections. Different patterns of immunostaining were encountered : thin or thick lining of the apical part of tumor cells, intracytoplasmic staining. Was also taken into account the percentage of stained area (<25%, 50%, >80%).

The statistical parametric analysis of our series did not detect any significant differences of expression of MUC1 mucin in the 3 groups except for intracytoplasmic tumor cell staining. All macropapillary carcinoma present significantly more intracytoplasmic staining in tumor cells than micropapillary carcinomas ($p<0.01$). No significant differences permitted to discriminate low aggressive carcinoma from the others.

Larger series from the 3 studied groups are needed to further explore the pronostic importance of MUC1 mucin expression and the impact of environmental factors on MUC1 deregulation.

330. P48 EXPRESSION PATTERNS OF ECM/BM COMPONENTS IN NATIVE AND CULTURED HUMAN THYROID TISSUE.

U. Bürgi, M.E. Bürgi, F. Simon, M. Paulsson, A. Sidiropoulos, D. Aeschlimann, C. Glaser, H. Wagner, Ch. Ruchti, H. Gerber, H.J. Peter.
Departments of Endocrinology, Visceral Surgery, Biomechanics, Pathology, University Hospital, CH-3010 Bern, Switzerland

The interactions between extracellular matrix (ECM), basement membranes (BM) and the follicular epithelial cells play a crucial role in the regulation of cell proliferation, differentiation and function and have become a topic of major interest. As a prerequisite for further elucidation of the mechanisms underlying these interactions, expression patterns of ECM/BM components in native and cultured human thyroid tissue were investigated. A 3-dimensional semisolid alginate culture system was used (J Biol Chem 266:15308,1991) which does not interfere with ECM/BM assessment (since it is devoid of ECM/BM components such as collagen) and which sustains morphology, proliferation (positive staining for MIB-1, 3-H-thymidine incorporation on autoradiographs) and TSH-dependent function (iodine uptake and organification) of follicular organoids obtained by collagenase dissociation of human thyroid tissue for up to 3 months. Thyroid tissue obtained at surgery from patients with benign uni- and multinodular goiters was processed for immunofluorescent and immunohistochemical staining both directly and after in vitro culture using poly- and monoclonal first antibodies directed against specific ECM/BM components such as type IV collagen, laminin, laminin A-, S- and M chain, fibronectin, osteonectin and perlecan and second antibodies coupled to either rhodamine or alkaline phosphatase. Specific staining for all the above ECM/BM components was obtained in nodular and extranodular native and cultured thyroid tissue without the need for prior pronase treatment of the tissue. The results show that distinct components of ECM/BM can be defined immunohistochemically and by immunofluorescence both in native human thyroid tissue as well as in human thyroid organoids cultured under defined in vitro conditions. The expression of specific ECM/BM components and the maintainance of a 3-dimensional architecture of human thyroid organoids cultured in an alginate system makes this system particulary attractive for ECM/BM studies in human thyroid tissue not feasible in vivo.

331. P49 **REGULATION AND METABOLIC ROLE OF PHOSPHOLIPASE D ACTIVITY IN HUMAN THYROID AND CULTURED DOG THYROCYTES.** **C. Lejeune, J. Mockel, M. Taton Institute of Interdisciplinary research (IRIBHN) and Dept. of Endocrinology, Hospital Erasme, Université Libre de Bruxelles, Brussels, Belgium.**

The actions of thyrotropin (TSH), adenosine-triphosphate (ATP), the ionophore A23187, the Ca^{2+}-ATPase inhibitor thapsigargin and phorbol dibutyrate (PDBu) on ^{3}H-cytidine-monophosphate phosphatidic acid (^{3}H-CMP-PA) accumulation were studied in human thyroid slices to evaluate PA generation and inositol recycling towards phosphatidylinositol (PtdIns) synthesis. The effects of the same agonists were also measured on phosphatidylbutanol (PtdBut) generation in ^{3}H-palmitate or ^{3}H-myristate prelabeled slices to assess the activity of phospholipase D (PLD). TSH 10mU/ml stimulated ^{3}H-CMP-PA accumulation in a LiCl and propranolol insensitive way, but had no detectable action on PLD activity. These effects of TSH were not reproduced by Bu_2cAMP or forskolin (FSK). The Ca^{2+}-ionophores increased both CMP-PA accumulation and PtdBut generation while ATP only stimulated PLD activity. The phorbol ester PDBu (5 10^{-7}M) increased PtdBut formation and ^{3}H-fatty acid incorporation into PtdCho, but had no effect on CMP-PA generation. Staurosporine (STSP) (5 10^{-6}M), an inhibitor of PKC, unexpectedly reproduced the effects of PDBu. We suggest that the PA generation induced by PLD stimulation could contribute to the stimulated H_2O_2 formation and iodide organification observed with the agonists inducing PtdBut accumulation. Indeed, Bu_2cAMP, FSK and choleratoxin (CTX) known to decrease iodide organification in human thyroid, inhibited the PLD stimulation induced by ATP and PDBu. In cultured dog thyrocytes, PDBu and STSP induced DNA synthesis and dedifferentiation, while thapsigargin inhibited TSH-induced growth and killed PDBu stimulated cells, suggesting a positive role of PLD stimulation towards dedifferentiated growth, whereas simultaneously raised $[Ca^{2+}]_i$ and stimulated PKC-PLD would lead towards growth arrest and cellular death.

332. P50 **SIMILAR CHANGES OF THE THYROCYTE ULTRASTRUCTURE FOLLOWING SMALL INCREASES OF TSH OR IODINE DEFICIENCY: A MODEL FOR GOITROGENESIS**

C. Hoang-Vu[1,2], G. Brabant[2], W. Sierralta[3], H. Leitolf[2], A. von zur Mühlen[2], H. Dralle[1]. [1]Klinik für Allgemeinchirurgie, MLU Halle, [2]Depts Clin Endocrinology, Med. Hochschule Hannover and [3]Electron Microscopy Laboratory, Max-Planck-Institute, F.R. Germany

Stimulation of thyroid function by TSH is generally accepted but its effects on thyroid proliferation and goiter formation are still controversial. In iodine deficiency a higher sensitivity of the thyroid to TSH has been discussed to form the basis of hyperplasia and proliferation (1). However, no direct evidence for this hypothesis has been reported by directly comparing the effects of a small increase in circulating TSH to the effects of iodine deficiency. In the present study we investigated on the ultrastructural level the impact of a doubling of TSH plasma levels (4.2 ± 0.2 vs. 1.9 ± 0.4 ng/ml in controls[C], $p<0.001$) induced by pulsatile application of TRH (2μg every 2h over 5 days;TRHP) in chronically catheterized rats to the effects of low iodine diet(<300 ng/kg for 7 weeks;[LID];TSH: 3.2 ± 0.7ng/ml). Thyroid hormones in both groups remained constant apart from a significant increase in T_4 in TRHP (3.5 ± 0.2 vs. 2.8 ± 0.1 μg/dl in C, $p<0.001$;). Thyroid weight significantly increased in LID (22.5 ± 2.5 vs. 16.1 ± 3.6 in C, $p<0.001$ or 18.5 ± 3.6 mg in TRHP,ns). Ultrastructural analysis of the thyrocytes by electron microscopy revealed a comparable appearance in TRHP and LID with an enlarged size of the endoplasmic reticulum (ER) cisternae (Fraction of cytoplasm: TRHP: 61.4 ± 16.9; LID: 58.6 ± 21.3; C: 16.2 ± 4.8%, $p<0.001$). Postembedding immunogold staining for thyroglobulin (Tg) demonstrated that Tg was a major secretory product within the ER both in LID and TRHP. The number of gold particles was significant higher in TRHP and LID when compared to those from C (TRHP:374 ± 92; LID:381 ± 78; vs C: 197 ± 59 gold particles/μm^2, $p<0.001$). The similarity of the findings in both situations supports the idea that the ultrastructural effects observed in LID may be mediated by a higher sensitivity of the thyrocytes to TSH. 1:Bray J Clin Invest 1968;47:1640-1647.

333. P51 REGULATION OF THE THYROID JUNCTIONAL COMPLEX BY PROTEIN KINASES. DIFFERENT EFFECTS OF PKC-INHIBITORS ON THE FUNCTION AND DISTRIBUTION OF E-CADHERIN. H. Fagman, L. Rödjer, L.E. Ericson. Institute of Anatomy & Cell Biology, Göteborg University, Göteborg/Sweden.

Thyroid epithelial cells are firmly connected by the Ca^{2+}-dependent cell adhesion molecule, E-cadherin, which is expressed at the entire lateral cell surface but concentrated in the circumferential adherens junction. Cadherin-mediated adhesion is positively controlled by TSH via cAMP/protein kinase A, although the effector molecule(s) is still unidentified. In contrast, mitogenic phorbol esters abrogate both thyroid epithelial barrier and adhesion via activation of protein kinase C (PKC), which thus may serve as a negative regulator of epithelial junctions. Here we have examined the ability of commonly used protein kinase inhibitors, isoquinolinyl-sulfonyl-methyl-piperazine (H-7) and staurosporine, and a new agent, bisindolylmaleimide (GF 109203X; Molecular Probes, OR), which exhibits a more specific inhibition of PKC than the others, to prevent dissociation of the thyroid epithelial junction complex.

Tight and polarized monolayers of pig thyrocytes in Transwell culture rapidly lost the transepithelial resistance and became leaky to [^{3}H]inulin when depleted of extracellular Ca^{2+}. The junction-breaking effect of Ca^{2+} removal was dose-dependently prevented or reversed by TSH (and forskolin/8-Br-cAMP), staurosporine (0.1-10 μM) and H-7 (1-50 μM) but not by GF 109203X (0.1-10 μM). The effect of H-7 was additive to those of TSH and staurosporine, whereas staurosporine was dominant over TSH. Staurosporine per se had a late (hours) disrupting effect of junctions not noted for the other kinase inhibitors nor TSH. Preceding this, staurosporine-treated cultures showed blebs positive for F-actin and cadherin at the apical cell surface, despite a normal location of the tight junction protein ZO-1; these membranous protrusions, which were not seen after H-7 or GF 109203X, were morphologically different to TSH-induced apical pseudopods. GF 109203X antagonized the negative effect of phorbol ester (TPA) on junctional integrity.

In conclusion, protein kinase inhibitors known to affect mainly PKC have distinct but different effects on tight junctional permeability and E-cadherin distribution in pig thyrocytes. Together, the data suggest that the thyroid junctional complex is dynamically regulated by several protein kinases, possibly isoforms of PKC, in addition to the adenylate cyclase/cAMP/PKA pathway. Specifically, the translocation of E-cadherin to apical parts of the cells in response to staurosporine, which occurred before loss of the epithelial barrier, might involve aberrant recruitment from an intracellular pool.

334. P52 THYROID FOLLICLE RECONSTRUCTION : CALCIUM, HEXAMETHYLENE BISACETAMIDE, COMPONENTS OF THE NCTC 109 MEDIUM AND COCULTURES WITH THE ENTEROBACTERIA ESCHERICHIA COLI. NEW DATA IN FAVOUR OF A cAMP-INDEPENDENT MECHANISM. G. Fayet, S. Hovsépian, T. Fazekas and A. Aouani, Laboratoire de Biochimie Médicale, Unité INSERM 38, 27 Bld Jean-Moulin, 13385 Marseille France.

To understand better the mechanism of thyroid follicle reconstruction we used two methods (1) the reorganization of freshly isolated porcine thyroid cells into follicles in the absence of any cAMP stimulator (2) a new system of in situ conversion of monolayer cells into follicle-associated cells with stimulators.

The serum- and TSH-free medium NCTC 109 allows follicle formation. We reconstituted this medium from Earle's salts (medium Ao) ; Ao + 24 aminoacids (medium Bo) ; Bo + 19 Vitamins (medium Co) ; Co+ 17 Other Components (medium Do i.e. NCTC 109). Earle salt solution without Ca++ and concentrated $CaCl_2$, $2H_2O$ solutions were also prepared.

Cells formed follicles spontaneously either in Do, Co, Bo for 15 days and even in Ao but only for 2 days. Ca++ was absolutely required, both for cell survival and to get follicles (0.22 - 1.8 mM). Hexamethylene bisacetamide (HMBA 0.025-5mM)) in Eagle serum-free medium induced (0.5 mM) or inhibited (2.5 mM) follicular reorganization depending on concentration. HMBA inhibited the effect of TSH (50 mU/ml) + forskolin (2.5 μM) + IBMX (0.125 mM), 3 stimulators, on cell morphology and on iodide trapping and organification, only for high concentrations. HMBA was also found to be a growth factor for an epithelial cell population not yet identified growing from a human thyroid (Grave's Basedow). When cells were cocultured with E. Coli the follicular architecture took place in a dramatic way. Iodide trapping and organification were silent in Ao, Bo, Co, Do, HMBA,+ E. Coli situations, despite the presence of follicles. The 3 stimulators re-established iodide activity (representing 50-70 % in Bo compared to Do) except in Ao.

Conversion of monolayer cells into follicle-associated cells was obtained after 24h treatment in Ca++ free Earle's salts but with the 3 stimulators. Cells detached from each others but not from the plastic support. When Bo medium was added with the 3 stimulators, cells reformed follicles in situ. Monolayer control cultures treated with Ca++ containing Earle's salts just developed domes. This suggest that the junctional complexes between monolayer cells had to be disrupted in order to reform other new junctions allowing folliculogenesis.

In conclusion we described simple media in which isolated porcine thyroid cells spontaneously form follicles. HMBA plays a role both in growth and differentiation of porcine and human thyroid cells. E. Coli in precise conditions provokes folliculogenesis. Iodopermease was silent in all these histiotypical cultures, but its function was reestablished by cAMP-stimulators. This indicates that follicle reconstruction takes place according to a cAMP-independent mechanism. Monolayer cells represent a different phenotype compared to cells from the tissue. Reorganization of these partly dedifferentiated cells would need both the destruction of abnormal junctional complexes and stimulators to re-induce the messages required to get folliculogenesis.

Acknowledgments : CEBMH1CT920081 and Laboratoires LAPHAL 13718 Allauch, Cédex, France.

335. P53 TSH-REGULATED EXPRESSION, GLYCOSYLATION AND SECRETION OF THE ALZHEIMER PRECURSORPROTEIN: POSSIBLE IMPLICATION FOR THE PROLIFERATION OF THYROCYTES.

G.M. Popp, K.S. Graebert, S. Rosentreter, P. Lemansky and V. Herzog, Institut für Zellbiologie der Universität Bonn, Ulrich-Haberland-Str. 61a, 53121 Bonn, FRG.

The Alzheimer ß-A4 amyloid precursor (APP) is a transmembrane protein which is ubiquitously expressed in the vertebrate organism. FRTL-5 cells were found to express 3 different transcripts of APP corresponding to APP 695, 751 and 770. After TSH stimulation the expression of APP was increased by a factor of about 1.3. Antibodies against the C-terminus of APP revealed a predominant localization of APP in the Golgi complex and in endocytic compartments including lysosomes. During transport through the Golgi complex, APP became O-glycosylated in a TSH-dependent fashion thereby increasing its molecular mass by 4 to 5 kDa (1). Upon reaching the cell surface, APP became strongly iodinated. A considerable proportion of APP appeared to escape glycosylation and to reach the cell surface as indicated by the iodination of immature APP (2). Part of APP was proteolytically cleaved thereby releasing the secretory N-terminal portion of APP (APPs). Observations with rat thyroid tissue and with FRT cells known to form a tight epithelial monolayer showed that APPs is released on the apical and the basolateral cell surfaces. The release of APPs which was stimulated about 4-fold by TSH coincided with a strong proliferative effect on thyrocytes. To test the possible effect of APPs on the proliferation of thyrocytes we treated FRTL-5 cells with conditioned culture media (CM) containing APPs. We observed a strong proliferative effect of CM which was partially inhibited after removal of APPs from CM by immunoprecipitation with an antibody directed against the N-terminus of APP. Increased rates of mitosis were observed only in thinly plated, non-confluent cell cultures. Various peptides from the N-terminal portion were synthesized and tested for the ability to induce proliferation. Only a 17-mer peptide previously shown to induce proliferation in fibroblasts (3) resulted in a 2-fold increase in the incorporation of BrDU detected by immunocytochemical techniques or of [3H]thymidine. These observations suggest that APPs is indeed able to induce proliferation of thyrocytes and that it may be a new candidate in the family of peptides active in regulating thyroid growth. (Supported by SFB 284 and by Fonds der Chemischen Industrie)

Literature:

(1) Graebert, K. S., G. M. Popp, T. Kehle and V. Herzog: Eur. J. Cell Biol. 66, 39, 1995;
(2) Graebert, K. S., P. Lemansky, T. Kehle and V. Herzog: Lab. Invest., 1995, in press;
(3) Ninomiya, H., J.-M. Roch, M. P. Sundsmo, D. A. C. Otero and T. Saitoh: J. Cell Biol. 121, 879, 1993.

336. P54 FOLLICLE FORMING CAT THYROID CELL LINES SYNTHESIZING EXTRACELLULAR MATRIX AND BASAL MEMBRANE COMPONENTS: A NEW TOOL FOR THE STUDY OF THYROIDAL MORPHOGENESIS.

C.Tognella, H.J. Peter, H. Wagner, J. Kaempf, F. Simon, C. Glaser, D.C. Ferguson, M.E. Peterson, H.J. Häuselmann, M.Paulsson, Ch. Ruchti, M.E- Bürgi-Saville, U. Bürgi, H. Gerber. Depts. of Clin. Chemistry, Visceral Surgery, Internal Medicine, Pathology and M.E.-Müller-Institute, University Hospital, CH-3010 Bern; Clinic of Surgery, District Hospital, CH-3600 Thun; Dept. of Physiol. and Pharm., Univ. of Georgia College of Vet. Medicine, Athens, GA 30602, USA; Dept. of Medicine, Animal Medical Center and Center for Res. Animal Resources, Cornell Univ., New York, NY 10012, USA

Interactions between follicular epithelial cells and extracellular matrix (EM)/basal membrane (BM) are supposed to play an important role in development and maintenance of thyroid tissue architecture. In the present study we have therefore investigated the synthesis of ECM/BM components by a feline thyroid cell line which is able to form follicle like structures in vitro and in 5 ras-transfected sublines. We have used a new semisolid culture system composed exclusively of polymerized alginate and therefore devoid of ECM/BM components. Cells were cultured as described (Peter et al, Thyroid 1: 331, 1991). Transfection was performed by lipofection with pZIP stre-ras (human Harvey ras; neo) and pSV2-neo (control, neo only) plasmids kindly provided by R. Fries (Univ. of Berne). Immunostaining was done using rabbit first antibodies directed against mouse collagen IV, human fibronectin, tumor laminin (EHS) and other ECM/BM components and second antibodies coupled either to a fluorescent system or alkaline phosphatase. For comparison, immunostaining was also performed in cryosections of nodular goiters of 6 hyperthyroid cats. Feline cells embedded in alginate gels as single cells and cultured for up to 30 days formed cell clusters within 10 days. Follicle like structures were formed in the original cell lines and also in the ras-transfected cells. Differences in proliferation rates were observed, the transfected cells growing up to 5-10 times faster than the non-transfected cells. The cell lines and their transfected clones were staining strongly positive for collagen IV and fibronectin, less so also for laminin. The cat goiter tissue stained positively for collagen IV, EHS-laminin, but negative for fibronectin and various other ECM/BM components. - In conclusion, cat cell lines grow threedimensionally in alginate over several weeks, they form follicle like structures and express with one exception the same ECM/BM components as the native cat goiter tissue. Transfection with ras does increase proliferation rate, but does not fundamentally alter formation of follicle like structures and ECM/BM expression. Alginate gel culture is a promising new tool for the study of follicular morphogenesis, polarity, the expression pattern of ECM/BM components and of the interaction between thyrocytes and ECM/BM, avoiding interference caused by gels composed of ECM/BM components.

337. P55 COLLAGEN AND FIBRONECTIN ARE GROWTH PROMOTING FACTORS IN NORMAL AND TUMOR THYROID CELLS

M. Vitale, M. Illario, A. Casamassima, V. Bassi, C. Sandomenico, V. Molese, GF. Fenzi, G. Rossi, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Dipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Università di Napoli; C.E.O.S., C.N.R.

The β_1 family of integrins is a group of receptors for extracellular matrix proteins (ECM) such as collagen, fibronectin, laminin and vitronectin. In normal thyroids, the majority of follicular cells expresses only the β_1 subunit associated with α_3, while less than 3% expresses α_1, α_3, α_5 and α_6. The expression of these molecules is regulated *in vitro* by cell-to-cell contact: the level of α_3 increases, while α_2 is expressed *de novo*. In thyroid tumors all α subunits are expressed. We studied the adhesion and the functional properties of integrins in normal and fetal thyroid cells and in tumors of thyroid origin.
Methods: Thyroid cells from normal glands, tumor cell lines (NPA, WRO) and fetal thyroid cells (TAD) were cultured in the presence of 10%FCS. Expression of the β_1 integrins was determined by flow-cytometry with antibodies against the various α subunits. Cells were incubated for 30 min in 96-wells plates coated with different concentrations of type I collagen or fibronectin (from 0.1 to 50 µg/ml). Non adherent cells were removed by gentle washes and adherent cells were fixed, stained by crystal-violet and counted. To determine the effect of substrates on cellular proliferation, cells were cultured in the absence of serum for 24-72 hours in 96-wells plates coated with type I collagen or fibronectin (from 0.1 to 100 µg/ml).(3H)-thymidine was added to the medium for the last 24 hours and the radioactivity incorporated in the cells was determined.
Results: Normal thyroid cells had high expression of $a_2\beta_1$ and $\alpha_3\beta_1$ integrins while in NPA cells all subunits were expressed. Both normal thyroid cells and NPA attached to collagen and fibronectin. Type I collagen and fibronectin induced a 3- to 6-fold increase of (3H)-thymidine incorporation in normal cells after 48 and 72 hours of stimulation. Also NPA and TAD cells, showed a 6- to 9-fold increase.
Conclusions: Type I collagen and fibronectin are growth promoting factors in normal, fetal and tumor thyroid cells. Growth stimulation was more effective in tumor and fetal cells. Adhesion and growth stimulation exerted by extracellular matrix can be mediated by their natural integrin receptors.

338. P56 SPONTANEOUS EARLY AGING OF THE RAT THYROID CELL LINE FRTL-5.T.Zimmermann-Belsing,Å.K.Rasmussen,U.Feldt-Rasmussen,University depart. of Endocrinology,Rigshospitalet,Copenhagen,Denmark.

FRTL-5 cells are widely used for the study of thyroid cell functions. The purpose of the present study was to characterize the passages 7-17 of the FRTL-5 cells in terms of growth and function. Growth was examined by cell counting, ^{3}H-thymidine incorporation and DNA content of the cells (diphenylamine method). The function of the cells was evidenced by iodine uptake (^{125}I), cAMP (competitive protein binding assay) and thyroglobulin (Tg) (ELISA) production. The growth and function was related to cell age (passage 7-17) and the influence of TSH (1 U/l). Kruskal-Wallis test was used for statistical analysis. TSH stimulated ^{3}H-thymidine incorporation and cAMP production of the cells ($p<0.05$, $n=17$), but no effect of TSH could be demonstrated on DNA content, Tg production or iodine uptake in passage 7-17. The TSH-stimulated Tg production during passage 7-10 was 84 ng/µg DNA (27-230), (median (range)), $n=24$, while it was significantly lower in passage 11-17 (median: 0 ng/µg DNA, range: 0-155, $n=33$, $p<0.05$). The marked decrease or loss of Tg production related to age was demonstrated both in FRTL-5 cells received from a European laboratory and from the ATCC cell bank. Also TSH-stimulated cAMP production and iodine uptake decreased in passage 11-17 compared to passage 7-10. Between passage 7 and 10 $_{3}$H-thymidine incorporation was decreased significantly decreased ($p<0.05$,$n=13$), while the growth evaluated as the DNA content of the cells increased significantly from passage 11 to 17 ($p<0.05$, $n=24$).

In passage 11-17 a distinct increase in growth rate was accompanied by a lower function of the cells. A total loss of the ability to produce Tg was demonstrated. FRTL-5 cells changed their biological properties already at passage 11. This study emphasizes the importance of characterizing FRTL-5 cells before using them for experiments.

339. P57 **THE EFFECT OF INSULIN LIKE GROWTH FACTOR-I (IGF-I) ON LIVER TRIIODOTHYRONINE (T_3)-DEPENDENT ENZYME ACTIVITIES AND NUCLEAR T_3 RECEPTOR NUMBER IN RATS.** **CG Pellizas, AH Coleoni, ME Costamagna, M Di Fulvio and AM Masini-Repiso. Depart. of Clinical Biochemistry. Faculty of Chemical Sciences. Nat. University of Cordoba. Cordoba. Argentina.**

The importance of thyroid hormones in the regulation of the Growth Hormone (GH)-IGF-I axis is well established. T_3 stimulates rat GH gene transcription and this effect is antagonized by IGF-I, a factor that is under GH control. In turn, T_3 stimulates the release and synthesis of IGF-I in the rat liver. Although the impact of GH or IGF-I administration on thyroid function has been studied with controversial results, the effect of these growth factors on the specific metabolic response of T_3 in target tissues has not been extensively explored. In previous studies we have demonstrated that IGF-I and GH incorporated to cultured rat hepatocytes induced a dose-dependent reduction of mitochondrial α-glycerophosphate dehydrogenase (α-GPD) and cytosolic malic enzyme (ME) activities, two useful biomarkers of thyroid hormone action. Since the maximal enzyme activity induced by a high T_3 concentration in the culture medium was significantly reduced in the presence of IGF-I, we speculated on the possibility that such response might be reflecting a diminished number of nuclear T_3 binding sites. Accordingly, using an in vivo model we aimed to further explore the mechanism by which IGF-I affects thyroid hormone action. Recombinant human IGF-I (60, 120, 240 and 480 ug/100g b.w.) was injected s.c. twice a day during two days to adult male Wistar rats. Mitochondrial α-GPD and cytosolic ME were measured in liver cellular fractions. Maximal binding capacity (MBC) of T_3 to nuclear receptors and the apparent affinity constant (Ka) were carried out in isolated liver nuclei. As shown in the table, IGF-I induced a significant dose-dependent reduction of α-GPD and ME activities.

	IGF-I dose (µg/100g b.w.)				
(n:4)	0	60	120	240	480
α-GPD	0.223 ±	0.186 ±	0.183 ±	0.103 ±	0.089 ±
ΔA/min/mgDNA	0.025	0.052	0.017 *	0.018 *	0.016 **
ME	0.691 ±	0.527 ±	0.398 ±	0.388 ±	0.355 ±
ΔA/min/mgDNA	0.209	0.089	0.139 *	0.057 *	0.059 *

Results expressed as mean ± SEM. *p<0.05; **p<0.01 vs. control (ANOVA)

In turn, IGF-I in a dose of 240µg/100g b.w. significantly ($p<0.01$) reduced de number of T_3 nuclear receptors (fmol T_3/100µgDNA ±SEM: 27.30 ± 6.50 vs. control 73.19 ± 14.07; n:7). The Ka of nuclear receptors for T_3 remain unchanged (10^9 M^{-1} ± SEM: 2.19 ± 0.74 vs. control 2.50 ± 0.77; n:7).

We conclude that IGF-I was able to impair the specific metabolic response of T_3 in the liver of the rat. The mechanism of this effect involves at least in part a reduction of the nuclear T_3 receptor number. These results are further evidences supporting a role of IGF-I in the regulation of the GH-IGF-I/thyroid hormone axis.

340. P58 ELECTRON MICROSCOPY FINDINGS ARE SUGGESTIVE OF THYROCYTE APOPTOSIS DURING TSH-SUPPRESSION. E.T. Kimura and P.A. Abrahamshon, Department of Histology & Embryology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

It is known that deprivation of a trophic hormone results in programmed cell death (apoptosis) in target tissues. In the thyroid gland the suppression of TSH secretion is followed by a marked size involution that has been regarded as flattening of the follicular epithelium. Recently apoptosis was identified in thyroid cell culture after TSH and growth factors were removed from the culture medium. To investigate the occurrence of similar patterns *in vivo*, male Wistar rats (~220g BW) were grouped as: (i) control; (ii) methimazole treated (MMI 0.03% in the drinking water), (iii) T3 pellet implant (~24µg/day; sc), (iv) T4 pellet implant (~2.4µg/day; sc). All animals were killed 21 days latter and the thyroid tissue was processed for electron-microscopy (EM). Tissue was fixed with Karnovsky's fixative followed by osmium post fixation. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a JEOL CX100. Thyrocytes of MMI-treated rats were homogeneously enlarged by a factor of ~2 with an otherwise preserved structure. In the T3-treated rats, thyrocytes were homogeneously flattened and single cells or small groups of degenerating cells could be spotted in an asynchronous fashion. These cells showed disarrangement of the cytoplasmic components with loss of spatial organization of the reticuli, enlargement of the cisternae filled with fine granular material, different degrees of mithocondrial vacuolization, irregular apical surface, fragmentation of the cytoplasm, and the presence of large cellular debris including nuclei and other cell organelle inside the colloid lumen. In addition, thyrocytes had an irregular nuclear outline and condensed chromatin. Similar structural findings were identifyed in T4-treated rats. To investigate further the presence of DNA fragmentation, *in situ*-end labeling of DNA (incorporation of biotinylated nucleotides by Klenow enzyme) was carried out in formalin-fixed, paraffin-embedded tissue. Labeled DNA was detected by streptoavidin-fosfatase alkaline and developed with ASMX salt-fast red. Signs of DNA labelling were not found under light microscopy analysis in any of the groups when compared with the positive controls (DNAse I [500 ng/ml] treated tissue). **Conclusion**: (i) the findings of structural degenerations under EM are suggestive of apoptotic death in the thyroids of T3/T4-treated animals and may contribute to thyroid involution during TSH suppression; (ii) the absence of nuclear fragmentation under ME and by DNA fragmentation by labelling analysis suggest that programmed cell death in the thyroid might have unusual characteristics as described in other tissues.

341. P59 **INDUCTION AND MODULATION OF NITRIC OXIDE GENERATION BY CYTOKINES IN CULTURED HUMAN THYROCYTES.** **S-I. Shimoda, Y. Hattori, N. Banba, S. Motohashi and K. Kasai, Department of Endocrinology, Internal Medicine,Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan**

Nitric oxide (NO) is involved in a variety of physiological and pathological processes. We now report on the induction of NO production by proinflammatory cytokines in primay cultures of human thyrocytes. Interleukin-1 (IL-1 α and β) induced the production of NO in the cells. Interferon-γ (IFN-γ) stimulated NO production synergistically with IL-1 α / β, but not when used alone. After IL-1 α /IFN-γ -stimulation, coexpression of mRNAs for NOS and GTP cyclohydrolase I (GTPCH), the rate-limiting enzyme for *de novo* synthesis of tetrahydrobiopterin (BH4; a cofactor for nitric oxide synthase), preceded the biosynthesis of NO and BH4, which required an induction period of 4-6 hr and continued for at least 48 hr. Actinomycin D abolished the expression of the two mRNAs and the production of both NO and BH4. The selective inhibitor of GTPCH, 2,4-diamino-6-hydroxypyrimidine (DAHP), inhibited BH4 production induced by IL-1 α /IFN-γ, leading to inhibition of NO production. Inhibition of NO synthesis by DAHP was completely reversed by sepiapterin, a substrate for BH4 synthesis *via* the pterin salvage pathway, indicating that BH4 is essentially required for NO production in thyrocytes. Of the cytokines tested, tumor necrosis factor-α (TNF-α) *per se* did not, but slightly stimulated NO production in the presence of IFN-γ. Interferon-α, interleukin-4 and transforming growth factor-β1 inhibited NO production. Thus, the induction of NO production upon treatment with IL-1, IL-1/IFN-γ or TNF-α/IFN-γ, and modulation of its production by other cytokines is clearly demonstrated in human thyrocytes. The role of relatively large amount of NO produced in the cells is now under study.

342. P60 BASIC FIBROBLAST GROWTH FACTOR (bFGF) EXPRESSION IS MEDIATED BY PROTEIN KINASE C FOLLOWING ACTIVATION BY IONIZING RADIATION IN HUMAN THYROIDS.

T. Hara, H.Namba, S. Yamashita, Nagasaki University School of Medicine, Nagasaki, Japan

bFGF is a potent mitogenic and chemotactic factor for a wide variety of cells. Recently, radioprotective effects of bFGF via autocrine mechanism have been reported in some type of endothelial cells. To determine the mechanism of bFGF induction, we investigated the bFGF mRNA expression using irradiated primary human thyrocytes. Northern blot analysis of total RNA isolated from irradiated (2Gy) primary human thyrocytes revealed elevated bFGF mRNA levels as early as 2hr after irradiation, peak at 4-12hr, and its level remains to be elevated at 48hr following irradiation. Irradiation (0.5-5Gy) promoted the induction of bFGF mRNA in a dose-dependent manner. To elucidate the signal transduction system in thyroidal bFGF gene expression, a protein kinase inhibitor, H7, was added 1hr prior to irradiation. Pretreatment with H7 (100mM) completely abrogated the radiation-mediated bFGF expression. Down-regulation of PKC following TPA (10^{-6}M) stimulation for 24hr resulted in attenuation of radiation-induced bFGK mRNA level. These results indicate the involvement of PKC in bFGF induction in human thyrocytes after radiation exposure.

343. P61 SERUM THYROID GROWTH-BLOCKING ACTIVITY IN PATIENTS WITH CHRONIC RENAL FAILURE ON HEMODIALYSIS. M.Nishikawa, A.Shouzu and M.Inada, Second Department of Internal Medicine, Kansai Medical University, Moriguchi, Osaka 570 JAPAN

To investigate the possible humoral factor (s) influencing thyroid cell growth in chronic renal failure (CRF) , we measured serum thyroid growth activity and thyroid growth-blocking activity using a cultured functioning rat thyroid cell line (FRTL-5 cells) in 17 patients on hemodialysis and 19 healthy controls. The underlying disease causing CRF was chronic glomerulonephritis in 9 patients and diabetic nephropathy in the remainig patients. Polyethylene glycol-treated serum was centrifuged and FRTL-5 cells were cultured with the supernatant. Thyroid growth activity was determined by [^{3}H] thymidine incorporation after incubation for 72 hours. There was no significant difference in [^{3}H] thymidine incorporation between cultures incubated with patient and normal serum, suggesting the absence of thyroid growth activity. However, when patient serum was added to cultures together with 20 or 50 uU/ml of thyroid-stimulating hormone (TSH) , TSH-stimulated increase in [^{3}H] thymidine incorporation was significantly decreased, indicating the presence of thyroid growth-blocking activity. Normal serum did not significantly affect TSH-stimulated [^{3}H] thymidine incorporation by FRTL-5 cells. This blocking activity was not significantly altered by hemodialysis. The mean serum levels of T4, FT4 and T3 were significantly lower in the patients than in the normal controls. The serum TSH concentrations of the patients ranged from 0.2 to 8uU/ml. The serum iodine concentration of the patients prior to hemodialysis ranged from 5.7 to 42 mg/dl (mean : 20.6 $\pm$ 4.4 μg/dl) , which was markedly higher than in normal controls (4-9 μg/dl) . However, no significant correlation was observed between this activity and serum levels of thyroid hormones or iodine concentration. Thus, patients with chronic renal failure have serum thyroid growth-blocking activity which is nondialysable and differs from iodine, suggesting that balance between unknown growth factors and this activity may determine the development of goiter.

344. P62 EFFECTS OF EPIDERMAL GROWTH FACTOR (EGF) ON THYMIDINE KINASE ACTIVITY IN THE TISSUE OF RAT THYROID LOBES INCUBATED *IN VITRO*. M. Karbownik[1], J. Brzeziński[2], A. Lewiński[1], H. Modrzejewska[3], J. Greger[3]. [1]Department of Thyroidology and [2]Department of Endocrine Surgery, Institute of Endocrinology and [3]Department of Biochemistry, Institute of Physiology and Biochemistry, The University School of Medicine at Łódź, Poland.

Epidermal growth factor (EGF) is believed to be a potent growth factor for thyroid follicular cells (TFC) (Westermark and Westermark, Exp. Cell Res. 138, 47, 1982). However, other authors failed to observe the proliferogenic effect of EGF on rat thyrocytes in monolayer culture (Ambesi-Impiombato et al., Proc. Natl. Acad. Sci. USA 77, 3455, 1980) or in suspension cultures of rat thyroid follicles (Smith et al., Endocrinology 119, 1439, 1986). We have measured the activity of thymidine kinase (TK - 2.7.1.21), the enzyme responsible for catalyzing the phosphorylation of thymidine and functioning as a part of the pyrimidine salvage pathway involved in DNA synthesis. Male Wistar rats were the donors of thyroid lobes. The thyroid tissue was incubated for 4 hours in RPMI 1640 medium (Gibco), containing Hepes buffer, 15% FCS and the examined substance - epidermal growth factor (EGF), used in five different concentrations (0.1 ng/ml, 1 ng/ml, 10 ng/ml, 100 ng/ml, 1000 ng/ml). The TK activity was measured by means of Cheng and Prusoff's method (Biochemistry 13, 1179, 1974) in modification by Greger and Dramiński (Z. Naturforsch. 44c, 985, 1989). The reaction products were separated by ascending chromatography.

It was shown that EGF, in all the examined concentrations, suppressed TK activity ($p<0.001$); a tendency towards diminishing TK activity could be observed parallelly to increasing EGF concentration. The inhibitory effect of EGF, when used in the highest concentration (1000 ng/ml), was significantly stronger in comparison with the effect of EGF in concentrations of 0.1 ng/ml, 1 ng/ml and 10 ng/ml. Our results do not speak in favour of the stimulatory effect of EGF on the thyroid growth processes in rats.

345. P63 Hormonal Regulation of the Level of DNA Polymerase β mRNA in Human Thyroid of Graves' Disease and Thyroid Tumors.

Motoko Kotake, Yoshikuni Sawai, Akira Nakai, Rumi Masunaga, Toshiki Mano, Keiko Shimazaki, Ritsuko Kato, Seiya Kato, Hifumi Nakagawa, Mitsuo Hukushima, Mitsuyasu Itoh and Akio Nagasaka.
Dept. of Internal Med. Fujita Health Univ. School of Med., Toyoake, Aichi, Japan.

We previously reported the relationship between the activity of DNA polymerase β and functions in various endocrine organs such as rat adrenal gland, testis, thyroid gland, and human thyroid gland. Here we examined the changes in DNA polymerase β mRNA in human thyroid tissue from Graves' disease and tumors. Human thyroid specimens were obtained by subtotal or total thyroidectomy from patients with various thyroid disorders, hyperplasia, thyroid adenomas, thyroid carcinomas, and normal thyroid surrounding tumors.

Total cellular RNA was extracted by phenol extraction. Equal amounts of total-RNA (20μg/well) were electrophoresed in denaturating 1% agarose gel. After electrophoration the gel was blotted onto nylon membrane.
The membrane was hybridrized with a ^{32}P-labeled rat β-pol cDNA probe, labeled by the random primer method. After exposure, the amounts of mRNA were counted in a densitometer.

The amount of β-pol mRNA in Graves' thyroid was significantly increased compared with that in the normal thyroid. In all thyroid tumors but one case of papillary thyroid carcinoma, the amount of β-pol mRNA was decreased compared with that in the normal thyroid. The change in the β-pol mRNA level was well correlated to that in the β-pol activities we reported previously. These findings indicate that DNA polymerase β activity is regulated by hormones at the mRNA level.

346. P64 **PREVALENCE AND SIGNIFICANCE OF THYROID AUTOANTIBODIES IN PATIENTS WITH CHRONIC HEPATITIS C.** MJ Huang, BY Huang, YF Liaw.
Division of Endocrinology and Liver Research Unit, Chang Gung Memorial Hospital and Medical College, Taipei, Taiwan

Studies have suggested an association between hepatitis C virus infection and autoimmunity, including thyroiditis. To determine prospectively the prevalence and clinical significance of thyroid autoantibodies in oriental patients with chronic hepatitis C, serum antimicrosomal antibody (AMA) and anti-thyroglobulin antibody (ATA) were assayed using hemagglutination tests in a consecutive series of 130 patients with chronic hepatitis C. A consecutive series of 143 patients with chronic hepatitis B seen during the same period were also studied as controls. All these patients were also tested for serum free thyroxine (FT4) using two-step radioimmunoassay and TSH using third generation chemiluminometric assay. Those with positive autoantibodies and/or thyroid dysfunction were then evaluated by an endocrinologist. Thyroid sonography, cytology and radioactive scintigraphy were performed where appropriate. The results showed that patients with chronic hepatitis C, especially female patients, had a higher prevalence of AMA, as well as ATA, than the patients with chronic hepatitis B (26.5 vs 5.7%; $P < 0.02$, in female patients). In addition, 16 of the 22 AMA positive patients with chronic hepatitis C, while only one of the patients with chronic hepatitis B, had an AMA titer >1:400. Of the 23 patients seropositive for thyroid autoantibodies, all had normal serum FT4 and TSH levels except two cases who had mild TSH elevation, 8 had thyroid enlargement including 7 Hashimoto's thyroiditis and one currently treated thyrotoxicosis. Of the remaining 15 patients who had no goiter, three had had subtotal thyroidectomy for thyrotoxicosis prior to this study. The results of this prospective study suggest that female patients with chronic hepatitis C have a high prevalence of thyroid autoantibodies and chronic lymphocytic thyroiditis as well as thyrotoxicosis as compared with chronic hepatitis B patients.

347. P65 A NEW TYPE OF THYROXINE-BINDING GLOBULIN DEFICIENCY. Y.Ueta, Y.Mitani, S. Taniguchi, T.Nawada, I.Manabe, A.Ohtahara, Y.Yamamoto and Y.Tanaka, First Department of Internal Medicine, Tottori University, Yonago Japan.

Complete Thyroxine (T4)-binding deficiency (TBG-CD) is inherited in an X-linked fashion. The TBG gene is located on the long arm of the X-chromosome. Several types of the mutations in TBG gene have been reported. Mutations, in these reports, are single base substitutions or single nucleotide deletions. We identified a new type of mutation in TBG gene. Two subsequent nucleotides in codon 28,29 are changed to another one nucleotide. This is the first case of this type of mutation in TBG abnormalities. One male patient with Graves' disease was suspected to have TBG-CD because of his normal serum concentration of T4 and triiodothyronine (T3), and high concentration of T3 uptake and free T4. His serum TSH level was low and serum TBG was undetectable. For Polymerase chain reaction (PCR),oligonucleotide primers were designed to amplify each of four coding exons. Genomic DNA was extracted from white blood cells and subjected to PCR and the generated DNA fragments were subcloned and sequenced. Two subsequent nucleotides *i.e.* the last nucleotide of codon 28 (C) and first nucleotide of codon 29 (T) in the common-type TBG molecule was deleted and substituted by another one nucleotide (A). The shift in the reading frame due to this mutation results in change of downstream encoding *i.e.* one different amino acid followed by frame shift in translation and premature termination in codon 51. This is the first report of this type of mutation in TBG abnormalities and this case is considered to be a rare case.

348. P66 **TSH MEASUREMENT IN SCREENING SUBCLINICAL HYPOTHYROIDISM AMONG HEALTHY PERSONS SEEKING ROUTINE CHECKUPS.** F. Akasu, J. Komatsu, M. Oritsu, H. Enomoto, T. Hiyoshi and M. Yoshitsugu, Division of Endocrinology, Japanese Red Cross Medical Center, Tokyo, Japan.

To obtain an estimate of the prevalence of subclinical hyper and, in particular, hypothyroidism among people seeking routine checkups, sensitive TSH measurement, as a single first-line test for thyroid evaluation was included in a physical checkup program. Individuals whose TSH values were outside stated limits were recalled for further thyroid function testing (free T4, TRH stimulation test, thyroid sonography and urinary excretion of iodine if applicable).

Results: In the period of May 1993 to January 1994, a total of 3533 (779 male; 2754 female: mean age 50 and 48, respectively) individuals were studied. Two hundred thirty-five subjects had TSH values outside reference limits (0.4-4.3 μIU/ml); they were subdivided into three groups based on their TSH values: TSH 4.3-10 (**group A**: 20 (2.6%)male; 140 (5.1%) female), TSH >10 (**group B**: 5 (0.6%) male; 20 (0.7%) female) and TSH < 0.4 μIU/ml (**group C**: 12 (1.5%) male; 21(0.8%) female. The number of subjects who participated in further evaluation were 89 (group A), 16 (group B) and 21 (group C), respectively, consisting of 53.6% among those who manifested abnormal TSH. In group C, 3 were diagnosed as having overt hyperthyroidism due to Graves' disease and 3 painless thyroiditis having fT4 values within the reference range. One patient received T4 therapy. Only 5 from group A showed low fT4. TRH test was therefore performed on 41 individuals from group A with elevated TSH, but without apparent signs of hypothyroidism. Peak TSH level in response to 500 μg TRH at either 30 or 60 min was variable, however 28 (32%) showed mildly high (peak 30-50 μIU/ml) and 36 (40%) showed remarkably high (>50) TSH response, resulting in only 25 (28%) with normal response. We tried to correlate the results of TRH testing with symptoms described by patients as well as their hematological and biochemical test results. Fatigue, hoarseness, sensitivity to the cold, edema and increased body weight were the major manifestations, however none of those correlates with the TRH test result. Moreover, CK, total cholesterol, Hb were not always abnormal even when peak TSH concentration exceeded 50. All the patients with overt hypothyroidism and 15 with subclinical hypothyroidism were treated with l-thyroxine. Two latent hypothyroid patients reported amelioration of chilblain, tinnitus, edema, darkening of the hair. Two women who showed a high TSH peak value were taking iodine rich sea tangle extract daily when the blood test was performed. After the cessation of the iodine rich diet, the TRH test gave normal response.

Conclusions: 1) Screening with TSH only is a cost efficient test for evaluating thyroid function of healthy persons; physical examination as well as routine laboratory tests are not reliable in screening for subclinical hypothyroidism. 2) Abnormal TSH values do not correlate with TRH testing; it is necessary to perform TRH test for the borderline patient. 3) Sensitivity of the single TSH assay in selecting the individuals who need thyroid supplement is 85% in female but only 20% in male patients.

349. P67 ADDITION OF PHENYTOIN TO HUMAN SERUM DISPLACES L-THYROXINE (T4) FROM SERUM BINDING PROTEINS. M.I. Surks and C.R.DeFesi, Montefiore Med. Ctr. and Albert Einstein College of Medicine, Bronx, N.Y. 10467.

Many studies have shown that phenytoin(P)-treated patients remain clinically euthyroid with normal serum TSH despite a 20-40% decrease in serum T4 and free T4 (FT4). Serum T3 and free T3 remain normal or minimally decreased. Most reports employed FT4 assays which required dilution of serum. This might decrease P concentration from a level that displaces T4 in vivo to an ineffective level in vitro. The results of two early reports, using undiluted serum to determine free T4 fraction (FT4F), might be limited because of lack of specificity in determination of T4 in ultrafiltrates. We re-investigated the effect of therapeutic concentrations (range: 16-20 ug/ml, mean ± SEM, 18.1±0.7 ug/ml) of P on FT4F determined by ultrafiltration of undiluted serum. Purified [125I]T4 was used and [125I]T4 in ultrafiltrates was identified by paper chromatography (Surks et al, JCEM 67:1031-1039, 1988). Addition of P resulted in a significant increase ($p<0.05$-$p<0.001$) in FT4F in each of six experiments (n=4 for vehicle; n=4 for P). The mean increase in free T4 fraction was 44.1±9.8% (range: 24.9-88%). Addition of phenytoin, therefore, resulted in an increase in serum FT4 from 1.57±0.07 to 2.27±0.12 ng/dl, $p<0.005$. When free T4 was determined in the same samples using Nichols Free T4-estimate (1:5 dilution of serum), no change in free T4 was observed (vehicle=1.28±0.07 ng/dl, P = 1.37±0.09 ng/dl. Our in vitro studies show that therapeutic concentrations of P significantly increase FT4F and free T4 in undiluted human serum. The magnitude of the change appears to be similar to the reported magnitude of the decrease in serum T4 in P-treated patients. It is therefore possible that P-treated patients have normal serum free T4, if measured by modern methods that employ undiluted serum.

350. P68 RELATIONSHIPS OF SERUM TSH TO FREE T_4 IN HEALTHY INFANTS AND CHILDREN, HEALTHY PREGNANT WOMEN, AND PATIENTS WITH UNTREATED THYROID DISEASE (PRIMARY AND SECONDARY) COMPARED TO HEALTHY NONPREGNANT ADULTS. JC Nelson, SJ Clark, RM Nelson, EI Carlton, DL Borut, DA Fisher and RB Wilcox. Loma Linda University School of Medicine, Loma Linda CA., and Corning Nichols Institute, San Juan Capistrano, CA.

In healthy children and pregnant women serum TSH is elevated and free T_4 (FT_4) is reduced, as compared to healthy nonpregnant adults (J. Pediatr 1993;123:899, JCEM 1990;71:276). In healthy neonates both TSH and FT4 are elevated. The objective of this study was to analyze these physiologic variations in the relationship of serum TSH to FT_4 and to compare them to the changes induced by thyroid disease, both primary and secondary. Healthy nonpregnant adults were used as the reference population.

SUBJECTS: Healthy neonates (N=43), healthy children 8 wk - 17 yr (N=283), healthy adults 18-44 yr (N=306), healthy pregnant women (1st Tri N=40, 2nd Tri N=106, 3rd Tri N=62), primary hypothyroidism, (N=83) "primary" hyperthyroidism (N=71), resistance to thyroid hormone (N=61), TSH-oma (N=1), and central hypothyroidism (N=7). Treated thyroid patients and individuals with NTI were excluded.

METHODS: TSH ICMA 3rd gen. and FT_4 direct equilibrium dialysis. AMA RIA was used to exclude unrecognized autoimmune thyroid disease in apparently healthy individuals.

RESULTS: For analysis the $\log_{10}$ [TSH] was plotted against [FT_4] (after Wehmann, 1994). A single domain of the data encompassed both healthy children and healthy adults and, when extended, included primary hypothyroidism and "primary" hyperthyroidism. That domain comprised the area between two lines; 1) a lower diagonal line, equation log [TSH] = 1.589 - 1.786 [FT_4] and, 2) an upper diagonal line, equation log [TSH] = 3.777 - 1.55 [FT_4], ending in a horizontal extension at 0.04 mU/L TSH between 3.4 and 30 ng/dL FT_4. This domain, which we termed the reference domain, included 583/589 of the normal data from children and adults and 150/154 of the primary thyroid disease data. Data from 68/69 of the individuals with secondary thyroid disorders fell outside this domain. All data from healthy neonates (1-4d) fell to the right of the reference domain, overlapping resistance to thyroid hormone data. 15% of the pregnancy data fell to the left of the reference domain into the domain characteristic of central hypothyroidism.

CONCLUSIONS: 1) This analysis of TSH relationships to FT_4 provided a unique perspective of the variations encounter in different clinical conditions. 2) It was especially informative in evaluating conditions with altered pituitary thyroid regulation; neonates, children, pregnancy, and secondary thyroid disorders. 3) Similarity of the data from the healthy neonates to the data from thyroid hormone resistance, and similarity of the pregnancy data to the data from central hypothyroidism, suggest the possibility that these two physiologic states may be associated with variations in TSH bioactivity like those reported in secondary thyroid disorders.

351. P69 Supression of Serum TSH Concentrations Following Oral Salsalate (Disalcid) Administration

Rong Wang, R. Bruce Wilcox, and Jerald C. Nelson. Loma Linda University, School of Medicine, Loma Linda, CA. And Corning Nichols Institute, San Juan Capistrano, CA.

Salsalate is a nonsteroidal anti-inflammatory drug that inhibits T4 binding to serum proteins without known effects on pituitary thyroid function. Chronic administration has been reported to reduce serum TT4 and TT3, sometimes to hypothyroid levels, without TSH elevation or other evidence of hypothyroidism. The objective of this study was to investigate the early time course of T4 and T3 responses to acute salsalate administration. Unexpectly, the predicted decrease of serum TT4 and TT3 was associated with a decline of serum TSH reaching a nadir at 9 and 24 h after salsalate.

DESIGN: Healthy volunteers (three, age 29 to 64) fasted for 12 h prior to and 2 h after drug administration. Salsalate was given orally in a single dose of 62-65mg/kg. Two basal blood samples were taken 0.5 h apart before salsalate and two samples at 24.0 and 24.5 h after salsalate. Additional samples were taken 1,2,3,4, and 9 h after salsalate. Sera were separated within 1 h and frozen (-20C) until analysis. The sera were assayed for TT4 and TT3 by RIA, FT4 and FT3 by equilibrium dialysis, and TSH by third generation ICMA. Data from both basal samples and both 24 h samples were combined. The pattern of T4 binding was examined by ^{125}I-T4 radioelectrophoresis.

RESULTS:

	0 h	1 h	2 h	3 h	4 h	9 h	24 h
TT4	9.9	8.2*	7.6	5.9**	5.4**	7.2*	7.3*
FT4	1.3	1.4	1.2	1.2	0.9	1.2	1.3
TT3	142	117**	93**	71**	69**	79*	81**
FT3	321	239*	181**	170**	154**	218	172*
TSH	1.99	1.25*	1.07*	0.92	0.82	0.63**	0.68**

* $p<0.05$, ** $p<0.01$ (p values were based on the ratio of subsequent time points to the basal time points.)

At 24 h serum FT4 was unchanged while sera TT4, TT3, FT3 and TSH were all reduced at 24 h. The lowest of TT4, TT3, FT3 occured at 4 h. The lowest of TSH occured at 9 and 24 h. Radioelectrophoresis demonstrated that a decrease in ^{125}I-T4 binding to both TBG and TBPA with the nadir at 3 h.

CONCLUSION: These preliminary data are consistent with the hypothesis that reduced serum TT4, TT3, FT3, and TSH with unchanged FT4, a pattern sometimes associated with acute nonthyroidal illness, can occur following an acute inhibition of serum T4 binding.

352. P70 CLINICAL COURSE OF YOUNG EUTHYROID STUDENTS WITH THYROID ANTIBODIES.

Y. Maeda[1], M. Nomaguchi[1], H. Tanaka[2] and S. Tojo[3] Health Service Center[1] and The First Department of Internal Medicine[2], Faculty of Medicine, Kagoshima University, and Tojo Hospital[3], 1-21-24 Korimoto, Kagoshima 890, Japan.

Euthyroid persons with thyroid antibodies (TA) are sometimes found on consultation. Some reports suggest that most of them may be expected to develop hypothyroidism in the future. However, we can not predict what clinical course each will run. We investigated thyroglobulin antibody (TGA) and thyroid microsomal antibody (TMA) in 588 students aged 18-24 to determine the frequency of thyroid disease. Seven of 153 males and twenty-eight of 435 females were found to be positive for TGA and /or TMA. Only one female of these 35 with TA had Graves' disease, but the rest of them were in the euthyroid state. Since then we have followed one male and 24 females of these 35 with TA over a period of ten years. In these ten years, we found another four females who developed Graves' disease. These five females who were found to have Graves' disease had not had children before the disease onset. Three of the remaining 19 who are still in the euthyroid state have had children, but none have developed hypothyroidism. The five females who were found to have Graves' disease had moderate levels of TMA and thyroid disease in their families. Three of them had B-22(B-54), CW-1 and DR-4 types of HLA. None of those who are still in the euthyroid state have such types of HLA.

We then investigated another 17 (male 6, female 11) students with Graves' disease and the family of one of these students many of whose members are thyroid disease patients. In this investigation, the students and the family members who had moderate levels of TMA also had B-22(B-54), CW-1 and DR-4 types of HLA, but other students who had low levels of TMA or had no TMA did not show such findings.

These data suggest that young females in the euthyroid state who have moderate levels of TMA and who have thyroid disease in their families and B-22(B-54), CW-1 and DR-4 types of HLA are more likely to develop Graves' disease than to develop hypothyroidism.

353. P71 IODIZATION OF WATER FOR CORRECTING IODINE DEFICIENCY : A SIMPLE AND EFFICIENT TECHNIQUE. P. Bourdoux[1], D. Yazipo[2], L. Fio-Ngaindiro[2], L. Namboua[2], J. Ndoyo[2], L. Barrière-Constantin[3], E. Pichard[4], ULB-Cemubac Brussels[1], Ministry of Health Bangui[2], Bangui[3], University of Bamako[4].

IDD are still a major problem in most developing countries. Recent estimates by WHO indicate that one billion people worldwide are at risk for iodine deficiency. Because of socioeconomic reasons, prophylaxis programs based on the use of iodized salt have been unsuccessful in most developing countries.

The aim of our study was to evaluate on a mass scale the efficacy of a technique of iodization of water. Briefly, for about 12 months, NaI incorporated in silicone matrices (Rhodifuse Iode®) is slowly but constantly released when matrices are immersed. The prefecture of Nana-Grebizi (Centralafrican Republic) with about 80,000 inhabitants was selected for the trial. Nine silicone-sodium iodide matrices were placed into each of the 198 wells throughout the prefecture. Urinary iodine concentration and the prevalence of goiter, the classical markers of iodine deficiency, were used to evaluate the efficacy.

Before intervention, uniform and severe iodine deficiency was demonstrated by a median urinary iodine concentration of 2.1 µg/dL in a representative sample (n=319) of the population living in the prefecture. The prevalence of goiter (n=3,090) was 60.9 % whereas that of visible goiter was 10.7 %. After 6 months, the median urinary iodine concentration increased significantly to 28.9 µg/dL (n=304; P<0.001). After 12 months, the median urinary iodine concentration was 17.4 µg/dL (n=278; P<0.001). It indicated an iodine intake in agreement with the daily requirements (150 µg/day). Concomitantly, the prevalence of total and visible goiter (n=2,645) dropped to 44.4 % and 2.5 % (a four fold decrease), respectively. Analysis of our data on urinary iodine, mainly those at 6 months, led us to adjust the iodine supply and to propose using 3 matrices for every 200 people.

Our data demonstrate that, in a large number of inhabitants, the iodization of water by the present technique is easily feasible. It is efficient for correcting iodine deficiency, offers an alternative to iodized salt and can be applied immediately where water supplies are available. Noteworthy is the possibility to apply it in countries where only small areas are affected by IDD, avoiding unjustified and useless treatment of the whole population.

354. P72 **CLINICAL EVALUATION OF A ONE-STEP, RAPID TSH ASSAY** J. Ehrenkranz, F. Schletter, J. Stock, K. Usiskin, Endocrine Section, Morristown Memorial Hospital, Morristown, NJ, USA.

Rapid, self-performing, semi-quantitative immunochromatographic (one-step) assay methods for measuring hCG, LH, and FSH are widely used around the world because of their diagnostic reliability, cost savings, and convenience. Although TSH shares considerably structural homology with hCG, LH, and FSH, a one-step assay for TSH has not been developed. A TSH assay which does not require instrumentation or trained personnel for test performance, provides a visual readout in <10 minutes and is able to detect TSH elevations of the degree found in primary hypothyroidism would be valuable as a screen for congenital, pediatric, and adult primary hypothyroidism, particularly in less developed parts of the world. The feasibility of such an assay has recently been shown using a monoclonal antibody pair with no hCG, LH, or FSH cross-reactivity that is able to detect TSH with a sensitivity in the range 5-20 µIU/ml and correlates with a reference EIA method. We now describe our initial experience using this assay in clinical practice. In the first study, TSH was measured in sera from 10 euthyroid (defined as a serum TSH < 5 µIU/ml as determined by second or third generation reference immunoassay) pregnant women with serum hCG ≥ 10,000 mIU/ml. and from 7 euthyroid and 4 hypothyroid postmenopausal females with serum LH and FSH ≥ 40 mIU/ml. In a second experiment, the one-step TSH assay results were compared to third-generation TSH measurements in 18 patients, ages 1 month to 86 years, with primary hypothyroidism and in 15 patients, ages 2 -73 years, with a variety of thyroid diseases and normal or suppressed TSH levels. In the final experiment, patient sera (n=4) containing TSH in concentrations ≥ 40 µIU/ml were serially diluted and the TSH concentration determined with the one-step assay at each dilution. These results, shown below, were used to calculate test sensitivity and specificity.

	true +	true -	false +	false -	sensitivity	specificity
hCG ≥ 10,000; LH, FSH ≥ 40 mIU/ml	4	17	0	0	100%	100%
Primary hypothyroidism	18	15	0	0	100%	100%
serial dilution	8	11	1	0	100%	92%

These preliminary results suggest that use of a rapid, self-performing, semi-quantitative TSH immunochromatographic assay outside the clinical laboratory is a reliable method to screen for elevations in TSH that are found in primary hypothyroidism.

355. P73 ACQUIRED INAPPROPRIATE THYROTROPIN (TSH) ELEVATION IN THYROXINE-REPLACED HYPOTHYROID PATIENTS. J.Takamatsu, S.Ueda, N.R.Farid, A.Kobayashi, F.Matsuzuka. First Department of Medicine, Osaka Medical College, Takatsuki ; and the Kuma Hospital, Kobe, Japan.

We report paradoxically elevated serum thyrotropin (TSH) levels [17.5 ± 10.3 mU/L (mean ± SD)] despite increased serum free thyroxine (T_4, 31.1 ± 4.5 pmol/L) and total triiodothyronine (T_3, 2.98 ± 0.40 nmol/L) levels in 16 of 232 (7 %) hypothyroid patients receiving T_4 replacement. Such inappropriate TSH secretion (SITSH) had not been observed before the patients had hypothyoidism. There have been no studies on the etiology.

A regimen of 75 μg T_3 per day for one week was added to the preexisting T_4 replacement therapy in 16 patients with hypothyroidism and SITSH, 20 patients with simple hypothyroidism alone, and 5 patients with resistance to thyroid hormone. Similarly, 30 μg T_3 per day for one week or 75 μg T_4 per day for four weeks was supplemented to the preexisting T_4 therapy.

Two groups of patients with hypothyroidism and SITSH and those with simple hypothyroidism had similar suppression of serum TSH level by either 75 or 30 μg of T_3 supplementation. In contrast, T_4 supplementation to patients with hypothyroidism and SITSH suppressed serum TSH much less than that in patients with simple hypothyroidism. In patients with resistance to thyroid hormone, serum TSH suppression was the least by either T_3 or T_4 supplementation. Serum cholesterol levels the SITSH patients at the time of normal serum free T_4 but high serum TSH were comparable to those in the simple hypothyroid patients when both free T_4 and TSH were normal(mean level, 4.78 and 4.81 mmol/L, respectively), and the similar finding was obtained in serum levels of sex hormone-binding globulin, another peripheral marker of thyroid function.

It is suggested that the inappropriate TSH elevation is related to acquired and selective reduction of type 2 but not type 1 iodothyronine deiodinase in the pituitary. Serum free T_4 concentration seems to be more accurate than serum TSH to monitor the thyroid function of these patients.

356. P74 **ELEVATED SERUM LEVELS OF ANTIBODIES AGAINST THYROGLOBULIN (TgAb) AND THYROID PEROXIDASE (TPOAb) IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION: IS IT A RISK FACTOR ?** M.M.Biancalana, J.H.Romaldini, C.S.Farah, D.I.Figueiredo, I.Rassi, D.Armaganijan, M.Shibata, M.Sampaio, L.A.Mattos, M.M.Santos. Endocrinology Department, HSPE-IAMSPE and Cardiology Institute, Dante Pazzanezze, São Paulo, Brazil.

It has been suggested that subjects with thyroid autoimmune disease are more frequently affected by acute myocardial infarction(M.I.). Therefore, studies were conducted in 143 patients, divided into four groups. Group I, 38 consecutive patients with M.I.(median age: 64.5 yr, range: 30-85 yr); group II consisting of 40 control subjects(34 yr, 19-50); group III comprising 26 subjects with symptoms of coronary artery disease but normal coronary angiography (56 yr, 42-77) and group IV, 39 patients with coronary artery disease documented by arteriography (61.5 yr, 35-90). All patients were euthyroid and goiter was not detected in anyone. TgAb and TPOAb were measured by a sensitive IRMA and the results expressed in % of binding. T_3, T_4 and TSH were measured by IFMA method (Delphia). The frequency of positive TgAb was higher in the groups I (8/38, 21%) and IV (14/39, 36%) compared with groups II (2/40, 5%) and III (3/26, 11.5%; $X^2 = 8.9$, $p < 0.003$). Positive TPOAb was also higher in the groups I (13/38, 34%) and IV (14/39, 36%) than the frequency observed in groups II (2/40, 5%) and III (2/26, 7.7%; $X^2 = 15.9$, $p < 0.001$). The median values of serum TSH were 1.42 μU/ml (0.25-5.91), 1.45 μU/ml (0.20-4.96), 1.45 μU/ml (0.20-4.50), and 1.20 μU/ml (0.20-3.50) in groups I, II, III and IV, respectively. Only TSH values in group I was higher ($p<0.05$) than values found in group IV. In contrast, the serum T_3 values of group I (0.9 ng/ml, 0.3-2.3) was lower than values of groups II (2.0 ng/ml, 1.2-2.9; $p<0.001$), III (1.4 ng/ml, 1.1-2.3, $p<0.001$) and IV (1.4 ng/ml, 1.0-2.3, $p<0.001$). Further, T_3 value of group IV was significantly lower ($p< 0.001$) than group II. The T_4 concentration of group I (7.5 μg/ml, 4.6-14) was lower than groups III (10.2 μg/ml, 5.6-14; ($p< 0.001$) and IV (10.6 μg/ml, 6.9-14.9; $p<0.001$). The T_4 value of group II (6.9 μg/ml, 3.6-11) was significantly lower ($p<0.001$) than group IV. Conclusions: Our data indicate that asymptomatic thyroiditis and thyroid dysfunction were common in both patients with M.I. and coronary artery disease. Thus thyroid autoimmunity may have influence on the pathogenesis of coronary atherosclerosis.

357. P75 CHRONIC LYMPHOCYTIC LEUKEMIA WITH THYROID INVOLVEMENT. W.J. Georgitis, N.H. Alex, M.M. Lieberman, and M.J. Johnson, Fitzsimons Army Medical Center, Aurora, Colorado.

A 68 year old Caucasian male with Rai stage II chronic lymphocytic leukemia (CLL) experienced dysphagia with painless enlargement of a multinodular goiter (MNG) during chemotherapy treatment at another medical facility. Radioiodine uptakes at 4 and 24 hours were 16.3 and 36.3%. Serum TSH was elevated at 58 uU/mL. The dysphagia resolved with thyroxine replacement. Another thyroid scan was compatible with MNG.

Further growth of the goiter caused recurrent dysphagia prompting Endocrine consultation. Neck palpation revealed a 50 gram, rock hard, multinodular goiter with adjacent mobile 1-2 cm cervical lymph nodes including a Delphian node. Diffuse lymphadenopathy was also present in the axillae and inguinal regions. A complete blood count showed WBC of 32,000, Hct 41, and platelets of 186,000. Thyroid tests included a TSH of 1.86, T4 13, T3 126, an undetectable thyroglobulin, and an antimicrosomal antibody of 1:1,638,400. Fine needle aspiration from three different sites in the goiter revealed a predominant population of small lymphocytes, accompanied by some mature forms, and a few plasma cells consistent with CLL. Automated flow cytometry with monoclonal antibody markers compared cells obtained from fine needle thyroid aspirations with his peripheral blood. A monoclonal B cell population (CD 19+ kappa+) with concurrent CD5+ CD20+ was present in the thyroid, similar to his peripheral blood and consistent with CLL.

Treatment with CHOP chemotherapy produced regression of the adenopathy and goiter with resolution of the patient's dysphagia. A literature review from 1960 to present failed to yield a case of CLL with thyroid involvement by the same clone of B cells causing the leukemia. This case should be distinguished from Richter's transformation in lymph nodes of a CLL patient. Lymphoid germinal center formation in the thyroid may have been a predisposing factor.

358. P76 **Gene analysis of Complete Thyroxine-Binding Globulin Deficiency(TBG-CD) in a Korean male**

Youn-Bok Chang*, Chang-Ho Back, Se-Chan Wee
Chang's Clinic*, Seoul, Department of Genetic Engineering, Hallym University, Chuncheon, Korea.

Thyroxine Binding Globulin(TBG) is the major transport protein of the thyroid hormones in human serum. The TBG gene is located on the long arm of the X-chromosome. Complete TBG deficiency(TBG-CD) is defined as undetectable TBG in serum with a sensitive assay. We found a TBG-CD in a Korean male and analyzed the TBG gene. Genomic DNA was extracted from the peripheral white blood cells. 4 exons of TBG genewere amplified with PCR. Each PCR products were ligated to pXT and pBluescript SK(+), and sequenced by the dideoxy chain termination method. A single nucleotide was deleted at the first base of the codon 352 of the TBG gene. As a consequence of frameshift, termination codon appeared 374. The deduced sequence of 22 amino acid was entirely different from that of TBG-C. These changes are same as those of TBG-CDJ in Japan. In addition, a nucleotide substitution was also observed at the third base of the codon 55 [GCA(Ala) to GCG(Ala)]. A nucleotide substitution was also observed at the first base of the codon 74 [GAG(Glu) to AAG(Lys)].

In conclusion, we found the changes of exon 4 of TBG gene in a Korean male are same as those of TBG-CDJ in Japan. In addition, we also found the silent mutation at codon 55 and substistution of amino acid at codon 74 of TBG gene.

359. P77 ACUTE PRIMARY HYPERPARATHYROIDISM AND THYROTOXICOSIS: WHEN "STORM" CLOUDS MEET

Gail Tolbert, Gaylord Walker, Martin Kenecker, Jocelyn Leveque, Jay W. Grosse, Barry Warner, University of South Alabama College of Medicine, Mobile, Alabama, USA

Severe life threatening and symptomatic hypercalcemia (↑Ca) caused by primary hyperparathyroidism (HPT) and associated with high morbidity and mortality represents parathyroid "storm" or crisis. ↑Ca in the setting of thyrotoxicosis (↑T4) seldom presents with acute, symptomatic or marked ↑Ca. Co-existing HPT and ↑T4 has been reported only 50 times. We present a case of co-existing parathyroid storm in a patient with ↑T4 and review the other 6 reported cases.

				Ca	P	PATH MORPHOLOGY	
Author	Year	OUTCOME	THYROID TX	MAX	MIN	THYROID	PARA
Noble	1936	Norm 1 wk	I,S	19.4	2.0	H	A
Gassmann	1960	Aut dx	S	17.8	2.7	M	A
Austoni	1965	----	ATM,S	14.0	2.7	M	3H
Soyannwo	1968	Aut dx	ATM	19.0	5.9	H	A
Parfitt	1970	Norm 9 yrs	ATM,S	19.0	1.9	N*	A
Piccione	1984	Norm 2 mos	I^{131} 5 mCi,S	15.0	2.6	H	A
Tolbert	1995	Norm 2 mos	ATM,S	16.0	1.5	H	2H

A=adenoma, ATM=antithyroid drugs, Aut dx=autopsy diagnosis, Ca=calcium, H=hyperplasia, I=iodine, M=multinodular goiter, S=surgery, Tx=treatment, N*=normal 24hr I^{131}uptake consistent w/Grave's Disease, Norm=normal, P=phosphorus, para=parathyroid.

EW is a 44 year old white female who presented with symptomatic ↑Ca, nausea, vomiting, abdominal pain, serum calcium 14.6 mg/dl (8.5-10.5), PTH 103 pg/ml (10-65). She was tachycardiac, tremulous, and diaphoretic with serum T4 22 ug/dl (4.7-13), TSH <0.1 uIu/ml (0.3-5). Treatment w/forced diuresis, propranolol, steroids, bisphosphanates and PTU were used in preparation for parathyroid/thyroidectomy. Conculsions: patients with co-existing parathyroid "storm" and ↑T4 have had fulminant ↑Ca (mean max >17 mg/dl) without prior I^{131} therapy. The common presence (5/7) of Grave's Disease and the disproportionate incidence of parathyroid hyperplasia (2/7) raises the likelihood of a causal relationship perhaps explained by: autoimmunity, prolonged adrenergic stimulation from excess thyroid hormone, a genetic mutation common to branchial structures or, calcitonin deficiency. This represents a surgically treatable acute endocrine emergency.

360. P78 ASSOCIATION BETWEEN THYROID AUTOANTIBODIES AND ANTI-HUMAN T-LYMPHOTROPIC VIRUS TYPE-I (HTLV-I) ANTIBODY IN NAGASAKI, JAPAN.

M. Tsuruta[1,2], N. Yokoyama[1,2], Y. Shibata[1], M. Akahoshi[1], T. Matsuo[1,3], M. Tomonaga[1,3], M. Izumi[2], [1]Radiation Effects Research Foundation, [2]The First Department of Internal Medicine, [3]Department of Hematology, Atomic Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan.

Virus infections are considered to be a trigger for several autoimmune diseases. HTLV-I, a retrovirus, infection could cause not only Adult T-cell Leukemia, HTLV-I Associated Myelopathy or HTLV-I Associated Arthropathy but also is observed at high frequency in Sjogren syndrome, one of the autoimmune diseases. Several reports suggest an association between HTLV-I and typical organ-specific autoimmune diseases such as Graves' disease or Hashimoto's disease. However, because they examined in hospitalized subjects and the number of subjects was too small, the association between HTLV-I and autoimmune disease has remained unresolved. Nagasaki is an endemic area of HTLV-I, favoring to examine the relationship between HTLV-I and autoimmune disease in these subjects. Therefore, we examined anti-HTLV-I antibody and thyroid autoantibodies, and analyzed the association between HTLV-I antibody and thyroid autoantibodies by cross classification in a community-based local population in Nagasaki. [Subjects and Methods] Thyroid autoantibodies and anti-HTLV-I antibody were measured in 2562 subjects (989 males and 1573 females), who had been examined biennially since 1958 in Nagasaki, from 1985 to 1987. Microsome and thyroid test were used to detect thyroid autoantibodies. Positiveness of thyroid autoantibodies was determined if either microsome or thyroid test was positive in more than 100 times dilution. The indirect-fluorescence-antibody method was used to detect anti-HTLV-I antibody. [Results] Of the total number of 2562, subjects 218 (81 males and 137 females), 8.5%, 371 (103 males and 268 females), 14.5%, were HTLV-I antibody positive and thyroid autoantibodies positive, respectively. Of the 371 thyroid autoantibodies positive subjects, 28 (7.5%) were HTLV-I antibody positive, whereas of the 2191 thyroid autoantibodies negative subjects, 190 (8.7%) were HTLV-I autoantibody positive. No statistically significant difference was observed between these two groups. No statistical differences were also found either by sex or age. [Summery] In a community-based local population, no association was found between thyroid autoantibodies and HTLV-I antibody.

361. P79 SOMATOSTATIN(S)TREATMENT ASSOCIATED WITH CORTICOTHERAPY(C)AND/OR RADIOTHERAPY(R) IN GRAVES OPHTHALMOPATHY(GO).Mihaela Simescu,Ioana Lancranjan,Emilia Nicolaescu and Mariana Sava,Institute of Endocrinology,Bucharest,Romania and Dept.of Neuroendocrinology Sandoz Pharm LTD.

Another therapeutical approach in GO beside those in use was performed considering the data about the presence of S receptors on the actived mononuclear leucocytes infiltrating the thyroid and orbit.The present study concerns a groups of subjects with GO(14 female and 1 male patients aged 42-68 yrs).The first group of 7 patients with evolutive severe GO(Class 2c,2b,4c,6b according to NOSPECS classification)had been previously submitted to C and R with no results as regards the disease severity.They were administred S(0.1 ng sc 3 times/day)for 2-6 months,associated with corticotherapy in doses of 6o-3o mg/day and with radiotherapy.The second group of 6 subjects with GO (Class 2b-c,3a,4a NOSPECS cls.was treated with S(the same amount as above)and C(3o mg/day)during 2 months.The patients was maintained euthyroid during the treatment. In the first group 5 out of 7 patients showed a post treatment improvement by 2 grades within the respective NOSPECS cls.-recovery of visual acuity,of ophthalmoplegy(if recent)and decrease in proptosis associated with eye ball luxation.In the second group 5 of 6 patients responded to the treatment by a 1-2 grade improvement within the respective NOSPECS cls.S associated with C and/or R are evident effects in a percent of patients GO;even in the cases with unsatisfactory response there is a stopping in disease aggravation. It is mentioning that none of patients with GO(n=9)followed up for 1-1.6 yrs.showed any aggravation of exophthalmic syndrome.In cases with goiter a significant decrease in volume under these therapies was noted.There may be a correlation between the positive response rate to treatment with S and C+R and the percentage of GO patients who manifest a orbital accumulation by of(lll- In-DTPA-DPhe) octreotide scintigraphy.

362. P80 GRAVES' OPHTHALMOPATHY IN AN INCIDENCE COHORT. G.B. Bartley, V. Fatourechi, E.F. Kadrmas, S.J. Jacobsen, D.M. Ilstrup, J.A. Garrity and C.A. Gorman, Mayo Clinic, Rochester, Minnesota.

The epidemiologic and clinical features of Graves' ophthalmopathy (GO) in a population-based setting have not been described. During a 15-year interval, 120 incident cases of GO were diagnosed among residents of Olmsted County, Minnesota. Seventeen (14.3%) of the patients were male and 103 (85.8%) were female (P = .00001). All patients were white. The age-adjusted incidence rates for females and males were 16.0 cases and 2.9 cases per 100,000 population per year, respectively (standardized rate ratio = 5.5; 95% CI 3.3 to 9.3). Peak incidence rates occurred in the age groups 40-44 years and 60-64 years in females and 45-49 years and 65-69 years in males. Approximately 90% of patients had Graves' hyperthyroidism, 1% had primary hypothyroidism, 3% had Hashimoto's thyroiditis, and 5% were euthyroid. The median age at the time of diagnosis of GO was 43 years (range, 8-88 years). Among patients with hyperthyroidism, GO developed in 61% within 1 year of the onset of thyrotoxicosis. Eyelid retraction was the most common ophthalmic feature, being present in >90% of patients at some point in the clinical course. Exophthalmos affected 60% of patients, restrictive extraocular myopathy was present in 40% of patients, and optic nerve dysfunction occurred in 6% of patients. Only 5% of patients had the complete constellation of eyelid retraction, exophthalmos, optic nerve dysfunction, extraocular muscle involvement, and hyperthyroidism. The most frequent ocular symptom at the time of diagnosis of GO was pain/discomfort (30%). Other symptoms included photophobia (20%), diplopia (17%), lacrimation (15%), and blurred vision (8%). Decreased vision from optic neuropathy was present in <2% of patients at the time of diagnosis of GO. Thyroid dermopathy and acropachy accompanied GO in 4% and 1% of patients, respectively. In 20% of patients, one or more surgical procedures were used to treat GO. The median time between diagnosis of GO and the initial operation was 2.7 years. The cumulative probability of undergoing ophthalmic surgery was 5% by 1 year after diagnosis of GO, nearly 10% by 2 years, 16% by 5 years, and 22% by 10 years. Patients older than 50 years at the time of diagnosis of GO were more likely to have surgery than patients 50 years or younger. Persistent visual loss from optic neuropathy occurred in 2 eyes, with final visual acuities of 20/30 and 20/60. The mean examination follow-up interval was 4.8 years and 22 patients (18.3%) had >10 years of examination follow-up. A long-term follow-up survey identified no patient with constant diplopia that was not correctable with prism spectacles. More than 50% of patients perceived their eyes as appearing abnormal, however, and 38% of patients were dissatisfied with their appearance.

363. P81 IDENTIFICATION OF PATIENTS WITH THYROID-ASSOCIATED OPHTHALMOPATHY WITH EARLY OPTIC NERVE INVOLVEMENT BY THE STUDY OF VISUAL EVOKED POTENTIALS.
M. Salvi, F. Neri, E. Spaggiari, C. Macaluso, E. Gardini, R. Minelli, J.R. Wall and E. Roti, Centro Tireopatie e Istituto di Oftalmologia, Università di Parma, Parma, Italy.

Active thyroid-associated ophthalmopathy (TAO) may progress to optic neuropathy in 3-5% of patients. To date there are no clinical or immunological markers predictive of the evolution to this complication. In order to detect early optic nerve involvement in TAO, in the present work we carried out a study of the visual evoked cortical potentials (VECP) in patients without evident signs of optic neuropathy. We studied 167 patients with TAO, of whom 88% had Graves' disease, 6% Hashimoto's thyroiditis and 6% primary myxedema. Fourty-two percent of patients were hyperthyroid, 49% euthyroid and 9% hypothyroid at the time of ophthalmological examination. Mean onset of TAO was 10.8±3.1 (SE) months after the diagnosis of thyroid disease. The ophthalmological assessment included: 1) lid fissure measurement; 2) Hertel; 3) color vision; 4) cover test and Hess screen; 5) visual acuity; 6) tonometry; 7) fundus examination; 8) visual field; 9) orbital CT scan or ultrasound. VECP were performed in 93 patients with TAO and in 25 normal subjects, as controls. The amplitude and latency of the cortical response to a visual stimulus were evaluated. Of all TAO patients 75 (50.3%) had active eye disease and 101 (68%) proptosis >21mm. Extraocular muscle involvement was present in 68 patients (46%) and increased tonometry in 30 (20%). Visual field defects were detected in 43 patients (30.4%). While the amplitude of the cortical response did not change significantly, the mean (±SE) latency in TAO patients was significantly different from that of normal subjects in both OD (105.5±0.6 vs 101.1±0.7msec; $p<0.0008$) and OS (105.8±0.7 vs 102.4±0.8msec; $p<0.01$). Seventeen patients (19%) had a positive test (>mean+2SD of normals). Latency was significantly increased in patients with proptosis ($p<0.01$) and visual field defects ($p<0.000003$), but not in those with active eye disease and altered ocular motility. A positive VECP test was significantly associated to the presence of soft tissue inflammation (chi square; $p<0.05$). We found no association between increased latency of VECP and a eye muscle abnormalities shown by orbital CT scan. In conclusion, VECP were shown to be altered in patients with TAO without other signs of optic nerve involvement. The presence of proptosis and active inflammatory orbitopathy was associated with an increased latency of the cortical response. The finding of a positive test in 45% of patients with visual field defects is suggestive of early optic neuropathy.

364. P82 **[In-111-DTPA-D-Phe-1]OCTREOTIDE (OCT) SCINTIGRAPHY (OCTREOSCAN) IN THYROIDAL AND ORBITAL GRAVES' DISEASE: FOLLOW-UP DURING TREATMENT.**
EP Krenning, PTE Postema, R Wijngaarde, WA Vandenbosch, PPM Kooij, RML Poublon, HY Oei. University and Eye Hospitals, Rotterdam, The Netherlands.

Thyroidal (thyr) and orbital (orb) Graves' disease can be visualized by Octreoscan. OCT uptake is correlated to disease-activity and severity in untreated patients. Preliminary longitudinal data are presented.
Planar head-neck and orb SPECT images were obtained 5 and 24h pi of 220 MBq OCT. Thyr OCT uptake was measured quantitatively (% of injected dose) and orb OCT uptake semi-quantitatively (grade 1-3). 12 thyrotoxic patients were treated with methimazole and L-thyroxine (mean follow-up 9 months). Thyr OCT uptake decreased in all patients ($p=0.02$). Thyr OCT uptake normalized at an average of 19 months after treatment was started (later if initial thyr OCT uptake was higher). Concomitant I-123-scintigraphy was performed in 5 patients. Neither initial nor decrease in thyr OCT uptake was correlated to thyroid size. 11 consecutive patients with orb Graves' disease were treated with retrobulbar irradiation. 7 responded to therapy (respons means at least decrease in highest, present NOSPECS-class). Disease-activity was scored by Clinical Activity Score (CAS n/7); disease-severity by Total Eye Score (TES n/120).

*) in grade	RESPONDERS			NON-RESPONDERS		P(resp/nonresp)	
PRE = pre-irradiation	PRE median	POST median	P(pre/post)	PRE median	POST median	PRE	POST
Orb OCT 5h pi	3*	2	0.009	2	2	0.066	0.282
Orb OCT 24h pi	2*	1	0.001	2	2	ns	0.011
CAS	4	1	0.002	3	3	0.185	0.037
TES	15	6	0.019	11	8	0.179	0.511

Normalization of 24h pi orb OCT uptake and CAS occurred both at an avarage of 8 months. Mean follow-up time was longer in responders (median 21 and 6.5 months resp., $p=0.012$). CAS > 2 had a sensitivity of 6/7 and a specificity of 1/4 in this study, with regard to respons to retrobulbar irradiation. Grade 2 or 3 Octreoscan(5h pi) had a sensitivity of 7/7 and a specificity of 0/4; grade 3 Octreoscan 4/7 and 4/4 resp.

OCT uptake decreases during successfull treatment of thyr and orb Graves' disease. High pre-treatment 5h pi orb OCT uptake is correlated to a respons to retrobulbar irradiation in patients with orb Graves' disease. The exact role of Octreoscan in predicting therapeutic outcome in thyr and orb Graves' disease remains to be proven.

365. P83 **IMAGING TECHNIQUES IN THE EVALUATION OF ENDOCRINE OPHTHALMOPATHY PATIENTS PRESENTING WITH DIPLOPIA.** **E. V. Nagy, J. Toth[1], I. Kaldi[2], J. Damjanovich[3], A. Leovey. Dept. I of Medicine, [1]Radiology and [3]Ophthalmology, University Medical School of Debrecen and [2]Ophthalmology Unit, County Hospital Debrecen, Hungary**

Endocrine ophthalmopathy (OP) is characterized by autoimmunity which targets orbital tissues. Involvement of the extraocular muscles often leads to diplopia, a frequent presenting complaint. The aim of the present series of investigations was to identify the most informative imaging technique to be used for diagnosis and follow up in patients presenting with diplopia. Forty three patients with OP and diplopia were studied. The size and character of extraocular muscles were examined using MR and ultrasonography. In some cases, patients had CT scans performed at the time of referral. Thyroid function tests and antibody levels were measured and compared to the findings of both the imaging techniques and ophthalmologic examinations. For the 315 muscles examined, muscle thickness detected by MR and ultrasonography correlated poorly (r=0.25). There was a tendency to underestimate muscle diameters on ultrasonography. When patients with different degree of eye movement restriction were studied separately (A: diplopia only at extremes of gaze, B: no diplopia at least in one direction and C: diplopia in all directions of gaze), analysis of the data has shown that the thicker the muscle and/or more restricted the free movement of the bulbus the less accurate the ultrasound. An additional information provided by the MR was the T2 time as the indicator of an active inflammatory process in the muscle(s). None of the laboratory test results (TSH, FT4, TRAb) showed correlation with muscle thickness or degree of inflammation (T2 value). However, 22/32 patients had thyroid function abnormality and 10/20 had elevated TRAb levels. In conclusion, the preferred technique for extraocular muscle imaging in OP is MR because both accurate muscle diameters are provided and the degree of inflammation estimated. Ultrasound may be used for follow up only in those infrequent cases when initial ultrasonographic diameters are comparable to those obtained by MR and there is no major restriction in eye movement.

366. P84 RECOVERY COURSE OF CHEMICAL TYPE AND IMMUNOLOGICAL TYPE REVERSIBLE HYPOTHYROIDISM. K.Sato, K.Okamura, H.Ikenoue, T.Kuroda, T.Mizokami, M.Fujishima. Second Department of Internal Medicine , Kyushu University, Fukuoka, Japan.

A spontaneous recovery frequently occurs in patients with primary hypothyroidism. We investigated the recovery course of various types of reversible overt hypothyroidism (serum thyrotropin >40mU/L) including (A) 26 patients with iodine induced hypothyroidism without antithyroid antibody, (B) 20 patients with postpartum hypothyroidism, (C) 5 patients following painless thyroiditis, (D) 20 patients with idiopathic hypothyroidism with antithyroid antibody and (E) 5 patients following subacute thyroiditis. The recovery course was evaluated by determining the days required for a 50 percent decrease in the serum thyrotropin concentration without any replacement therapy. On the basis of the sequential measurement of the serum thyrotropin concentration, the value was calculated from the regression equations after logarithmic transformation. The rate at which hypothyroidism recovered was (A) 6.1 ± 3.1 [mean$\pm$SD] days, (B) 16.2 ± 5.8 days, (C) 15.3 ± 5.2 days , (D) 16.8 ± 9.6 days and (E) 18.5 ± 8.9 days, respectively. Eleven of the 26 patients (42 percent) in group A showed a very rapid recovery from hypothyroidism as assessed by a recovery rate of less than 5 days. In contrast, none of the patients showed such a rapid recovery in the other groups. These data suggest that the recovery from immunological type reversible hypothyroidism (Group B-D) is almost the same as that from hypothyroidism following subacute thyroiditis (Group E) and is much slower than that from chemical type reversible hypothyroidism mainly induced by excess iodine (Group A). In conclusion, there are at least two types of reversible hypothyroidism, namely chemical type with rapid recovery and immunological or destructive type with slow recovery.

367. P85 CLINICAL SIGNIFICANCE OF ANTI-THYROTROPIN RECEPTOR ANTIBODIES IN THYROID-ASSOCIATED OPHTHALMOPATHY. I. Miyake, M. Koga, N. Nagasawa, Y. Hiromatsu, K.Kojima*, Y. Inoue**, K. Nonaka, Department of Medicine and *Department of Radiology, Kurume University School of Medicine, Kurume, and **Eye Division of Olympia Clinic, Tokyo; Japan.

Thyroid-associated ophthalmopathy (TAO) is an autoimmune disorder frequently associated with Graves' disease. However, the autoantigen in the orbit has not been fully elucidated. We have recently reported the presence of thyrotropin receptor (TSH-R) mRNA in orbit by reverse transcriptase polymerase chain reaction and in situ hybridization. In the present study, to evaluated a possible role of anti-TSH-R antibody in the development of ophthalmopathy, we measured anti-TSH-R antibodies and quantitatively assessed the severity of ophthalmopathy by magnetic resonance imaging (MRI) or computed tomography (CT). We studied two groups: (I) seventy-three patients with TAO (30 men and 43 women); (II) twenty-five patients (11 men and 14 women), who had surgery for severe TAO. The retroorbital volume was assessed as the area at the level of optic nerve in the horizontal planes, and the enlargement of eye muscles was estimated as the area in the coronal planes of MRI for group I and CT for group II. Anti-TSH-R antibodies [thyrotropin binding immunoglobulins (TBII), thyroid stimulating antibodies (TSAb)] were determined at the almost same time of MRI or CT. In group I, TBII actvity in serum significantly correlated to the swelling of the rectus muscles (sum of right rectus muscles, $r=0.30$, $p<0.05$; sum of left rectus muscles, $r=0.29$, $p<0.05$), especially the inferior rectus muscles (right, $r=0.29$, $p<0.05$; left, $r=0.29$, $p<0.05$). Neither TBII or TSAb correlated with proptosis or retroorbital volume, assesses by the MRI. In group II, TBII activity in serum significantly correlated with the proptosis, eye muscle enlargement and retroorbital volume ($r=0.51$, $p<0.01$; $r=0.79$, $p<0.01$; $r=0.68$, $p<0.01$; respectively). In conclusion, our data suggest that anti-TSH-R antibody play a role in the development of ophthalmopathy in TAO.

368. P86 NEW HPLC METHOD TO ANALYSE THE ABERRANT DISTRIBUTION OF GLYCOSAMINOGLYCAN IN GRAVES' OPHTHALMOPATHY. Ch. Hansen, B. Fraiture, R. Rouhi, K. Kuhlemann, E. Otto, J. Beyer and G. Kahaly, Dept. of Medicine III, University Hospital, Mainz, Germany

A disorder in the metabolism of glycosaminoglycans (GAG) seams to play an important role in the pathogenesis of endocrine autoimmune diseases, e.g. type I diabetes mellitus and Graves' ophthalmopathy (GO). In the present study a highly specific and reproducible method to determine the concentration and distribution of different GAG components was developed in order to obtain a sensitive test system for the activity of GO. This method was used to analyse the GAG excretion in 24h urine collections of 12 patients with clinically active GO (Thyroid 1992: 2: 235; 7fem., 23-71yrs, median 35yrs) and 12 controls (10fem., 16-70yrs, median 31yrs). The isolation of urinary GAG was achieved by sequential precipitation with cetylpyridinium chloride, potassium acetate in ethanol and elimination of protein impurities by precipitation with trichloroacetic acid, followed by sequential enzymatic hydrolysis with chondroitin lyase AC, ABC and hyaluronat lyase and high pressure liquid chromatograpy (HPLC)-analysis of the resulting a,ß-unsaturated disaccharides. By means of this method, the concentration of three GAG polymers, chondroitin sulfate A (CA), dermatan sulfate (DS) and hyaluronic acid (HA) could be determined in GO patients and controls with a high recovery rate of 76+/-14.9% for CA, 67.6+/-12.3% for DS and 72.9+/-7.8% for HA. Significant differences ($p<0.01$) between patients (pts) and controls (cts) in the urinary fraction of chondroitin sulfate A (Wilcoxon two sample test, pts: 25.5, 20.8, 32.4mg/24h, cts: 19.2, 14.3, 27.2mg/24h, median, 25th, 75th percentile) and hyaluronic acid (pts: 4.8, 4.4, 5.3mg/24h, cts: 2.6, 2.1, 3.1mg/24h) could be detected. In contrast, urinary dermatan sulfate concentration exhibited no significant increase (pts: 20.4, 12.7, 22.2mg/24h, cts: 16.0, 12.3, 19.7mg/24h). Furthermore, the total urinary GAG excretion of patients (48.2, 40.3, 54.6mg/24h) was elevated compared to controls (35.4, 31.1, 39.2mg/24h). GAG excretion decreased to normal range under immunosuppressive therapy, whereas GAG values augmented markedly 2-3 months after dropping prednisone therapy. The new method represents a sensitive test system to determine the GAG concentration and distribution in different body fluids providing a specific method to determine the nature of the excreted GAG, reflecting the distinctive and aberrant pattern of fibroblast GAG production in GO patients.

369. P87 HIGH PREVALENCE OF ACHLORHYDRIC ATROPHIC GASTRITIS IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE M. Centanni, M. Marignani, A. Casini, S. Terracina, G.F. Delle Fave, B. Annibale. Endocrinology Section, Dept of Experimental Medicine and Gastroenterology Unit, University "La Sapienza" Roma, Italy.

The achlorhydric atrophyc gastritis (AAG), a risk condition for developing gastric cancer, is a simptomless disease, often of autoimmune origin, which is frequently underdiagnosed. A relationship between autoimmune thyroid disease (AITD) and gastric immunological disorders has been already suggested; however, the atrophyc gastritis was not clearly characterized being used the anti-parietal cell antibodies (APC) detection and/or the presence of a pernicious anemia to define AAG. Aim of this study has been to screen for the presence of AAG a cohort of subjects, diagnosed as having AITD in our outpatient section, showing high levels of anti-TPO antibodies. To this end 40 subjects (36 F and 4 M) aged from 21 to 74 were divided by classes of age as follows: A) 25 to 35 years (n= 11); B) 36 to 50 years (n= 14); C) 51 to 75 years (n=15). All these subjects showed anti-TPO antibodies highly positive(>2 x cut off levels): 6 of them were euthyroid, 8 were hyperthyroid 26 were hypothyroid. The diagnosis of AAG was based on hypergastrinemia and pentagastrin-resistent achlorhydria confirmed by hystological examination of the corpus obtained by gastroscopy. TPO antibodies were measured by a radioligand assay (intra-assay var. 3.6% inter-assay var. 4.6%; Radim Techland, Liege, Belgium). Out of 40 subjects 21 (52.5%) were diagnosed as having AAG. 4 of them were euthyroid, 2 hyperthyroid and 15 hypothyroid both clinical or subclinical. Significantly high level of anti-parietal cells antibodies (PCA) have been found in 55% of the subjects. In the younger group (A) 4/11 subjects (36.4%) had atrophic gastritis compared to 7/14 (50%) in the group B and to 10/15 (66.6%) in the group C. These results indicate that achlorhydric atrophyc gastritis is quite often associate with autoimmune thyroid disease but no relation seems to exist with the functional aspects of AITD. In particular, it has to be mentioned the early occurrence of AAG in young patients with AITD showing high levels of anti-TPO antibodies. Therefore an early screening for the presence of AAG would be advisable in these subjects.

370. P88 FOLLOW-UP STUDY AND MRI FINDINGS IN PATIENTS WITH HASHIMOTO'S THYROIDITIS AND WITH HYPERPROLACTINEMIA. C. Takagi, K. Notsu, M. Imaoka and Y. Kato*, Department of Medicine, Shimane Prefectural Central Hospital and First Division, Department of Medicine, Shimane Medical University, Izumo, JAPAN

Serum prolactin (PRL) levels in some patients with primary hypothyroidism increase because of excess of thyrotropin-releasing hormone (TRH) and/or of impaired PRL clearance. Every hypothyroid patient, however, has not increased serum levels of PRL. Is it necessary to measure serum PRL levels in patients with primary hypothyroidism ? We performed TRH loading tests and measured serum PRL levels in patients with Hashimoto's thyroiditis (HT). Magnetic Resonance Imaging (MRI) of sella and serum PRL levels were followed. One hundred and fourteen women with HT and 15 normal women (Cs) participated in this study. The mean (+SD) age in all subjects was 54.7 (+12.3) yr. Serum samples were collected at 0, 30, 60, 90, 120, 150 and 180 min after iv injection of TRH. Serum PRL, TSH and free triiodothyronine (FT3) levels were measured at every time. The increased dose of serum PRL and FT3 were revealed as dPRL and dFT3, respectively. Serum free thyroxine (FT4) levels were measured at 0 min. There was a significantly negative correlation between basal FT3 and dPRL, and between dFT3 and dPRL ($r=-0.54$, $n= 114$, $p<0.0001$ and $r=-0.38$, $n=66$, $p=0.013$, respectively). There was a significant correlation between basal TSH and dPRL ($r=0.35$, $n=114$, $p=0.0003$). Patients were divided into three subgroups; 62 with both normal FT4 and TSH (euthyroid:Group A), 28 with normal FT4 and increased TSH (latent hypothyroid:Group B) and 24 with supressed FT4 and increased TSH (primary hypothyroid:Group C). The prevalence of hyperprolactinemia (over 13.4 ug/L) was 6 of 62 (9.7%), 2 of 28 (7.1%) and 9 of 24 (37.5%) in groups A, B and C, respectively. Nine in Group C, whose range of serum PRL was 16.0-123 ug/L, received replacement therapy with l-T4 and serum PRL in 6 of 9 gradually improved. Four of 9 had pituitary enlargement in MRI and the size gradually decreased in 3 patients. The other one had PRL producing tumor and the therapy with bromocriptine was effective. Two of the other 8 patietns had drug-induced hyperprolactinemia and another two had liver cirrhosis. A 23-yr-old woman was pregnant. The causes in the other 3 still remain unclear. These results may show that (1) there is a significant correlation between thyroid function and serum PRL levels, (2) the natural course in major parts of patients with hyperprolactinemia are well and the size of pituitary gradually decrease and (3) the patient(s) with PRL producing tumor can be detected. The measurement of serum PRL levels in patients with HT is necessary because the causes of hyperprolactinemia are valiable and unexpected, and specific treatment may become necessary.

371. P89 UNUSUAL DEVELOPMENT OF ACTIVE GRAVES' OPHTHALMOPATHY IN AN IMMUNOSUPPRESSED-PATIENT. G. Höfle, G. Finkenstedt, R. Moncayo*. Department of Internal Medicine and *Nuclear Medicine, University of Innsbruck, Austria.

Graves' ophthalmopathy is a disease that is most frequently associated to immunogenic hyperthyroidism. In spite of this known association individual cases have shown exceptions to this pattern. We present a female cases with an unusual course of Graves' ophthalmopathy.
A 52 year-old woman was referred in 1993 to our Institution because of active eye disease. Hyperthyroidism had developed one year before and was treated with anti-thyroid drugs as well as with radioiodine (185 MBq 131I). The patient had had a heterotopic heart transplantation in 1984 because of dilatative cardiomyopathy; thyroid function was normal at that time. After transplantation she was maintained under constant immunosuppression with prednisolone and cyclosporin-A. During the first visit she was moderately hyperthyroid under methimazole. The serum levels of TSH-receptor antibodies, neopterin and sIL-2R were elevated, whereas the levels of TNF and IL-6 were normal. One month later hyperthyroidism worsened, and the levels of IL6 and sIL-2R also increased. A normalization of thyroid function was achieved 4 weeks later with an increase of the dose of anti-thyroid treatment. Neopterin levels have remained elevated, whereas sIL-2R levels normalized. Eye signs improved due to local radiotherapy. An initial scintigraphic evaluation of the retrobulbar space using 111 Mbq of the 111-Indium-labelled Octreotide (OctreoScan ®, Mallinckrodt) showed a strong tracer accumulation, thus suggesting a localized immune reaction. Eye muscle thickening was also evidenced on CT. Actually she is euthyroid under a combined treatment with propylthiouracil and thyroxine. This cases demonstrates an unusual disease course leading to active ophthalmopathy. It rules out the ability of constant immunosuppression (cyclosporin-A and prednisolone) to prevent the development of an autoimmune disease.

372. P90 SERUM CONCENTRATIONS OF NEOPTERIN, BETA-2 MICROGLOBULIN AND SOLUBLE INTERLEUKIN-2 RECEPTOR IN PATIENTS WITH GRAVES' OPHTHALMOPATHY. M. Herold, I. Baldissera*, W. Mayer*, University of Innsbruck, Dept. of Internal Medicine, *Dept. of Ophthalmology, A-6020 Innsbruck, Austria

Neopterin, a molecule secreted by activated macrophages, ß2-microglobulin (ß2-micro), a product of nucleated cells, and soluble interleukin-2 receptor (sIL-2R), a protein released by activated T-lymphocytes, are three sensitive serum markers of immune activation. We measured their serum concentrations in patients with Graves' ophthalmopathy to evaluate whether general immune activation occurs and whether serumcon centrations of neopterin, ß2-micro and sIL-2R receptor reflect disease activity.

Twenty-six patients (21 women, 5 men) aged between 22.7 and 69.2 years (median = 43.0; mean ± SD = 45.2 ± 11.7) were tested within a routine control at our hospital. All patients were diagnosed 1 to 3 years before in an initially hyperthyroid state. At the time of investigation the majority of patients were euthyreot (data provided by R. Moncayo, Thyroid Unit).

Blood was collected by venapuncture, serum separated, portioned and kept frozen at -20 °C until further analysis. All samples were analysed together in one assay run.

Commercially avaylable immunoassays (radioimmunoassay, RIA; enzyme immune-linked immunoassay, ELISA) were used according to the instructions of the manufacturers (neopterin: RIA from Henning, Berlin, Germany; ß2-micro: RIA from Pharmacia, Uppsala, Sweden; sIL-2R: ELISA from Immunotec, Marseille, France).

All serum concentrations (median; mean ± SD) of neopterin (5.9; 6.5 ± 1.8 nmol/L) were within the normal range (below 10 nmol/L). ß2-micro (normal range: below 2.4 mg/L) was slightly elevated (2.7 mg/L) in one patient (female, 22.7 years of age) and sIL-2R (normal range: below 4.8 ng/mL) was increased (5.0 ng/mL) in a second case (female, 69.2 years of age). Both patients had no symptoms of any accompanying disease. Serum concentrations of ß2-micro (1.7; 1.6 ± 0.4) and of sIL-2R (2.8; 2.7 ± 1.1) were distributed similarly to healthy adults. Compared to 20 healthy controls, we found no statistically significant differences (Kruskal Wallis U-test) in any of the three serum concentations.

We conclude that determination of sensitive humoral immune activation markers like neopterin, ß2-micro and sIL-2R does not seem to be useful in staging or examining disease activity in Graves' ophthalmopathy.

373. P91 THYROID HORMONE AUTOANTIBODIES (THAA) IN A CASE OF CHRONIC THYROIDITIS WITH LARGE GOITER: EFFECTS OF VARIOUS TREATMENTS ON THAA TITERS.

S. Fujii, M. Oomori, U. Miwa, T. Seta, T. Ohka. Ishikawa Prefectural Central Hospital, Kanazawa, JAPAN

The presence of thyroid hormone autoantibodies (THAA) has been demonstrated in patients with various thyroidal diseases. With regard to changes in THAA activity during treatments (e.g. thyroid hormone replacement, subtotal thyroidectomy and hydrocortisones), only a few reports have been published. And the correlations between the circulating THAA and the free hormone binding substances [e.g. anti-thyroglobulin (anti-TG) and human anti mouse immunoglobulin antibody (HAMA)] by RIAs have not been clarified. We describe a patient associated with circulating anti-T_4 and anti-T_3 THAA in whom the effects of various treatments on the titer of THAA were investigated.

A 73-year-old woman was referred to our hospital for further examination of large goiter, body weight gain and dyspnea. Serum T_4, T_3 and TSH levels as measured by single antibody RIA were 3.6 μg/dl, 107 ng/dl and 97.9 μU/ml, respectively. Serum free T_4 and free T_3 levels as measured by single antibody RIAs (^{125}I-T_4 and -T_3 analog Kit) were 9.8 ng/dl and 6.2 pg/ml, respectively. But after 20% polyethylene glycol treatment, free T_4 and free T_3 levels were decreased to 0.4 and 1.8, respectively. Serum free T_4 level measured by equilibrium dialysis (ED) was 0.4. Specific bindings of ^{125}I-T_4, -T_3 and TSH to serum were 45, 36 and 6.2 %, respectively. Both titers of TGHA and those of MCHA were 1 : 5120 x 5120. Serum anti-TG level by RIA was 2819 ng/ml. After absorption of the patient's serum with mouse immunoglobulin, binding of ^{125}I-T_4 was not decreased. The patient was diagnosed as having hypothyroidism due to chronic thyroiditis. So, thyroid hormone replacement therapy was started with levothyroxine sodium (50 μg/day for two weeks and 100 μg/day for eight weeks). After this re-placement therapy, serum free T_4 measured by ED method and TSH were 1.9 and 1.74, respectively. So the patient became to be euthyroid. Specific bindings of ^{125}I-T_4 (41.4) and -T_3 (34.8) were not decreased, though serum anti-TG level decreased to the value of 263.2. Then, she underwent subtotal hemithyroidectomy. Though she was euthyroid, specific bindings of ^{125}I-T_4 and -T_3 and serum level of anti-TG were decreased to the values of 31.4, 16.0 and 68.4, respectively. Eight months after the operation, she had a asthma attack. Seven day's hydrocortisones therapy (400mg/day) in addition to levothyroxine sodium resulted in decrease in the serum specific bindings of ^{125}I-T_4 (29.2) and -T_3 (8.2) and serum anti-TG (26.7).

Thus the serum THAA, unchanged by thyroid hormone replacement, decreased by both subtotal thyroidectomy and further treatment with hydrocortisones. There was neither significant correlation between titers of THAA and the HAMA, nor titers of THAA and anti-TG level.

374. P92 **THE EFFECT OF SOMATOSTATIN VERSUS CORTICOSTEROID IN THE TREATMENT OF DYSTHYROID EYE DISEASE.** S.C. Yeung, Annie W.C. Kung, John Michon, S.H. Cheung, K.S. Tai and F.L. Chan. Departments of Medicine and Ophthalmology, University of Hong Kong and Department of Diagnostic Radiology, Queen Mary Hospital, Hong Kong.

Uncontrolled study has demonstrated the usefulness of somatostatin in the treatment of mild dysthyroid eye disease. We performed a prospective study to evaluate the usefulness of somatostatin versus corticosteroid in the treatment of mild/moderate dysthyroid eye disease. All patients were rendered euthyroid and observed for three months to exclude spontaneous improvement without active treatment. They were randomised to receive either somatostatin (octreotide 200 μg q8h subcutaneously) or corticosteroid (prednisone 1 mg/kg/day in decreasing doses). Assessment on soft tissue inflammation, exophthalmos, palpebral aperture, intraocular pressure, diplopia, cornea and visual acuity were made every four weekly. Treatment was assessed at the end of 3 month.

Interim analyses was performed on 7 patients treated with octreotide and 9 patients with prednisone (8M, 8F; age 41±18 yr). Ophthalmopathy index before and after treatment for somatostatin was 10.56±3.11 vs 9.96±3.99, corticosteroid 10.23±5.72 vs 6.80±5.95 (both p=NS).

In terms of individual patient response, somatostatin group: complete improvement (CI) 1/7, partial improvement (PI) 3/7, no improvement/deterioration (NI) 3/7; corticosteroid group: CI 3/9, PI 2/9, NI 4/9. These finding were confirmed by MRI assessment of eye-muscle score and degree of proptosis. In conclusion, somatostatin is effective in some patients with dysthyroid eye disease but the result is inferior when compared with corticosteroid.

375. P93 THE EFFECT OF A COMBINDED THERAPY OF TOTAL THYROIDECTOMY AND 131I ABLATION ON MALIGNANT EXOPHTHALMOS -A LONG TERM FOLLOW-UP STUDY-, Y. Matsumoto, S. Kubota, S. Fukata, A. Kobayashi and K. Kuma. Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University (Y.M., S.K.), Fukuoka 812, Kuma Hospital (S.F., A.K., K.K.), Kobe 650, Japan

Twenty patients with malignant exophthalmos who received total thyroidectomy and 10-50 mCi of 131I for ablation of residual thyroid tissue more than one month after operation between 1972 and 1983, and who were followed for 10-years after the combinded therapy were the subjects of this study. After the combinded therapy, thyroid function was kept euthyroid using L-T4. TBII values, anti-thyroglobulin antibody titers, and anti-microsomal antibody titers of all subjects 10 years after total thyroidectomy were negative. The degree of exophthalmos was evaluated before and 1, 5 and 10 years after total thyroidectomy. A decrease of greater than or equal to 2 mm in the degree of exophthalmos 10 years after total thyroidectomy was judged as improved and an increase of the same magnitude as worsened. Over all, 21 eyes (52.5 %) showed improvement, 16 eyes (40 %) were unchanged, and the remaining 3 eyes (7.5 %) had worsened. Furthermore, 11 of the improved 21 eyes had already improved one year after total thyroidectomy. The degree of exophthalmos before total thyroidectomy was greater in the improved group (23.5±3.3 mm) than that in the unchanged and the worsened groups (21.1±2.7 mm) ($p<0.05$). The goiter size in the improved group (31.3±18.2 ml) was significantly smaller than that in the unchanged and worsened groups (59.7±43.5 ml) ($p<0.05$). According to the degree of exophthalmos before total thyroidectomy, the improved rate was 80 % of 10 eyes (≥25 mm of degree of exophthalmos), 50 % of 24 eyes (20-24 mm of degree of exophthalmos) and 17 % of 6 eyes (≥19 mm of degree of exophthalmos). In conclusion, as there are no useful standard therapeutic methods for management of malignant exophthalmos, the combined therapy of total thyroidectomy and 131I ablation is useful for malignant exophthalmos associated with small goiter.

376. P94 FACTORS ALLEVIATING THE HYPERTHYROIDSM IN GRAVES' PATIENTS WITH EXTRAOCULAR MUSCLE ENLARGEMENT. N. Hamada, J. Y. Noh,* Y. Okamoto,** M. Ohno,*** and K. Ito,* Sumire Hospital, Osaka Social Welfare Fndn., **Osaka City General Hospital, ***Osaka Ekisaikai Hospital, Osaka, and *Ito Hospital, Tokyo 150, Japan.

Graves' ophthalmopathy occurs in euthyroid patients, and its severity is not necessarily related to the severity of the hyperthyroidism in those with Graves' disease who are hyperthyroid. Therefore, clinical characteristics of Graves' patients with ophthalmopathy were identified by analysis of 204 untreated hyperthyroid patients with Graves' disease. All patients underwent orbital computed tomography (CT) and were examined by an ophthalmologist. Patients were subdivided into a control group of 22 patients without proptosis or CT abnormalities, an EOM group of 57 patients with extraocular muscle enlargement, and an OF group of 125 patients in whom proptosis may have been due to increased orbital fat. Dunnett's method was used in comparisons with controls. The mean age of the OF group was significantly less than that of the control group, but that of the EOM group was about the same as in the controls. The serum free T4 and free T3 concentrations in the EOM group (5.38 ± 2.70 ng/dl and 13.58 ± 6.88 pg/ml; mean ± SD) were significantly less than those in the controls (7.22 ± 2.65 and 17.57 ± 6.38); these values in the OF group (6.16 ± 3.27 and 15.65 ± 7.48) were little different from control values. The level of TSH binding inhibiting immunoglobulin (TBII) was significantly lower in the EOM group (37.04 ± 23.13%) and the OF group (35.58 ± 22.75) than in the controls (52.77 ± 20.60). The thyroid gland in the EOM group was significantly lighter than in the controls. These findings indicate that the degree of hyperthyroidism was slighter in the EOM group than in the control group. To find whether factors influencing the TBII and TSAb values were present in the EOM group, we added EOM serum to control serum and assayed TBII and TSAb again. However, those values in control serum were not influenced by the addition. The TSAb to TBII ratio was significantly higher in the EOM group than in the controls. The hyperthyroid Graves' patients with extraocular muscle enlargement have factors that make the degree of hyperthyroidism slighter, and those factors may be related to the lower TBII values in these patients.

377. P95 HORMONE-DEPENDENT INTERACTIONS OF THYROID HORMONE RECEPTORS WITH THEIR ANTIBODIES. M. K. Bhat, P. McPhie+ and S.-y Cheng, Laboratory of Molcular Biology, NCI and +Laboratory of Biochemical Pharmacology, NIDDK, NIH, Bethesda, MD, USA. 20892-4255

. Thyroid hormone receptors (TRs) are ligand-dependent transcription factors. TRα1 and TRβ1 are two TR isoforms which are highly homologous in their DNA and hormone binding domains, but have no sequence similarity in the amino terminal A/B domain. To understand the molecular basis of ligand-dependent transcriptional activation of TRs, we mapped the regions of TRs which interact with the thyroid hormone, 3, 3',5-triiodo-L-thyronine (T3). Using human TR subtype α1 (h-TRα1) purified from *E. coli* as an immunogen, we screened for clones which secrete monoclonal antibodies (mAb) to inhibit binding of T3 to h-TRα1 and h-TRβ1. A mAb C4 which recognized both h-TRα1 and h-TRβ1 was obtained. Analysis of the binding data indicates that binding of T3 to both isoforms was competitively inhibited by mAb C4. Using a series of truncated mutants and synthetic peptides, we mapped the epitope of mAb C4 to the conserved C-terminal sequence Glu^{457}-Asp^{461}. Mutant PV, which has a frame shift mutation in this region and does not bind T3, was not recognized by mAb C4. Thus, part of T3 binding sites is located in this 5-amino acid region. Based on the known crystallographic structure of the thyroid hormone binding site in transthyretin, we expect mutiple interaction sites of T3 with the hormone binding domain. Consequently, we developed other mAbs which could inhibit T3 binding. Using the hormone binding domain of h-TRβ1 purified from *E. coli* as an immunogen, mAb C3 which recognized both TR isoforms was obtained. mAb C3 competitively inhibited the binding of T3 to both h-TRα1 and h-TRβ1. Using a series of synthetic peptides, the epitope of mAb C3 was mapped to the conserved region of Glu^{248}-Val^{256}. Thus, another T3 interaction site is located in this 9-amino acid region. Using circular dichroism spectrocopy, we showed that the sequence of the C-terminal Glu^{457}-Asp^{461} was capable of forming an amphipathic α-helix. Deletion of this sequence or replacement by one which could not form an α-helix, destroyed the structural integrity of the hormone binding domain. In contrast, isolated Glu^{248}-Val^{256} peptide showed no potential of forming secondary structure, suggesting that it may form a loop on the surface of the hormone binding domain. The present studies identified two regions in the hormone binding domain which interacted with T3. These studies also show that the interactions of TRs with their mAbs were regulated by T3. This may mimic the T3-dependent interactions of TRs with as yet unidentified bridging factors/ adaptors in the transcriptional activation pathway.

378. P96 TRANSTHYRETIN (TTR) LIGAND COMPLEXES: CRYSTAL STRUCTURE OF RAT TTR-EMD21388 COMPEX. Vivian Cody, Andrzej Wojtczak,+ Joseph R. Luft and Walter Pangborn, Hauptman-Woodward Medical Research Institute, 73 High St., Buffalo, NY 14203 and +N. Copernicus University, Torun, Poland.

Human transthyretin (TTR), a tetramer of equivalent 127 residue monomers, has two equivalent thyroid binding sites, and is one of three plasma proteins responsible for the serum transport of thyroid hormones such as thyroxine (T4), or products of its enzymatic degradation. Sequence analysis of TTR from various species shows that more than 85% of the residues are identical to human. In the case of rat TTR there are 22 substitutions in the 127 residue monomer compared to the human sequence. Comparison of the environment near these 22 residues permits evaluation of their influence on hormone binding interactions and tetramer assembly. As part of a program to understand the molecular basis for binding selectivity of thyroid hormones for TTR, we report the crystal structure of rat transthyretin bound with the bromoflavone, EMD21388 (3,5-dibromo-3-methyl-6,2',4'-trihydroxyflavone), a strong competitor for the binding of T4. Crystals of rat TTR are tetragonal, space group $P4_32_12$, and diffract to 2.5Å resolution for the flavone complex and have a complete tetramer in the asymmetric unit of the crystal lattice. In contrast, the structure of the human TTR-EMD 21388 complex crystallizes in the orthorhombic space group $P2_12_12$, and has two independent monomers in the asymmetric unit. This arrangement results in a statistical disorder in the position of the ligand in the binding domain of the human structure. Because the rat TTR crystals have one complete tetramer in the asymmetric unit, as was also observed for the rat TTR-T4 complex, these data provide the first observation of the unambiguous ligand binding environment not complicated by the presence of crystallographically imposed symmetry constraints present in human structures. Refinement of the rat TTR crystallographic data show that the secondary and tertiary structures of rat and human TTR are similar with only small differences in the orientation of their respective monomers. Difference electron density maps reveal two density peaks corresponding to the bromine positions for a single orientation of the flavone in both TTR binding domains, unlike the multiple modes of binding observed for the structure of the human TTR-EMD21388 complex. Data for the human structure revealed not only that the flavone binds deeper in the channel than T4, but also that it bound in a reverse orientation with the bromophenolic ring near the channel entrance. These data show that differences in side chain conformations result in small changes in the monomer-monomer contacts and permit observation of a unique TTR tetramer structure. Supported in part by DK-41009, FOGARTY TW00226 (VC) and Polish State Committee for Scientific Research, PB 0470/P2/93/05 (AW).

379. P97 Antisense-mediated blockade of "spot 14" expression specifically inhibits induction of lipogenic enzymes by triiodothyronine (T3) and glucose. William B. Kinlaw and Jori L. Church, Department of Medicine, Division of Endocrinology and Metabolism, Dartmouth Medical School, Lebanon NH, USA.

. Spot 14 protein (pS14) and the enzymes of fatty acid synthesis are rapidly induced in rat liver by T3 injection or glucose feeding. We previously showed that pS14 is primarily nuclear in location, and that its zonal distribution in liver is identical to that of the lipogenic enzymes. In the current studies we used an antisense oligonucleotide (ON) to inhibit the induction of pS14 in cultured rat hepatocytes exposed to T3 and high glucose levels to examine the function of pS14 in lipid metabolism. Hepatocytes grown in low glucose (5.5 mM) media without T3 (LO) overnight containing S14 or control ONs were treated for 48 hours with either LO or HI (50 nM T3, 27.5 mM glucose) media and ONs. HI medium caused induction of pS14 within 4 h, and maximal accumulation by 24 h. Transfection of the S14, but not the control, ON abrogated pS14 induction. Incorporation of 14-C-acetate into lipids likewise increased twofold in HI-treated cells, and this was inhibited ($p < 0.05$) by the S14, but not the control, ON. Chromatography showed that reduced triglyceride formation accounted for the change in net lipogenesis. We analyzed expression of two major lipogenic enzymes, fatty acid synthase and ATP-citrate lyase, by western blot to determine whether reduced enzyme expression accounted for the lowered lipogenesis in antisense-treated cells. Both enzymes were induced by exposure to HI medium, and the induction was inhibited by the S14, but not the control, ON. In contrast, the S14 ON had no effect on expression of either pyruvate carboxylase or propionyl carboxylase, enzymes of the gluconeogenic and fatty acid-oxidative pathways, respectively. Specificity of the S14 ON effect was further demonstrated by its inability to alter lipid synthesis in a rat hepatoma cell line that does not express pS14. We conclude that pS14 participates in a tissue-specific pathway that signals for increased expression of cellular lipogenic enzyme content in response to T3 and increased substrate availability.

380. P98 ABNORMAL EXPRESSION OF T3 RECEPTOR ISOFORMS IN A NON-T3 RESPONSIVE THYROTROPIC TUMOR. V. D. Sarapura, T. M. Bright, W. M. Wood, B. R. Haugen, D. F. Gordon and E. C. Ridgway, University of Colorado Health Sciences Center, Denver, Colorado, USA.

MGH101A, a mouse thyrotropic tumor that no longer expresses the TSHβ-subunit gene, is not responsive to thyroid hormone (T3), as manifested by a lack of inhibition of α-subunit gene expression and unregulated growth. A permanent cell line (αTSH) derived from this tumor exhibits the same phenotype. In transient transfection studies with αTSH cells, T3 failed to inhibit the activity of both the TSHβ- and α-subunit gene promoters, in contrast to a 50-80% inhibition in T3-responsive thyrotropic tumor cells (TtT97). When T3 binding studies were performed using intact αTSH cells, high affinity T3 receptors (TR) were found in αTSH cells ($Ka=2x10^{10}M^{-1}$), with a similar number of sites per cell (~15000) as in TtT97. Northern blot analysis of polyA(+)RNA from αTSH and TtT97 cells revealed that TRα1, TRβ1 and TRβ2 isoforms were present in TtT97 cells, whereas αTSH cells contained similar levels of TRα1 transcripts, while those for TRβ1 were 2.6-fold lower and TRβ2 message was not detectable. Southern blot analysis of the TRβ gene locus showed an identical restriction enzyme patterns in MGH101A and TtT97, suggesting that there are no gross deletions or rearrangements. Subsequent Northern blot analysis showed that, in TtT97 tumors, the TRβ1/actin mRNA ratio was 6-fold higher for total RNA than for polyA(+)RNA, suggesting differential losses of TRβ transcripts during the polyA(+) purification process. When total RNA from αTSH cells was analyzed, the level of TRβ1 transcripts was 3.6-fold lower and TRβ2 transcripts were now detectable at a level 2.5-fold lower than in TtT97. PCR analysis of total RNA also detected a TRβ2 signal in αTSH cells that was 4.3- and 16.7-fold lower in two different samples when compared to TtT97 cells. Western blot analysis of whole cell protein extracts from αTSH cells using specific antibodies for TRα1 (PA1-211, Affinity BioReagents), TRβ1 (kindly supplied by S. Cheng) and TRβ2 (kindly supplied by M. Lazar) detected proteins of the appropriate size for each of the isoforms. Co-transfection of αTSH cells with vectors expressing either mouse TRα1, TRβ1 or TRβ2 under the direction of a strong promoter restored T3 inhibition to both the TSHβ- and α-subunit promoters. A dose-response study with increasing amounts of co-transfected TR resulted in accordingly higher degrees of inhibition, up to 60-80%. In summary, the non-T3 responsive thyrotropic tumor (MGH101A/αTSH cells): 1) contains a lower level of TRβ1 and TRβ2 but not TRα1 when compared to the T3 responsive thyrotropic tumor (TtT97), 2) contains appropriately-sized proteins that bind to TRα1-, TRβ1- and TRβ2-specific antibodies, and 3) acquires T3 inhibition of the TSHβ- and α-subunit gene promoters by overexpression of either TRα1, TRβ1 or TRβ2. We conclude that abnormal TRβ gene expression may contribute to the lack of T3 responsiveness of this tumor, but defects in nuclear localization or interaction with other transcription factors cannot be excluded.

381. P99 **EFFECT OF AMIODARONE ON RAT HEART mRNA CODING FOR Ca^{2+} ATPase OF THE SARCOPLASMIC RETICULUM.** Lea Maria Zanini Maciel, Nassim Iazigi. School of Medicine of Ribeirão Preto, Ribeirão Preto-SP, Brazil.

A number of the cardiovascular effects of Amiodarone (AM) resembles those of hypothyroidism. It has been reported to cause bradycardia, prolonged systolic time interval and a decreased in Ca^{2+} ATPase activity of cardiac myosin. The speed of diastolic relaxation is markedly decreased in the heart of hypothyroid rats and this change has been linked to diminished uptake of Ca^{2+} into the sarcoplasmic reticulum (SR) and a decreased activity of Ca^{2+} ATPase pumps of the SR. To determine if AM may produce similar change in Ca^{2+} ATPase activity of SR we quantitated the mRNA of SR Ca^{2+} ATPase pump comparing control, hypothyroid and amiodarone treated male Wistar rats. RNA was prepared from hearts and quantitated on Northern blots. RNA blots were hybridized with a 2.3 Kb cDNA clone corresponding to the 3' end of the rat heart SR Ca^{2+} ATPase. To assure equal RNA loading, all blots were hybridized with a clone to rat 28S cDNA. Densitometric scanning of autoradiogram showed that heart SR Ca^{2+} ATPase mRNA levels were decreased by 40±3% in AM treated rats and by 70±6% in hypothyroid animals vs control group. T_3 treatment of AM-treated rats reversed all the changes induced by AM. Serum thyroxine (T_4) was higher in AM treated than in control rats, while serum T_3 was similar to control animals. These results show that 1) AM induced changes that resemble those of hypothyroidism. 2) AM causes hypothyroidism-like changes despite normal serum T_3 and increased serum T_4 and 3) T_3 reverses the effects of AM. These data support the hypothesis that AM inhibits the action of thyroid hormone on the heart.

382. P100 RELATIONSHIP BETWEEN THE THYROID HORMONE TRANSPORT SYSTEM AND THE Na^+/H^+ EXCHANGER IN CULTURED RAT BRAIN ASTROCYTES. A. Beslin, F. Chantoux, J.P. Blondeau and J. Francon, Unité Thyroïde, INSERM U96, Kremlin-Bicêtre, Paris, France.

Thyroid hormone (TH) transport into rat brain astrocytes is carrier-mediated. In the present work we studied the effect of extracellular Na^+ depletion on the initial velocity (V_i) of 0.1 nM [^{125}I]labeled-TH uptake (2-min incubation, 22 °C), in HEPES buffered (pH 7.4), isosmotic (320 mOsmol/L) solutions. When NaCl was replaced by choline-Cl or mannitol, V_i decreased by up to 25%. Monensin did not inhibit T_3 transport, and Na^+ substitution for K^+ or Li^+ had weak effect (≤ 5% inhibition), indicating that there was no T_3/Na^+ cotransport. Amiloride (1 mM) inhibited T_3 transport by ≈ 30%, suggesting a relationship with the Na^+/H^+ exchanger which participates in the regulation of the intracellular H^+ concentration ($[H^+]_i$) in astrocytes. To confirm this hypothesis, astrocytes were acid-loaded using the NH_4^+ (5 to 40 mM NH_4Cl) prepulse technique, followed by incubation in mannitol-containing buffer, in order to block the Na^+/H^+ exchanger activity. Intracellular pH (pH_i) was monitored using the BCECF fluorescence method. In Na^+-containing buffer, the resting pH_i was ≈ 7.1. In mannitol-containing buffer, pH_i was ≈ 6.9, and after acid-loading, pH_i ranged from 6.8 to 6.2. Under these conditions, the decrease (up to 40%) of T_3 or T_4 transport was linearly related (r = 0.98) to the decrease of pH_i. Acid-loading decreased the Vmax of transport, while the Km was not changed. Membrane depolarisation by high extracellular K^+ concentration (50 mM KCl replacing NaCl) had no effect on T_3 uptake, suggesting that depolarisation resulting from $[H^+]_i$ increase was not involved in the decrease of TH transport. Finally, addition of the H^+ ionophore FCCP in Na^+-containing buffer produced pH_i drop to ≈ 6.4, but had no effect on T_3 uptake.

Incubation of acid-loaded astrocytes in mannitol-containing buffer, in the presence of increasing Na^+ concentrations (0 to 135 mM) produced a parallel increase in both the V_i of H^+ extrusion and the V_i of T_3 uptake, up to the control values. Half-maximum effects were obtained at ≈ 10 mM Na^+.

In conclusion, TH transport is in close relationship with the Na^+/H^+ exchanger activity in cultured rat brain astrocytes. It was reported that TH are preferentially transported under the phenolic protonated form. The exchanger might extrude H^+ dissociating from the hormone at (within) the inner leaflet of the plasma membrane, thus facilitating dissociation of TH from their carrier. $[H^+]_i$ increase, when the exchanger is blocked, may reduce TH dissociation and thus decrease uptake.

383. P101 **TREATMENT WITH PHYSIOLOGICAL DOSES OF L-THYROXINE PREVENTS THE OVARIECTOMY-INDUCED BONE LOSS IN RATS.** C.H.A. Gouveia and A.C. Bianco, Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil.

Thyroid hormones accelerate bone metabolism and favor bone resorption, leading to decreased bone mineral density (BMD). However, the clinical significance of a low-dose L-thyroxine (T_4) therapy and its recognition as a potential risk factor for osteoporosis remains controversial. In the present study, it was investigated the effects of T_4 in the BMD of different bone sites of ovariectomized (OVX) Wistar adult rats. A total of 12 animals (210±7 days; 248±22 g) were grouped as: (i) **control** (n=4), (ii) **OVX** (n=2), (iii) **OVX+1xT$_4$** (n=3; s.c. implant of T_4 pellet releasing 2.4 μg/day; ≈physiological dose) and (iv) **OVX+2xT$_4$** (n=3; s.c. pellet releasing 4.8 μg/day). The rats were subjected to bilateral ovariectomy and to pellet implant one day after they were first scanned. BMD was measured by dual-energy X-ray absorptiometry (DEXA, Lunar; Madison, WI) under anesthesia with xylazine and ketamine (15/90 mg/kg sc, respec.) at baseline and at the end of the study (days 0 and 84, respec.). DEXA was performed in the high resolution mode in which ≈5x10^4 0.6x1.2 mm samples were accumulated at 1/16 sec intervals. The lumbar (L2-5) and caudal vertebrae (C2-5), both tibias and femurs were selected manually from the total body scan. The results indicated that OVX blocked the increase of BMD observed in practically all skeletal areas of the intact rats over the experimental period; an example is the 9±3% increase in the femur BMD of the control group versus the 4±3% decrease in OVX group (p<0.05). Treating OVX rats with 1xT_4 prevented the generalized decrease in BMD, keeping it within the control range, mainly at the L2-5 segment and the femur regions. On the other hand, doubling the dose of T_4 caused the BMD to decrease even further in almost all bone sites examined, specially in the L2-5 segment where it was observed a 7±1% decrease. **Conclusion:** in OVX adult rats, bone mass is particularly sensitive to thyroid hormones in a dose-dependent fashion. The effect can be either positive or negative depending on minimal variations of the dose used: T_4 prevents the OVX-induced BMD decrease when given in physiological doses and potentiates it when a double dose is used.

Plasma T4 and changes (Δ) of body weight, area and BMD. Effects of OVX ±T4 treatment.

	control	ovx	ovx+1xT_4	ovx+2xT_4	*P* (ANOVA)
T_4 (ng/ml)	43±3	45±1	55±3†*	73±6†*,**	<0.001
Δ Body Weight (g)	52±27*	54±1*	29±25	35±31	0.60
Δ Skeleton area (cm^2)	1.8±0.4*	2.3±1.1	3.7±1.5*	1.7±1.1	0.19
Δ Skeleton Regions (BMD)					
Total body (mg/cm^2)	12±6*	4±1	7±7	-1±5	0.10
Total body (-head)	13±5*	-1±3†	8±6	-2±6†	0.02
Vertebrae: Lumbar(L2-5)	18±14	-5±4†	19±4*,**	-18±3*†*,**	0.01
Caudal (C2-5)	17±10*	-2±1	16±9	-6±7†**	0.02
Femur: Total	26±8*	-11±1†	17±11*	-6±8†**	0.01
Diaphysis (2/4 lenght)	36±12*	-3±4†	12±11	4±6†	0.01
Distal (1/4 lenght)	5±14	-34±6†	21±3*	-17±21**	0.01
Tibia: Total	5±4	4±6	7±3*	-1±4	0.18
Proximal (1/4 lenght)	18±10*	-6±1†	13±11	-12±8†**	0.01

†vs. control, *vs. ovx; ** vs. ovx+T4; * p<0.05 by paired t-test; the values are means± standard deviations.

384. P102 REGULATION OF HIGH-AFFINITY THYROID-HORMONE BINDING IN HUMAN KIDNEY AND LIVER CYTOSOL BY THE REDOX STATE OF NADP. CYTOSOLIC-NUCLEAR INTERRELATIONSHIP. M.P. Vié, M. Samson, A. Beslin, J. Francon and J.P. Blondeau, Unité de Recherches sur la Glande Thyroïde, INSERM U96, Kremlin-Bicêtre, Paris, France.

The cytosoluble T_3 binding (CTB) present in human kidney and liver was studied at 0° C by a charcoal-adsorption method using [^{125}I]T_3 as a ligand. CTB was activated by NADPH (maximum effect at 3 x 10^{-7} M), and NADPH activation was inhibited by NADP$^+$ (half-maximum inhibitory effect at ≈ 10^{-5} M). These results suggest that an important function of CTB is the buffering of intracellular thyroid hormone free concentrations and that this function is regulated by the redox state of cytosolic NADP.

A unique 38-kDa polypeptide was identified in the cytosol of human kidney and liver by photoaffinity labeling, using underivatized [^{125}I]T_3 as the UV-activated probe, SDS-PAGE and autoradiography. Its functional identification was based on the correlation between the characteristics of CTB and those of the 38-kDa polypeptide photolabeling.The latter was dependent on NADPH and was suppressed by unlabeled T_3 at concentrations that suppressed CTB. A polypeptide with similar properties, excepted the molecular weight (35 kDa), was identified in rat kidney cytosol. The affinity of CTB is similar to that of nuclear receptors, but it is ≈ 200 times more abundant, and its subcellular localization, molecular weight, and iodothyronine specificity are different.

[^{125}I]T_3 binding to purified human kidney nuclei was characterized at 22° C. Equilibrium binding was obtained after a 2-h incubation. The T_3 Kd was 0.40 ± 0.03 nM. Unlabelled ip-T_2 or T_3 were equally potent to compete with [^{125}I]T_3 for binding.

The effect of CTB sites on T_3 binding to human kidney nuclear receptors was studied at 22° C under conditions where all reactants were at binding equilibrium. Cytosol was without effect on nuclear binding in the absence of nucleotides, as well as in the presence of NADP$^+$. In contrast, when activated by NADPH, the CTB sites strongly competed with nuclear receptors for the binding of T_3. The effect of NADPH was dose-dependent, showing parallelism to CTB activation, and was suppressed by an exogenous NADPH-oxidization system (GSSG + glutathione reductase). These observations suggest a role of the cytosoluble binding sites in regulating the amounts of T_3 that reach cell nucleus, by the buffering mechanism described above, related to the intracellular redox state of NADP.

385. P103 HYPOTHYROIDISM REDUCES GLUT I EXPRESSION DURING RAT TESTICULAR DEVELOPMENT.

MM Godbole, J Virmani, AN Srivastava*, A Mandal, M Shukla and A Mithal. Sanjay Gandhi PG Institute of Medical Sciences, Lucknow, *Avadh University, Faizabad, India.

The energy required in the seminiferous microenvironment for germ cell maturation comes through glucose oxidation and lactate production in Sertoli cells. FSH and thyroid hormones are considered to be the main physiological regulators of glucose transport and lactate production in Sertoli cells. We have studied the effect of hypothyroidism on the expression of glucose transporter isoform (GLUT I) mRNA during rat (*Sprague dawley*) testis development. Pups from normal animals (EUT) were maintained on chow and tap water while neonatal hypothyroidism was induced in dams by providing lactating mothers with 6-propyl-2-thiouracil (PTU, 0.006%) and methimazole (MMI, 0.025%) through their drinking water. The pups continued to be on PTU and MMI after weaning till sacrifice on day 50. The animals were severely hypothyroid at the time of sacrifice at D-50 (S-T4: 77.62 ± 2.07 nmol/l, S-TSH: 7 ± 0.43ng/ml in EUT group; S-T4: <32.53 nmol/l, S-TSH: 25 ± 0.43ng/ml in MMI group; and S-T4: <32.17 nmol/l, S-TSH: 28 ± 0.70 ng/ml in PTU group). Tissue LDH-X levels were decreased by 30-35 % in both the hypothyroid groups compared to the EUT group. Testicular weights were decreased by 60% in hypothyroid rats. Compared to EUT group, significant decrease in serum FSH levels in both the groups of hypothyroid animals were observed (S-FSH: 4 ± 0.05 ng/ml in EUT, <1.0 ng/ml in both MMI and PTU groups). Serum testosterone levels remained unchanged in all the groups. Northern blot analysis of total RNA from 50 day old rat testis using GLUT I cDNA probe showed a 2.7kb signal in EUT group. A 5 fold reduction in signal intensity was observed in testis of age matched rats in PTU and MMI groups as compared to EUT group. Since thyroid hormone is the main regulator of GLUT 1 synthesis, which is important for glucose transport and lactate production in Sertoli cells, we conclude that the effects of hypothyroidism on testicular function is partly attributable to decreased GLUT I glucose transporter gene expression and/or suppression of FSH. These results suggest that the thyroid hormone is essential for postnatal maturation of testicular function and may suggest a regulatory role of the hormone on gametogenic development. However the precise mechanism whereby the regulation takes place still remains conjectural.

[This work is a part of Ph.D. work of Ms. Jyoti Virmani]

386. P104 GENERATION OF NONESTERIFIED FATTY ACIDS WITHOUT HEPARIN IN SERA FROM CRITICALLY ILL AND NORMAL SUBJECTS: IMPLICATIONS FOR FREE THYROXINE MEASUREMENTS.

C.-F. Lim, D. Dutta, D.J. Topliss, J.R. Stockigt. Ewen Downie Metabolic Unit, Alfred Hospital, Melbourne, Australia.

The in vivo use of heparin causes the release of lipases into the circulation which generate nonesterified fatty acids (NEFA) in vitro and can artifactually elevate serum free thyroxine (T_4) (*Clin Endocrinol 1983; 19:591*). Critically ill patients are reported to have high serum concentrations of phospholipase A_2 (*Crit Care Med 1994; 22:956*), but it is unclear whether heparin was used. In this study, we questioned whether sera of normal subjects and critically ill patients not given any heparin have the potential to generate NEFA during routine equilibrium dialysis, and so artifactually influence serum T_4 protein binding. Initial samples (pre-heparin) from nine critically ill patients (six multi-trauma) were studied. Laboratory volunteers (n=6) served as controls. Serum NEFA concentrations (Wako, Osaka, Japan) were measured before and after 20 h, 37 C; free T_4 fraction was measured by equilibrium dialysis using [^{125}I]T_4 (serum 1:10; 20 h, 37 C; $MgCl_2$ pptn). **Results**: The mean NEFA:albumin molar ratio in the patients was 2.3 (range 0.91 - 3.4). * Paired t-test: pre vs. post, $p<0.005$; ** Triglycerides; Mean±SD

	NEFA,pre mM	NEFA,post mM	Free T_4 %x10^2	TG** mM	TT_4 nM	FT_4 pM
Normals	0.26±0.10	0.44±0.11*	1.82±0.19	1.4±0.7	96±17	17.3±2.7
Crit Ill	0.84±0.40	1.24±0.41*	2.76±0.99	1.9±2.0	59±19	15.4±5.3
vs. Crit Ill	$p<0.005$	$p<0.001$	$p<0.05$	N.S.	$p<0.005$	N.S.

In a normal serum, concentration of NEFA (mM) increased progressively during storage at 4 C: Day 0, 0.21; Day 3, 0.33; Day 7, 0.39; Day 10, 0.51; Day 14, 0.56. **Conclusions**: Our results indicate time-dependent generation of NEFA during incubation in the absence of heparin in ***both*** normal sera and sera of critically ill patients. The marked increases of serum NEFA after incubation in critical illness suggest increased circulating lipases active in vitro. The high NEFA concentration in critically ill patients could therefore result in artifactual elevation of free T_4 fraction during incubation of equilibrium dialysis. These findings throw doubt upon previous free T_4 estimates obtained in sera after incubation at 37 C or after prolonged storage at 4 C.

387. P105 EFFECT OF DIIODOTHYRONINES ADMINISTRATION ON RESTING METABOLISM AND TISSUE OXIDATIVE CAPACITY OF HYPOTHYROID RAT. Fernando Goglia, Maria Moreno, Assunta Lombardi and Antonia Lanni. Dipartimento di Fisiologia Generale ed Ambientale, Napoli, Italy.

Previous studies have shown that two diiodothyronines (3,3'-diiodo-L-thyronine (3,3'-T2) and 3,5-diiodo-L-thyronine (3,5-T2) (T2s)) are able to rapidly stimulate both *in vivo* and *in vitro* rat liver oxidative capacity. The same diiodothyronines interact directly with isolated cytochrome-c-oxidase. This interaction leads to a stimulation of the activity of isolated enzyme and to a conformational change as shown by modified visible spectra of the oxidized enzyme [1]. More recently, the presence of mitochondrial specific binding sites for T2s have also been shown [2,3]. However, only a small effect on resting or basal metabolic rate has been described so far.

We have studied the effect of daily i.p. administration of T3 (2.5 μg/100 g b.w.) or 3,3'-T2 and 3,5-T2 (2.5; 5.0; 10 μg/100 g b.w.), for 3 weeks, on resting metabolism (RM) of hypothyroid rats. On the same animals we have measured the oxidative capacity of tissues which are metabolically very active, such as liver, brown adipose tissue (BAT), skeletal muscle and heart.

As largely expected, resting metabolism was significantly decreased in hypothyroid rats (-32%). Both 3,3'-T2 and 3,5-T2 administration were able to enhance RM of hypothyroid animals in a dose-dependent way. Upon treatment with a dose of 10 μg/100 g b.w., the RM was about 30% higher than in hypothyroid rats and not significantly different from the values observed in normal animals. At tissue level these results were correlated to the oxidative capacity for the all considered organs. The 10 μg/100 g b.w. dose induced the highest stimulation of oxidative capacity (for both T2s), with more relevant values in muscle (+58% on average), liver (+50% on average) and BAT (+45% on average) . T3 administration (2.5 μg/100 g b.w.) to hypothyroid rats gave comparable effects.

Conclusion: 3,3'-T2 and 3,5-T2 mimik T3 effects on RM and oxidative capacity of tissues from hypothyroid rat.

1. F. Goglia et al. (1994) *FEBS Lett.* 346:295-298
2. F. Goglia et al. (1994) *J. Mol. Endocrinol.* 13:275-281
3. A. Lanni et al. (1994) *FEBS Lett.* 351:237-240

388. P106 Changes in catecholamine and its metabolite concentrations in cerebral cortex, cardiac muscle, adrenal gland and serum from hyper- or hypothyroid rats.

Toshiki Mano, *Hideo Sakamoto, Takehiko Mokuno, Yasutoshi Itoh, Ritsuko Kato, Michiko Hamada, Yuko Nishida, Mitsuo Hukushima, Kiyoshi Asano, Takako Miyamoto, Tetsuya Kawabe, *Ko Fujita, *Hiroshi Kuzuya.
Dept. of Intern. Med., and *Division of Molecular Biology, Inst. for Comprehen. Med. Sci., Fujita Health Univ., School of Med. Toyoake, Aichi, Japan.

Object. To clarify the relationship between catecholamine concentrations in the cerebral cortex, cardiac muscle, adrenal gland and sera in hyper- and hypothyroid states, we measured the levels of CA and its metabolites in those organs from hyper- and hypothyroid rats. **Materials and Methods.** Male Wistar rats weighing about 200g received SC injections of l-thyroxine (T4) (200μg) every other day or methimazol(MMI) (1mg) daily in the morning. The concentrations of CA and its metabolites in the tissue homogenates of the organs and in the sera were measured by electrochemical detection with CA analyzer(Neurochem, ESA, INC, USA). **Results.**

hypothyroid rat

	adrenal gland	cardiac muscle	cerebral cortex	serum
dopamine	→	→	→	→
norepinephrine	↑	↓	↑	→
epinephrine	→	N.D.	N.D.	→
normetanephrine	↑	↓	↑	N.D.
metanephrine	↓	→	→	N.D.
vanillylmandelic acid	→	→	↓	→
Homovanillic acid	↑	→	→	↓

hyperthyroid rat

	adrenal gland	cardiac muscle	cerebral cortex	serum
dopamine	→	N.D.	↓	→
norepinephrine	↑	↑	↓	→
epinephrine	→	N.D.	↑	→
normetanephrine	→	↓	↓	N.D.
metanephrine	→	↓	→	N.D.
vanillylmandelic acid	→	→	↓	↑
Homovanillic acid	→	↓	↑	↓

↑ significantly increased, compared with normal control.
↓ significantly decreased, compared with normal control.
N.D. not detectable

Discussion. The concentration of norepinephrine (NE) which mainly acts in cardiac muscle and may not be supplied from capillary blood was changed inversely in hypothyroid and hyperthyroid states, probably inducing cardiac dysfunction. In the cerebral cortex the changes in epinephrine concentrations were also inverse in hypothyroid and hyperthyroid states, which may have some role in cerebral function.

389. P107 SUBCELLULAR ALTERATIONS OF CARDIAC FIBERS IN RATS SUBJECTED TO HYPOTHYROIDISM AND AFTER RESTORING EUTHYROIDISM. F.J.C.Stamato, M.J.Simões, O.A.Moura, A.C.Lopes and R.P.Furlanetto, Disciplines of Endocrinology, Histology and Medicine Urgency, Universidade Federal de São Paulo, São Paulo, Brazil.

Thyroid hormones (HT) have a direct effect on cardiac function, increasing cardiac contractility, pulse rate and cardiac protein synthesis. Mitochondria, the cellular organelles responsible for ATP production, show an increase in size and number during hyperthyroidim. HT deficiency results in alterations in cardiac frequency and myocardial contractility with cardiomegaly and diminished cardiac output. Our aim was to investigate subcellular alterations induced by hypothyroid in atrial cardiomyocytes of albino rats and the evolution of these modifications after restoring euthyroidism. We studied 36 rats (*Rattus norvegicus albinus*) divided in three groups: controls (C), experimental (E) and "*per-feeding*" (P). Group E rats were submitted to hypothyroidism by the administration of methimazole (0,1mg/100g of body weight) during 90 days. Four rats of each group were then sacrificed and their cardiomyocytes analyzed by electron microscopy. The drug was interrupted and a new evaluation of cardiac fibers in the different groups was made 1 and 3 months later. In the hypothyroid state, severe alterations were observed: a) swollen mitochondria, decrease in their number and lysis of their cristae; b) lesser concentration of atrial granules in the perinuclear area; c) myofibrils with marked heterogeneity of patterns, some with rupture and loss of continuity of their myofilaments; d) abundant granular interstitial tissue separating the myofibrils. These alterations were not observed in C and P rats neither in the E rats evaluated 3 months after withdrawn of methymazole. In E rats analyzed one month after drug withdrawn, myofibrils modifications were less marked but still present. Our results show severe subcellular alterations in rat atrial cardiomyocytes during hypothyroidism and their total recovery once restoring the euthyroid state. This finding suggests that hypothyroid patients may have complete normalization in their cardiac function after replacement hormone therapy.

9:00 A.M.
390. **THYROID-DERIVED FIBROBLASTS SUPPORT SURVIVAL, DIFFERENTIATION AND IMMUNOGLOBULIN SECRETION OF CULTURED GRAVES' THYROID-DERIVED B-LYMPHOCYTES.** A. M. Bichlmair[1], B. E. Wenzel[2], P. C. Scriba[1], A. E. Heufelder[1]. Molecular Thyroid Research Unit[1], Medizinische Klinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität, München, and Cell- and Immunobiology Lab[2], Med. Universität zu Lübeck, Germany.

The mechanisms of B-cell accumulation within the thyroid gland in Graves' disease and the factors promoting their local expansion and differentiation to immunoglobulin secreting plasma cells have not been explored in detail. We hypothesized that failure of intrathyroidal B cells to undergo apoptosis may extend their survival, propagate local antigen presentation and prevent resolution of the intrathyoidal immune process. Evidence of enhanced B cell survival in co-cultures with thyroid-derived fibroblast monolayers led us to investigate whether paracrine factors or direct cell contact between B cells and fibroblasts may account for this effect, and whether modulation of B cell apoptosis may be involved. As demonstrated by DNA agarose gel electrophoresis, acridine orange staining and in situ nick translation, B cells derived from the thyroid gland of five untreated patients with active Graves' disease exhibited significantly lower rates of apoptosis compared to matched peripheral blood B cells (9% vs 32%; $p<0.01$). Co-culture of intrathyroidal B cells with irradiated thyroid-derived fibroblast monolayers reduced apoptosis of cultured B cells, enhanced their survival and stimulated their capacity to secrete IgG. Compared to B cells cultured alone, co-culture for 14 days with thyroid-derived fibroblasts in the presence of IL-2 and IL-10 resulted in a 15-fold stimulation of IgG production by polyclonally activated B cells ($p<0.001$). Dual chamber experiments revealed that this effect partially required direct cell contact, but was critically dependent on soluble paracrine factors produced by intrathyroidal fibroblasts, as demonstrated by neutralization experiments using antibodies to GM-CSF, IL-1, IL-6 and IL-10. Taken together, these data suggest that both direct cell contact and paracrine factors produced by intrathyroidal fibroblasts affect B cell differentiation to high-rate immunoglobulin secreting plasma cells. Modulation of B cell terminal differentiation and apoptosis through signals delivered by the intrathyroidal microenvironment may represent an as yet unrecognized mechanism that facilitates the survival and maturation of intrathyroidal B cells in Graves' disease. *Supported by DFG (He 1485/5-1).*

9:15 A.M.
391. **ANATOMIC SITE-SELECTIVE INDUCTION OF CYCLOOXYGENASE-2 AND PGE_2 IN HUMAN ORBITAL FIBROBLASTS.** **H.-S. Wang, V.D. Winn, C.H. Evans, D.A. Young and T.J. Smith. Albany Medical College and VAMC, Albany NY 12208, NCI, Bethesda, MD 20892 and University of Rochester School of Medicine, Rochester, NY 14642**

Orbital fibroblasts, implicated in the pathogenesis of Graves' ophthalmopathy (GO), are substantially different from other human fibroblasts with regard to their protein and macromolecular synthetic repertoires and responses to small regulatory molecules. We now report that PGHS-2, the newly identified and cloned inflammatory cyclooxygenase (COX), is highly responsive in orbital fibroblasts to induction by leukoregulin (LR), an activated T lymphocyte product. Steady-state levels of PGHS-2 mRNA are increased more than 30-fold within 6 hrs. of cytokine exposure. The induction is completely attenuated by dexamethasone (10^{-8} M). The abundance of PGHS-1 (the constitutively expressed COX) mRNA is unaffected by cytokine treatment. LR causes a dramatic increase (up to 500-fold) in PGE_2 production and massive cAMP generation in orbital fibroblasts. The magnitude of these increases is substantially greater than that seen with IL-1, TGF-ß or interferon-γ treatment and is greater than that observed in dermal fibroblast cultures. LR treatment of orbital fibroblasts also causes a dramatic change in their morphology that was not observed in dermal fibroblasts. Cells become stellate with the development of several long cytoplasmic processes. The shape-change can be blocked by indomethacin and dexamethasone and is reproduced with exogenous PGE_2 (10^{-7} M) and 8-Br-cAMP (1 mM). These anatomic-site selective effects of a lymphokine on endogenous prostanoid production in orbital fibroblasts may constitute the molecular basis for the inflammatory component of GO. Coupled with our recent findings that LR also increases hyaluronan synthesis in orbital fibroblasts (Am J Physiol 268:C382, 1995), this cytokine is a prime candidate molecular trigger for GO. **(supported by NIH grants EY 08976 and DK 16177 and VA Merit Review)**

9:30 A.M. 392. **T-CELL-RECEPTOR V GENE SELECTION IN HUMAN THYROID ORGANOIDS.** Andreas Martin, Jin Zhang, Pamela Unger* and Leonard D. Shultz#, Departments of Medicine and Pathology*, Mount Sinai School of Medicine, New York, NY, USA and The Jackson Laboratory#, Bar Harbor, ME, USA.

We have used a human thyroid organoid model in the *scid* mouse to examine the interaction of T cells and their thyroid target cells in an *in vivo* environment. Campath-1 monoclonal antibody was used to remove lymphocytes from human thyroid monolayer cells by complement-mediated lysis and the resulting thyrocytes were resuspended in a basement membrane gel and transplanted into *scid* mice where they re-organized into thyroid neo-follicles. To study the long-term effect of thyrocytes on the human peripheral T-cell repertoire, Graves' disease thyrocytes were Campath-1 treated and mixed with autologous peripheral blood mononuclear cells (PBMC) prior to organoid construction and transplanted s.c. into *scid* mice (2×10^6 thyrocytes and 3×10^6 PBMC/organoid). Five weeks later, organoids were explanted, total cellular RNA extracted and analyzed for human T-cell receptor (hTcR) mRNA using primers for the hTcR constant region (Cß2) as well as hTcR variable (18 Vα and 20 Vß) gene families. In comparison to peripheral blood and control organoid (PBMC/fibroblasts) where most hTcR V genes were expressed there was a marked reduction in the numbers of hTcR Vα and hTcR Vß families in PBMC/thyrocyte organoids (mean number of both Vα and Vß families in PBMC/thyrocyte organoids, 7.0 ± 2.2). Organoids constructed with Campath-1 treated thyrocytes alone contained no detectable hTcR transcripts. Among both hTcR Vα and Vß families, certain hTcR V genes were predominant: Vα1 ($42 \pm 15\%$), Vß4 ($19 \pm 8\%$) and Vß5 ($29 \pm 9\%$) differed significantly from other hTcR V gene families ($p < 0.01$, ANOVA, Tukey's protected t-test). Both Vα1 and Vß5 were consistently present in 5/5 mixed cell organoids and were also present in the original thyrocytes, suggesting intra-organoid selection for certain hTcR V gene families from the peripheral repertoire. This model permits the reconstruction of hTcR V gene selective events in an *in vivo* environment and offers a potential window on the early stages of human autoimmune pathogenesis.

9:45 A.M. 393. **THYROID TISSUE FROM PATIENTS WITH GRAVES' DISEASE CONTAINS CLONALLY EXPANDED T CELLS WITHIN A RESTRICTED BUT HETEROGENOUS T CELL POPULATION - EVIDENCE FOR A DUAL MECHANISM OF IN-SITU T CELL EXPANSION.** T.F. Davies and M. Nakashima, Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA.

We have reported restricted use of human T cell receptor (hTcR) Vα and Vβ gene families expressed by the intrathyroidal lymphocytes of patients with autoimmune thyroid disease and in murine models of spontaneous and antigen-induced thyroiditis. In the present study we have used a sensitive radioactive PCR method to observe the pattern of V gene use within the different families expressed. This method is based on the different lengths of the CDR3 (N-D-N) region which is subject to random nucleotide additions and deletions. Hence each T cell clone is likely to have CDR3 regions of differing length which can be visualized as distinct bands of labelled PCR product. In practice, PBMC showed 8-12 bands per V gene family and served as the autologous controls. Total cellular RNAs were extracted from paired thyroid tissue and PBMC and reverse-transcribed. The labelled PCRs were performed with 18 Vα and 21 Vβ oligonucleotides as forward primers and a ^{32}P-labelled C region oligonucleotide as reverse primer. PCR products were then subjected to sequencing polyacrylamide gels and exposed. We observed reduced numbers of intrathyroidal hTcR Vα (mean = 8.6/18) and Vβ (mean = 13.6/21) gene families in 3 of 4 patients with Graves' disease. Limited PCR banding patterns (< 6), representing within-family restriction ($n = 12$) and enhanced individual bands, representing clonal expansion ($n = 8$), were observed in the thyroid tissues when compared to autologous PBMC. In agreement with the labelled PCR technique, sequencing of selected PCR amplified V gene families showed evidence for the presence of both T cell clonal expansion and a heterogenous T cell population sharing the same V gene family. These results support our hypothesis that the human intrathyroidal T cell population in Graves' disease is selected by mechanisms associated with the TcR V gene invariant product as well as specific antigen recognition by the CDR3 region.

9:00 A.M. 394. CLONING AND ANALYSIS OF RAT THYROID TRANSCRIPTION FACTOR-1 (TTF-1) GENE.

M. Ohno, M. Nakazato, T. Kogai, T. Endo and T. Onaya, Third Department of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi, 409-38, Japan.

TTF-1 is a homeodomain-containing thyroid transcription factor which activates the gene of thyroid specific proteins, thyroglobulin, thyroid peroxidase and thyrotropin receptor. Thus, TTF-1 is one of the key molecules which mediate thyroid specific functions.

In order to understand the expression mechanism of TTF-1 itself, we have screened 1.2 x 106 plaques of the rat liver genomic library with 331 bp of 5' end of rat TTF-1 cDNA, and obtained a positive clone. Then we determined the exon/intron organization and the structure of the 5' flanking region. The clone is 14 kbp long and contained 5.2 kbp upstream sequence from translation initiation site. We found that the gene has a single intron, 882 bp long, in the coding sequence between 373th and 374th nucleotides of the rat TTF-1 cDNA. The 5' flanking region is GC rich (68%), but characteristic TC-repeat was found from -440 to -379. There are no sequences corresponding to a consensus TATA box, but CAAT box was located at position -171. We could not find consensus sequences of CRE or TRE, but found that a TTF-1 binding consensus sequences, CTCAAGC, at a position -175 bp, which overlaps the consensus CAAT box. Consensus sequences for SP1 binding sites are found at positions -1551, -1473, -1106, -1098, and -966 bp. Multiple AP2 binding consensus sequences were found at positions -1005, -887, -782, -525, and -194 bp.

DNase I foot print analysis to the -80 to -250 bp of TTF-1 upstream has demonstrated that the regions, from -91 to -118 bp, -126 to -143 bp, -161 to -176 bp, and -192 to -209 bp, are protected by nuclear extract from thyroid cells but not by the extract from the liver. The region, -161 to -176 bp, contained the binding site for TTF-1 and CAAT box. These results suggest that TTF-1 protein itself might bind TTF-1 gene, thus, expression of TTF-1 is autoregulated by TTF-1.

9:15 A.M. 395. THYROID HORMONES REGULATE MESSENGER RNA LEVELS OF THE THYROID-SPECIFIC TRANSCRIPTION FACTORS : TTF-1 AND PAX-8. S. Selmi-Ruby, C. Watrin and B. Rousset.

INSERM U 369, Faculté de Médecine Alexis Carrel, Lyon, France

Among the identified thyroid-specific transcription factors, two of them, TTF-1 and Pax-8 are now known to control several aspects of thyroid cell differentiation. In our search for genes expressed in the thyroid that could be subjected to transcriptional and/or post- transcriptional controls by thyroid hormones, we have analyzed the potential regulatory role of T3 and T4 on the expression of TTF-1 and Pax-8 by pig thyroid cells in culture.

Pig thyrocytes were cultured without or with TSH (1mU/ml) from the outset of culture to obtain monolayers (M) or reconstituted thyroid follicles (RTF). After 24 h, cells were placed in a serum free medium and at day 3, cultured without or with increasing concentrations of T3 or T4 (1 nM to 1000 nM) for 2 to 48 h. Total RNA was extracted by the guanidinium thiocyanate method and analyzed by Northern blot using a 0.6 kb cDNA fragment corresponding to the 3' UTR of rat TTF-1 mRNA or a 0.3 kb cDNA fragment corresponding to the paired domain of rat Pax-8 (the probes were kindly supplied by R. Di Lauro, Naples, Italy). mRNAs were quantified by densitometry. TTF-1 and Pax-8 mRNAs of 2.6 kb and 3.1 kb, respectively, were detected in both M and RTF.TTF-1 mRNA levels were low in M and 2.3 times higher in RTF. Similarly, the Pax-8 mRNA content of RTF was markedly higher (x6) than that of M. These differences between M and RTF were, at least in part related to an action of TSH since a treatment of M by TSH (1 mU/ml) from day 1 to day 5 induced a marked elevation of Pax-8 mRNAs. When cultured in the presence of 100 nM T3 for 48 h, M exhibited a 2.6- and 3.5- fold increase of their TTF-1 and Pax-8 mRNA content. These T3 actions were reproduced by 100 nM T4. The T3-induced effect was concentration dependent. A significant elevation of both TTF-1 and Pax-8 mRNAs was observed at 1 nM T3. Pax-8 mRNA levels were significantly increased after 2-4 h of T3 treatment, near maximum after 8 h and sustained for up to 48h. The T3-induced TTF-1 mRNA increase was only seen after 8 h of T3 treatment. Treatment of RTF by 100 nM T3 caused only minor changes in TTF-1 and Pax-8 mRNA levels. Experiments are in progress to document the site(s) of action of T3 and T4.

In summary, we have identified two potential targets, TTF-1 and Pax-8, for T3-dependent ,T3 nuclear receptor-mediated regulation in the thyroid. Our data lead us to postulate that thyroid hormones could exert diverse autocrine regulatory functions by controlling the expression of transcription factors to which is linked the maintenance of thyroid cell differentiation.

9:30 A.M. 396. DYNAMIC CHANGES OF TIGHT JUNCTION PROTEIN ZO-1: POSSIBLE ROLE IN TGFß1-INDUCED JUNCTIONAL DISASSEMBLY-REASSEMBLY RELATED TO EMIGRATION OF THYROID EPITHELIAL CELLS.

M. Nilsson. Institute of Anatomy & Cell Biology, Göteborg University, Göteborg/Sweden.

Several peptide growth factors are known to promote the migration of thyroid epithelial cells. An important early step in this process, whether natural or part of tumor infiltration, is cell detachment from the epithelial sheet. Very little is generally known about molecular mechanisms controlling epithelial cell emigration, although changes in the junctional complex including cadherin-mediated cell-cell adhesion is likely involved. In this study on polarized, pig thyrocyte monolayers cultured in Transwell chambers, we focused on growth factor effects on ZO-1, a phosphoprotein (225 kD) located at the cytoplasmic aspect of epithelial tight junctions, and homologous to the tumor suppressor gene product, dlg, in Drosophila. The distribution of ZO-1 was compared with those of cadherin, vinculin and F-actin by immunofluorescence and correlated with electron microscopic observations.

Confluent cells treated with transforming growth factor beta-1 (TGFß1; 10 ng/ml) for 2-4 days showed reorganization of F-actin in the apical cytoplasm and had a variable, although still epithelial-like shape. The size of individual cells in the monolayer was increased, as if the number of cells attached to the filter was reduced, but the culture DNA content was the same as in controls. Ultrastructurally, the TGFß1-treated cells in monolayer were distended and tilted with respect to the apicobasal axis and, importantly, flat cells appeared between the continuous monolayer and the supporting filter. Although the majority of cells in the monolayer had a normal, linear ZO-1 distribution along the cell border, clusters of cells showed complex ZO-1 changes, which comprised fragmentation, complete disintegration and signs of reassembly. Specific alterations of cadherin, vinculin and actin distribution appeared in some of the cell profiles with a changed ZO-1 distribution. TGFß1 also induced a progressive reduction of transepithelial resistance and an increase of paracellular [^{3}Hinulin] flux. All these effects of TGFß1 were augmented by EGF (10 ng/ml) but antagonized by IGF-1 (100 ng/ml). On the other hand, TGFß1 equally inhibited the cell proliferation induced by EGF or IGF-1.

In conclusion, the present study provides the first example of TGFß1-induced regulation of the junctional complex in epithelia, and suggests that ZO-1 is a target protein of this growth factor. The dynamic changes of ZO-1 may serve to regulate the dissociation and reassembly of junctions when thyrocytes are stimulated by TGFß1 to disconnect from the epithelial layer in a migratory process.

9:45 A.M. 397. Serum deprivation activates apoptosis in Ki-ras but not in polyoma transformed or in differentiated thyroid cells.

B. Di Jeso, L. Racioppi, A. Feliciello, F. Pacifico, P. Giuliano, L. Ulianich, E. Consiglio, S. Formisano, and E. V. Avvedimento
Centro di Endocrinologia ed Oncologia Sperimentale and Dip. di Biologia e Patologia Cellulare e Molecolare, Via S. Pansini 5, 80131 Napoli, Italy

Ki-ras, the oncogenic variant of c-ras transforms thyroid cells in culture. The transformed phenotype is associated with the complete loss of the thyroid differentiation markers, i. e., thyroglobulin (Tg), thyroperoxidase, iodide carrier and TSH receptor. On the contrary, polyoma middle-T (MT), although fully capable of transforming thyroid cells, does not cause a complete dedifferentiation of these cells. In fact, a residual expression of Tg, iodide carrier, and TSH receptor is detected in MT transformed thyroid cells (MT cells).

Both cell lines (ki and MT cells) are no longer dependent on TSH for growth. Instead, they become highly sensitive to serum. Even trace amount of serum (0.5%) fully stimulates the growth of these cells. However, serum deprivation, i. e. starvation in 0.2% BSA, causes a complete growth arrest of MT cells and normal FRTL-5 cells but cell death of ki cells. We have determined by fluorescence-activated cell sorter analysis that the death of ki cells starved in 0.2% BSA was in fact apoptotic (about 20% of cells were apoptotic after 24 hours in BSA, and about 50% after 48 hours). c-myc is involved in the activation of the apoptotic pathway in ki cells since these cells show a two-fold increase in c-myc mRNA levels after 6 hours starvation in BSA, while both MT cells and normal FRTL-5 cells show a downregulation of c-myc mRNA levels after the same starvation time. In an attempt to better understand the molecular basis of the different behaviour of ki and MT cells we dissected the MT signal transduction pathway. Transfection of MT cells with H17, a transdominat negative mutant of c-ras, reactivates Tg expression. This suggests that MT acts by activating endogenous c-ras. The activated c-ras exerts its inhibitory effect downstream the cAMP levels since treatments of MT cells with cholera toxin, forskolin or 8Br-cAMP were unable to activate Tg expression.

In conclusion, our data suggest that coupling a growth-arrest signal (serum deprivation) with a constitutive active signal (activated ras) causes apoptosis. They also indicate that the oncogenic conversion of c-ras to v-ras is critical to activate the apoptotic pathway in transformed thyroid cells.

10:30 A.M. 398. CLONING AND EXPRESSION OF THE HUMAN SELENOPROTEIN, TYPE 3 IODOTHYRONINE DEIODINASE. D. Salvatore, S.C. Low, A-L. Maia, J.W. Harney, W. Croteau, D. St. Germain and P.R. Larsen. Thyroid Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 and Departments of Medicine and Physiology, Dartmouth Medical School, Hanover, NH 03756

Type 3 iodothyronine deiodinase (D3) catalyzes the conversion of T4 and T3 to inactive metabolites. D3 is highly expressed in placenta and may thus play a role in the regulation of the level of circulating fetal T3. Based on similarities between the rat D3 and the rat type 1 iodothyronine deiodinase (D1), degenerate oligos were used to amplify a 200bp fragment from human placental mRNA by RT-PCR. This fragment was used as a probe to screen human placental cDNA libraries. Five positive clones were identified, one of which contained an open reading frame of 836 bases, which predicts a protein of 279 amino acids (assuming the in frame UGA codon encodes selenocysteine). The functional activity of hD3 was demonstrated by transient transfection of the cDNA into 293 HEK cells. Following transfection, cells were harvested and incubated in the presence of ^{125}I T3 or reverseT3 (rT3). HD3 exhibits high affinity inner ring deiodination of T3 (Km 1.2 nM) and very low Vmax for 5' deiodination of rT3. The Kcat for T3 of D3 was 100 fold lower than the Kcat of D1 for rT3. Sequences in the 3' untranslated region of the mRNA are required for expression of activity. As expected, hD3 was resistant to inhibition by both propylthiouracil (1mM) and aurothioglucose (Ki for ATG 5.2 μM). To determine the size of the translated protein, we incubated extracts of 293 HEK cells transiently transfected with hD3 with bromoacetyl ^{125}I T3 and analyzed the labelled proteins. A single band of ~ 34 kDa was observed which could be competed with unlabelled bromoacetyl T3 and specific competitors of the D3 reaction. We also demonstrated that D3 is a selenoprotein by incubating HtTA cells (derived from HeLa cells) transiently transfected with D3 in the presence of ^{75}Se. A single protein band of ~ 34 kDa was observed, and the selenium labelling of D3 was greatly augmented by co-transfection of the human selenium donor protein cDNA, selD, recently cloned in our laboratory. Northern blot analysis using human tissue poly A$^+$ RNA revealed a single mRNA band of 2.0kb in human placenta and not in seven other human tissues, including liver, kidney and brain. In conclusion, we have cloned the human D3 and demonstrated that while it is a selenoprotein, it has very different kinetic and biochemical properties from those of the human D1.

10:45 A.M. 399. REGULATION OF TYPE 1 5'DEIODINASE BY INFLAMMATORY CYTOKINES IN RAT LIVER CELLS PH DAVIES, MC SHEPPARD, JA FRANKLYN. DEPARTMENT OF MEDICINE, UNIVERSITY OF BIRMINGHAM, EDGBASTON, BIRMINGHAM, B15 2TH, UK.

Type 1 5'deiodinase enzyme (5'D-I) is a major determinant of tissue thyroid hormone action, essential for the peripheral conversion of T_4 to T_3. Decreased 5'D-I activity has been implicated in alterations in circulating thyroid hormones seen in non-thyroidal illnesses (NTI), but the mechanism for reduced 5'D-I activity is these circumstances is not known. The administration of the cytokines tumor necrosis factor α (TNFα) and interleukin-1 (IL-1) to experimental animals and human volunteers result in changes in circulating thyroid hormone concentrations similar to those seen in NTI. It has been postulated that inflammatory cytokines, commonly elevated in many types of NTI, might mediate the euthyroid sick syndrome by inhibiting 5'D-I activity. We have reported a stimulation of 5'D-I activity by interleukin-6 (IL-6) (Thyroid 1994 4:S32) and now present the results of further investigation of the effects of IL-6, along with those of TNFα and IL-1 upon 5'D-I expression, measuring changes in mRNA by Northern hybridization and 5'D-I enzyme activity in a substrate conversion assay (^{125}I release with reverse T_3 as substrate), in rat Φ_1 liver cells. All 3 cytokines significantly increased 5'D-I enzyme activity, following 24 hours treatment of cells: TNFα, control 100 ± 8%, 0.05μg/l 166 ± 15%, 0.5μg/l 212 ± 25%, 5μg/l 259 ± 34, n = 6, $p<0.01$ (by one-way ANOVA); IL-1, control 100 ± 3%, 0.1μg/l 165 ± 9%, 1μg/l 166 ± 5%, 10μg/l 180 ± 5%; n = 6, $p<0.01$; IL-6 control 100 ± 11%; 2.5μg/l 203 ± 11%; 5μg/l 254 ± 24%; 10μg/l 272 ± 5; n = 6, $p<0.01$. Timecourse studies (1-48 hours) showed that this effect of cytokines began after 4 hours and was maximal at 18 hours. Stimulation of 5'D-I activity by IL-6 was still apparent after 48 hours (205 ± 5% control), wheras the effect of TNFα and IL-1 declined after 18 and 24 hours respectively, with little stimulation apparent at 48 hours (127 ± 4% and 118 ± 7% control activity respectively). Co-incubation with cycloheximide 10μM abolished stimulation of 5'D-I activity by all 3 cytokines, suggesting that new protein synthesis is required for this effect. Kinetic analysis revealed that stimulation of 5'D-I enzyme activity was a result of increased V_{max}, with K_m unchanged (K_m 94-99nmol/l for rT_3; V_{max} control 2.6, TNFα 5.2, IL-1 4.3 and IL-6 5.8 pmol/mg/min). 5'D-I mRNA abundance (signal relative to 18s rRNA) was not significantly changed by any of the cytokines studied (control 100 ± 4%, TNFα 5μg/l 105 ± 16%, IL-1 10μg/l 112 ± 12%, IL-6 10μg/l 71 ± 21% (n = 9; NS)). Our data suggest that the inflammatory cytokines TNFα, IL-1 and IL-6 all stimulate 5'D-I activity in the absence of an effect upon pretranslational 5'D-I expression in Φ_1 rat liver cells. These findings are contrary to the hypothesis that inflammatory cytokines may mediate the ESS by causing inhibition of 5'D-I activity. The stimulation of 5'D-I enzyme activity reported is dependent on new protein synthesis and independent of an increase in 5'D-I mRNA abundance, but the exact nature of this effect remains unknown.

11:00 A.M. 400. PRESENCE OF GROWTH FACTORS-INDUCED 5 DEIODINASE ACTIVITY IN CULTURED BROWN ADIPOCYTES. A. Hernández, M.-J. Obregón. Inst. Investigaciones Biomédicas (CSIC). Madrid. Spain

Inner ring deiodination is a key pathway of thyroid hormones metabolism that leads to inactivation of thyroid hormone action. This action is acomplished by the iodothyronine 5 deiodinase enzyme (5D) (inner ring deiodinase or type III). 5D activity has been found in most tissues: liver, kidney, brain, placenta and skin, but has never been described to be present in brown adipose tissue. 5D is regulated by thyroid status, it is high in fetal tissues and low in the adult ones. 5D is induced in cultured astroglial cells by growth factors (GF), phorbol esters and cAMP. Recently, a cDNA encoding a protein with 5D activity has been cloned.

We found very low cellular T3 concentrations in brown adipocytes (BA) differentiated in culture, despite they were grown in the presence of 10% euthyroid serum. The **aim** of this work is to test the presence of serum-dependent 5D activity in BA, and to identify which GF may be involved in 5D induction of 5D.

Brown preadipocytes were isolated from 20-days-old rats, seeded and cultured in DMEM+10 mM Hepes, 15 µM ascorbic acid, 3 nM insulin and 10% newborn calf serum (NCS). Cells were used during differentiation. 5D specific activity (1U= 1pmol/h/mg protein) was determined using 20 mM DTT, pH=7.5 and 25 nM T3 using inner ring labelled T3 (80 µCi/ug) as tracer (gift from Dr. Thoma & Rokos, Henning, Berlin).

Results: Cultured BA showed a high 5D activity (2.5 U) after 24 h exposition to fresh 10% NCS. This activity decreased to 25% or 10% when 1% NCS or serum-free medium were used. The effects of several GF was tested in the presence of serum-free medium (20 h). Platelet-derived growth factor (PDGF, 50 ng/ml), vasopressin (20 nM), Insulin-like growth factor I (IGF-I, 2 nM) induced significant 5D activity (2-6 fold). Epidermal growth factor (EGF, 10 ng/ml), basic and acidic fibroblast growth factor (bFGF, 10 ng/ml and aFGF, 4 ng/ml) strongly induced 5D activity (25-, 45- and 50-fold, i.e. 23.4, 32.2 and 45.3 U, respectively). The doses indicated above led to maximal inductions. Time-course experiments showed a maximum effect at 24 h for EGF induction and 8h for both aFGF and bFGF inductions. The presence of heparin (50 µg/ml) results in a displacement of the maximal effect from 8h to 14h for FGF's inductions, and potentiated 8-fold the effects of aFGF. Heparin did not affect 5D activity itself. All aFGF, bFGF, EGF and serum stimulations of 5D activity were abolished in the presence of actinomycin D (5 µg/ ml) or cycloheximide (25 µM). Kinetic studies disclose a Km of 22.5 nM ± 2.8 for T3 in the assay conditions described. BA were also able to express the specific marker uncoupling protein when holding high, or very high, 5D activities.

Summary: 1) To our knowledge these are the first data reporting that high 5D activities are present in cultured brown adipocytes. 2) These activities are induced by the presence of serum. 3) EGF, aFGF, bFGF and PDGF strongly stimulate 5D activity and may contribute to the serum effect at physiological doses. 4) Gene transcription and *de novo* protein synthesis are required for 5D induction. The low T3 concentrations resulting from these GF effects should be taken in account when using cell cultures to test thyroid hormone-dependent effects. *Grants from PGC 92/0061, FISS 94/0274 and FISS 92/0888*

11:15 A.M. 401. **REGULATORY MECHANISM OF IODOTHYRONINE 5' DEIODINASE ACTIVITY AND mRNA OF CULTURED RAT MYOCARDIAL CELLS.** T. Yonemoto, A. Gondou, Y. Mori, H. Matsubara, M. Nishikawa, The Second Dept. of Internal Medicine, Kansai Medical University, Moriguchi, Osaka 570, JAPAN

We previously demonstrated that iodothyronine 5'-deiodinase (I-5'-D) activity is increased by triiodothyronine (T3) and angiotensin II (A II) in cultured rat cardiac myocytes. To evaluate further the stimulatory mechanism of I-5'-D, we investigated the role of protein kinase C and intracellular Ca^{++} on myocardial I-5'-D activity. Moreover, to elucidate the molecular mechanism of the stimulatory effect of A II , we quantified the changes in mRNA levels, using a competitive reverse-transcriptase polymerase chain reaction. I-5'-D activity was increased about 1.6 folds over the basal level by adding BAY-K 8644, Ca^{++} channel agonist, to the culture medium . This stimulatory effect of BAY-K 8644 was completely blocked by nifedipine. 12-O-tetradecanoylphorbol-13-acetate, protein kinase C activator, similarly stimulated I-5'-D activity and this effect was blocked by H-7. The addition of high concentration (20-40mM) of K^+, which caused the depolarization of membrane had significant stimulatory effects on I-5'-D activity about 1.4-3.5 folds over the basal level. This result suggests that depolarization of plasma membrane leads to the increase in this enzyme. The addition of A II induced a 2.3-fold increase in I-5'-D mRNA level. A significant increase in the I-5'-D mRNA level was evident 8hr after A II addition. This increase reached a maximal level at 12hrs (2.3-fold) and remained for at least 24hrs, consistent with the change in the I-5'-D activity. Cardiac fibroblasts did not express the I-5'-D mRNA. These findings indicate that (1) I-5'-D activity in myocardial cells is increased by activating voltage sensitive Ca^{++} channel and protein kinase C, (2) the mRNA encoding the I-5'-D is present in cardiac myocytes, and (3) A II induces the I-5'-D mRNA accumulation. Thus, this study suggests that A II could affect the action of thyroid hormone on the heart by modulating the I-5'-D gene expression.

11:30 A.M. 402. **EXPRESSION OF THE TRANSMEMBRANE THYROID HORMONE TRANSPORT PROTEIN FROM RAT LIVER IN *XENOPUS LAEVIS* OOCYTES.** R. Docter, E.C.H. Friesema, P.G.J. van Stralen and G. Hennemann. Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands.

Our studies as well as that of others have shown that transport of thyroid hormones across the plasma membrane involves a stereospecific, saturable, temperature-, ATP- and Na^+ gradient-dependent transport mechanism. The exact nature of this transporter is not yet known, but is most likely a membrane protein. Therefore, we have initiated studies to express this transport protein in *Xenopus laevis* oocytes.

Oocytes (stage V/VI) were injected with 23 nl poly-A^+ RNA (mRNA) isolated from livers of adult male Wistar rats (23 ng mRNA/oocyte), or with 23 nl water (negative control), or with 23 nl rBAT cRNA (0.23 ng cRNA/oocyte; positive control). rBAT expresses Na^+-independent transport of L-arginine, L-cysteine and L-leucine (PNAS 89 (1992) 5601). Uptake of [^{125}I]-T_3 was measured to test for T_3 transport and of [^{3}H]-L-Arg for rBAT cRNA expression. Injected oocytes were kept at 18 C in modified Barth's solution ((mM) 88 NaCl, 1 KCl, 0.82 $MgSO_4$, 0.4 $CaCl_2$, 0.33 $Ca(NO_3)_2$, 2.4 $NaHCO_3$, 10 Hepes pH 7.4, 10 IU/ml penicillin and 10 μg/ml streptomycin) for 3 to 4 days, with daily change of medium. To measure transport, groups of 10 oocytes per injected material were incubated at 25 C in 100 μl incubation buffer ((mM) 100 NaCl or choline chloride, 2 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes / Tris pH 7.5) containing 5 nM [^{125}I]-T_3 or 50 μM [^{3}H]-L-Arg. After 1 h oocytes were washed with buffer with 0.5 % BSA and counted individually.

Results: Injection of rBAT results in a rise in L-Arg uptake from 6 pmol (H_2O inj.) to 230 pmol L-Arg / oocyte without a change in T_3 uptake. Injection of 23 ng total liver mRNA results in a Na^+-dependent rise in T_3 uptake (see table). This uptake can be inhibited by 1 μM unlabeled T_3. Gradient centrifugation of total mRNA on 6-20% sucrose, results in a 2- to 4-fold enrichment of activity in fractions containing mRNA species between 1 and 2.5 kb.

	fmol T_3 /oocyte (mean ± SEM)	
	Na^+	choline$^+$
H_2O	3.8 ± 0.3	4.1 ± 0.2
mRNA (A)	7.4 ± 0.3	4.6 ± 0.4
mRNA (B)	6.3 ± 0.3	4.4 ± 0.3
mRNA (C)	6.3 ± 0.6	4.3 ± 0.3
mRNA (D)	6.2 ± 0.5	4.4 ± 0.4
all vs H_2O	$p < 0.001$	NS

A-D livers of different rats; 10 oocytes /exp.

Conclusions: 1. Injection of total mRNA from rat liver into *X. laevis* oocytes results in a stimulation of saturable Na^+-dependent T_3 transport. 2. There exists at least one species of mRNA in rat liver which codes for a protein capable of inducing membrane transport of T_3. 3. Therefore, it seems possible for hepatocytes to regulate the expression of this protein via increased or suppressed transcription of the gene for this messenger RNA. 4. This regulation may be of importance in the determination of overall thyroid hormone bio-activity in health and disease.

11:45 A.M. 403. SERUM "COMPOUND W" CONCENTRATIONS IN FETUSES AND THEIR MOTHERS AT VARIOUS GESTATIONAL STAGES. P. Beck-Peccoz, D. Cortelazzi, A.M. Marconi°, A.M. Baggiani°, M. Buscaglia°, D.H. Polk*, D.A. Fisher* and S.Y. Wu^, *Institutes of Endocrine Sciences, Ospedale Maggiore IRCCS, and °Obstetric&Gynecology, S. Paolo Hospital, University of Milan, Milan, Italy; *Perinatal Lab, Harbor-UCLA Medical Center, Torrance, CA, and ^Thyroid Research Lab, VA-UC Irvine Medical Center, Long Beach, CA, USA.*

The important role of sulfation in thyroid hormone metabolism during fetal development is increasingly recognized. Among others, recent studies showed that the concentrations of 3,3'-diiodothyronine sulfate (T_2S) circulating in fetal and maternal sheep are very high, as they are in human maternal and cord sera. Interestingly, in pregnant women a substance (Compound W), immunologically and chemically similar to T_2S but that does not cochromatograph with synthetic T_2S in HPLC, has been discovered (J Clin Endocrinol Metab 78: 1505, 1994).

The present study was undertaken to determine whether elevated serum compound W levels occurred in normal human fetuses and to correlate fetal and maternal levels in paired samples taken from 19 week to the end of gestation. Fetal samples were obtained by cordocentesis carried out for rapid kariotyping and diagnosis of infection or hematological disorders.

In 22 normal fetuses, a progressive increase of Compound W concentrations from 19 to 39 weeks of gestation was recorded and a significant positive correlation was observed between Compound W levels and gestational age ($P<0.001$). Serum levels of Compound W increased from 75 to 260 ng/dl, as expressed in T_2S equivalent. In 20 pregnant women, serum Compound W levels also increased during the above gestational period but such an increase did not reach statistical significance ($P=0.08$). However, a significant correlation ($P<0.05$) between fetal and maternal Compound W levels was recorded in 14 paired samples taken at various gestational stages. Finally, a highly significant correlation ($P<0.001$) was found between serum concentrations of Compound W in 50 paired cord and maternal samples obtained at delivery.

In conclusion, circulating levels of Compound W are very high in fetal circulation and increase during fetal development. This increased Compound W concentrations in fetuses may account for the high levels recorded in mothers and, therefore, the measurement of Compound W levels in maternal circulation may be a potential marker for fetal thyroid function.

10:30 A.M. 404. IMPORTANCE OF ASPARTATE-474 IN THE FIRST EXOPLASMIC LOOP OF THE THYROTROPIN RECEPTOR (TSHR) IN RECEPTOR ACTIVATION. S. Kosugi and T. Mori, Department of Laboratory Medicine, Kyoto University, Japan.

It has been suggested that the long extracellular domain of glycoprotein hormone receptors plays a major role in high-affinity ligand binding. However, how conformational change in the transmembrane domain of the receptor occurs following ligand binding to the extracellular domain remains to be elucidated. On the basis of reciprocal mutagenesis experiments, Ji & Ji have postulated that interaction of Lys-91 of the α-subunit of hCG and Asp-397 in the first exoplasmic loop of the lutropin receptor is necessary for receptor activation. Because α-subunit is common to all glycoprotein hormones and this Asp in the first exoplasmic loop is conserved only among all glycoprotein receptors, we hypothesized that this phenomenon might be common to all glycoprotein hormones and their receptors. As a first step, we evaluated the role of Asp-474 in the TSHR by using mutagenesis, transfecting mutants in Cos-7 cells and measuring TSH binding and TSH- and Graves' IgG (TSAb)-stimulated cAMP responses.

Cells with wild type (WT) TSHR showed maximal TSH-cAMP response of 6-fold and EC_{50} of $2x10^{-10}$M. Cells with Asp-474 to Glu mutant (D474E) receptor showed 40% of WT TSH binding capacity without significant change in TSH binding affinity. However, TSH-stimulated cAMP response of D474E was severely impaired. Maximal response to TSH was less than 2-fold and EC_{50} was higher than $2x10^{-8}$M. TSAb-stimulated cAMP response was also decreased in parallel. The fact that cells with comparably decreased expression of WT TSHR exhibited normal EC_{50} and maximal TSH-cAMP response suggests that the effect of Asp to Glu mutation cannot be attributed to decreased receptor number, but rather is due to impaired function of the mutant receptor. These results clearly indicate that Asp-474 in the first exoplasmic loop of the TSHR does not have a major contribution to TSH binding but has a significant role in TSH- and TSAb-induced signal transduction.

10:45 A.M. 405. ADDITIVE EFFECT OF ACTIVATING POINT MUTATIONS OF THE TSH RECEPTOR GENE. M. Tonacchera, J. Parma, F. Cetani, S. Costagliola, R. Paschke, *G. Vassart. IRIBHN, *Service Genetique Medicale, Université Libre de Bruxelles, Brussels, Belgium.

Recently somatic and germline mutations conferring a higher constitutive activity to the TSH receptor (TSHr) have been described as a cause of toxic adenoma or hereditary/sporadic toxic thyroid hyperplasia. In order to approach the undestanding of the mechanism of TSHr activation the effect of combining previously described mutations within a single receptor was studied. Double mutants harboring the aminoacid substitution V509A (3° transmembrane segment) and A623I (3° intracellular loop), named V509A-A623I, and the double mutants V509A and C672Y (7° transmembrane segment), named V509A-C672Y, were constructed. Constructs were subcloned in the expression vector pSVL and after transient expression in COS-7 cells basal and TSH induced cAMP and inositolphosphate (IP) production was determined together with 125-I binding kinetics.

Our results show that transfected cells with single mutant receptors (V509A; A623I; C672Y) exhibit a high basal cAMP production, as expected. Cells transfected with the individual double mutants increased basal cAMP values which showed intermediate values with respect to the individual constructs harboring a single mutation. Cells transfected with the double mutants showed 10 times less receptor expression with respect to the constructs harboring a single mutation.Constructs harboring one or double mutations exhibited a higher affinity for bTSH binding with respect to the wild type TSHr. After bTSH challenge the double mutant V509A-A623I exhibited a maximal cAMP response similar to that of the wild type receptor, while the V509A-C672Y showed a blunted response. bTSH elicited a reduced maximal stimulation of IP production in the construct V509A-A623I, while cells transfected with V509A-C672Y construct loose completely the ability to increase IP after bTSH challenge.

In conclusion our results support previous suggestions that different conformations of the receptor are involved in activation of cAMP or IP cascades. They demonstrate a relationship between constitutive activity and affinity for TSH binding which implies an interplay between the effector and binding domains of the receptor.

11:00 A.M. 406. MUTUAL ANTAGONISTIC INTERACTIONS BETWEEN THE PROTEIN KINASE A AND PROTEIN KINASE C/TYROSINE KINASE PATHWAYS IN HUMAN THYROID c-*jun* AND c-*fos* PROTO-ONCOGENE EXPRESSION, CELL PROLIFERATION AND DIFFERENTIATION. Z. Kraiem, G. Gorenstein, E. Sobel, O. Sadeh and R. Heinrich, Endocrine Research Unit, Carmel Medical Center, Haifa 34362, Israel.

Our aim has been to delineate the role of the major signal transduction pathways believed implicated in the control of human thyroid cell growth and differentiation: the protein kinase A (PKA), protein kinase C (PKC) and tyrosine kinase (PTK)-mediated mechanisms. The experimental model used was our system of thyroid follicles of human origin cultured in suspension under serum-free conditions in which the follicular three-dimensional structure is retained. The data demonstrated mutual antagonistic interactions between the signal transduction pathways: TSH (acting via PKA) attenuated phorbol ester (acting via PKC) as well as EGF (PTK)-mediated human thyroid cell proliferation whereas the PKC and PTK pathways inhibited PKA-mediated human thyroid cell differentiation (*Endocrinology*, 136:585, 1995). In order to explore possible underlying mechanism(s) causing these antagonistic interactions, we examined the involvement of the proto-oncogenes c-*jun* and c-*fos* in our system. Both EGF (1-50ng/ml) and the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA 10^{-11}-10^{-7}M) dose-dependently stimulated c-*jun* and c-*fos* mRNA expression. As expected of immediate early genes, c-*jun* and c-*fos* mRNA levels rose in a rapid, transient fashion with a peak following 1 hour exposure to the phorbol ester or growth factor. Moreover, c-*jun* and c-*fos* mRNA superinduction was observed in the presence of an inhibitor of protein synthesis, cycloheximide. The c-*jun* and c-*fos* mRNA effects elicited by TPA could not be mimicked by the inactive phorbol ester, 4α-phorbol-12, 13-didecanoate and were reversed by the PKC inhibitor, chelerythrine, whereas the effects induced by EGF were reversed by the PTK inhibitor, genistein. This indicates a PKC- and PTK-mediated pathway triggered by the phorbol ester and growth factor, respectively. TSH caused a marginal increase in c-*jun* and c-*fos* mRNA. Addition of TSH (0.1-0.5mU/ml), however, to either TPA or EGF dose-dependently inhibited (up to 65%) the c-*jun* and c-*fos* mRNA elicited by these agents. The repressive action of TSH on the TPA and EGF mRNA effects were mimicked by forskolin and 8-BrcAMP, suggesting inhibition at sites both proximal and distal to cAMP generation. The TSH inhibitory action seems to require *de novo* protein synthesis since it was abrogated in the presence of cycloheximide. The above effects on c-*jun* and c-*fos* mRNA elicited by the PKA, PKC and PTK pathways, observed in normal human thyroid cells, were also reproduced in an established human thyroid carcinoma cell line (MRO-87-1). In conclusion, it would seem that the TSH-TPA as well as the TSH-EGF interactions regarding c-*jun* and c-*fos* mRNA parallel their effects on cell proliferation and differentiation. Thus, increased human thyroid cell proliferation and decreased cell differentiation is accompanied by, or is possibly a consequence of, enhanced c-*jun* and c-*fos* gene expression.

11:15 A.M. 407. EXPRESSION OF FUNCTIONAL TSH RECEPTOR (TSHR) IN RAT EPIDIDYMAL FAT CELLS AND 3T3L1 CELLS.
K. Haraguchi, T. Endo and T. Onaya, Third Department of Internal Medicine, University of Yamanashi Medical School, Tamaho, Yamanashi, Japan.

It has been thought that the expression of functional TSH receptor has been found only in thyroid tissues. However, we found that mRNA for TSHR is rich in rat epididymal fat tissue. Sequencing of cDNA for TSHR in fat cells revealed that the aminoacid sequence is exactly same as that of TSHR in thyroid. To explore the function of TSHR in fat cells, we studied several aspects of TSH-dependent signal transduction using rat fat cells and 3T3L1 cells. Rat epididymal fat cells were prepared by the method of Rodbell, later modified by Honnor et al. Glycerol and cAMP production were measured using commercially available kits. TSH binding study using Chinese hamster ovary (CHO) cells was done as described by Foti et al. TSH binding study using solubilized TSHR was performed as described by Smith et al. Results: (1) Fat cells increased cAMP production in response to high concentrations (1 mU/ml<) of TSH. (2) High concentrations (1 mU/ml <) of TSH stimulated lipolysis as measured by glycerol production in rat fat cells in the presence of adenosine deaminase. (3) TSHR solubilized from fat cells showed high- and low- affinity TSH binding sites comparable to those of FRTL-5 cells. (4) IgGs from patients with Graves' disease bound solubilized TSHR. (5) CHO cells which were transfected with TSHR cDNA from fat cells showed same TSH binding characteristics and cAMP response to CHO cells transfected with TSHR cDNA from human thyroid. (6) 3T3L1 mouse fibroblasts which were brought to fat cell differentiation but not the cells before differentiation showed an increased cAMP production in response to high concentrations of TSH.

The results indicate that (a) TSHR is functional in rat fat cells. (b) Expression of TSHR in fat cells is related to the differentiation of cells. Furthermore, the discrepancy between the results ((1) (2) vs (3) (5)) in terms of TSH concentration required for the responses suggests the existence of an unknown mechanism(s) which regulates TSH-dependent signal transduction in fat cells.

Our system should provide further insights in understanding the mechanism of expression and the roles of TSHR in extrathyroidal tissues.

11:30 A.M.
408. **INCREASED cAMP STIMULATION BY THE HUMAN TSH RECEPTOR VARIANT WITH THE PRO52THR SUBSTITUTION IN THE EXTRACELLULAR DOMAIN**

S. Hagner, C.J. van Koppen, U.R.M. Bohr, K.H. Jakobs, and U. Loos, Dept. of Internal Medicine I, University of Ulm and Institute of Pharmacology, University GH Essen

Recently, we identified a naturally occurring variant of the human TSH receptor with a Pro52Thr substitution in its N-terminal extracellular domain. Secondary structure prediction analysis suggested a major change with the loss of a loop in the N-terminus of the receptor protein. To determine the functional significance of this substitution, cDNAs of wild-type (wt) and variant receptors were stably expressed in Chinese hamster ovary cells. The Pro52Thr substitution did not affect synthesis and membrane localization of the receptor, as evidenced by TSH binding analysis to intact cells. Variant and wt receptor were expressed in equivalent numbers on the cell surface and displayed identical binding affinity for TSH as analysed by Scatchard analysis: The number of high affinity binding sites were 34 ± 3 and 32 ± 3 fmol/mg protein for wt and variant receptor, respectively, with $K_{d(H)}$ values of 178 ± 33 and 184 ± 32 pM. However, maximal TSH stimulation of cAMP synthesis was about 3-fold higher in cells expressing the variant receptor as compared to wt expressing cells (increase of 500 ± 24 pmol cAMP/mg protein versus 181 ± 24 pmol cAMP/mg protein). Basal and cholera toxin- or forskolin-stimulated cAMP levels were not different. In conclusion, the Pro52Thr substitution in the N-terminal region of the human TSH receptor gives rise to a receptor protein with enhanced signalling efficiency to adenylyl cyclase. This naturally occuring hyperactive TSH receptor may participate in hyperthyroidism in patients with Graves' disease.

11:45 A.M.
409. TRANSFORMING GROWTH FACTOR β SPECIFICALLY INHIBITS THE TSH/cAMP-DEPENDENT PROLIFERATION OF DOG THYROCYTES IN PRIMARY CULTURE: ITS EFFECTS ON CELL CYCLE REGULATORY PROTEINS. F. Depoortere, R. Burikhanov, M. Baptist and P.P. Roger, I.R.I.B.H.N., Free University of Brussels, Campus Erasme, B-1070 Brussels, Belgium.

TGFβ1 has been demonstrated to be expressed in thyroid gland. The activity of this growth factor is considered as a paradigm of negative growth control in normal epithelial cells and is the subject of intense investigations. Various growth and differentiation effects of TGFβ have been reported from various in vitro thyroid cell systems and are especially controversial in FRTL-5 cells. We have previously shown that TGFβ is a general antagonist of TSH and cAMP responses in human thyrocytes in primary culture (Taton et al., Mol. Cell. Endocrinol. 95, 13 (1993)).

Using dog thyroid primary cultures, we unexpectedly observed that TGFβ1 strongly inhibited the triggering of DNA synthesis by TSH and cAMP, but weakly affected the action of cAMP-independent mitogens including EGF, HGF, TPA and serum. Unlike observations from human thyrocytes, TGFβ1 action was quite specific in dog cells since it did not inhibit the cAMP-dependent morphological effects of TSH, nor TSH-dependent iodide transport. Kinetic analyses and utilization of immunofluorescent PCNA staining as a cell cycle marker revealed that TGFβ inhibited cell cycle progression in G1, either before or just after PCNA appearance 6 hrs before S phase initiation. Immunoblotting experiments and immunofluorescence microscopy also showed that the specific inhibition of the cAMP-dependent cell cycle involved the inhibition of expression or intracellular translocation of various proteins that control the cell cycle. TGFβ strongly inhibited the induction by TSH of cdc2, cyclin A and PCNA, but less the expression of CDK2 and CDK4, which were already present in quiescent cells. However, TGFβ prevented previously undescribed nuclear translocations of CDK4 and CDK2 that precede DNA synthesis initiation. We are currently investigating the effects of TGFβ on CDK inhibitors and D-cyclins that control CDK4 and CDK2 activities.

The demonstration of differential and quite specific inhibitory effects of TGFβ in dog thyrocytes, concerning mainly the cAMP-dependent cell cycle, may thus prove invaluable in the characterization of the divergent cAMP-dependent and cAMP-independent mitogenic pathways.

410. P1 **A THYROTROPIN RECEPTOR CODON 52 POLYMORPHISM AND HLA-DR3 ARE INDEPENDENTLY ASSOCIATED WITH INCREASED RISK FOR THE DEVELOPMENT OF AUTOIMMUNE THYROID DISEASE IN CAUCASIAN FEMALES, WHILE HLA-DRB3 AND DQA_1*0501 ARE NOT.** **R.M. Cuddihy, D.J. Schaid, and R.S. Bahn. Division of Endocrinology and Section of Biostatistics, Mayo Clinic, Rochester, MN.**

We have described previously a polymorphism (C→A) in the first position of codon 52 of the human TSH receptor (hTSHr) gene that is associated with an increased incidence of autoimmune thyroid disease (AITD) in women (Thyroid, 1995 (in press)). This polymorphism results in an amino acid substitution (proline→threonine) at this position, significantly altering the conformation of the receptor protein. In order to determine whether this polymorphism or other susceptibility alleles for AITD confer the greater risk, we screened our Caucasian female population for alleles encoding the DR3 antigen (DRB_1*03 alleles), DRB_3 alleles, and the DQA_1*0501 allele. Sequence specific PCR primers were used to detect the presence or absence of each allele. Results were compared between the cases (97 Caucasian females with AITD) and controls (65 Caucasian females with documented normal thyroid function) using a logistic regression analysis. Only the Pro^{52}→Thr^{52} hTSHr gene polymorphism and the HLA-DR3 (DRB_1*03) alleles were independently associated with AITD; with a χ^2-p-value of 0.006 and odds ratio of 8.4 for the hTSHr polymorphism, and a χ^2-p-value of <0.001 and odds ratio of 6.1 for DR3. There were no independent associations between DRB3 (p=0.72) or DQA_1*0501 (p= 0.34) positivity and AITD, adjusted for the effects of the hTSHr polymorphism and DR3. Thus only the Pro^{52}→Thr^{52} hTSHr gene polymorphism and the HLA-DR3 (DRB_1*03) alleles were shown to be independent markers of AITD. We suggest that, of the four genetic susceptibility markers tested, these 2 genetic loci confer the major susceptibility towards the development of AITD.

411. P2 HUMAN THYROTROPIN RECEPTOR TRANSLATED *IN VITRO* AS A NASCENT PROTEIN BINDS AUTOANTIBODIES FROM GRAVES' DISEASE PATIENTS. N.G.Morgenthaler, J. Tremble, G.C. Huang, W. A. Scherbaum and J. P. Banga. Department of Medicine, King's College School of Medicine, London, UK and Medizinische Klinik III, Universitat Leipzig, Leipzig, Germany.

The pathogenic autoantibodies to the thyrotropin receptor (TSH-R) in Graves' disease patients are usually recognised by biological or radioreceptor binding assays. Due to their low concentration they have proved difficult to characterise by conventional immunoprecipitation analysis. We have used a novel, highly sensitive assay employing hTSH-R polypeptide expressed in an *in vitro* translation system to evaluate the binding and characterisation of autoantibodies to TSH-R from Graves' disease patients. *In vitro* translation of the full length (764 amino acids) and extracellular region of the TSH-R (TSH-R.E) (393 amino acids, no signal sequence) gave stable transcripts of 85kD and 50kD respectively, as assessed by SDS polyacrylamide gel analysis. The nature of the translated products was confirmed by immunoprecipitation with three different, well characterised murine monoclonal antibodies (mabs) or rabbit polyclonal antisera generated to recombinant hTSH-R.E. The epitopes recognised by the mabs and polyclonal antisera were localised to the amino terminus, the central region and the carboxyl terminus of TSH-R.E using internal, methionine start sites for translation of TSH-R.E polypeptide fragments and immunoprecipitation. Graves' disease sera from 49 patients (and 10 normal sera) with varying levels of TSH binding inhibitory immunoglobulin activity (TBII) were examined for their ability to immunoprecipitate the translated hTSH-R.E protein. A proportion (>30%) of sera immunoprecipitated the translated receptor which was not recognised by any control sera. There was no correlation between TBII activity and the ability to immunoprecipitate the nascent translated receptor protein. Our results provide compelling evidence that TSH-R autoantibodies (i) immunoprecipitate the nascent, translated TSH-R and (ii) some do not require post-translation modification of the receptor such as glycosylation or proteolytic processing for binding. Furthermore, our preliminary studies on epitope mapping indicate that the autoantibodies that immunoprecipitate the nascent receptor recognise at least three separate antigenic sites on the extracellular region of the molecule.

412. P3 POLYCLONAL ANTISERA TO RECOMBINANT THYROTROPIN RECEPTOR; BIOLOGICAL PROPERTIES AND BINDING TO NATIVE RECEPTOR PROTEIN. J. P. Banga, M. R. Kim, A Gardas and M Gupta. Department of Medicine, King's College School of Medicine, London, UK and The Department of Clinical pathology, The Cleveland Clinic Foundation, Cleveland, Ohio, USA.

In order to begin to identify the regions on the human thyrotropin receptor (TSH-R) that interact with thyroid stimulating antibody (TSAb) and antibodies capable of blocking TSH mediated cAMP stimulation (blocking antibody, TBAb) in Graves' disease patients, we have characterised polyclonal antisera to the human TSH-R . For immunisations, recombinant, extracellular region of TSH-R (amino acids 1-414, numbering includes the signal sequence) generated in insect cells was used for injections; although the immunogen does not bind TSH, some native structure is conserved as assessed by the binding of a proportion of Graves' disease autoantibodies. The activity of pre-immune sera obtained before injection of the two rabbits was compared to the immune sera in all the experiments described.

High titre antisera were generated readily after three injections in adjuvant, but multiple immunisations were a pre-requisite to generate antibodies with significant TSH binding inhibitory immunoglobulin (TBII) activity in a radioreceptor binding assay. In an *in vitro* transcription and translation assay using ^{35}S-methionine labelled translated receptor, epitope mapping of the antisera using internal methionine start site transcripts showed, as expected for polyclonal antisera, that multiple regions of the receptor protein were being recognised. Interestingly, both the antisera react by FACS analysis with native TSH-R stably expressed in CHO cells. Binding with functional TSH-R was further confirmed by the demonstration that the antisera inhibit TSH mediated cAMP production in FRTL-5 cells. However, none of the antisera stimulated cAMP production in FRTL-5 cells; pre-immune serum was also found to have significant TSAb activity and therefore emphasises the critical importance of comparing thyroid stimulating activity in xenogeneic antibodies with appropriate control sera. The ability to generate xenogeneic antibody to TSH-R that reacts with native receptor protein and mimic some functional properties of Graves' disease autoantibodies such as TBAb activity but fail to reproduce other functional properties such as TSAb activity has important implications for understanding the generation of these pathogenetic autoantibodies in human disease.

413. P4 KINETICS OF ANTIBODY RESPONSES TO THYROTROPIN RECEPTOR IN MICE THAT ARE SUSCEPTIBLE (BALB/cJ) AND RESISTANT (SJL/J) TO THE INDUCTION OF HYPERTHYROXINEMIA. Sai A. Patibandla and Ji-Lao Fan, Department of Microbiology/Immunology, University of Texas Medical Branch, Galveston, TX 77555-1019.

Thyrotropin receptor (TSHR) serves as a target antigen in a number of autoimmune diseases of the thyroid. The extracellular domain of the human TSHR (ETSHR) protein, expressed in our laboratory, has been used to immunize different strains of mice to show that only BALB/cJ mice had elevated levels of thyroxine in their sera. In the present study, we immunized BALB/cJ and SJL/J mice with the ETSHR protein and analyzed their antibody responses against TSHR. Pre-immune sera and sera obtained at different time intertvals after immunization (days 11, 22, 33, 44 and 55) were tested for the presence of antibodies to ETSHR in an ELISA. After only two immunizations (day 22) with ETSHR, SJL/J mice had significant antibody responses. In contrast, BALB/cJ mice did not show significant antibody titers until day 33. Antibody titers reached highest levels in both strains after 4-5 injections of ETSHR (day 44-55). The sera were analyzed for subclass specific anti-TSHr antibodies at different time intervals. BALB/cJ showed predominantly IgG1 subclass specific anti-TSHr antibodies. Whereas, SJL/J mice showed predominantly IgG1 and IgG2b subclass specific anti-TSHR antibodies. Next, we analyzed sera from both groups for reactivity against a panel of 26 synthetic peptides of TSHR. On day 33 BALB/cJ mice showed reactivity predominantly to peptide 1 and by day 55 it showed reactivity to peptides 1 and 23. SJL/J mice showed reactivity initially (on day 33) to only peptide 1 and then, by day 55, the antibody repertoire spread to include peptides 21, 23, 24, 25, and 26. These data suggest that the processing and presentation of TSHR are different in these two strains of mice and that different subsets of T-cells may be involved in the immune responses. Differences in the anti-TSHR responses between the two strains of mice could account for the differences in their susceptibility to the induction of hyperthyroxinemia.

414. P5 **DEFINING THE MAJOR ANTIBODY EPITOPES ON THE HUMAN THYROTROPIN RECEPTOR.** H. Vlase, P.N. Graves, Y. Tomer, J. Morris* and T.F. Davies, Department of Medicine, Mount Sinai School of Medicine, New York, New York and Department of Medicine*, Mayo Clinic, Rochester, MN, USA.

We have used either non-glycosylated prokaryotic (E.coli) or glycosylated eukaryotic (insect cell) recombinant hTSH receptor extracellular domain (hTSHR-ecd) to immunize BALB/c and CBA/J mice. After receiving either 1 or 3 antigen boosts all mice developed highly specific hTSHR-ecd-Abs as detected by direct enzyme immunoassay and confirmed by western blot analyses. The B cell epitopes recognized by these murine hTSHR-ecd-Abs were mapped using 26 synthetic overlapping peptides encompassing the entire hTSHR-ecd (amino acids (aa) 22-415, i.e. without the signal sequence). While all BALB/c and CBA/J mice antisera recognized peptide #1 (aa 22-41), only the hyperimmunized mice (CBA/J) demonstrated recognition of additional peptides (#21 to # 26), clustered towards the carboxyl terminus of the hTSHR-ecd (aa 322-415). Furthermore, sera from hyperimmunized mice blocked the binding of ^{125}I-bTSH to soluble native TSHR, while sera from mice which only received a single antigen boost were inactive. In addition we used a similar approach to map the B cell epitopes of two antisera from NZW rabbits immunized and boosted repeatedly with eukaryotic hTSHR-ecd (provided by Dr. P . Banga, University of London). As with the murine sera, these rabbit antisera significantly blocked the binding of ^{125}I-bTSH to soluble native TSHR, recognized peptide #1, and additional peptides again clustered near the carboxyl terminus of the hTSHR-ecd. These data provide the first comprehensive B cell epitope mapping study of induced hTSHR-ecd-Abs. While the N-terminal region was highly antigenic, repeated immunization induced antibodies targeted to a region of the hTSHR which appeared to be critical for TSH binding.

415. P6 TRANSFER OF THYROIDITIS WITH SPLEEN CELLS FROM MICE IMMUNISED WITH RECOMBINANT HUMAN THYROTROPIN RECEPTOR (TSHR). S. Costagliola, M-C.Many *, M. Ludgate. IRIBHN,ULB and Lab. Histology, UCL * ; Brussels, Belgium.

In a previous study we have induced thyroiditis in 100% and thyroid binding inhibiting immunoglobulins in 50% of female BALB/c mice immunised with the bacterially produced TSHR. The aim of the present study was to determine whether disease could be transferred to naive recipients using immune cells from primed mice.

8, six week female BALB/c mice were immunised intra-peritoneally with the TSHR in an adjuvant of alum plus attenuated Bordetella pertussis, on days 0 (100ug), 14,28 and 35 (50ug). These and two untreated mice were sacrificed on day 43 and their spleens and thyroids removed, the latter to verify the presence of thyroiditis in receptor treated animals. Spleen cells were mechanically disrupted and cultured for 64 hours at $3x10^6$/ml in RPMI supplemented with 20ug/ml TSHR. 9,six week female BALB/c mice received 10^7 spleen cells from TSHR treated mice and 6 from untreated animals, in both cases by immunisation into the tail vein. Animals were sacrificed sixteen days later and their thyroids were examined by immunohistochemistry. The six mice which received splenocytes from untreated mice had normal thyroids.In contrast,seven of the nine mice receiving splenocytes from TSHR treated mice revealed an extensive lymphocytic infiltration of their thyroid glands with follicular destruction. In common with antigen treated mice, the infiltrate contained large numbers of activated T cells expressing the receptor for IL2. However, there were differences, since macrophages and dendritic cells were plentiful and B cells were very scarce in the spleen treated animals and vice versa in those receiving antigen. Despite the follicular destruction, the mice showed no evidence of production of antibodies to mouse thyroglobulin when tested by ELISA.

In conclusion we have been able to transfer thyroiditis to naive recipients with immune cells from primed mice and the infiltrate induced differs from that with direct immunisation of antigen.

416. P7 **VARIABLE REGIONS OF IMUNNOGLOBULIN GENES ENCODING "blocking type" ANTITHYROTROPIN RECEPTER ANTIBODIES OF PATIENTS WITH PRIMARY MYXEDEMA** J. Okuda, T. Akamizu, H. Li, F. Matsuda+
Department of Laboratory Medicine, Faculty of Medicine, and +Center for Molecular Biology and Genetics, Kyoto University, Kyoto, Japan

We have previously reported EBV-transfected human B cell clones from patients with primary hypothyroidism producing monoclonal "blocking type" anti-thyrotropin receptor antibodies (TSH-R BAb) [JCEM **79**:60, 1994]. In the present study, we isolated and characterized imunnoglobulin heavy chain (Ig H) genes of 6 B cell clones (3 IgG type and 3 IgM type clones) and imunnoglobulin light chain (κ) genes of 4 clones (2 IgG type and 2 IgM type clones). mRNAs were exracted from B cell clones and cDNAs were synthesized by the reverse transcription method. Variable regions of immunoglobulin genes were amplified by PCR with specific oligonucleotide primers, subcloned into pcDL vecter and then sequenced. V_H gene families to which 6 clones belong were V_{H-3} in 3 clones, V_{H-4} in 2 and V_{H-1} in 1. Comparing nucleotide sequences of the V_H genes with known germline V_H segments, the most homologeous V_H segments of at least two clones have been reported to be used in an autoantibody or in the fetal liver repertoire. A number of replacement (R) and silent (S) mutations were found in both CDRs and framework regions (FRs) although the R/S ratios were not significantly different between CDRs and FRs. The frequency of somatic mutations of one IgG type clone was higher than that of the other clones. V_κ gene families of the 4 clones were all $V_{\kappa 3}$. The most homologeous V_κ segments of 2 clones have been reported to be used in autoantibodies. In the other 2 clones (one was IgG type and the other was IgM type clone) which have been obtained from different patients, the same germline V_κ segment was used and the frequency of of somatic mutations of the IgG type clone was higher than that of the IgM type one. A number of R and S mutations of the V_κ segments were also found in both CDRs and FRs and the R/S ratios of the CDR were considerably higher than those of the FR. In summary, we isolated and characterized Ig heavy and light chain genes of monoclonal TSH-R BAbs, including IgG type clones. These findings suggested the restricted usage of V_κ segments and the importance of somatic mutations in Ig variable regions for TSH-R BAb activity.

417. P8 **DISCRETE CHARACTERISTICS OF ANTIBODIES RAISED AGAINST TSH RECEPTOR-RELATED PEPTIDES WHOSE SEQUENCES ARE NOT CONSERVED IN THE LH/CG RECEPTOR.** M.Murakami, K.Miyashita, Y.Hosoi, T.Negishi, T.Michimata, M.Yamada, T.Iriuchijima and M.Mori, First Department of Internal Medicine, Gunma University, School of Medicine, Maebashi, Japan.

The antibodies for TSH receptor are known to have two different types of biologic activities in the thyroid: thyroid-stimulating antibody (TSAb) and thyroid stimulation-blocking antibody (TSBAb). In order to identify the specific regions in the human TSH receptor for these discrete characteristics, we produced rabbit antibodies raised against four different peptides of the extracellular domain of the human TSH receptor which sequences are not conserved in the LH/CG receptor, and measured the TSAb and TSBAb activities of those antibodies using Chinese hamster ovary cells expressing human TSH receptors. Two to three rabbits were immunized with each peptide, and specific antibodies were obtained as assessed by the immunoprecipitation assay using radioiodinated peptides. Only antisera from rabbits which were immunized with a peptide of amino acids 32-56, including the small insertion near the N-terminal end of the extracellular domain, showed apparent TSAb activities and have been shown to be significantly precipitated by IgG of patients with Graves' disease. TSAb activity correlated with the antibody titers against the peptide in those rabbits ($p<0.05$). In contrast, antisera from rabbits immunized with a peptide of amino acids 352-378, including a part of the large insertion near the C-terminal end of the extracellular domain, showed the obvious TSBAb activities. TSBAb activity correlated with the antibody titers against the peptide in those rabbits ($p<0.05$). This peptide was significantly immunoprecipitated by IgG of hypothyroid patients who had TSBAb ($p<0.05$), and a significantly positive correlation was observed between the immunoprecipitation of this peptide and TSBAb activity ($p<0.01$).

These results suggest that the epitope for TSAb is quite different from that for TSBAb in the human TSH receptor, and that the heterogeneous characteristics of the biologic activities of TSH receptor antibodies may be attributable to the differences in the regions of the receptor recognized by the autoantibodies.

418. P9 SPECIFIC EFFECTS OF RADIOIODINE (RAI) ON THYROID STIMULATING (TSAb) AND BLOCKING (TBAb) ANTIBODIES IN GRAVES' DISEASE.

V.P.Michelangeli, C.Poon, D.J.Topliss, P.G.Colman. Dept. Diabetes and Endocrinology, Royal Melbourne Hospital and Ewen Downie Metabolic Unit, Alfred Hospital, Victoria, Australia.

RAI treatment, in patients with Graves' disease, has been reported to cause increases in TSH binding inhibitory immunoglobulins (TBII), but the varied effects on TSAb and TBAb in a series of patients has never been reported. Our aim was to investigate these effects in a large series of patients with Graves' disease and to establish clinical correlates.

TSAb, TBAb and TBII were measured in 33 patients (27 females and 6 males) who received RAI. All had been treated for at least 6 months with a thionamide drug and had blood taken on at least 4 occasions post RAI. Patients were defined as developing 'early' hypothyroidism if their TSH exceeded 4.0mU/L within 6 months of RAI. TSAb and TBAb were bioassayed using JPO9 cells.

Following RAI, TBII levels fell in 7 (20%) (group 1), rose in 16 (48%) (group 2) and remained unchanged but elevated in 10 patients (32%) (group 3). In group 1, TSAb values dropped or were already normal in all 7 (100%); none had TBAb and none became hypothyroid. In group 2, TSAb increased in 7 (44%), TBAb increased in 3 (19%) and both increased in 3 patients (19%); 3 (19%) had neither TSAb or TBAb and 6 became hypothyroid (2 with elevated TBAb, 3 with elevated TSAb and 1 with neither elevated). In group 3, five (50%) had only TBAb, 2 (20%) only TSAb and 3 patients (30%) had both; 5 (50%) became hypothyroid (all had TBAb).

There are diverse changes in TBII, TSAb and TBAb following RAI. Early hypothyroidism occurred in 33% of patients, 75% of whom had TBAb. When TBAb were present in the absence of TSAb, hypothyroidism occurred in 6 of 8 versus 3 of 14 where TSAb were present alone ($p<0.05$). Development of hypothyroidism was not related to continuation or dosage of antithyroid drugs and was transient in 50% of patients. Thus early hypothyroidism following RAI therapy can be associated with increased TBAb in some patients. Recognition of this clinical situation may help avoid unnecessary life-long thyroxine replacement.

419. P10 **IN VITRO SPONTANEOUS PRODUCTION OF THYROTROPIN RECEPTOR ANTIBODIES (TRAb)**

J.Aguayo, R.Arellano, G.Pineda. Endocrine Section, Dept of Medicine, U of Chile and I.E.M.A. Endocrine Lab. Santiago,Chile

It has been postulated that in Thyroid Autoimmune Diseases, antibodies are produced by intrathyroidal lymphocytes. Graves'Disease(GD) is characterized by the production of TRAb that are considered to be responsible of the clinical expression of the disease, contrary to what happens in Toxic Nodular Goiter(TNG).In order to determine the site of their synthesis we studied its *in vitro* spontaneous production by thyrocytes(T) and peripheral lymphocytes(L) cultures obtained by usual procedures from 13 pts [10 GD, all TRAB(+) and 3 TNG, all TRAb(-)].Cells were cultivated separatedly for 5 d after which they were transferred to NUNC plates for culturing them alone or together (co-cultures,T+L) for other 10 d. Simultaneously, 1% Pokeweed(Pw), Concavaline A (ConcaA, 6 ug/ml) and Propylthiouracil(PTU,0.1 mM) were added in different combinations to other co-cultures in order to determine its effect on antibody synthesis. At the end of the 10-d-incubation period, supernatant was put aside and kept frozen until analyzed.TRAb was measured by a RIA using as standard MRC LATS B Reference Preparation dissolved in culture media + negative human sera. Results are expressed in U/L ± SD

	L	T	T+L	T+L+Pw	T+L+Pw+ConcaA	T+L+Pw+PTU
GD	18.3 ± 2.95	39.3 8.63	38.8 7.75	36.4 10.04	38.3 8.93	41.6 11.69
TNG	11.1 ± 3.32	13.2 2.54	14.0 3.86	14.1 2.01	14.4 2.19	14.2 2.19
p	<0.02	<0.001	<0.001	<0.001	<0.001	<0.001

Our results tend to demonstrate that TRAb are mainly of intrathyroidal origin occurring only in GD, probably due to the presence of intrathyroidal lymphocytes in thyroid tissue cultures. Lack of enhanced synthesis in co-cultures or by mitogens could be explained because antibody productor cells are in their maximal capacity. These results also show that PTU does not affect antibody production.

420. P11 DEMONSTRATION OF THE THYROACTIVE SMALLER COMPONENTS RELEASED FROM TSAb-IgG BY PROTEASE DIGESTION OR REDUCTION. Y. Ochi, T. Inui, W. Chen, T. Kouki, M. Ogura, T. Hachiya and Y. Kajita, Dept. Clin. Lab. Med., Shiga Uni. of Med. Sci., Shiga; 2nd Dept. Int. Med., Kobe Univ., Hyogo; 2nd Dept. Int. Med. and Kyoto Pref. Univ. of Med., Kyoto, Japan.

The thyroactive fragment released from TSAb-IgG that had the TBII activity, anti-thyroglobulin (anti-Tg) and anti-TPO activity was examined by papain digestion or by dithiothreitol (DTT) reduction. The unbound fraction (UF) and the bound fraction (BF) were separated using Protein A-Sepharose column after papain hydrolysis of TSAb-IgG. When these both fractions were gel-filtrated on Sephadex G-100 column, the protein peak showed Fab fragment (Mr 50 KD) in the UF and Fc fragment (Mr 50 KD) with tracer amounts of Fab fragment in the BF. The thyroid stimulating (TS) activity (cAMP production in porcine thyroid cells) and TSH binding inhibition (TBI) activity (determined by TSH receptor assay) were found in both Fab fraction and the retarded fraction (between Mr 50 KD and 20 KD) in the UF. Neither TS nor TBI activity was observed in Fc fragment.

In pepsin hydrolysis the UF from Protein A column consisted of both $F(ab')_2$ fragment (Mr 100 KD) and pFc' fragment (CH_3, Mr 25 KD). The biological activities were found in both $F(ab')_2$ fragment and the retarded fraction (less than 50 KD). The biological activity of TSAb distributed in not only Fab or $F(ab')_2$ fragment but also the thyroactive smaller components (TSC) which had neither anti-Tg nor anti-TPO activity.

The UF from Protein A column after papain hydrolysis was applied to Protein L-sepharose column (specifically binds with κ chain). Then, the UF (Fab(λ) fragment) and the BF (Fab(κ) fragment) were separated. The Fab(κ) fragment was reduced with DTT, and then Fd fragment was separated from κ -chain by Protein L column. The TS and TBI activities were found in Fd fragment that had neither anti-Tg nor anti-TPO activity.

The present study showed the existence of the biologically active smaller components with failure of antibody nature in TSAb-IgG molecule (such as the TSC and Fd fragment) than Fab fragment (Mr 50 KD). We suggest that these TSC may be released from Fab fragment of TSAb-IgG by treatment with protease or reduction and that TSAb may be TSC binding immunoglobulin (IgG).

421. P12 **INFLUENCE OF ADJUVANTS ON DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNITY TO THE THYROTROPIN RECEPTOR.** G.S.Seetharamaiah and B.S.Prabhakar, Department of Microbiology/Immunology, University of Texas Medical Branch, Galveston, TX 77555.

Thyrotropin receptor (TSHR) is the target antigen in several autoimmune disorders including Graves' disease (GD). Earlier, we immunized four different strains of mice with a recombinant extracellular domain of the human TSHR (ETSHR) in Complete Freunds Adjuvant (CFA) and only BALB/c mice developed hyperthyroxinemia. In order to determine the effect of adjuvant composition, we immunized BALB/c mice four times with ETSHR protein in three different commercially available adjuvants, i.e. CFA, Titer Max and Gerbu. CFA and Titer Max are water-in-oil emulsions containing killed *mycobacterium tuberculosis* and block copolymer CRL 89-41 respectively. Gerbu contains GMDP (N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine). Mice immunized with ETSHR in CFA and Titer Max showed high ELISA titers of antibody to ETSHR. Antibody subclass distribution was heterogeneous with higher levels of IgG1. However, mice immunized with ETSHR in Gerbu developed lower titers of antibody against ETSHR which was predominantly restricted to the IgG1 subclass. These mice were subsequently challenged twice with porcine thyroid membrane (PTM). Mice primed with ETSHR in CFA and then challenged with PTM in incomplete freunds adjuvant (IFA) showed elevated serum TSH binding inhibitory immunoglobulins (TBII) and total thyroxine (T4) levels. Mice primed with ETSHR in Titer Max and then challenged with PTM in IFA showed elevated TBII but normal T4 levels. The third group of mice primed with ETSHR in Gerbu and then challenged with PTM in Gerbu, continued to show low levels of anti-ETSHR antibody and TBII activity, but showed elevated levels of T4. Mice immunized with a control antigen along with each of the three adjuvants and then boosted with PTM showed normal levels of TBII and T4. These studies showed that adjuvant composition could greatly influence autoimunity to TSHR.

422. P13 **FUNCTIONAL RELEVANCE OF DIFFERENCES IN FINE SPECIFICITY OF THYROTROPIN RECEPTOR SPECIFIC ANTIBODIES IN MICE WITH AND WITHOUT EXPERIMENTAL HYPERTHYROXINEMIA,** Bellur S. Prabhakar, Sai A. Patibandla, John S. Dallas, John C. Morris and Neelam M. Wagle, Departments of Microbiology/Immunology and Pediatrics, University of Texas Medical Branch, Galveston, TX 77555 and Division of Endocrinology, Mayo Clinic, Rochester, MN.

Earlier, studies showed that BALB/cJ (H-2d) mice are susceptible to the induction of hyperthyroxinemia, whereas, SJL/J (H-2s), C57BL/6J (H-2b) and B10BR (H-2k) mice did not show any perturbation in thyroxine levels. Lack of elevated thyroxine levels in the latter groups of mice was not due to an absence of or quantitative difference in antibody response against the TSHR. Therefore, to understand the immunological basis for the different outcomes in various strains of mice, we characterized anti-ETSHR antibodies in sera from susceptible (BALB/cJ) and resistant (SJL/J) mice. These two strains of mice showed that there were no significant differences in the TBII activity, titers, relative affinities and isotypes of antibodies against the TSHR. Next we tested sera from these mice against a panel of peptides that span the entire ETSHR. BALB/cJ mice reacted with two peptides, whereas, SJL/J mice showed reactivity with seven peptides. One and six of the reactive peptides reversed the ability of sera from BALB/cJ and SJL/J mice, to block TSH binding to TSHR respectively. SJL/J sera reacted with peptides which either bind or induce blocking antibodies, but did not show hypothyroidism. These data, together with reversal of bioactivity of sera with various peptides showed that antibodies in SJL/J mice were heterogeneous and likely contained a mixture of blocking and stimulating antibodies which negated the effects of each other. In contrast, the TSHR specific antibodies in BALB/cJ were relatively homogeneous and most likely represented stimulating antibodies directed against conformational epitopes.

423. P14 EXPRESSION OF SECRETED FORM OF RECOMBINANT HUMAN THYROTROPIN RECEPTOR. Y. Okamoto, N. Hamada*, T. Sato, S. Tanaka, Y. Nishizawa**, S. Fujii, H. Morii**, Department of Internal Medicine, Osaka City General Hospital, *Sumire Hospital, and **Second Department of Internal Medicine, Osaka City University, Osaka, Japan.

The extracellular domain (ECD) of the thyrotropin receptor (TSHR) is thought to be the main target of an autoimmune response in patients with Graves' disease. The development of an efficient expression method of TSHR-ECD has been awaited for a better understanding of immunological phenomenon in Graves' disease. In this study we attempted to express chimeric TSHR-ECD fused with human Ig G1 constant region (Ig Cγ1). Expression vector of CD8-ECD fused with Ig Cγ1 (CD8 Rg) using pCDM8 expression vector was kindly provided by Dr. A. Aruffo (Seattle). CD8-ECD cDNA was replaced with human TSHR-ECD cDNA (TSHR Rg). TSHR Rg, CD8 Rg, or pCDM8 vector was transfected to COS7 cells using liposome method. Culture supernatants of transfected COS7 cells were harvested on day 5. Secreted form of TSHR Rg or CD8 Rg was detected by enzyme linked immunosorbent assay (ELISA) as follows. Ninety-six well plates were coated with goat anti-human IgG (Fc) antibody for overnight at room temperature. Culture supernatants were added to each coated well, and incubated for overnight at 4℃. After washing wells, each well was incubated with mouse anti-human TSHR peptide (amino acid #30-49) antibody or mouse anti-CD8 monoclonal antibody. Then assay was performed by using peroxidase labeled anti-mouse IgG (Fc) antibody and o-phenylenediamine dihydrochloride. ELISA values are shown below (mean ± SD):

	transfected plasmids		
antibody used in ELISA	pCDM8	TSHR Rg	CD8 Rg
anti-TSHR peptide Ab	0.482 ± 0.073	0.649 ± 0.041*	0.521 ± 0.020
anti-CD8 monoclonal Ab	0.140 ± 0.020	0.184 ± 0.020	1.446 ± 0.024**

*$p<0.05$; *vs.* pCDM8 and CD8 Rg, **$p<0.01$; *vs.* pCDM8 and TSHR Rg

These data indicate that secreted form of TSHR-ECD can be expressed as chimeric protein fused with Ig Cγ1.

424. P15 EFFECTS OF ANTI-CANCER DRUGS ON TWO HUMAN THYROID CELL LINES WITH DIFFERENT STAGES OF DIFFERENTIATION. [1,2]A. Hishinuma, [2]T. Yamanaka, [2]K. Kasai, [1]T. Ieiri and [2]S.-I. Shimoda, Departments of [1]Clinical Pathology and [2]Endocrinology, Dokkyo University School of Medicine, Mibu, Tochigi, Japan.

The hPTC cells and the hAG cells are two human thyroid cell lines established from the tissue of papillary carcinoma and multinodular goiter, respectively. These cell lines express mRNA for TSH receptor and respond to TSH with increased synthesis of cAMP. However, growth control of these cell lines is different. Activation of protein kinase A inhibits growth of the hPTC cells and activation of protein kinase C does not affect replication of the hPTC cells; but activation of protein kinase A and C stimulates growth of the hAG cells. Using these cell lines we studied effects of anti-cancer drugs (doxorubicin; DXR, cisplatin; CDDP, nimustine; ACNU, bleomycin; BLM, cyclophosphamide; CPA, and aclarubicin; ACR). First, the hPTC cells were incubated with the drugs for 48 hours, and the cellular DNA of the live cells were measured with diaminobenzoic acid. Of the drugs tested DXR showed the lowest ED50 (0.029 μ g/ml) and the blood concentration in humans with administration in the usual dosage was 13.8 times of the ED50. The clinically-expected blood concentrations divided by ED50's for the other anti-cancer drugs were, in the order of higher values, 2.3 for CPA, 1.7 for BLM, 1.2 for CDDP, 0.5 for ACR, and <0.1 for ACNU. DXR showed long-lasting effects since 2-hour exposure of DXR to the hPTC cells resulted in significant reduction of cellular DNA content of the live cells even after 48 hours. We then studied difference in the effects of DXR on the hPTC cells and the hAG cells. ED50 for hPTC was significantly lower (0.035 μ g/ml) than that for hAG (0.460 μ g/ml). Since formation of free radicals is one of the major anti-cancer mechanisms of DXR, effects of free radicals on ED50's for hPTC and hAG were assessed by adding glutathione (GSH), N-acetylcysteine (NAC), buthionine sulfoximine (BSO), and α-tocopherol (α-Toc) into the culture media. GSH reduces free radicals in the extracellular fluid. NAC promotes production of GSH in the cytoplasm, but BSO interferes production of GSH in the cytoplasm. α-Toc reduces free radicals in the plasma membrane. GSH and α-Toc did not affect ED50's for hPTC and hAG. However, NAC increased ED50's for hPTC and hAG, and BSO reduced ED50's for hPTC and hAG. The effects of NAC and BSO on ED50 for hPTC were greater than those for hAG. These studies show that DXR was one of the most potent anti-cancer drugs for the human thyroid cell lines. The cytocidal activity of DXR was more effecient to hPTC than to hAG. Such DXR effects were at least partly due to free radical formation in the cytoplasm.

425. P16 THE CLINICAL FEATURES OF ADVANCED DIFFERENTIATED THYROID CARCINOMA UNDERGOING RADIOIODINE THERAPY. H.Koshiishi, T.Mimura, O.Ozaki, K.Sugino, W.Kitagawa, K.Ito,Jr., and K.Ito, Ito Hospital, Tokyo, Japan.

During a 16-year period from 1978 to 1994, 202 patients with differentiated thyroid carcinoma were treated with radioactive iodine-131 (RI) for distant and local metastasis. The response to the therapy and survival rates were studied retrospectively.

The patients consisted of 52 males and 150 females with a mean age of 50.2 (range, 6-82) years. Histologic types were papillary carcinoma in 131, follicular carcinoma in 71 cases. Metastases were noted in lung in 116 (combined with regional nodes in 34), bone in 31 (combined with regional nodes in 4), combined with lung and bone in 16, and regional and/or mediastinal lymph nodes in 39 cases.

RI therapy was applied to the unresectable metastatic lesion. Total therapeutic dose of I-131 ranged from 29 to 945 mCi, the frequency was from 1 to 14. Surgery of primary lesion and metastatic lesion was performed in 199, whereas chemotherapy in 30 and external irradiation in 40 cases were added.

The therapeutic response and survival rates are shown below.

Site of metastasis	Positive rate of RI uptake	Response rate		Survival rate	
		Radiographic	Clinical	5Y	10Y
Lung (116)	75.0%	26.7%	53.4%	49.7%	38.9%
Bone (31)	83.9%	25.8%	48.4%	29.9%	0
Lung+Bone (16)	75.0%	0	31.3%	25.0%	0
Lymph node(39)	87.2%	23.1%	48.7%	60.9%	33.8%

Response rates were estimated by the radiographic findings, and by the subjective clinical symptoms. The rate of clinical response was higher than that of radiographic response. That of 10-year survival rate of patients with bone metastasis was significantly lower than that of lung and lymph node metastasis ($p < 0.01$). Another important factor of survival rate was age at RI therapy. The 10-year survival rate was 70.7% for those under 40 but 12.5% for those over 40 ($p < 0.01$).

426. P17 CLINICAL OUTCOME OF HIGH-RISK PATIENTS WITH DIFFERENTIATED THYROID CANCER.

R. Vassilopoulou-Sellin, E.S. Delpassand, T. P. Haynie, M.D. Anderson Cancer Center, Houston, Texas

Among patients with differentiated thyroid cancer, who generally have an excellent prognosis with near ,normal lifespan, there exist subsets with significant risk for morbidity and mortality from the disease. It is important to define the patterns of disease progression and the clinical outcome of such patients so that effective surveillance and treatment strategies can be developed. We describe the course of two such subsets of adult patients, treated at the M.D. Anderson Cancer Center: (A) Patients with recurrence after therapeutic radioiodine administration for papillary thyroid cancer: there have been 61 such cases, age at diagnosis (median, range) was 50 years (20-78), first recurrence occurred 21 months (3-114) after initial diagnosis, 19 patients died from thyroid cancer at 59 months (9-156), while 31 patients remain without evidence of disease for 112 months (25-234) of available follow-up and 5 cases are alive with disease 61 to 153 months. Cervical lymph node metastases were present in 39 cases and extrathyroidal or extranodal tumor invasion in 28 at initial diagnosis; distant metastases developed in 14 patients (lung, bone, brain). Radioiodine uptake by recurrent tumor deposits occurred in a minority of cases and only in the cervical region. (B) Patients with follicular thyroid cancer and distant metastases: there have been 44 such cases, age at diagnosis (median, range) was 57 (23-80) years. Symptomatic bone metastases were the chief complaint of almost all 24 cases in whom distant metastases were present at diagnosis, and developed after 53 months (8-216) in the remainder (bone and lung). The primary tumor was limited to the thyroid in most cases. There are 13 patients alive with disease 43 months (4-230) after diagnosis while 27 patients died from thyroid cancer after 83 months (4-238). Symptomatic spinal cord compression occurred in 13 cases and preceded death by 4 to 34 months. Persistent radioiodine uptake was seen in most bone metastases which were so treated on multiple occasions; in contrast, radioiodine uptake was rarely demonstrated in lung metastases. Radiotherapy was palliative and useful for bone metastases. Conclusion: Subsets of patients with differentiated thyroid cancer can be identified with characteristic clinical course and increased risk for disease-related morbidity and mortality. Surveillance and treatment should be adapted to their disease category and should include more broadly oncologic principles.

427. P18 THE CORRELATION BETWEEN PAPILLARY THYROID CARCINOMA AND LYMPHOCYTIC INFILTRATION IN THE THYROID GLAND.

S. Matsubayashi, Y. Matsumoto and F. Matsuzuka, Department of Psychosomatic Medicine, Faculty of Medicine, Kyushu University (S.M., Y.M.), Fukuoka 812, Kuma Hospital (F.M.), Kobe 650, Japan

Ninety-five patients with papillary thyroid carcinoma (PTC) who received primary surgically treatment in 1983 at Kuma Hospital and who were followed-up through 1992 were the subjects of this study. Initial therapy was tumor resection for 5 patients, lobectomy for 23 patients, total thyroidectomy with one side modified neck dissection for 60 patients and total thyroidectomy with bilateral modified neck dissection for 7 patients. According to lymphocytic infiltration in the thyroid gland, the recurrence of the tumor was evaluated. Group A was 36 patients with lymphocytic infiltration, 26 with infiltration surrounding the tumor, 3 with infiltration inside the tumor, and 7 with both. Group B consisted of the remaining 59 patients with no lymphocyic infiltration. Age, sex, initial tumor size, and initial treatment were not different between both groups of A and B. Anti-thyroglobulin antibody and/or anti-microsomal antibody were positive in 16 patients from group A and 4 patients from group B ($p<0.001$). Local tumor invasion into contiguous neck structures at the time of initial surgery was not found in group A, but was found in 7 patients from group B. The recurrence of the tumor was found in only one patient of group A (2.8%), but in 11 patients of group B (18.6%). The duration between initial treatment and recurrence was 2 to 10 years. The percent of patients free from recurrence over the 10-years follow-up from group A was significantly higher than that from group B ($p<0.01$; Cox-Mantel test). In conclusion, lymphocytic infiltration surrounding the tumor or into the inside of the tumor in PTC might be a means for predicting a good prognosis.

428. P19 **Preferential Expression of the Cell Adhesion Molecule CD44 in Papillary Thyroid Carcinoma.** J. Figge, G. Gerasimov*, I. Dedov*, M. Bronstein*, K. Troshina*, G. Alexandrova*. Albany Medical College, Albany, NY; and *The Russian Endocrinology Research Center, Moscow, Russia

CD44 is a polymorphic family of immunologically-related integral membrane glycoproteins associated with cell adhesion, lymphocyte homing, and tumor metastasis. At least 20 different CD44 isoforms have been described that arise from the differential splicing of 10 variant exons (termed v1-v10). We studied CD44 expression in 114 formalin-fixed paraffin-embedded thyroid tumors using the A3D8 anti-human CD44 monoclonal antibody. Intense plasma membrane immunoreactivity was observed in 65 of 67 papillary carcinomas (98%; 30 cases were from Russia and 37 from New York); 9 of 16 follicular adenomas (56%); 4 of 8 Hurthle cell neoplasms (50%); 5 of 15 medullary carcinomas (33%); and 3 of 8 follicular carcinomas (38%). These results show that among thyroid neoplasms, papillary carcinomas preferentially display the CD44 antigen ($p <= 0.001$, Fisher exact test). Non-neoplastic follicular epithelium exhibited a low to moderate level of staining compared with adjacent papillary carcinoma cells. To further characterize the CD44 isoform, we tested a subset of cases with the 2F10 monoclonal antibody, which recognizes a CD44 variant exon (CD44v6) implicated in tumor metastasis in a rat model. Eleven of 11 papillary carcinomas tested were 2F10 positive, and one of the follicular carcinomas was positive. These results suggest the hypothesis that deregulated CD44v6 expression on the plasma membrane of papillary carcinoma cells contributes to the ability of those cells to metastasize to regional lymph nodes and then to remain dormant for years.

429. P20 EXCESSIVE EXPRESSION OF CYTOPLASMIC C-MYC PROTEIN IN THYROID PAPILLARY CARCINOMAS

Y.Nakamura[1,2], H.Kamma[1], T.Yazawa[1], K.Kakudo[2] and T.Ogata[1]

1)Department of Pathology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, and 2)Wakayama Medical Collage, Wakayama, Japan.

The nature of cytoplasmic expression of c-myc protein has been not confirmed yet, though the excessive expression of nuclear c-myc protein is in general believed to be closely related to cell growth. The objective of this study is to clarify what means the cytoplasmic expression of c-myc protein in thyroid cancer. Forty surgical samples of papillary carcinomas(PTCs) were used for this study. The follicular cells in non-tumorous areas of each sample were applied as a control. Immunohistochemical studies were performed using 9E10 and MIB-1 antibodies against c-myc protein and Ki-67, respectively. Furthermore, a western blot analysis of c-myc protein was performed by SDS-PAGE and chemiluminescence detection method. The follicular cells were positive in 14.5% and 39.8% for c-myc protein in nuclei and cytoplasm, respectively. In PTCs, the cytoplasmic expression was observed in 95.5% ($p<0.01$, vs control) of tumor cells and the nuclear expression was in 28.8% of tumor cells. Ki-67, a proliferating marker, was positive in 0.6% of follicular cells and 2.7% of tumor cells. There was no significant relation between the expressive grades of cytoplasmic c-myc protein and nuclear Ki-67. Western blot analysis of cytoplasmic c-myc protein revealed that all five samples examined expressed an alternative 64 kDa c-myc protein in the cytoplasmic fraction in addition to 67 kDa c-myc protein. Conclusively, thyroid cancers over-expressed c-myc protein in cytoplasm and there may be no relation between its cytoplasmic protein and tumor cell growth. The expression of unusual c-myc protein in cytoplasm of the tumor cells may be caused by alternative splicing of c-myc.

430. P21 THE ROLE OF RADIATION THERAPY IN DIFFERENTIATED THYROID CANCER. R. W. Tsang, J. D. Brierley, W. J. Simpson, T. Panzarella, M.K. Gospodarowicz, and S. B. Sutcliffe, University of Toronto, Princess Margaret Hospital, Toronto.

The purpose of this analysis was to determine the prognostic factors for local failure and cause-specific survival in papillary and follicular thyroid cancer, and the role of external beam radiation therapy (RT) when used as part of initial management.

We reveiwed records of 382 patients with differentiated thyroid cancer (papillary-262, follicular-120) managed at the Princess Margaret Hospital (PMH) between 1958 and 1985. The median age was 49 years (range, 10 - 85 years), with M:F ratio of 1:3. When staged according to UICC-1987, there were 220 patients with stage I or II, 102 with stage III, and 33 with stage IV disease (27 Stage X). The median duration of follow-up was 10.8 years (range, 0.03 - 33.3 years) for alive patients.

In papillary tumors, the 10-year cause-specific (CS) and overall survival rates were 93% and 85% respectively, with the corresponding rates for follicular patients being lower at 69% and 56%. The local relapse-free rate (LRFR) for both histologies were 86% at 10 and 15 years. Older age, metastatic disease at presentation, post-operative residuum, tumor size > 4 cm, and poor differentiation were identified in multivariate analysis as significant factors for CS death. Age, tumor size > 4 cm, bilaterality, nodal involvement, less extensive surgery (< near-total thyroidectomy) and the lack of use of radioiodine were important for local-regional failure. The use of external RT was associated with more advanced local disease. After adjusting for important prognostic factors, there were no statistically significant differences in CS survival or LRFR between patients who received RT and those who did not. The 33 patients with gross residual disease who received post-operative RT had a 5 year LRFR of 62% (±9%) and CS survival of 65% (±9%).

The prognostic factors for differentiated thyroid cancer have been clearly identified. Most patients at risk for local recurrence received postoperative RT. Therefore, the role of postoperative RT in patients with differentiated thyroid cancer remains unclear. Patients with gross residual disease post-surgery appeared to benefit from radiation therapy (±radioiodine). A phase III trial in patients at high risk for local recurrence is required to assess the potential benefit of RT.

431. P22 INFLUENCE OF 3,5,3'-TRIIODOTHYROACETIC ACID TREATMENT ON TSH SUPPRESSION AND SURVIVAL OF PATIENTS WITH DIFFERENTIATED THYROID CANCER. *P. Pujol, JP. Daurès N. Nsakala, R. Martinel, J. Bringer, Department of Endocrinology and Epidemiology, University Hospital of Montpellier, France.*

TSH suppression is required for the post-operative treatment of patients with differentiated thyroid carcinoma. This is usually achieved using high doses of L-thyroxine (L-T4). However, some patients have intense side effects due to hyperthyroidism. We investigated the influence of 3,5,3'-triiodothyroacetic acid (Triac) combined with L-T4 treatment on TSH suppression and survival in patients with differentiated thyroid carcinoma that showed poor tolerance to L-T4.

Between 1970 and 1993, 141 patients with differentiated thyroid carcinoma underwent thyroid surgery and were managed for post-operative medical treatment. The follow-up included a yearly check-up involving clinical examination, plasma TG and TSH level assessment, chest X-ray, as well as I^{131} total body scan the first 3 years after surgery. 26 patients under L-T4 treatment presented a poor tolerance to LT4 and 13 of them have nonsuppressed TSH. These 26 patients were treated with a combination of L-T4 and Triac (mean dose 800 µg/D), and a significant reduction of the daily dose of L-T4 was obtained (mean reduction : 25 µg/D, $p<0.05$). In the subgroup with nonsuppressed TSH (n=13), combination of L-T4 plus Triac resulted in a significant improvement of TSH suppression (mean TSH decreased from 1.6 to 0.2 mU/l, $p<0.05$). Univariate and multivariate analysis of disease free survival were performed, and no statistical difference were found between the group of patients treated with combination of L-T4 and Triac (n=26) and the group treated with L-T4 alone (n=106). Based on clinical chart review, there was an apparent improvement in peripheral side effects in 18 (79%) patients .

In patients with differentiated thyroid carcinoma, combination of L-T4 and Triac may improve TSH suppression and therapeutic tolerance, without affecting the outcome of patients.

432. P23 Total thyroidectomy and preservation of parathyroid gland function in operation of childhood thyroid papillary carcinoma. H. Funahashi, T. Imai, Y. Tanaka, M. Wada, T. Morita, Department of Surgery II, Nagoya University School of Medicine

It is a controversial point if total thyroidectomy should be performed to the patients with thyroid papillary carcinoma, especially children patients, and how parathyroid gland function is preserved. We have performed total thyroidectomy and parathyroid autotransplantation after total resection as a routine method. Total thyroidectomy is desirable because incidence of intraglandular metastasis is originally high in papillary carcinoma and the incidence in children is not low compared with that in adults. Operation to reduce the possibility of recurrence is even more desiable because prognosis of the disease is favorable. In regard to the parathyroid gland, we find out 4 glands as much as possible, resect the glands one by one and transplant them into the pocket in the sternocleidomastoid muscle. It is not so difficult to find out the parathyroid gland if technical skill is obtained. The resected gland is made muddy and transplanted into the pocket in the sternocleidomastoid muscle exposed by handling from the cervical wound. The pocket is closed with nylon suture. PTH va $\leq$0.3% returns to the preoperative value in about 10-14 days. Supplementation of calcium is required for at least 10 days immediately after the operation. We performed these operations to 18 children patients and supplemented thyroid hormone with Levothyroxin 150 μ g/day in all the patients. Only 1 patient fell into permanent hypoparathyroidism but showed nomal serum calcium value after Vit D and calcium lactate were given. Follow-up period ranged from 27 to 168 months and the mean was 95.5 months. Recurrence was not observed. Seventeen out of 18 patients were female, 6 of which reached the marriageable age and married. Three out of the 6 patients were pregnant and gave birth to children by normal delivery. Since favorable results have been obtained also from the viewpoint of Quality of Life, we believe that these operative procedures are acceptable.

433. P24 SOME MORE INFORMATION ON THE VALUE OF OUTPATIENT ABLATION OF THYROID TISSUE WITH <30 mCi of I-131 IN PATIENTS WITH DIFFERENTIATED THYROID CANCER. I.R. McDougall, Stanford Health Services, Stanford, California

57 patients with differentiated thyroid cancer were treated with 29.9 mCi of I-131 to ablate functioning thyroid tissue in the region of the thyroid bed. All patients had previous thyroid surgery, from subtotal thyroidectomy to total thyroidectomy. Patients were off thyroxine (T_4) for 4 weeks or studied 4 weeks after the operation without having received T_4. 56 patients had WBS and uptake, 48-72 hours after a 2 mCi tracer dose of I-131. 1 patient had a scan of the cervical area after I-123. All patients had measurement of TSH and thyroglobulin coincidentally with WBS. Based on clinical information, age, gender, size of cancer, and scintigraphic findings, a decision was made to treat with radioiodine and these patients were judged to be treatable with an outpatient dose of 29.9 mCi of I-131. The therapy dose was scanned after 5-7 days, and all patients had follow-up diagnostic WBS 6-12 months later. Prior to I-131 therapy, uptake was 8.06% $\pm$ SEM 0.94%. Follow-up was considered negative if repeat WBS showed no evidence of abnormal uptake in the lateral cervical area and the uptake in the region of the thyroid bed was $\leq$0.3% Uptake measurements on repeat scan were 0.49% $\pm$ SEM 0.17%. (P= <0.0001.) 47 patients were judged to have a negative follow-up scan, and their pre- and post-treatment uptakes were 6.73% $\pm$ 0.9% and 0.11% $\pm$ 0.01% (P= <0.0001). Of those who were judged to have an abnormal follow-up scan, the pre- and post-uptakes were 14.9% $\pm$ 2.4% and 2.3% SEM $\pm$ 0.8%. 8 of these were women, 2 men. 2 had pulmonary lesions, 9 of 10 were retreated, 1 patient died of cancer, and 1 from other reasons. 1 patient with pulmonary lesions did not have WBS before treatment. 7 of 10 patients who had abnormal scintiscan had a pre-therapy uptake of >10% compared with 17 of 47 with successful treatment. In this selected group of patients, 82.5% had successful ablation of residual thyroid tissue with 29.9 mCi of I-131. Age or gender did not influence the outcome; however, there was a significant difference between the pre-treatment uptake of those who had successful ablation versus those that did not. 94% of those patients whose uptake was $\leq$10% before I-131 therapy were successfully treated with a single dose of 29.9 mCi of I-131.

434. P25 TREATMENT OF MICRONODULAR PULMONARY METASTASES OF PAPILLARY THYROID CARCINOMA WITH 131-I. J. C. Sisson, S. Zempel, and S. Spaulding. University of Michigan, Ann Arbor, MI, USA.

Efficacy of 131-I therapy on papillary thyroid cancer remains controversial. Investigations of outcomes from treatment have been limited by requirements of many patients and many years of followup. However, if 131-I can reduce life-threatening, distant metastases, the results should be apparent in months. Outcomes of 10 patients with micronodular pulmonary metastases, each treated in the past 10 years, were investigated. Ages of patients at therapy were 5-45 years. Evaluations were 9-12 months after therapy. The three indices of effect were: Xray/CT; scintigraphic image; and serum thyroglobulin level (Tg).

From the initial dose of 131-I (60-260 mCi) aimed at lung tumors: 1) 0/10 had normalization of all 3 indices; 2) 2/6 had Xray/CT abnormalities reduced (3 had initially normal Xrays plus no CT comparisons and one had followup Xray elsewhere); 3) 5/8 had scintigraphic abnormalities reduced (one had only post-therapy abnormality and was not retreated and one was reevaluated elsewhere); 4) 3/6 had Tg values that were 53-75% of baseline, and 3/6 had rises in Tg (3 did not have serial tests and one had antibodies to Tg).

A second treatment (123-350 mCi) was given to 9 patients. Data are incomplete but: 1) one patient had normalization of all indices; 2) 4/7 had Xray/CT abnormalities disappear, but in 3/7 abnormalities were unchanged or worse.

Dosimetry, performed only after 1993, gave 8 measurements in 6 patients. Absorbed doses (rad) were: body 49-135; and blood 87-200. In 4 subjects lung tumor volumes were estimated, and, even allowing for a therapeutic dose less than predicted by dosimetry, tumors received >14,000 beta rad. Hematologic toxicities: leukopenia in three subjects, including neutropenia in one, and thrombocytopenia in two were minor and transient.

Conclusions. 1) 131-I therapy will reduce micronodular pulmonary metastases of papillary carcinoma in over half of the patients; 2) more than one large (often >200 mCi) appear to necessary to achieve maximum effects; and 3) more than one large dose can be given without serious shortrange toxicity.

435. P26 IS MORE AGGRESSIVE TREATMENT NEEDED FOR FOLLICULAR THYROID CANCER? L.J. DeGroot, E.L. Kaplan, and F. Straus, Thyroid Study Unit and Departments of Pathology and Surgery, The University of Chicago, Chicago, IL 60637

We evaluated the course of 49 patients with follicular thyroid carcinoma (FTC) and compared them to prior experience with 249 patients with papillary carcinoma (PC). FTC occurred in an older age group (avg. onset 36 yrs), but patients died at a younger age (avg. 44 yrs in FTC). Overall deaths were 16%, double that in PC. There were fewer recurrences, and in FTC almost all recurrences were followed by death. Recurrences and death from FTC did not occur more than 13 years after the original diagnosis, in contrast to the continued recurrences and deaths throughout the 40 years observation in patients with PC. No deaths occurred in patients who were under age 45 at diagnosis with tumors < 2.5 cm in diameter and with intrathyroidal disease or only positive neck nodes. Mortality was increased with increasing size of tumor, distal spread of disease, and age over 45. Prognosis in individuals with a Hurthle cell pathology did not differ from the overall group. In contrast to PC, more extensive surgery and postoperative ^{131}I ablation did not clearly influence deaths in patients with intrathyroidal disease or neck nodes. Of 8 deaths, 5 were in Class I, and most of these patients received near-total thyroidectomy and most were ablated post-operatively. Some of these individuals were under age 40, or had tumors < 2.5 cm. Thus, should patients with FTC, with intrathyroidal disease or neck nodes, who are above age 45 and have tumors > 2.5 cm in size have more aggressive therapy? We consider that more aggressive treatment with ^{131}I, radiotherapy to the neck, or chemotherapy, may be appropriate to improve survival in this subgroup of patients.

436. P27 STRUCTURAL DETERMINANTS OF THYROTROPIN RECEPTOR INTERNALIZATION. Yufei Shi, Minjing Zou, Nadir R Farid, Molecular Endocrinology Laboratory, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.

Following TSH binding to its receptor, the hormone-receptor complex is internalized. What proportion of bound receptor undergoes internalization, the routes involved, and the role of endocytosed receptor in the mediation and/or termination of TSH action are unclear. In order to elucidate the structural basis of TSH receptor internalization, we have investigated the role of the cytoplasmic tail and that of tyrosine 678 of the motif NPXXY at the boundary between the VII transmembrane helix and the proximal cytoplasmic tail in the process of internalization. The NPXXY motif is implicated as an endocytosis signal in a variety of receptors and is conserved in all seven transmembrane segment G protein coupled receptors. TSH receptor constructs truncated by up to 56 residues ($TSHR^{708}$) of the C-terminue of the cytoplasmic tail or mutated at tyrosine 687 to alanine were stably transfected in CHO cells which were tested for TSH binding, adenylyl cyclase activation and internalization. $TSHR^{708}$ was associated with TSH binding capacity and affinity, as well as TSH-stimulated adenylyl cyclase comparable to that of wild type receptor. By contrast, the rate of receptor internalization was enhanced. The replacement of tyrosine 678 by alanine did not impair high affinity TSH binding, but did abolish hormonally responsive adenylyl cyclase activity. The mutation reduced the rate of TSH internalization.

We conclude that 1) the C-terminal 56 residues of the cytoplasmic tail of the TSH receptor inhibit internalization, and 2) tyrosine 678 promotes the coupling of the receptor to G protein as well as enhances its internalization. Tyrosine 678 may bear important spatial relationship to residues in the cytoplasmic aspect of III transmembrane helix crucial to the receptor coupling to G protein. The cytoplasmic tail of the TSH receptor may contain sequence domains which modulate the effect of the NPXXY motif on internalization. The roles of residues other than tyrosine 678 in this process need to be defined.

437. P28 COMPARISON OF THE CONSTITUTIVE ACTIVITY OF THE TSH AND LH RECEPTORS USING DIFFERENT ASSAY CONDITIONS. F. Cetani, M. Tonacchera. *G. Vassart. I.R.I.B.H.N, *Service de Genetique Medicale, Universitè Libre de Bruxelles, Brussels, Belgium.

The TSH receptor (TSHR) and the LH receptor (LHR) are members of the family of G protein coupled receptors. Recently, point mutations conferring constitutive activity to the TSHR and LHR have been observed as cause of toxic adenoma and familial male precocious puberty, respectively. Common molecular activating mechanisms have been proposed but while the wild type (WT) TSHR has the capacity to activate cAMP production in the absence of agonist, the LHR seems, when studied in the same assay conditions, to be "silent". In order to compare both receptors we performed functional studies. Human TSHR and LHR cDNAs (the latter a kind gift of Prof. Minegishi) subcloned in the expression vector pSVL were transiently expressed in COS-7 cells. 48 hours after transfection with each construction, cells were tested for their ability to produce cAMP accumulation in medium containing normal Na+ concentration (medium 1) or an isoosmolar concentration of sucrose (medium 2).

Cells transfected with the WT TSHR showed a 6.5 fold increase in basal cAMP accumulation over cells transfected with pSVL alone in medium 1 and about a 12 fold increase in basal cAMP in medium 2. Cells expressing the LHR exhibited a 1.6 fold increase in basal cAMP over cells transfected with pSVL alone in medium 1 and, surprisingly, elicited a marked increase in basal cAMP production of about 12 fold when assayed in medium 2 showing a clear constitutive activity. A third receptor of the same family (melanocortin MC5) was transiently expressed in COS-7 cells and the cAMP production was the same in both conditions without constitutive activity.

In conclusion, the results presented show that i) the TSHR has a clear constitutive activity after transient expression in COS-7 cells and assayed in the presence or absence of salt; ii) the LHR, in the same conditions, shows a faint constitutive activity in the presence of salt and a clear constitutive activity in the absence of salt. In agreement with the known effect of salts on affinity for hormone binding, the absence of salt, in vitro, may play a role in driving the receptor into an active conformation able to activate the G-proteins in the absence of agonist. The TSHR seems to be easily autoactivated which could explain the wide diversity of the activating mutations described to date (12) and might play a role in its susceptibility to activation by autoantibodies in Graves' disease.

438. P29 PROTEOLYTIC CLEAVAGE OF THE HUMAN TSH RECEPTOR: ANALYSIS USING EXTRACELLULAR REGION CHIMERIC TSH-LH RECEPTORS. G.D. Chazenbalk, Y. Nagayama S.M. McLachlan and Basil Rapoport. Thyroid Molecular Biology Unit, V.A. Medical Center and the University of California, San Francisco, California 94121.

.The TSH receptor, as well as the LH/CG and FSH receptor, belongs to a subfamily of G protein-coupled receptors with large extracellular regions. Unlike the LH/CG receptor, there is evidence that the extracellular domain of the TSH receptor undergoes endoproteolytic cleavage when expressed in both thyroid and non thyroid cells. Previous studies involving human TSH receptor mutants have localized the proteolytic cleavage site to be closely upstream to amino acid 317. However, site-directed mutagenesis of three clusters of positively charged amino acids in this vicinity, which were potential endoproteolytic sites, did not abolish TSH receptor cleavage. Because the LH receptor is not cleaved, in the present study we examined whether substituting segments of the LH receptor for homologous regions of the TSH receptor between amino acids 262-316 (domain D) would abolish TSH receptor endoproteolytic cleavage. The amino acids substitutions were at positions 262-268 (chimeric receptor TSH-LHR-D1), 270-278 (TSH-LHR-D2), 287-297 (TSH-LHR-D3) and 309-316 (TSH-LHR-D5). Chimeric receptor TSH-LHR-D4 (amino acid residues 298-308) was not studied because of its lower affinity for TSH. Other amino acids within the TSH receptor D domain are identical to those in the LH/CG receptor and, therefore, could not be substituted. Covalent cross-linking of radiolabeled TSH was performed to monolayers of Chinese hamster ovary (CHO) cells stably transfected with the wild-type TSH receptor or TSH-LHR chimeras D1, D2, D3 and D5. When analyzed by SDS/ PAGE under non-reducing non reducing conditions, both the wild-type TSH receptor and all chimeric TSH-LHR receptors were visualized as ~ 95-100 kD bands. Under reducing conditions, all receptors yielded TSH binding fragments of about 54 kD. In summary, the present data indicate that within amino acid residues 261-316 homologous substitution of small regions of the TSH receptor with the corresponding amino acids of the LH/CG receptor does not prevent proteolytic cleavave of the TSH receptor. Further studies are necessary in order to localize the proteolytic cleavage site of the human TSH receptor.

439. P30 TSH-RECEPTOR EXPRESSION AND HUMAN THYROID DISEASE: RELATION TO THYROIDAL TRANSCRIPTION FACTORS S. Deiters, F. Schuppert, G.F.W. Scheumann*, H. Dralle**, A. von zur Mühlen. - Department of Clinical Endocrinology, Department of Abdominal and Transplant Surgery*, Hannover Medical School, D-30625 Hannover, FRG; Center for Surgery I**, Halle-Wittenberg University, D-06120 Halle/Saale, FRG

It was the goal of this study to investigate the role of thyroid specific transcription factors in the regulation of thyroid function. Thyroid transcription factor 1 (TTF-1) and paired box-gene 8 (Pax-8) were investigated in context with thyroid peroxidase (TPO), thyroglobulin (TG) and thyrotropin receptor (TSH-R) expression in the human thyroid gland. Expression levels of TTF-1 and Pax-8 were investigated in a total of 24 human thyroid glands with the help of Northern blot analysis. Results were related to expression levels of TPO, TG (both assessed in 33 human thyroids) and TSH-R (assessed in 66 thyroids). In relation to 6 healthy controls whose mean expression levels were arbitrarily defined as 1 relative unit, Pax-8 expression was increased in Graves' thyroids (3.791 units, n=8, p=0.0019) and decreased in Hashimoto thyroids (0.364, n=2, p=0.0455) and anaplastic carcinoma (0.054, n=6, p=0.0039). TTF-1 was decreased in Graves' thyroids (0.868, n=8, p=0.0389). TPO expression correlated with Pax-8 (p=0.0001, n=24), but not with TTF-1 expression (p=0.0984, n=24). Despite the reduced TTF-1 expression levels there was a correlation with TSH-R (p=0.0251, n=24) and TG expression (p=0.0471, n=24). We conclude that TPO expression is largely regulated by Pax-8 and that neither TTF-1 nor Pax-8 are decisive regulators of TSH-R expression suggesting that presently unknown factor(s) are important in the regulation of the TSH-R gene in the human thyroid gland.

440. P31 TSH REGULATION OF THYROID HORMONE SYNTHESIS AND PROTEIN KINASE C EXPRESSION. M.C Eggo, E.G Black, H. Chahal and *J.M Lord. Departments of Medicine and *Immunology, University of Birmingham, Edgbaston, Birmingham B15 2TH United Kingdom

We have examined the regulation of the expression of protein kinase C (PKC) isozymes in human thyroid cells in culture induced to differentiate with TSH. We have correlated their expression with the regulation of T3 and T4 synthesis in these cells and have examined the effects of selective PKC activators on thyroid function. Human thyroid tissue removed at surgery was collagenase-digested and cultured for 7 days in mF12M medium containing insulin and TSH. At this time T3 and T4 synthesis were dependent on the addition of exogenous iodide (10^{-7}M). Thyroid hormone synthesis was stimulated by TSH, the optimal concentration being 300μU/mL. At high TSH concentrations (10mU/mL), accumulation of T3 and T4 in the medium and iodine uptake and organification were much reduced. Activation of PKC with the pan PKC activator tetradecanoylphorbol 13-acetate, resulted in rapid inhibition of iodine uptake, organification and T3 and T4 synthesis. The phorbol ester 12-deoxyphorbol 13-phenylacetate-20-acetate which *in vitro* selectively activates PKCβ1 was equally effective in assays of inhibition of iodide uptake and organification at early time points (5h). Sapintoxin A which activates all isozymes with the exception of PKCδ was the most effective inhibitor in these assays which may imply a positive role for this isozyme in thyroid function. We examined the regulation by TSH of the expression of specific PKC isozymes ($\alpha,\beta1,\beta2,\gamma,\epsilon,\eta,$) with polyclonal antibodies raised in rabbits using the techniques of Western blotting (ecl detection) and confocal microscopy. We found that all isozymes examined with the exception of PKC γ were present in human thyroid cells. At 10mU/mL TSH, the expression of PKC β1 and β2 was strongly inhibited compared to their maximal expression at 1mU/mL. The expression of PKC α was apparently unchanged but Western blotting revealed that this isozyme was in an activated state and that TSH increased the amount of the proteolysed fragment of PKCα with a corresponding decrease in the intact molecule. PKC δ was present in the nucleus as well as the cytoplasm and was negatively regulated by TSH. These data show that TSH regulates PKC isozyme expression which may be related to functional roles of these enzymes in regulating thyroid hormone synthesis.

441. P32 **SPECIFICITY OF SYNTHETIC TSH ANTAGONISTS:** A.A.K.Hassoun, E.R.Bergert, J.C.Morris. Mayo Clinic, Rochester, MN 55905

In our continuing efforts to create potent and specific inhibitors of TSH receptors, we generated twenty new peptides by modifications of our previous most potent and specific antagonist. These were synthesized using an Advanced Chem Tech 350 automated multiple peptide synthesizer, purified by HPLC and their structure was characterized by amino acid composition analysis and mass spectroscopy. The peptides included sequences from Determinant Loop (DL; β88–95), Carboxyl terminus (C; β101-112), Intercystine Loop (I; β31-50), and α26-46 in various arrangements and positions. The potency of these peptides in inhibition of TSH binding and biological activity (presented at the Endocrine Society Meeting) was enhanced by adding α42-45 sequence, by changing Cys to Gly at α32, or by changing the position of the Intercystine Loop within the peptide. In the current study, we evaluated the specificity of these peptides. Using hCG and FSH radio-receptor assays (RRA), we tested the peptides' ability to inhibit ^{125}I-hCG and ^{125}I-hFSH binding to LH/CG and FSH receptors respectively. We found that the addition of α42-45 resulted in a similar enhancement of the binding inhibition potency to LH/CG receptors, but not to FSH receptors, and was thus not specific to TSH receptors. Five of the six peptides with this additional α sequence have EC_{50} that ranged between 12.8 ± 4.7 μM to 22 ± 4.8 μM, and between 164 ± 138 μM and no inhibition activity in hCG and FSH RRA respectively. We also found that a peptide with no α sequence, that was a highly potent TSH biological activity antagonist (EC_{50} = 5.7 ± 1.8 μM), had much reduced binding inhibition in both hCG and FSH RRA, with EC_{50} of 273 ± 12 μM and > 500 μM, respectively. Changing the Intercystine Loop position within the peptide, that previously created a highly potent inhibitor of TSH biological activity (EC_{50} = 4.3 ± 2.4μM), did not adversely affect the specificity having EC_{50} in hCG and FSH RRAs of 153 ± 62 μM, and 731 ± 157 μM, respectively. Increasing the separation between the Intercystine Loop and the α sequence created two peptides with potent and specific inhibition of hCG binding activity (EC_{50} = 21.2 ± 8 μM, and 29.7 ± 8.2 μM). These data demonstrate the potential for creation of potent and specific antagonists of TSH using a synthetic peptide chemistry and a peptidomimetic approach.

442. P33 ACTIVATION OF PHOSPHOLIPASE D BY THYROTROPIN IN FRTL-5 THYROID CELLS. J Ginsberg, S Gupta, A Gomez-Munoz, W C Matowe, D Brindley, Signal Transduction Laboratories, University of Alberta, Edmonton.

Although thyrotropin (TSH) has been demonstrated to activate protein kinase C (PKC) in vitro, the mediator(s) of PKC action remain unknown. Phospholipase D (PLD) activation is recognized to occur as a consequence of PKC activation. To determine whether TSH activates PLD in vitro, FRTL-5 thyroid cells were treated with TSH (Thytropar; 100 μU/ml) or phorbol ester (PMA, 100 nM) prior to assessment of PLD activity by measurement of [^{3}H]phosphatidylethanol from [^{3}H]phosphatidylcholine. Both PMA and TSH significantly stimulated PLD after 1 min ($p<.05$) with maximal responses of 600% and 680% of control values ($p<.05$) occurring at 60 min. Purified bovine TSH could reproduce the effects of Thytropar. To determine whether PLD stimulation occurred as a consequence of PKC activation, TSH- and PMA-mediated PLD activity was studied in FRTL-5 thyroid cells that either had been pre-treated with the PKC inhibitor, chelerythrine (1 μM, 10 min) or PMA (100 nM, 24 h) to down-regulate PKC. Both these manoeuvres abolished the PLD stimulation seen with PMA or TSH. To determine the effect of PLD activation on thyroid function, FRTL-5 thyroid cells were treated with the PLD agonist, lysophosphatidic acid (10 μM, 30 min) which enhanced iodide uptake to that seen by TSH stimulation. Prior treatment with the PLD inhibitor, ceramide (10 μM, 90 min) abolished this effect. In summary, in FRTL-5 thyroid cells: 1. TSH- and PMA-mediated stimulation of PLD activity occurs via PKC activation, and 2. PLD activation leads to increased iodide uptake. We postulate that PLD may serve to perpetuate the PKC signal in the thyroid via further production of diacylglycerol and that PLD may be an important regulator of thyroid function in vitro.

443. P34 **THE AMINO TERMINUS OF THE HUMAN THYROTROPIN RECEPTOR IS A POTENT LINEAR EPITOPE.** P.N.Graves, V.Nguyen and H.Vlase, Department of Medicine, Mount Sinai School of Medicine, New York, New York, 10029.

We have found that the N-terminus of the human TSH receptor extracellular domain (hTSHR-ecd) (amino acids 22-41) is a dominant epitope when tested as a synthesized antigenic peptide. We have now evaluated whether this epitope can also be recognized within the context of the entire hTSHR-ecd, using protein unfolding and refolding conditions. Unfolded and refolded hTSHR-ecd proteins were resolved using a PAGE/SDS system and were equally reactive with murine antisera recognizing amino acids 22-41, as well as murine monoclonal antibody (MAb A10, P. Banga, London) recognizing hTSHR amino acids 22-35, consistent with recognition of a linear epitope equally accessible in the folded and unfolded state. Further evidence was obtained by mutagenesis of cysteine residues within the epitope, as these may be implicated in folding via S-S bond formation. Cys to Ser substitutions at positions 24, 29, and 31 (from wild type to the triple substitution) were generated using a mixed oligonucleotide PCR-based strategy to produce the mutant hTSHR-ecd cDNAs. Clones of expression-positive *E. coli* transformants were identified by immunoblotting, using an antipeptide rabbit serum to hTSHR amino acids 397-415 and the blots were reprobed with MAb A10 (recognizing amino acids 22-35). Antibody recognition was unaffected by the substitutions, again consistent with the linear epitope hypothesis. We conclude that the N-terminus of the hTSHR is a dominant linear epitope.

444. P35 **PRODUCTION OF SOLUBLE PROKARYOTIC HUMAN TSH RECEPTOR EXTRACELLULAR DOMAIN BY DISULFIDE BOND FORMATION IN THE CYTOPLASM OF E. COLI.** Y. Bobovnikova and V. Nguyen, Department of Medicine, Mount Sinai School of Medicine, New York, NY 10024.

The reducing potential of the E. coli cytoplasm prevents the formation of protein disulfide bonds, in contrast to the periplasmic compartment, which allows such bonds to be formed. Recently, E. coli mutants of the thioredoxin reductase (trx) gene were identified which allowed disulfide bonds to form in the cytoplasm (Derman et al., Science, 1993). We have taken advantage of this system to increase the yield of soluble human thyrotropin receptor extracellular domain (hTSHR-ecd). hTSHR-ecd cDNA (amino acids 21-414) was inserted into the vector pGEX-2TK by directional cloning and used to transform the trx mutant E. coli AD494. Expression of hTSHR-ecd was induced with IPTG and cell sonicates were fractionated into insoluble and soluble fractions. The expressed hTSHR-ecd fusion protein was detected by Western blot analysis using a murine MAb directed against hTSHR amino acids 21-35. Similar amounts of hTSHR-ecd protein was present in the soluble and insoluble fractions. This is in strong contrast to our own and other previously published reports using other E. coli expression systems, where most of the expressed hTSHR-ecd was in the insoluble fraction. In addition, analysis using reducing and nonreducing PAGE/SDS systems showed that the majority of the soluble hTSHR-ecd migrated as a refolded, disulfide bond-stabilized tetrameric species. The insoluble material migrated as unfolded monomers. This new expression system offers unique advantages for future studies of structure/function of the receptor, as well as the binding of autoantibodies in autoimmune thyroid disease.

445. P36 **DECREASED SENSITIVITY OF THYROID STIMULATING ANTIBODY ASSAY WITH CHINESE HAMSTER OVARY CELLS TRANSFECTED WITH CLONED HUMAN TSH RECEPTOR USING HYPOTONIC INCUBATION MEDIA WHICH IS DUE TO DECREASED VIABILITY OF CELLS DURING INCUBATION.** **Bo Youn Cho, Won Bae Kim, Jae Hoon Chung*, Hong Kyu Lee and Chang-Soon Koh. Department of Internal Medicine, Seoul National University College of Medicine, *Department of Medicine, Samsung Medical Center, Seoul, Korea.**

We investigated the optimal condition of thyroid stimulating antibody (TSAb) assay using Chinese hamster ovary cells transfected with cDNA of human TSH receptor (TSHr-CHO) stably expressing functional human TSH receptors. The extracellular cAMP responses of TSHr-CHO cells to the stimulation of bTSH or Graves' IgG were observed in three different incubation media. Extracellular cAMP stimulated by 10 U/L of bTSH or by 5 g/L of Graves' IgG were 4923.6±305.4% and 700±13.5% of basal when sucrose containing NaCl-free isotonic Hank's balanced salt solution (HBSS) (media A) was used, but those were 1129.2±228.1% and 245.5±17.1% of basal when NaCl-free hypotonic HBSS (media B) was used as incubation media ($p<0.01$). Same results were obtained with different concentrations of bTSH or of Graves' IgG. The incubation of TSHr-CHO cells in media B caused marked increase in the basal cAMP level without concomittant fold-increase in the stimulated cAMP level at various doses of bTSH and Graves' IgG. Use of media B failed to detect TSAb activities of two known "TSAb-positive" Graves' IgG tested, whereas TSAb activities were detected in both samples when the media A was used. In case of NaCl containing isotonic HBSS (media C), extracellular cAMP responses were poor at 0.001 and 0.1 U/L of bTSH and at all doses of Graves' IgG tested (0.5, 1, 5 g/L). The incubation of TSHr-CHO cells in media B caused significant increase in the number of trypan blue-stained, nonviable cells (5.7 ± 1.5, 7.6 ± 1.9 and 8.5 ± 1.6% at 1, 2 and 3 h of incubation, respectively; $p<0.01$) comparing to those incubated in media A or media C (about 2-3% in both media). Those decrease in the viability of TSHr-CHO cells when incubated in hypotonic incubation media may explain the decrease in the stimulation index of extracellular cyclic AMP with the use of media B, which is known to increase the sensitivity of TSAb assay using other thyroid cells (FRTL-5, human thyroid cells or thyroid adenoma cells). TSAb assay with TSHr-CHO cells is a sensitive and physiologically relevant assay system to measure TSAb activities merely through measurements of extracellular cAMP provided that the cells are incubated in NaCl-free isotonic incubation media.

446. P37 **ANALYSIS OF THE BIOACTIVITY OF RECOMBINANT HUMAN THYROID STIMULATING HORMONE IN TT_4 SUPPRESSED MICE.**

R. J. Mattaliano, B. G. Whittaker, N. M. Losapio, L. Cheney, E. M. Cole and B. M. Pratt.
Genzyme Corporation, Therapeutic Protein Development, Framingham, MA 01701

Recombinant human TSH (rhTSH) has been produced in Chinese hamster ovary cells for use as an adjunct in procedures used to detect metastatic thyroid carcinoma. An assay which measures cAMP production in response to exogenous TSH using a bovine thyroid microsome preparation has been developed to determine rhTSH activity. The specific activity of highly purified rhTSH (>98% by SDS-PAGE) is approximately 5 U/mg when compared to the WHO/NIBSC 84/703 reference standard. The routine measurement of TSH activity in vivo has been hindered by the availability of a robust assay system. Recently, East-Palmer et al (Thyroid. 5:55-59) have described an in vivo TSH bioassay, based on the McKenzie assay principles, which measures an increase in TT_4 level in response to exogenous TSH. In this assay, endogenous TSH and TT_4 levels are suppressed by giving mice (Crl:CF-1, 22-30 gr.) T_3 (3 ug/mL) in drinking water for 4 days. The assay endpoint is the measurement of the plasma TT_4 level 6 hours post intraperitoneal TSH injection. In support of product development, we have used this assay to determine the in vivo response of several rhTSH lots. For mice fed T_3 containing water, the average half-maximal TT_4 response (ED_{50}) was 0.4 ug rhTSH/mouse for 3 representative rhTSH lots (2-3 assays/lot). We have replaced oral T_3 delivery with subcutaneous time released T_3 containing pellets (0.1 mg T_3/21 day release) to ensure consistent TT_4 suppression and obviate T_3 solution preparation/stability concerns. The use of this T_3 delivery vehicle has in our laboratory proven to be a simple and efficient alternative means of consistently suppressing TT_4 to $\leq$ 1 ug/dL. For mice with implanted T_3 containing pellets for 6 days, the ED_{50} averaged 0.6 ug rhTSH/mouse for the 3 lots of rhTSH (3-4 assays/lot). This assay has demonstrated utility for making comparative in vivo measurements and offers a convenient alternative to more labor intensive methods.

447. P38 LONG-TERM FOLLOW-UP OF CONTRALATERAL LOBE IN HEMITHYROIDECTOMIZED WOMEN DUE TO SOLITARY FOLLICULAR ADENOMA. H. Niepomniszcze, A. Castellanos, E. Faure, A. García, M.C. Zalazar, G. Bur and B. Elsner. Services of Endocrinology and Pathology, Complejo Médico "Churruca-Visca", and Divisions of Endocrinology and Pathology, Hospital de Clínicas "José de San Martín", School of Medicine, University of Buenos Aires, Buenos Aires, Argentina.

Since there are not enough studies on the long term evolution of the remaining thyroid tissue after hemithyroidectomy, in patients who underwent surgery for solitary follicular adenomas, we decided to look for such cases in our records. This search was carried out following a number of specific requisites: a) all patients, prior operation, should have a single thyroid nodule with normal palpation of the rest of the gland; b) they should be clinically euthyroid at the time of surgery; c) the histological diagnosis of the nodule should be follicular adenoma; d) the operation should be a hemithyroidectomy, leaving intact the contralateral lobe; e) the macroscopic aspect of the contralateral lobe should be normal at the time of surgery; f) the minimum period of follow-up, after operation, should be 4 years. With the fulfillment of all these requirements we found 47 cases of women aging 23 to 79 years ($\bar{x}$= 45.9) at the time of surgery, with a consecutive follow-up period of 4-32 years ($\bar{x}$= 7.2). According to the performed TRH-TSH test just before operation, as well as the presence or absence of antithyroid antibodies, and also considering the histology of the extranodular tissue removed during surgery, we classified patients into two groups: normal extranodular thyroid (NET) [n=32] and abnormal extranodular thyroid status (AETS) [n=15]. Eleven women with AETS had thyroid autoimmunity, with or without overt thyroiditis, and the other four patients had subclinical hypothyroidism with negative titers of antithyroid antibodies. From all patients, 28 have received treatment with L-T4 immediately after surgery, while the other 19 had not. None of the patients receiving L-T4 had any alteration in the remaining lobe. However, 6 women without treatment (31.6%) have developed abnormalities in such lobe. Among them are included the only 3 patients with AETS who had not received L-T4 treatment. The other 3 women were two patients with NET, that have shown hyperresponse of TSH to TRH after surgery (peak TSH >25 μU/ml). and a woman who developed a new follicular adenoma 32 years later. None of the patients with NET, which had normal TRH-TSH test after surgery, have presented any kind of alteration in the contralateral lobe, even in cases without L-T4 prophylactic treatment. The abnormalities found in the remaining lobe of the 6 patients mentioned above were: 2 follicular adenomas, 1 benign nodule which shrinked after treatment with L-T4, 2 diffuse enlargement (goiter) and 1 papillary carcinoma. It is concluded: 1) follicular adenomas would have a greater chance to develope in a gland subjected to thyroid autoimmunity than in a previously normal gland. 2) treatment with L-T4 prevents the formation of new nodules or the development of goiter in the contralateral lobe. 3) the remaining lobe of patients with thyroid autoimmunity and/or previous subclinical hypothyroidism develope morphological abnormalities if the patient has not received prophylactic treatment after surgery. 4) only those patients, who have healthy extranodular tissue before and after surgery, would be eligible for not taken thyroid medication.

448. P39 RECURRENCE OF SUBACUTE THYROIDITIS LONG TERM AFTER THE FIRST ATTACK.

H. Fukazawa, K. Yoshida, K. Abe, M. Yamamoto, S. Saito and H. Kurihara, Narugo National Hospital, Narugo-Machi: The Sec. Dep. of Inter. Med. Tohoku Univ. School of Med. and JR Hospital, Sendai: Kurihara Thyroid Clinic, Morioka, Japan.

Recurrent episodes of subacute thyroiditis (SAT) in the painful form after complete recovery have been rarely documented in the literature. We saw recurrent episodes of SAT in 18 patients (2 men, 16 women: aged 36-64 years) out of 321 patients (37 men, 284 women: aged 27-80 years), diagnosed in our clinic from 1979 to 1994. Among 18 patients with recurrent SAT (Group I), recurrences were occurred in 2 patients (0.6%) after 1~2 years, one (0.3%) after 2-5 years, 4 (1.2%) after 5-10 years and 11 (3.4%) after 10-16 years. The frequency of recurrence occurred in more than 5 years after first episodes was significantly higher ($p<0.01$) than that occurred in less than 5 years. The thyroid function and HLA typing were studied in Group I, and these data were compared with those of 15 patients with SAT, who had been followed for more than 10 years and had no episode of recurrence (Group II). In Group I, the mean erythrocyte sedimentation rate (ESR) in patients with the recurrent episodes was 71 mm/h, and significantly lower than that (99 mm/h) in the first episodes. However, all the symptoms were not necessarily milder in the recurrent episodes, and all recurrent episodes were accompanied pain in the neck. The ESR in the first episodes of Group I was not significantly different from that in Group II. The serum T_4 concentration in the first episodes in Group I was not significantly different from that in the recurrent episodes, and was not significantly different from that in Group II. With regard to the HLA typing, HLA -A, B, C, DR, DP and DQ antigens were determined, and the data were compared with a normal control group comprising 1264 unrelated Japanese subjects. The frequences of HLA-A24, B35 and CW3 were higher in the entire group of SAT (Group I and II) than in the control subjects. The frequency of HLA-A26 in Group I was also higher than in the control subjects. However, no significant difference was observed between Group I and II. The patients in Group I had a significantly higher ($p<0.05$) incidence of HLA-DR4 than in Group II and in the control subjects. In conclusion: Painful recurrence of SAT may be rare but dose occur more than 10 years after the first episode. The frequency of recurrence is 5.6% in our clinic. Group I had a significantly higher incidence of HLA-DR4 than in Group II, suggesting that the association of HLA-DR4 may be related to the predisposition to the recurrece of SAT.

449. P40 THE CHARACTERIZATION AND RESPONSE TO TREATMENT OF THYROID NODULES IN PATIENTS WITH GRAVES' DISEASE.

N.E. Carnell and W.A. Valente, Division of Endocrinology, University of Maryland, Baltimore, Maryland, USA.

The pathogenesis of thyroid nodules in patients with Graves' disease (GD) is poorly understood. We hypothesize that many "nodules" in GD are, in fact, pseudonodules and will respond favorably to non-surgical therapy. In addition, we propose any cold nodule not responding to therapy carries a high risk of carcinoma. Therefore, to clarify the etiology of thyroid nodules in GD and assist in risk stratification for thyroid carcinoma, we report on 468 Graves' patients, ages 12-75, followed for 1-31 years (mean 5.1 years) treated with radioiodine (n=345), surgery (n= 19), antithyroid drugs (n=88) or observation (n=18). Sixty patients (12.8% of the total) had nodules noted by palpation, nuclear scan and/or ultrasound and were classified as: 1) GD with a solitary hypofunctioning nodule (n= 29, 6.2%), 2) GD with multiple nodules (n= 19, 4%), 3) GD with a solitary autonomous nodule (n= 4, 1%), and 4) patchy irregular GD (PIGD) (n=8, 1.7%). Six patients (1.3%) were found to have thyroid carcinoma (5 in group 1 and 1 in group 4). Of the 29 patients with solitary cold nodules, 6 were not palpated and all disappeared after ^{131}I treatment. Of the 23 patients with a palpable cold nodule, 7 went directly to surgery and 3 were found to have thyroid carcinoma. The remainder of the patients were treated with ^{131}I (9) or PTU (7). Eight nodules (4 in each group) vanished and 3 nodules decreased in size. Of the 5 patients with a residual solitary cold nodule, 2 were found to have carcinoma and the rest had benign fine needle aspiration biopsy (FNAB). The 19 patients with multiple nodules were differentiated from Plummer's disease by the presence of orbitopathy or positive anti-TPO antibodies with ^{131}I uptake over 50%. All but 1 of these patients became hypothyroid after ^{131}I therapy and 16 (84%) had resolution (8) or significant decrease in size (8) of the nodules. Three patients (16%) are unchanged. The 4 patients with autonomously functioning nodules were all treated with ^{131}I. Three of the nodules disappeared. The one remaining nodule is still autonomous. The 8 patients with PIGD all had irregularities on examination, scan and ultrasound without clearly defined nodules. Seven of these patients had typical Hashitoxicosis. Of the 3 treated with ^{131}I, 2 developed a palpable nodule post-ablation, one of which turned out to be a carcinoma. In summary, "nodular" Graves' disease, as expected, represents other common forms of thyroid disease (from solitary nodules to Plummer's disease) superimposed upon GD. There are, however, a significant number of cases (33%) found in all 4 of the above categories which represent "pseudonodules" born out of the autoimmune process itself. Some of these cases can be identified, *a priori*, such as those patients with Hashitoxicosis. However, many such patients are identified only after treatment. These are the cases of hot, warm, or even cold nodules which disappear easily after modest doses (80-100 mCi/g) of ^{131}I or even after a trial of thionamide therapy. Therefore, for those patients not identified as having a suspect thyroid carcinoma by FNAB, it is safe to proceed with non-surgical therapy. Any cold nodule which remains unchanged (or becomes apparent) after treatment needs careful re-evaluation due to the high risk of malignancy (3 of 7, 43%).

450. P41 CHILDHOOD THYROID DISEASES AROUND CHERNOBYL. K.Ashizawa[1],S.Yamashita[2],M.Ito[2],T.Nishikawa[1], K.Hara[2],H.Namba[2],M.Izumi[1],Y.Shibata[3],M.Hoshi[4],S.Nagataki[1]. [1]The First Department of Internal Medicine [2]Atomic Disease Institute,Nagasaki University School of Medicine, [3] Radiation Effects Research Foundation, Nagasaki,[4]Hiroshima University, Hiroshima, Japan.

Since the thyroid gland of children is especially vulnerable to the carcinogenic action of ionizing radiation,attention has been focused on the high incidence of childhood thyroid cancer around the Chernobyl .However it is important to clarify the prevalence of various thyroid diseases as well as the radiation dose before establishment of the effect of radiation on thyroid.Therefore we have screened 50,000 children,who were younger than 10 years old at the time of the accident from May 1991 to December 1993 at the five diagnostic centers around the Chernobyl (Mogilev,Gomel,Kiev,Korosten,Klincy).

【Methods】 Uniformal methods of investigation include 1) exact history of each subject,2) whole body radioactivity (^{137}Cs) including the measurements of radioactivity (^{137}Cs) in soil and water, 3) thyroid ultrasonic scanning , 4) measurements of serum levels of free thyroxine and thyrotropin and titers of thyroid autoantibodies, 5) measurements of urinary iodine, 6)fine needle aspiration biopsy in subjects with goiter and/or nodule.

Goiter was defined as a thyroid volume exceeding the upper limit calculated by the following formula:

$$LIMIT=1.7\times10^{0.013\times age+0.0028\times height}\times(\text{body weight})^{0.1}$$

【Results】 Prevalence of goiter based on this formula of the LIMIT is higher in Kiev and thyroid autoantibodies in Korosten than those in Mogilev where is less contaminated and not iodine deficient.The reason of high prevalence of goiter in Kiev may be due to iodine deficiency.In Gomel 19 cases of thyroid cancer were observed,which is significantly higher than other four oblasts.So far there was no relationship between the occurrence of childhood thyroid diseases and the whole body ^{137}Cs(Bq/Kg) levels/the soil ^{137}Cs radio contaminated levels (Ci/Km^2).

【Summary】 Based on these screening system,the incidence of childhood thyroid diseases around Chernobyl is clarified to vary among the regions. A long- term prospective study (case-control/cohort) should be conducted using an established diagnostic criteria and analyzed with an accurate estimation of exposure dose to external and/or internal radiation.Supported by Sasakawa Memorial Health Foundation.

451. P42 TREATMENT OF TOXIC THYROID NODULE BY PERCUTANEOUS ETHANOL INJECTION DURING PREGNANCY. D. Cortelazzi, D. Castagnone°, B. Tassis*, E. Venegoni*, R. Rivolta°, and P. Beck-Peccoz, Institute of Endocrine Sciences and °Department of Radiology, Ospedale Maggiore IRCCS, and *Obstetrics and Gynecology Clinic I, University of Milan, Milan, Italy.

A 35-year old pregnant woman (13th week of gestation) was referred to us for overt hyperthyroidism. Physical examination revealed tachycardia, tremors and weight loss. The thyroid gland was palpable with a nodule inside the right lobe. No exophthalmos was present. Thyroid function tests showed high levels of free thyroxine (24.8 pmol/L; normal values: 9-20 pmol/L) and suppressed TSH (<0.05 mU/L; normal values: 0.26-5 mU/L). Anti-thyroglobulin (AbTg) and anti-thyroperoxidase (AbTPO) auto-antibodies were negative. Thyroid scintigraphy was not carried out because of pregnancy, but the diagnosis of autonomous toxic thyroid nodule was confirmed by ultrasound color-Doppler that showed increased vascolarity confined to the nodule area. The volume of the nodule was 8 ml and the cytological evaluation, performed by FNA, showed a benign papillary lesion.

Antithyroid drugs are the primary modality for maternal hyperthyroidism during pregnancy, but it is well known that, besides the potential maternal toxic effects, the transplacental passage of thioamides can induce fetal hypothyroidism, as well as goiter. Surgical or radioiodine ablation of the thyroid nodule is excluded because of pregnancy. Therefore, we decided to treat the toxic nodule by ultrasound guided percutaneous ethanol injection (PEI). Four injections of 3 ml of sterile ethanol (95%), one every 3 days, were performed. After the first two ethanol injections, thyroid hormone circulating levels fell into the normal range. The woman complained only a transient pain and burning sensation. The pelvic ultrasound control showed normal fetal growth and heart rate. After 3 weeks from the first ethanol injection, the woman was completely euthyroid (FT4: 9.9 and FT3: 4.1 pmol/L) and at ultrasound color-Doppler control, the nodule was reduce in size by about one third with normal vascolarity. TSH levels became detectable after two months (0.4 mU/L). The obstetric examination was normal, as were fetal growth and heart rate. Unfortunately, one month later the woman spontaneusly aborted for obstetric reasons.

In conclusion, these data suggest that during pregnancy the percutaneous ethanol injection might be an effective and quik therapy for toxic thyroid nodule and a safe alternative to the administration of anti-thyroid drug.

452. P43 RECURRENCE OF SUBACUTE THYRODITIS AFTER A LONG LATENT PERIOD.
Makoto Iitaka, Naoko Momotani, Jun Ishii, Kunihiko Ito, Department of Medicine 4, Saitama Medical School, Saitama, Ito Hospital, Tokyo, Japan

Subacute thyroiditis is considered to be a viral disease and the report of the late recurrence after complete recovery is quite rare. We examined 3,344 patients with subacute thyroiditis seen at Ito Hospital from 1970 to 1993. Subacute thyroiditis recurred in 48 patients 14.5±4.5 years after the first episode. Five patients had the third episode 7.6±2.4 years after the second episode. The mean age of the patients at the first, second, and third episode was 38.1±6.2, 52.7±9.0, and 57.8±10.1 years old, respectively. The mean recurrent rate from 1973 to 1985 was 2.3±0.9% per year. The erythrocyte sedimentation rate(ESR) at the first, second, and third episode was 75±27, 57±26, and 68±36 mm/h. Thyroid radioactive iodine uptake (RAIU) at 24h was 1.6 ±1.9, 2.5±3.0, and 6.0±6.0, respectively for the three episodes. There was a significant difference in ESR or RAIU between the first and second, or the first and third episode, respectively.There was no difference in serum thyroid hormone levels among the three episodes. Nor was there any difference in leukocyte counts. However, there was significant difference in the duration of the treatment between the first and the second episode (3.6±2.6 vs 1.9±0.9 month). The number of the patients taking steroid decreased and that of the patients taking nonsteroidal antiinflammatory agents increased at the second episode. These findings were compatible with the milder clinical manifestations in the recurrent patients. The recurrence must be related to the decline of antibody titers. However, the immune system must react more quickly to the virus than did before, which may account for the milder clinical manifestations at the second or third episode.

453. P44 **COMPARISON OF CONVENTIONAL VERSUS SONOGRAPHY-GUIDED FINE NEEDLE ASPIRATION OF THYROID NODULES.**
D. Danese*^, S. Sciacchitano^, R. Bocale^, D. Pesaresi^, M. Andreoli^ and A. Pontecorvi°. *Forensic Medical Institute of Rome, Italian Air Force, ^Departement of Experimental Medicine, "La Sapienza" University of Rome, °Institute of Medical Pathology, Catholic University.

Fine needle aspiration (FNA) citology is an accurate, slightly invasive and cost-effective method for the diagnostic evaluation of thyroid nodules. Recently, ultrasound guidance has been introduced as an aid to enhance FNA diagnostic accuracy, allowing the investigation of non palpable nodules, as well as a more appropriate sampling of larger nodules. A retrospective study was performed to compare the accuracy of the conventional FNA (C-FNA) technique with that of sonography-guided FNA (SG-FNA). During a 14-year period, 9683 patients with thyroid nodules were evaluated in our outpatient clinic: 4986 patients underwent C-FNA, and 4697 were investigated by SG-FNA. FNA was performed using a 20cc syringe in a Cameco syringe holder and a 23 gauge needle. A 10 MHz transducer (ESAOTE Biomedica AU4 IDEA ultrasonographer) was used to determine the size, morphology and localization of nodules. FNA was carried out by the same medical staff and cytologic specimens were reviewed by the same cytologist throughout the entire period. Thyroid nodules investigated by C-FNA showed a prevalent hyperechoic (73%), hypoechoic (9.5%) or mixed echogenic (17.5%) sonographic morphology, with maximal diameter ranging between 0.5 and 8.1 cm (mean 2.2 cm). SG-FNA nodules appeared with a hyperechoic (48.5%), hypoechoic (15.1%) or mixed echogenic (36.4%) sonographic pattern, nodule size ranging between 0.5 and 8.9 cm (mean 2.6 cm). Diagnostic FNA included a clear cytologic picture of either a benign or malignant lesion, while non-diagnostic FNA comprehended the cytologic pattern of follicular neoplasia or inadequate specimens. FNA was diagnostic in 91.3% of C-FNA and in 96.5% of SG-FNA cases, with a 1.6% and 2.1% prevalence of thyroid cancer, respectively. A cytologic pattern of follicular neoplasia was observed in 238 C-FNA cases (5%) and in 272 patients (5.4%) of SG-FNA group. Specimens were inadequate for cytologic diagnosis in 433 cases (8.7%) of C-FNA group but only in 167 SG-FNA patients (3.5%). False-negative results occurred in 9 C-FNA nodules (0.19%), 3 of them having maximal diameter shorter than 1.5 cm. On the contrary, only 3 false-negative diagnoses (0.06%) were obtained among SG-FNA group, 2 of them involving occult carcinomas. The lower incidence of false-negatives when FNA was performed under sonographic guidance, was even more pronounced in view of the higher prevalence of cystic lesions, which present a higher risk of false-negative diagnosis, in SG-FNA compared to C-FNA group (15.1% vs. 9.5%, respectively),. The overall sensitivity of C-FNA and SG-FNA was 93% vs. 100%, and the accuracy 91.3% vs. 96.4%, respectively. SG-FNA had a positive predictive value of 100% and a negative predictive value of 99.9%.

In conclusion, sonography-guided FNA of the thyroid appears as a simple and reliable technique which can greatly improve diagnostic accuracy in the preoperative evaluation of thyroid nodules.

454. P45 **CALCITONIN HORMONE RESERVE IN AUTOIMMUNE THYROIDITIS. L.A. Verbruggen, B. Velkeniers, J.J. Body*, E. Finné, L. Vanhaelst. Dept. of Rheumatology and Endocrinology, AZ-VUB, and *Service de Médecine et Laboratoire d'Investigation clinique, Institut Bordet, ULB, Universities of Brussels (VUB-ULB), Brussels, Belgium.**

Primary hypothyroidism with autoimmune thyroid is the first reported condition in which spontaneous calcitonin (CT) deficiency occurs. Progressive decrease of thyroid hormone reserve has been demonstrated in different stages of autoimmune atrophic thyroiditis (AAT). The present study was conducted to evaluate the CT hormone reserve in patients affected with AAT of different severity grades. Basal CT and CT response (area under the curve(AUC)) to calcium infusion were considered as parameters of CT secretory response. We studied 49 female patients with autoimmune thyroiditis divided in 4 groups on the basis of their basal and peak TSH values after oral TRH, as well as their fT_3 and fT_4 values. All patients had positive thyroid antibodies. The control group comprised age-matched female persons, euthyroid with undetectable antithyroid antibodies. Group 1 (n = 11) had thyroid parameters comparable to normal controls, group 2 (n = 11) had normal basal TSH but significantly increased peak TSH in response to oral TRH, group 3 (n = 14) had increased basal TSH, group 4 (n = 14) were hypothyroid with increased basal TSH and infranormal fT_3 and fT_4. Basal CT was higher in normal women compared to patients with AAT, stage 1, 2, 3 and 4. Basal CT was not significantly different between groups 1, 2, 3 and 4. Stimulated CT levels (as measured by the AUC) were significantly lower in all groups of patients compared to control, with significantly lower stimulated CT values in group 4 compared to the other groups. Basal and postinfusion Ca levels were not different among the various groups. The results of the present study confirm the presence of CT deficiency, as measured by basal and stimulated values in association with AAT. The degree of CT deficiency is more severe in hypothyroid patients, but is already present in the first stages of the disease. Therefore, it is probable that C cells are the sites of either autoimmune destruction, or failure secondary to lymphocytic infiltration rather than the consequence of fibrosis of the gland, characteristic of the end stage of the disease.

455. P46 ACUTE SPONTANEOUS HEMORRHAGIC DEGENERATION OF THE THYROID NODULE WITH SUBACUTE THYROIDITIS-LIKE SYNDROME. T.Mizokami, K.Okamura, T.Hirata*, K.Yamasaki*, K.Sato, H.Ikenoue, and M.Fujishima. Second Department of Internal Medicine, Faculty of Medicine, and *Division of Diagnostic Ultrasound, Department of Radiology, Kyushu University, Fukuoka, Japan.

Subacute thyroiditis-like syndrome (STLS), characterized by a tender thyroid gland, transient thyrotoxicosis, a low thyroidal radioactive iodine uptake (RAIU), and an accelerated erythrocyte sedimentation rate (ESR) has been reported in patients with either acute suppurative thyroiditis, an acute exacerbation of Hashimoto's thyroiditis, primary or metastatic thyroid carcinoma, or thyroid amyloidosis (JCE&M **67**:41,1988). In this paper, the clinical features of STLS were evaluated among patients with a hemorrhagic degeneration of the thyroid nodule.

Seventeen consecutive patients (including 3 men and 14 women, aged 14 -75 years) with a hemorrhagic degeneration of the thyroid nodule were treated at the Second Department of Internal Medicine, Kyushu University Hospital in 1989 and 1990. The thyroid nodules measured 1.8-5.0 cm in the long axis, and were characterized by a fluid volume of greater than at least 50% of the total nodule volume based on the findings of an ultrasound evaluation. Fresh or old hemorrhagic fluid was demonstrated by a fine-needle aspiration in all of the patients. Four patients (2 men and 2 women) demonstrated an abruptly swollen painful thyroid nodule without any history of previous trauma. Anti-thyroid antibodies were detectable in none of these patients. Interestingly, one patient demonstrated high serum levels of thyroid hormones (free T4; 43 pmol/L) and thyroglobulin (Tg; 1250 ng/mL), a suppressed serum TSH level, a low RAIU (1.8 %/24h), and an accelerated ESR (31 mm/h) at 8 days after onset, without any symptoms or signs of infection. Among the other 3 patients examined 5-18 days after onset, the serum free T4 level increased in one patient (26 pmol/L), the serum Tg increased notably in all three (360, 1990 and 18000 ng/mL), the RAIU was low in two (3.1 % and 7.5 %/24h), and the ESR was accelerated in one (28mm/h). In all 4 patients, the serum level of the thyroid hormones and the ESR returned to normal, while the serum level of Tg decreased remarkably within a few months. On the other hand, 13 patients (aged 22-75 years, mean 45±19) with a hemorrhagic cyst of the thyroid had not previously experienced any spontaneous pain before they came to our hospital. In addition, the serum Tg increased in 8 patients (50-290 ng/mL), the ESR was slightly accelerated in 2 (25 and 30 mm/h), and the serum level of the thyroid hormones and the RAIU were all within the normal ranges.

In conclusion, the hemorrhagic degeneration of the thyroid nodule itself may thus occur unnoticed, while an acute expansion of the hemorrhagic cyst volume may be accompanied with transient STLS.

456. P47 **FINE NEEDLE ASPIRATION BIOPSY ON NODULAR THYROID DISEASE. FOUR YEARS OF EXPERIENCE IN A GENERAL TEACHING HOSPITAL.** JJ Castro[1], F Baptista[1], L Lopes[1], R Madureira,[2] E Oliveira,[2] A C Fernandes,[2] A Galvão-Teles.[1] Endocrine Unit[1] and Pathology Department[2] of Sta. Maria Hospital. University of Lisbon.

Several large studies have confirmed the efficacy of fine needle aspiration biopsy (FNAB) in the evaluation of nodular thyroid disease (NTD), although the rate of false-negative results varies between 2% and 8%. In our 1200 bed general teaching hospital we started routine FNAB in 1987. We now report the preliminary results of our experience with the **aim** of assessing the accuracy of FNAB in the diagnosis of NTD. **Methods:** We studied retrospectively the clinical and pathological notes of 87 patients (81 women and 6 men, aged 49.3±12.6 years old) from the Endocrine Clinic with the diagnosis of NTD (solitary nodules, 61%; dominant nodule on multinodular goiters, 32% and nodule on simple goiters, 7%). We included all patients submitted to FNAB since1989 till 1992 because of NTD, who were submitted to surgery or have a follow-up of five years after the first biopsy. Forty-seven patients had results of ultrassonography (US). FNAB were performed on an outpatient basis by a cytopathologist. Cytological specimens were reviewed by any of the two staff cytopathologists. Specimens were categorized as benign, suspicious and malign. **Results:** Nodules were classified as benign (n=44), suspicious (n=35) and malign (n=8).. Apart from mild discomfort at the site of biopsy no complications occurred. Eleven patients with benign FNAB were submitted to surgery and 35 had regular follow-up for 5 years (benign outcome with multiple FNAB). In the group of 35 patients with suspicious FNAB, 32 were submitted to surgery and 3 refused surgery and had regular follow-up. All patients with malign results on FNAB were operated. The outcome is shown on the table:

	Surgery		No surgery
Outcome→	Malign	Benign	Benign
Cytology↓			
Benign	0	9	35
Suspicious	8	24	3
Malign	6	2	-

The results of FNAB were as follows: Sensitivity 100%; specificity 60%; positive predictive value 32.5%, negative predictive value 100% and accuracy 68.6%. Nodule measurements by US (28.3±11 mm) were well correlated with measurements by palpation (27.6±11.2 mm) ($r=0.59; p<0.05$). **Conclusions:** Our results confirm that FNAB is an accurate method for diagnosing NTD. The relatively low specificity found in our study may be explained by the small number of patients or attributed to the criteria in diagnosing suspicious cases. Routine US of the thyroid doesn't seem to add any important information to clinical examination on the evaluation of palpable nodules.

457. P48 DEVELOPMENT OF A SOFT TISSUE ABSCESS IN THE NECK : A COMPLICATION OF FINE NEEDLE ASPIRATION OF THE THYROID IN A BACTEREMIC PATIENT. D.H. Sarne University of Illinois at Chicago Medical Center Chicago, Illinois

Fine needle aspiration (FNA) is the diagnostic method of choice for the evaluation of thyroid nodules and may also be used in the evaluation of goiters. It is usually performed without any significant complications. This report describes a patient who developed a soft tissue abscess in the neck following FNA of the thyroid gland. **CASE HISTORY** A 33 year old black male with AIDS and a history of staphylococcal osteomyelitis presented with fever, tachycardia and an enlarged, tender goiter. Blood cultures were drawn and he underwent FNA of the thyroid. He was thyrototoxic with T4 = 46.5 ug/dl, T3 = 576 ng/dl and his TSH was < 0.1 uU/ml. FNA revealed groups of follicular cells and inflammatory cells consistent with sub-acute thyroiditis. He was treated with Propranolol and Prednisone with marked improvement of his neck pain within 24 hrs. His blood cultures were positive for staphylococcus aureus and he was treated with cephalexin. His steroids were rapidly tapered and his antibiotics were later discontinued after a month of therapy. Within a week, he developed a large painful red swelling of the anterior neck. An aspirate of the lesion returned gross pus with numerous polymorphonuclear cells and coccoid bacteria. A culture of the aspirate grew staphylococcus aureus. CT imaging of the neck revealed the lesion to be a large, soft tissue abscess. The large abscess cavity was incised and drained and he was treated with a second course of antibiotics without recurrence. While FNA of the thyroid is usually without significant complications, this immuno-compromised patient developed a neck abscess when an FNA was performed at a time when he was also bacteremic. Development of a local abscess is a possible complication of FNA especially in patients who are bacteremic.

458. P49 **Subclinical autoimmune thyroiditis as a determinant for rapid cycling of bipolar psychosis**

H.A. Drexhage[1], H.A.P.C. Oomen[1,2], A.J.M. Schipperijn[2]
[1]Dept. of Immunology, Erasmus University, Rotterdam, [2]Langeveld Center for psychiatry, Noordwijk, The Netherlands

Objective: to determine: I. The prevalence of (subclinical) thyroid abnormalities in a psychiatric population, II. Whether (subclinical) thyroid abnormalities are associated with the occurrence of affective disorders, most notably with rapid cycling of psychosis (DSM3R).
Design: for objective I serum was collected 2-3 weeks after hospitalization of 3756 psychiatric patients. Sera were stored and later assayed for the presence of TPO-antibodies and for abnormal TSH levels. Data were compared to data obtained in a healthy control group (n=1877).
For objective II the prevalence study was followed by a case-control study in which three groups of cases (Group I = patients with TSH levels <0.4 mU/L 2-3 weeks after hospitalization, n=45; Group II = patients with TSH levels >4.0 mU/L, n=39; Group III = TPO-antibody positive patients at hospitalization, n=32) and one controlgroup (patients lacking such thyroid parameter abnormalities, n=83) were randomly formed out of the patients of one of the clinics. Cases and controls were studied for the presence and/or later development of affective disorders (mainly rapid cycling of psychosis). Outcomes were corrected for lithiumusage prior to serumcollection.
Results: the percentage of patients positive for TPO-Abs was 10.0% (331/3316); the TPO-antibody data were dependent on the age and sex of the patients (older>younger; females>males). TSH abnormalities were found in 10.0% (332/3316) of the patients, e.g. 5,9% (198/3316) with TSH levels and 4.1% (134/3316) with raised TSH levels. Abnormalities in serum thyroxine-levels were only found in 9.8% of patients with TSH abnormalities. In comparison with the controlgroup of healthy individuals, the prevalences of "positive TPO-antibodies" and abnormal TSH levels were the same.
The case-control study showed that affective disorders were found/developed equally frequent in cases and controls. However the character of the affective disorder differed between cases and controls: rapid cycling of psychosis was more frequently found/developed more frequently in patients with an affective disorder and TPO-antibodies (also in the absence of a raised serum TSH). Correction for prior lithiumusage had no effect on this outcome.
Prior lithiumusage was particularly evident in the group with a raised serum TSH. Although there was an association between raised serum TSH and rapid cycling of psychosis, this association was lost after correction for prior lithium usage.
Conclusion: Subclinical forms of autoimmune thyroiditis (characterized by TPO-antibodies with or without a raised serum level of TSH) change the character of an affective disorder, e.g. the condition induces a shift from mania and vital depression towards rapidly cycling of psychosis.
Raised serum levels of TSH in the absence of TPO-antibodies are also frequently found in rapid cyclers, however this abnormality is mainly due to the thyroid intoxicating effects of lithiumtherapy often given to such patients.

459. P50 THE VALUE OF ASPIRATION NEEDLE BIOPSY IN PREOPERATIVE EVALUATION OF THYROID NODULES. A. Carpi, E. Ferrari, °G. Di Coscio, Departments of Internal Medicine and °Pathology. University of Pisa, Pisa, Italy.

From 1980 to 1992, 5,403 consecutive euthyroid patients with thyroid nodule(73% with single and 27% with multiple nodules) were examined by FNA cytology for preoperative selection.1,668 of these patients(31%)had nodules also suitable for evaluation by large needle biopsy histology(Aspiration Needle Biopsy,ANB).No significant complications occurred following ANB.The proportion of inadequate specimens was 22% for ANB and 11% for FNA,however a definite diagnosis was obtained with ANB in 88 patients with inadequate FNA finding.Diagnostic sensitivity was higher for FNA than for ANB(93% vs. 64%)whereas specificity was better for ANB diagnoses(82% vs. 57%).Nonetheless ANB contributed to the increase of overall sensitivity as four of all the malignant nodules diagnosed as benign by FNA were correctly identified by ANB.Analysis of the postoperative results of 102 nodules with FNA and ANB finding of benign nodule or of suspected cancer showed that the addition to the same FNA finding(benign nodule or suspected cancer) of a different ANB diagnosis(suspected cancer or benign nodule)greatly changed the probability of finding a malignant nodule at postoperative histology(from 6% to 32%,p<0.05).ANB was also useful in showing a macrofollicular component in 47% of 245 nodules diagnosed by FNA as 'pure microfollicular nodules'.This differentiation,by ANB,between true microfollicular neoplasms and hyperplastic ones was used to reduce the number of surgical excisions.These data show that ANB cannot replace FNA for preoperative selection of thyroid nodules because most palpable nodules are not suitable for ANB examination,the number of inadequate samples is rather high and ANB sensitivity is relatively low;however these data indicate that the addition of ANB to FNA greatly increases the overall accuracy as the two techniques are complementary.The principal benefit of ANB seems to derive from a better diagnosis of the benign nodules,particularly of the microfollicular nodule.

460. P51 **ALTERATIONS IN THYROID FUNCTION DURING AND AFTER CARDIOPULMONARY BYPASS IN PEDIATRIC PATIENTS: CLINICAL IMPLICATIONS** B. Murzi, G. Iervasi, R. Moschetti, V. Vanini, S. Berti, A. Clerico, L. Salutini, S. Masini, and A. Biagini CNR Institute of Clinical Physiology, Pisa, Italy

Cardiopulmonary bypass (CPB) has been considered responsible for causing the changes in thyroid hormone metabolism in patients (pts) undergoing open heart surgical procedures. Because of its similarity to the euthyroid sick syndrome the replacement of T3 has suggested as a possible new way to treat the low cardiac ouput after CPB. So far investigations have mainly been restricted to adult pts. The purpose of this study was to assess whether CPB affects thyroid function in children during CPB and to evaluate the duration of the phenomenon. The concentrations of thyroid hormones were measured in 14 pediatric pts before, during, and after CPB. The ages of the pts ranged between 18 months and 14 years. Pts were kept normothermic, or moderate or deep hypothermia was induced depending on the specific pathologic condition involved. Basal values of TT4 (8.7±2.0 μg/dL), TT3 (164±39 ng/dL), FT4 (13.6±1.8 pg/mL), FT3 (5.7±1.3 pg/mL), and TSH (2.9±1.0 μU/mL) were all in the normal range. A marked reduction in the levels of TT3, TT4, FT3, and TSH, and in the ratio of FT3 to FT4 was detected during the time frame of the study. The minimun levels of each hormone (TT4= 72%, TT3= 40%, FT4= 77%, FT3= 39% of the baseline values) were reached between 12 and 48 hours after CPB, indicating that changes in thyroid function and in the conversion of T4 to T3 are triggered by CPB and represent specific phenomena, and that these changes are progressively exacerbated during the post-operative period. The TSH level was markedly reduced versus its baseline values 48 hours after operation (24%, $p<0.01$), despite low levels of both total (40%, $p<0.01$) and free (39%, $p< 0.01$) T3: it tended to return to its preoperative level by the third postoperative day, but both the total (75%,$p< 0.01$%) and free (74%, $p<0.01$) T3 levels remained below their baseline values for 7 days postoperatively. Neither hemodilution nor hypothermia was responsible for the alteration observed. We conclude that pediatric pts undergoing CPB manifest changes in hormone metabolism similar to those seen in adult pts. These changes increase progressively during the postoperative period, and are still present 7 days postoperatively. The exact mechanism responsible for causing these changes is not thoroughly understood. Our data suggest that T3 replacement therapy may be beneficial in pediatric pts undergoing open heart surgical procedures.

461. P52 **THE EVOLUTION AND SIGNIFICANCE OF VENTRICULAR LATE POTENTIALS PARAMETERS DURING THYROID REPLACEMENT TREATMENT IN HYPOTHYROIDISM.** **S.A.Kontoyannis, D.A.Kontoyannis, G.D.Piperingos, A.Kalabalikis, P.Konaxi, and D.A.Koutras. Athens University, Department of Clinical Therapeutics, ALEXANDRA General Hospital, Athens, Greece.**

We have previously reported the high incidence of ventricular late potentials (LP) in hypothyroidism (HPO). The aim of this study was to examine the possible evolution of these LP during thyroxine replacement therapy and their relation to thyroid state. The study population consisted of 46 patients with documented HPO. The signal-averaged electrocardiograms (SAECG) were obtained before thyroid replacement therapy, every two weeks for the next two months, and finally while euthyroid 6 months after starting treatment. On SAECGs automated measurements were provided for QRS duration (QRSD), low amplitude signals (LAS) and root mean square (RMS). The LP defined as QRSD >114 ms, LAS >38 ms and RMS <20 μV. The results as $\bar{x} \pm$ SD are as below:

TIME	QRS (ms)	LAS (ms)	RMS (μV)
before - 0 weeks	97.9 ± 11	32.7 ± 9.7	33.2 ± 17.2
8th week	96.1 ± 12	29.8 ± 7.9*	38 ± 20
>25th week	93.4 ± 13	27.8 ± 6.6**	44 ± 20**
* = $p<0.05$	** = $p<0.01$	vs values before treatment	

The values of LAS and RMS improved in paralled to the thyroid state, but the improvement of LAS was earlier and of higher significance. LP were present in 7/46 before and disappeared in all but one patient. In conclusion, given the association between arrhythmiogenic propensity and ventricular late potentials, the disappearance LP decreases the likelihood of arrhythmia. It should be noted that the single patient who died during this treatment, had LP before death.

462. P53 A NOVEL 4-BASE PAIR DELETION MUTATION OF Gsα GENE IN A JAPANESE PATIENT WITH PSEUDOHYPOPARATHYROIDISM. M. Yokoyama[1)], K. Takeda [1)2)], K. Iyota[1)], M. Sasaki [2)], K. Hashimoto[1)], 2nd Dep. of Internal Medicine[1)] and Clinical Laboratory Medicine[2)], Kochi Medical School, Nankoku, Japan.

Mutations in the guanine nucleotide binding protein α subunit (Gsα) are associated with either constitutive activation or loss of function in human disease. The generalized deficiency of Gsα in patients with Albright's hereditary osteodystrophy is associated with reduced responsiveness of many tissues to hormones and neurotransmitters that act by stimulating adenylyl cyclase. We have screened the Gsα gene for mutations in three unrelated Japanese patients with pseudohypoparathyroidism (PHP). Two of them have resistance to both PTH and TSH. Exons 2-13 of the Gsα gene were amplified in vitro using specific primers and screened for mutations by denaturing gradient gel electrophoresis (DGGE). The DGGE analysis of PCR products from co-amplification of exons 7 and 8 revealed two additional bands in one patient who had reduced responsiveness to PTH, TSH and ADH. We identified a novel 4 base pair deletion at exon 7 in codons 189-190. This deletion causes a frameshift and the synthesis of a truncated form of Gsα of 201 rather than 394 amino acids, which is very likely to be biologically inactive. The patient was heterozygous for this deletion. The patient's mother had the same mutation and although her serum calcium, PTH, thyroid hormone and TSH levels were normal, she had subcutaneus and renal calcifications. The unaffected brother and the other two PHP patients did not have this mutation. Thus, this mutation appears to be necessary but not sufficient to produce the complete PHP phenotype. Thus, other factors, either genetic or acquired, may be necessary for the full syndrome to occur.

463. P54 DIRECT DETERMINATION OF FREE TRIIODOTHYRONINE (T_3) IN UNDILUTED SERUM BY EQUILIBRIUM DIALYSIS / RADIOIMMUNOASSAY (RIA)

PHILIP TAING, LANCE MIKUS, INDER J. CHOPRA; DEPARTMENT OF MEDICINE; UCLA CENTER FOR HEALTH SCIENCES; LOS ANGELES, CA 90024

We have devised a practical, sensitive, reliable and reproducible assay for measurement of free T_3 concentration in serum. The assay employs a convenient and disposable plastic equilibrium dialysis cell and a buffer that resembles the in vivo biochemical environment (Nelson J.C., R.T., Tomei, Clin Chem 34:1737, 1988). A 200 µl aliquot of serum was dialyzed against 2.4 ml buffer at 37 C for 18 ± 2 h and T_3 was quantified by RIA of 800 µl aliquot of the dialysate buffer. The detection threshold of the RIA approximated 2-3 pg/ml permitting accurate measurement of >300pg/dl of free T_3 directly. Serum specimens that may contain less free T_3 were spiked with 200 ng/dl of nonradioactive T_3 prior to dialysis. Free T_3 in the dialysate of these samples was divided by total T_3 in serum(after spiking) to determine % free T_3. Free T_3 was calculated by multiplying % free T_3 and serum total T_3 (before spiking). Free T_3 concentration (pg/dl) did not differ appreciably in two serum pools when tested both with and without spiking with exogenous T_3. The between assay coefficient of variation of two specimens tested over a two month period approximated 19%. Serum free T_3 concentration was [mean ± SD(n), range, p] [585 ±149 (39), 309-880] in normal subjects. It was significantly increased [1484 ± 626 (13), 1050-3400, $p<0.001$) in hyperthyroidism, and decreased in nonthyroidal illness [NTI, 276 ± 154(n=9), 107-640, $p<0.001$], cord blood serum [247 ± 51 (n=11), 184-361] and pregnancy [427 ± 144(8), 185-705, $p<0.02$]. Serum free T_3 concentration varied widely in hypothyroidism [547± 579(10), 20 - 1846, NS). Conclusions: We have described a practical method and initial results of direct measurements of free T_3 concentration in health and disease.

464. P55 THYROID HORMONES DO NOT ONLY REGULATE CHOLESTEROL, BUT ALSO TRIGLYCERIDE METABOLISM. H. Engler, W.F. Riesen, Institute for Clinical Chemistry and Hematology, Kantonsspital, CH-9000 St.Gallen, Switzerland

The effect of tyroid hormones on cholesterol metabolism is well studied. The thyroid hormones are affecting the mRNA synthesis for the LDL receptor. This leads to an increased number and activity of the LDL receptor in hyperthyroidism. The effect of thyroid hormones on triglycerides is less well understood. A similar stimulatory effect of thyroid hormones on synthesis and activities of the lipolytic enzymes would cause a decrease in triglyceride-rich lipoproteins and decreased levels of the protein moieties of these lipoproteins in hyperthyroidism. To our knowledge, no data concerning alterations in apolipoprotein C-II, C-III, and E levels during thyroid dysfunctions are published. We measured cholesterol, triglyceride, LDL-, HDL-cholesterol and apolipoprotein A-I, A-II, B, C-II, C-III, and E in 46 hyperthyroid patients (FT4: 39.8±11.5 pmol/l; TSH < 0.07 mU/l), in 36 hypothyroid patient (FT4: 7.1±4.2 pmol/l; TSH 40.5-100 mU/l), and in 43 euthyroid controls (FT4: 16.3±4.0 pmol/l; TSH 0.5-2.8 mU/l). The results are summarized in the following table:

Group	Cholest. mmol/l	Triglyc. mmol/l	LDL-C mmol/l	HDL-C mmol/l	apo B g/l	apo C-II mg/l	apo C-III mg/l	apo E mg/l
hyperthyr.	4.6±1.1	1.4±0.5	2.8±0.9	1.4±0.8	0.95±0.24	28.2±11.9	74.2±22.8	26.9±6.6
euthyr.	6.1±1.1	1.6±0.7	3.9±1.1	1.5±0.5	1.25±0.30	34.5±12.9	94.3±27.5	32.2±14.5
hypothyr.	7.6±2.2	1.9±1.0	4.8±2.2	1.8±1.5	1.49±0.56	38.0±21.4	107.8±49.5	30.1±12.2
statistics	p<0.0001	p<0.005	p<0.0001	n.s.	p<0.0001	p<0.02	p<0.0001	n.s.

Our data demonstrate an effect of thyroid hormones on triglyceride and apolipoprotein C-II and C-III levels. We observed significantly higher levels of apo C-II and particularly of apo C-III in hypothyroidism compared to those in the euthyroid or hyperthyroid states. As these two proteins are regulators of the lipoprotein lipase (C-II as activator, C-III as inhibitor), these changes, which are associated with the thyroid function, might indicate an effect of the thyroid hormones on the activity of the lipoprotein lipase. No significant differences were observed between the apo E and HDL-C levels in hyper- and hypothyroidism. However, the mean HDL-C levels were clearly higher in hypothyroidism and the apo E levels lower in hyperthyroidism compared to the euthyroid state. The parallel changes of apo B and apo E might reflect the increased activity of the LDL (apoB/E) receptor. Our data suggest that thyroid hormones regulate not only the cholesterol metabolism by their effects on apoB and the LDL (apoB/E) receptor, but also the triglyceride metabolism through their effects on the lipolytic enzymes and/or their regulators.

465. P56 **THYROID DYSFUNCTIONS AFTER ALLOGENIC BONE MARROW TRANSPLANTATION WITHOUT THE USE OF PREPARATIVE TOTAL BODY IRRADIATION. M.E. Toubert[1], G. Socié[2], S. Aractingi[3], A. Devergie[2], P. Ribaud[2], H. Bourdeau[2], N. Parquet[2], E. Gluckman[2], P. Vexiau[4]. Services de Médecine Nucléaire[1], de Greffe de Moëlle[2], de Dermatologie[3], d'Endocrinologie[4] Hôpital SAINT-LOUIS, 1, av Claude Vellefaux, 75010 - Paris, France.**

Following allogenic Bone Marrow Transplantation (BMT), thyroid abnormalities have been described, including peripheral hypothyroidism (PHT) and Graves's disease and, more recently, we described the incidence and poor prognosis value of the euthyroid sick syndrome* (ETS). These abnormalities have been mostly linked to the use of preparative Total Body Irradiation (TBI). In this study, we prospectively followed 77 patients (pts) who underwent BMT without the use of preparative TBI. Primary diagnosis included Chronic Myeloid Leukemia [#26], Acute Myeloid Leukemia [#19], non-malignant diseases [#=22 : aplastic anaemia in 18 cases and ß Thallassemia in 4] and other malignant diseases [#10]. All but 6 patients were grafted from an HLA-identical sibling donor. Conditioning regimens consisted in the association of busulfan and cyclophosphamide (Cy)[#=60] or alkeran [#=9] or Cy plus Anti-Thymocyte Globulin [#=8]. FT3, FT4 and US TSH was schedulded to be mesured before, 3 months and 12 months after BMT. As in our previous study, pts were classified in 3 categories, ie : normal, PHT (US TSH>4 mIU/l) and ETS*(low FT3 [<3.6 pmol/l] or low FT4 [<8.5 pmol/l]). Before BMT, thyroid dysfunction was diagnosed in 15 patients [PHT, 9; ETS, 6 pts]. At 3 months, 7 pts were no evaluable (early death).; among evaluable pts[#=70], 41 (59%) had thyroid dysfunction [PHT, 6; ETS, 35 pts]. At 12 monts, 35 pts were evaluated (42 pts died), 12 had thyroid dysfunction [PHT, 4; ETS, 8 pts]. Of interest, we confirm in this analysis the poor prognosis of pts developing an ETS, since 36 months projected survival is 94% for normal profil, 80% for PHT, and 30% for ETS (<0.001). Finally, PHT was diagnosed in 6 pts post-transplant who had not received any kind of therapeutical irradiation before transplant. This underlines the fact that beside radiotherapy, other factors lead to the development of peripheral hypothyroidism after allogenic BMT.

***Br. J. Haematol, 1993, 85, 778-82**

466. P57 LEAN BODY MASS AS A DETERMINANT OF THYROID VOLUME IN HEALTHY ADULTS IN A NON-IODINE DEFICIENT AREA

MFT Wesche, NJ Smits. Academic Medical Center, Depts. of Endocrinology and Radiodiagnostics, University of Amsterdam, Amsterdam, the Netherlands

Thyroid volume of males is larger than of females. The sex difference in thyroid volume is thought to be related to the difference in body weight, because previous studies of Hegedüs et al. and Berghout et al. showed no sex difference in the ratio of thyroid volume to body weight. In view of the different body composition of men and women, the aim of our study was to investigate if lean body mass was a better determinant of thyroid volume than body weight.
Methods: 44 healthy volunteers (21 males, 23 females, with equal distribution of sexes in age groups between 20 and 70 years) were studied. All subjects lived in the non-iodine deficient area of Amsterdam and had a body mass index lower than 30; none used medicines and all had normal thyroid function tests. Thyroid volume (TV) was measured by ultrasonography, and lean body mass (LBM) with a body impedance analyzer (BIA 109 Akern, Florence). Student's t-test was used to evaluate differences between two group means. Single linear regression analysis was applied for the correlation of parameters. **Results** are given in the table (values as mean ± SD).

	all	males	females	p
TV (ml)	8.5 ±3.6	10.3 ±3.4	6.9 ±2.9	0.0009
BW (kg)	70.9 ±10.6	76.6 ±10 .6	65.7 ±7.5	0.0003
LBM (kg)	52.8 ±10.4	60.9 ±8.7	45.4 ±4.6	<0.0001
TV/BW (ml/kg)	0.120 ±0.046	0.135 ±0.04	0.106 ±0.046	0.034
TV/LBM (ml/kg)	0.160 ±0.058	0.169 ±0.051	0.152 ±0.063	n.s.

The TV/BW ratio differed between males and females, but no sex difference was observed in the TV/LBM ratio. TV was directly related to BW (r=0.42, p=0.005); the correlation between TV and LBM was stronger (r=0.55, p=0.0001).
Conclusion: in healthy adults lean body mass rather than body weight appears to be a major determinant of thyroid volume.

467. P58 ULTRASONOGRAPHIC VERSUS SCINTIGRAPHIC MEASUREMENT OF THYROID VOLUME IN THYROID PATIENTS

MMC Tiel-van Buul, MFT Wesche, NJ Smits, WM Wiersinga. Academic Medical Center, Depts. of Nuclear Medicine, Endocrinology and Radiodiagnostics, University of Amsterdam, The Netherlands.

In the treatment of thyroid disease with radioactive iodine, the dose of I-131 is in general calculated from the thyroid gland weight and the 24-hour radioiodine uptake. The scintigraphic volume is usually taken as an estimate of thyroid weight. The aim of the study was to compare ultrasonographic and scintigraphic measure-ments of thyroid volume in patients referred for I-131 treatment.
Methods: Included were 40 patients referred for I-131 treatment because of Graves' hyperthyroidism (GH, n=20), toxic multinodular goiter (TMG, n=10) or sporadic nontoxic goiter (SNG, n=10). Excluded were patients with retrosternal goiter. Ultrasonographic volume was determined by obtaining transverse thyroid scans at 5-mm intervals; the sum of all volumes between two successive scans equals the total thyroid volume. Scintigraphic volume of each lobe was calculated by the formula: ¶/6 x height x width x depth, using anterior and lateral views. Both imaging techniques were obtained within one day. Statistical differences between groups were analyzed using the Mann-Whitney U test.
Results: Thyroid volumes (TV) are given in the table.

	ultrasonographic TV (ml)		scintigraphic TV (ml)		p	difference (%)	
	median	range	median	range		median	range
GH	18	11-46	21	10-42	0.9	-1	-47 to +122
TMG	50	14-74	29	13-45	0.089	-43	-60 to +65
SNG	61	22-198	26	11-79	0.045	-52	-77 to -14

Ultrasonographic TV was in GH lower than in TMG (p=0.009) and in SNG (p=0.003). Scintigraphic TV was not significantly different in the three patient groups.
Conclusions: 1. Thyroid volume measured by scintigraphy is underestimated with a factor 2 in SNG.
2. Thyroid volume by scintigraphy usually equals that by ultrasonography in GH. These findings are relevant for the dosimetry of I-131 in thyroid disease.

468. P59 Changes of thyroid function by prophylactic application of thyrostatic drugs in patients with autonomy during iodine contamination. M. Hüfner, R. Müller, W. Nolte, D. Emrich*, Dept. of Intern. Med. and Nuclear Medicine*, University of Göttingen, Germany

Thyroid autonomy is frequent in endemic goiter areas. Patients with advanced autonomy are considered to be at risk for developing thyrotoxicosis after iodine contamination. We have performed a placebo controlled study to investigate whether perchlorate or methimazole have some protective effect when given prophylactically starting before iodine contamination. Patients who had to undergo coronary angiography (CA) were screened for TSH. In patients TSH < 0,3 mU/l a TRH test, fT3 I/fT4 I, techn. uptake and urinary iodine excretion was measured. Patients with Δ TSH < 2,5 mU/l (moderate degree of autonomy), normal fT3I and fT4I, 10 min techn. uptake > 1,2 % and normal iodine excretion were randomized into three groups: I. placebo, II. 900 mg perchlorate/d, III. 20 mg methimazole/d. Treatment was started 12 hours before CA and continued for 14 days. 30 days after CA the thyroid investigation was repeated. 51 patients were included out of more than 1200 screened. 4 mild, transient cases of hyperthyroidism (elevated fT3I and/or fT4I) were observed, two with clinical symptoms: 1 case in group II and III each (one with clinical symptoms), 2 cases in group I (one with clinical symptoms). Techn. uptake decreased by 50 % after 30 days in group I, no significant change was observed in group II + III. Urinary iodine excretion increased significantly in group I (from 57 to 115 μg/g creat.) but was essentially unchanged in group II + III. We conclude that patients with moderate degree of thyroid autonomy (low basal TSH, normal fT3I/fT4I) are at low risk to develope clinical relevant thyrotoxicosis after CA. Prophylactic application of 20 mg methimazole or 900 mg perchlorate for 14 days can not prevent mild hyperthyroidism in some cases. However, changes of techn. uptake and urinary iodine excretion indicate some protection of the thyroid against excess iodine incorporation.

469. P60 HYPOTHYROIDISM WITH LONG-STANDING GOITER AND RESISTANCE TO REPLACEMENT THERAPY. M.-F. Langlois and D. Bellabarba, Service d'endocrinologie, Centre Hospitalier Universitaire, Sherbrooke, Québec, CANADA.

A 63-year old female patient was referred for a preoperative evaluation of a pituitary tumor. She had been seen in our clinic in 1973 for a slight, firm goiter and symptoms of hyperthyroidism. Serum T4 and T3 were normal, thyroid antibodies negative. No definite diagnosis was made at the time. In 1979 she consulted again for symptoms suggesting hypothyroidism. The goiter was unchanged. T4 was 140 nmol/L (N: 60-170), T3 1.75 nmol/L (N: 1.3-3), TSH 3.6 mUi/L (N:1.5-6.5). Follow-up was suggested. She returned in 1984 complaining of cold intolerance, fatigue, dullness. The goiter had increased in size and she had a slight palpebral edema, dry skin and slow relaxation of Achilles tendon reflex. T4 was 69.5 nmol/L, TSH 34.4 mUi/L and positive (1/1, 600) TPO antibodies. Autoimmune thyroiditis with hypothyroidism was diagnosed and she was started on L-T4 0.1 mg daily. In 1985 and 1986 she had improved on medication taken regularly, but was complaining of fatigue and of nervousness and dysphagia. Goiter was unchanged. TSH had decreased, but remained at the upper limits of normal (6.7-8 mUi/L), whereas T4 was at the upper limits of normal or frankly elevated (160-194 nmol/L). Thereafter she was followed by her family physician and in the ensuing years the TSH increased to 18-26 mUi/L (N: 0.3-6 mUi/L) with T4 at 170-195 nmol/L. These values did not change during 1990-1993, while L-T4 was increased from 0.1 to 0.15 mg daily. On the highest dose she complained of slight symptoms of hyperthyroidism. In 1993 a thorough investigation, off thyroid therapy, revealed a TSH of 23.7 mUi/L (N: 0.3-3.8), T4 170 nmol/L and a ^{131}I thyroid uptake of 52%. A CAT scan of the pituitary showed a large tumor invading the cavernous sinus and surrounding the right internal carotid artery. There was a suprasellar extension reaching the sphenoidal sinus. Preoperative work-up showed a TSH of 40 to 51 mUi/L and T4 at 99 nmol/L. TSH levels did not change after TRH. TPO antibodies were still positive at 1/1, 600. We also found increased prolactine at 54 μg/L, low plasma cortisol of 155 nmol/L AM and very low FSH-LH, which did not rise after LHRH. Histology of the tumor showed a chromophobe adenoma with positive immunostaining for TSH. After surgery TSH promptly decreased to 2.4 mUi/L and the free T4 to 9.1 pmol/L (N: 10-27). The clinical and biological observations infer that: 1) this patient had an autoimmune thyroiditis with hypothyroidism several years before the development of a TSH producing adenoma, and 2) the first disease could have contributed to development of the second. Furthermore the presence of these two diseases could explain why she did not develop frank hyperthyroidism, a situation that delayed the diagnosis of the tumor.

470. P61 COMPUTER SYSTEM FOR SEMIAUTOMATIC EVALUATION OF RELATIVE VOLUMES OF HISTOLOGICAL COMPARTMENTS IN THE THYROID GLAND. M. Klencki, D. Słowińska-Klencka*, A.Lewiński, Department of Thyroidology and *Department of Experimental Endocrinology and Hormone Diagnostics, Institute of Endocrinology, the University School of Medicine at Łódź, Poland.

The measurement of the ratio of volumes of the epithelium, colloid and stroma (connective tissue and blood vessels) is one of the methods frequently used in the morphometric analysis of the thyroid gland. The changes in the relative volumes of the above mentioned histological compartments in the thyroid closely follow the changes in the functional state of that gland. The common approach in practical estimation of these relative volumes is based on the technique named "point-counting". Since the methods of maeasurements were very laborious and time-consuming, we decided to develop a computer system called by us "Compartments" for the quantitative analysis of the thyroid histological structures, The "Compartments" system consists of a Windows 3.1 application, running on an AT personal computer with a 80386 or 80486 microprocessor. A very important part of the hardware is an image acquisition card, which enables digitisation and transmission of a 24-bit image from the microscope via a TV camera to computer memory. We used a PC TVC4512-8 card with 1MB RAM (Logitex, Łódź - Poland) and a WV-CL352E CCD colour camera (Panasonic, Japan) but any other equipment with similar parameters would be satisfactory. The applied method of measurements, based on the „point-counting" technique, requires the recognition of the compartments, corresponding to the selected points of image. The neural network was employed to facilitate the process of classification of the examined points. The error-free compartment recognition was not achieved because of some overlapping of the pattern classes. Nevertheless, the semi-automation of assigning the examined points to histological compartments makes the measurements less laborious and time-consuming. The system was applied for examination of thyroid slides stained with hematoxylin and eosin, but it can be used for slides stained with any other technique. There is also a possibility of performing measurements based on „point-counting" technique with use of our system on other organs and tissues.

471. P62 OBSTACLES TO REDUCED LENGTH-OF-STAY FOLLOWING THYROIDECTOMY. F.D. Moore, Jr., Brigham & Women's Hospital, Harvard Medical School, Boston, MA.

A systematic approach to morbidity has been used to reduce hospitalization after thyroid surgery safely. Initially, routine calcium supplements were studied, reducing symptomatic hypocalcemia to <1% and allowing safe discharge after a two night stay. Patients with a serum calcium ≥8.0 mg/dL 18 hours after thyroid surgery exhibited no further decline and no symptomatic hypocalcemia. A program of one night hospital stay was started on 1/1/94 with these calcium levels as a criteria for discharge. This study reports results of this attempt, comparing consecutive patients from 1993, with an expected two night length of stay, to an equal number of consecutive patients from 1994. Patients undergoing urgent thyroid surgery were excluded from analysis.

		Nights in Hospital (mean)	
		1993	1994
Bilateral Thyroidectomy	n=40	2.15	1.45
Thyroid Lobectomy	n=6	1.00	1.17

Little variation in case mix was noted between 1993 and 1994. Significant obstacles to the overnight stay were encountered when it was reduced to 24 hours:

Obstacle to projected discharge	1993(two night stay)	1994(one night stay)
Major co-morbid illness	5	4
Postoperative Morbidity	2	11
Asymptomatic hypocalcemia	1	4
Pain	1	2
Systemic malaise	0	2
Other	0	3
Transportation	0	2
Patient refusal	0	1

These results suggest that further length of stay reduction can be achieved only with more effective prevention of early hypocalcemia and reduction of the after-effects of general anesthesia.

472. P63 SURGERY FOR ALL: IS IT WISE TO DO MAJOR SURGERY IN REMOTE AFRICA? T.S.Harrison, D. Downing, J.F. Seaton, J. Teeter, M. Teeter, A.O. Longombe, P. Bourdoux, D.G. Koivunen, and G.W. Geelhoed, Pennsylvania State University, Hershey Medical Center, Hershey, Pennsylvania and University of Brussels, Belgium.

In today's world, surgically approachable Iodine Deficient Goiter is seen most often in remote places. Will Lipiodol remove the need for surgery? With encouragement from the ICIDD, we offer experiences with a previously untreated, by surgery or Iodine, severely Iodine deficient tribe in North Central Zaire. Over 400 patients received intramuscular Lipiodol; 213 were followed as longitudinally as possible for at least 6 years. Clinical; including goiter circumference, measured planimetrically from outlines inked directly on tissue paper; serum T-4 and TSH, and Iodine and Thiocyanate excretion were analyzed statistically. Most patients are frail, anemic, dry and not well nourished. After Lipiodol, in 215 patients, 194 thyroids shrank quickly: at 6 months post Lipiodol, thyroids remained small $P<.001$, $n = 86$. At 3 years, T4 levels were elevated and TSH suppressed, $P<.001$ for both. Iodine excretion remained high, $P<.001$. Moderate urinary thiocyanate was always present. Data were comparable at 6 years after 2 Lipiodol injections. Shrinking thyroids regrew to original size in 3 and/or at 6 years in 50 patients. At 3 and 6 years post Lipiodol; T4, TSH, and I excretion differed significantly compared to 50 untreated subjects ($P<.02$-$.001$). Thirty eight patients were operated on; mostly with local and cervical block. Indications: impending airway obstruction 10, recurrent laryngeal nerve paralysis 2, massive glands not regressing after Lipiodol 21, chronic draining sinuses 2, cosmetic dissatisfaction 3. In 6 Iodine deficient patients with no Iodine supplement pre-op, surgically exposed glands wept plasma copiously. All required massive I.V. fluid for 12 hours and then were normal. Oral Lugol's preoperatively, at least 4 days, made a dramatic difference; in Grave's Disease doses, 5 drops thrice daily in 50 ml water, glands up to 1.8 k'g handled easily; there was no weeping fluid, anatomical details stood out easily. Resections of any size were easy. Preop Lugol's in intense Iodine deficient goiter needs more emphasis. Surgical Complications; 2: Both easily managed. No wound infections. Five deaths; 3 Surgical; Postop airway obstruction in a Cretin; probable pulmonary embolus 5 days post op; sudden collapse 8 days Postop ? Cause: Two medical deaths: Suffocation from massive goiter 3-1/2 years post Lipiodol and uncontrolled thyroid storm, the latter 2 years after Lipiodol. We conclude: 1) Most glands shrink after Lipiodol, but regrow gradually, maintaining thyroxine production; 2) Surgery is probably worthwhile; patient selection needs refinement; 3) Surgery must be part of an overall Iodine Deficiency Control Program; and 4) At least 4 days pre-op Lugol's is imperative.

473. P64 A CASE OF THYROID Histiocytosis X WITH SEVERE IMMUNODEFICIENCY. Shinji Kitahama, Makoto Iitaka, Jun Ishii, Saburo Murakami, Yoshihiko Simizu. Saitama Medical School,Saitama, Japan.

Adult type Histiocytosis X with thyroid involvement is extremely rare in the literature. Usually the clinical course of Histiocytosis X is mild and self limiting. We encountered a 60 year-old man who complained a progressively increasing large goiter and polyuria. Laboratory data showed subclinical hypothyroidism,liver dysfunction,and severe immunodefficiency. (CD4 $94/mm^3$: CD8 $70/mm^3$) Antibodies to HIV was negative. The water restiction test and pitressin loading test revealed the presence of central diabetes inspidus.We temporarily made a diagnose of an anaplastic cancer or a malignant lymphoma from the clinical course.The fine needle aspiration cytology suggested an poorly differented papillary cancer or involvement histiocytes. Ultrasound demonstrated diffuse enlargement goiter without descrete nodules. Trachea was shifted to the right and extremely stenotic. The computed tomography showed the occlusion of the left internal carotid vein. The bone lesion was detected by the bone scintigraphy. The fibrotic change was noted in the bone lesion. Because of easy bleeding and extensive involvement to the ajacent area by the tumor, a surgical removal of the tumor was unsuccessful. The irradiation therapy with a little amounts of doxorubicin HCl(20mg) was performed. The thyroid was decreased in size and the tracheal stenosis was improved. Pathologically,many histiocytes were infiltrated in the thyroid. CD1,CD4,CD68,S-100 Protein and lyzyme were positive in the infiltrating cells. Birbeckgranules were detected with an electron microscope. Histiocytosis X, although very rare, must be considered as a differential diagnosis when the thyroid gland progressively increased in size.

474. P65 SERUM CONCENTRATIONS OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) DETERMINED BY A HIGHLY-SENSITIVE CHEMILUMINESCENT ASSAY IN THYROID DISEASES.

S. Sakane, Y. Murakami, Y. Yamano, N. Ohsawa
First Department of Medicine, Osaka Medical College, Takatsuki 569; The St. Maria Hospital, Ibaraki 567; The Kuma Hospital, Kobe 650; Japan.

Granulocyte colony-stimulating factor (G-CSF) concentrations in serum were determined by a newly developed and highly sensitive chemiluminescent enzyme immunoassay (CLEIA) in the clinical course of Graves' disease (GD) with agranulocytosis and subacute thyroiditis (SAT). The sensitivity of CLEIA was 0.5 pg/ml and the intra- and inter- assay coefficients of variation were less than 5%. In normal subjects (n=116), the average value of G-CSF was 13.8±7.7(SD) pg/ml.

Six GD patients with methimazole(MMI)-induced agranulocytosis (Agra; circulating neutrophil counts < 500/μl) were studied. All patients were treated with recombinant human G-CSF (rhG-CSF), and the sample for G-CSF determination was obtained just before the subcutaneous injection of rhG-CSF. Before oneset of Agra, serum G-CSF levels were within normal range (19.0 ±7.8 pg/ml) during therapy with MMI. But when circulating neutrophil counts decreased to less than 500/μl , G-CSF levels significantly elevated to 113.3±79.2 pg/ml. In the therapeutic course with rhG-CSF, serum G-CSF further increased to more than 1000 pg/ml preceding the recovery of neutrophils. The maximum levels of G-CSF were correlated with individual duration of Agra.

In SAT before therapy, neutrophil counts significantly increased to 5.15 ±2.07×10^3/μl compared with the convalescent phase (2.94±1.07×10^3/μl), and the data correlated with individual serum G-CSF levels (r=0.854, p<0.01). Serum concentrations of other cytokines including IL-1 α, IL-3 and GM-CSF were less than the detectable threshold of ELISA. During two weeks of glucocorticoid therapy, although neutrophils increased to 7.73±1.64×10^3/μl , serum G-CSF levels were depressed from 25.1±15.3 to 13.8±13.9 pg/ml (p<0.01).

These data indicate that G-CSF is the one of the mediators of increase of circulating neutrophils in the recovery phase of agarnulocytosis and the acitive phase of subacute thyroiditis, while G-CSF does not contribute to steroid-induced neutrophilia.

475. P66 THE SUSPENSORY LIGAMENT OF BERRY: ITS RELATIONSHIP TO THE RECURRENT LARYNGEAL NERVE.

S. Sasou and S. Nakamura , Division of Pathology, Iwate Medical University, Morioka, Iwate, Japan

The ligament of Berry (posterior suspensory ligament) affixes the thyroid gland to the larynx and trachea and is closely located laterally to the distal course of the recurrent laryngeal nerve. Some authorities, however, emphasize a variation that the recurrent laryngeal nerve runs medially to the ligament of Berry in approximately 25% of all cases. It is very important to know exactly the course of the recurrent laryngeal nerve to the ligament of Berry so as to prevent nerve injury in thyroid surgery.

The purpose of this paper is to make clear the anatomical relationship between the ligament of Berry and the recurrent laryngeal nerve, and how to prevent the surgical complication of paralysis of the nerve, using 484 cases of thyroid surgery. Topography of the laryngo- tracheo-esophageal region and the histology of the ligament of Berry were studied in detail in 25 cadavers at autopsy.

Among the 484 cases having thyroid surgery, 562 recurrent laryngeal nerves (296 in the right side, 266 in the left side) were identified and the course was determined. All nerves were identified when we tried to find them located latero-dorsally to the ligament of Berry. They were clearly separated and no nerve penetrated through the ligament nor medially located to it. In addition, there was no nonrecurrent laryngeal nerve among them and no paralysis occurred as a resulting complication after surgery.

In the examination of 25 cadavers, the ligament of Berry was strongly connected to the trachea and easily identified as a whitish connective tissue band when the thyroid gland was lifted anteriorly and separated by forceps. The length of the ligament was 8 to 14 mm (mean: 11.5 mm) and the width 2 to 7 mm (mean: 3.1 mm). The upper border of this ligament was at the entrance level of the recurrent laryngeal nerve to the larynx, and the lower border extended to the second or third tracheal ring. The distance from the midline of the anterior tracheal wall to the ligament varied from 10 to 20 mm (mean: 14 mm) depending upon the diameter of the trachea. In all cases, the ligament was situated medio-anteriorly to the recurrent laryngeal nerve and independently identified. No recurrent laryngeal nerve penetrated the ligament or the thyroid gland. In three cases, the recurrent laryngeal nerve was partially covered by the proliferated connective tissue near the ligament of Berry. However, this tissue was clearly different from the ligament observed in the microscopical study. It seemed possible to differentiate the connective tissue from the ligament by a careful operation during surgery. Microscopically, this ligament consisted of the fibrous tissue in which blood vessels and lymphatics were frequently found.

In conclusion, the ligament of Berry consists of dense connective tissue containing blood vessels and lymphatics, and is located medio-anteriorly to the recurrent laryngeal nerve. The nerve never penetrated the ligament or thryoid gland. This relationship of the ligament and the recurrent laryngeal nerve is very impotant for the prevention of recurrent nerve injury during thryroid surgery.

476. P67 **TSH-RECEPTOR EXPRESSION AND HUMAN THYROID DISEASE: RELATION TO CLINICAL AND ENDOCRINE PARAMETERS** F. Schuppert, S. Deiters, G.F.W. Scheumann*, H. Dralle**, A. von zur Mühlen. - Department of Clinical Endocrinology, Department of Abdominal and Transplant Surgery*, Hannover Medical School, D-30625 Hannover, FRG; Center for Surgery I**, Halle-Wittenberg University, D-06120 Halle/Saale, FRG

It was the goal of this study to characterize transcript levels of the thyrotropin receptor (TSH-R) in human thyroid tissue in relation to established clinical and endocrine parameters. TSH-R mRNA expression was investigated in a total of 66 human thyroid glands with the help of Northern blot analysis. In relation to 6 healthy controls whose mean TSH-R expression level was arbitrarily defined as 1 relative unit, expression was increased to 2.213 units in Graves' thyroids (p=0.0098, n=35) and decreased to 0.3 in Hashimoto's thyroids (p=0.0281, n=5) and 0.073 in anaplastic carcinoma (p=0.0033, n=6). No significant changes were seen in endemic goiters (n=8) and in thyroid autonomy (n=6). There was a tendency towards a negative correlation between TSH-R expression and serum levels of TSH binding-inhibiting immunoglobulins (TBII). All other clinical or endocrine parameters including TSH-R stimulating antibodies (TSAb) showed no clear relation to TSH-R expression. Preoperative treatment of the Graves' patients with iodide was associated with high intrathyroidal iodide and iodine concentrations and was also associated with low TSH-R expression levels. We conclude that the responsiveness of the TSH-R to different stimuli could be actively regulated through TSH-R expression levels. The fact that in Graves' thyroids TSH-R expression was significantly increased may indicate an important regulatory principle in the pathogenesis of hyperthyroidism. It remains to be shown whether increased TSH-R mRNA levels truly lead to an increase of TSH-R protein levels.

477. P68 **RADIOIODINE TREATMENT OF THYROID CARCINOMA IN PATIENTS ON DIALYSIS FOR CHRONIC RENAL FAILURE** Ch. Daumerie, S. Vynckier, J. Caussin, M. Jadoul, J.P. Squifflet and A. Wambersie, Cliniques Universitaires St Luc, Brussels, Belgium

The current treatment for thyroid carcinoma is thyroidectomy followed by administration of radioiodine. Radioiodine treatment of patients undergoing chronic hemodialyis requires an adjustment of the dose and timing of administration of the isotope. Despite an obvious clinical interest, no optimal strategy for such an adjustment has been clearly defined.

We followed six [I-131] treatments in three patients who underwent thyroidectomy for papillary carcinoma and who were under dialysis for chronic renal disease. The administered [I-131] activity was 925 MBq (25 mCi) i.e., one fourth of the usual activity, in order to deliver the same body dose as in non dialysis subjects. Dialyses were performed on the day prior to iodine administration and repeated at 2-3 days intervals. Radioactivity in the blood and dialysate as well as the whole body dose were measured during 3 posttreatment dialyses. Data from 18 posttreatment dialyses were normalized and pooled for analysis.

During dialysis, the blood activity decrased with a halflife 3.58±0.51 (mean±1SD) hours.The mean absorbed rate at a distance of 1 m during the three successive dialyses were respectively 57.0±1.7, 18.5±2.5 and 7.5±2.5 μGy/hour. Analysis of the variations of activity in arterial blood in a control and dialysis patients showed that whole body irradiation when dialysis was carried at 48 hours was 1.9 times higher than if dialysis was performed immediately after radioiodine administration. Radioactivity monitoring showed that the hazard to personnel involved in patient management was minimal (130 μSv per dialysis).

It has been observed that [I-131] uptake in residual thyroid cell clusters occurs within one hour after administration. In order to minimize whole body irradiation, the optimal strategy for [I-131] treatment in hemodialysis patients is to give half the current dose and to perform the first dialysis 2 to 3 hours after isotope administration. Proper disposal of dialysis fluid and equipment decontamination should be carried out during the 3 dialyses that follow radioiodine treatment.

478. P69 ASSESSMENT OF REMISSION IN GRAVES' DISEASE BY MEASUREMENT OF THYROID ARTERY BLOOD FLOW USING ULTRASOUND PULSE DOPPLER

Taniyama M, Nagakura H, Kawauchi A, Kaname M, Ban Y. Depts. of 3rd Internal Medicine, Surgery, and Clinical Pathology, Showa University, Tokyo, JAPAN

Recent advances in Doppler ultrasonography have enabled to non-invasively measure the blood flow of the thyroid arteries, which has been shown to be increased in hyperthyroid Graves' disease. The increment of the blood flow does not return to the normal level shortly after becoming euthyroid with anti-thyroid drug (ATD) treatment. This may suggest that the patients with high blood flow who are going to withdraw ATD are not remitted.

We have evaluated the usefulness of the measurement of blood flow of superior thyroid arteries by ultrasound pulse Doppler in the assessment of the remission in Graves' disease. We performed ultrasound pulse Doppler and T3 suppression test before stop medication in the 24 patients with Graves' disease who were euthyroid with minimal dose of ATD. Among 15 patients who remained euthyroid, only three patients had mean velocity of the blood flow in the superior thyroid artery more than 20 cm/sec, two of who had very low ^{123}I-uptake after T3 administration, whereas 7 out of 9 patients who recurred had mean velocity higher than 20 cm/sec. Two patients with low blood flow recurred at 9 and 23 month after drug withdrawal respectively. Four patients who recurred within six months had high blood flow even though T3 suppression test was positive. The patients whose mean velocity is less than 20 cm/sec are expected to remain remission at least a year regardless of the results of T3 suppression test. On the other hand, those whose mean velocity is more than 20 cm/sec are at high risk for recurrence unless T3 suppressibility is prominent. Thus, measurement of the blood flow of the superior thyroid artery by ultrasound pulse Doppler is useful for detection of the remission.

479. P70 LACK OF STIMULATION BY THYROTROPIN RECEPTOR ANTIBODY OF NORMAL THYROID GLANDS IN MOST FETUSES AT TERM.

J.Y.Noh, A.Nagata,* N.Momotani, and K.Ito, Ito Hospital, Shibuya-ku, Tokyo; *Yamasa Shoyu Co., Choshi, Chiba, Japan

TSH receptor antibody (TRAb) seems to cause hyperthyroidism in Graves' disease, and there is significant but weak correlation between levels of FT4 and TSH-binding inhibitory immunoglobulin (TBII). The weakness of the correlation may arise in part to individual differences in the response of thyroid tissue to TRAb in this disease. If so, there may be stronger correlation between TRAb and FT4 in normal thyroid tissue than in Graves' thyroid tissue. In an examination of this hypothesis, the function of thyroid glands of fetuses at term of mothers with TRAb but who were euthyroid after thyroidectomy was investigated. We studied 89 patients after subtotal thyroidectomy for Graves' disease who gave birth in the euthyroid state without taking any drugs, and their 89 infants' TBII, thyroid stimulating antibody (TSAb), and FT4 in sera from umbilical cord blood (UCB) were assayed, and the correlation of FT4 with TBII and with TSAb was calculated. For comparison, TBII, TSAb, and FT4 were measured in sera from 175 untreated patients with Graves' disease. TBII was assayed with a TRAb kit (Cosmic Co.), and TSAb was assayed with a TSAb kit (Yamasa Shoyu Co.). In the TSAb assay, the intra-assay and interassay coefficient of variation of cAMP was 6.81% (n=10) and 7.83% (n=5), respectively. In untreated Graves' patients, correlation between FT4 and TBII was significant ($r=0.401$, $p<0.001$), but there was weaker correlation between FT4 and TSAb ($r=0.249$, $p<0.001$). The correlation between TBII in the sera of mothers at birth and sera from UCB was strong ($r=0.927$, $p<0.0001$). TBII, TSAb, and FT4 of sera from UCB were 21.1±13.2% (mean±SD; reference range, -3.1 to 10.7%), 186±105% (reference, <180%), and 1.18±0.24 ng/dl (reference, 0.76 to 1.40), respectively. Here, correlation between TBII and TSAb was weak ($r=0.408$, $p<0.001$), and correlation between FT4 and TBII or TSAb was not significant ($r=0.244$ and $p<0.03$; $r=0.264$ and $p<0.02$, respectively). Of the 73 samples of UCB in which TBII was detected, the FT4 level was in the reference range in 61 samples (84%), and of the 29 samples in which TSAb was detected, the FT4 level was in this range in 21 samples (72%). Contrary to expectation, the correlation between TRAb and FT4 in fetuses at term with normal thyroid glands was not significant. Although Graves' thyroid glands are stimulated by TRAb, most normal thyroid glands are not.

480. P71 GRAVES' HYPERTHYROIDISM, WHICH WAS AGGRAVATED AFTER DELIVERY, SUBSEQUENTLY AMELIORATES TO ITS ORIGINAL STATUS. Y. Gomi, N. Momotani, and N. Ishikawa, Ito Hospital, Tokyo, Japan.

It is generally accepted that Graves' hyperthyroidism is often aggravated after delivery though it tends to ameliorate during pregnancy. However, it is not known whether the natural course of Graves' hyperthyroidism undergoes decisive changes after the patients go through pregnancy or not. We, therefore, investigated changes in TBII levels and thyroid status during and after pregnancy, and compared these a year after delivery with those around the time of conception in 18 women who had maintained a remission status after surgery for Graves' disease for at least 9 months after the operation and who still showed positive TBII around the time of conception. Four of the 18 women had been taking l-thyroxine for postoperative hypothyroidism and continued it throughout the observation periods. In six of the other 14 women, transient thyroid dysfunction developed within one year after delivery, but thionamides were not administered. During pregnancy, TBII levels decreased in 11 (61%) and increased in 4 (22%) women, and the levels showed little change in the remaining 3 (17%). After delivery, TBII levels increased in 9 (50%), decreased in the other 5 (28%), and they did not show unequivocal changes in the remaining 4 (22%). Interestingly, the greater the decrease in TBII levels during pregnancy, the more they rose after delivery. Moreover, when the levels rose after delivery, they subsequently declined during the postpartum period. Consequently, the mean TBII levels around a year after delivery (28.4±24.5%) were not significantly different from that about the time of conception (33.2± 24.3%). Neither the mean FT4 level nor the mean TSH level was significantly different between a year after delivery and around the time of conception (FT4, 15.4±3.1 vs. 16.3±3.3 pmol/L; TSH 3.2±2.8 vs 11.4±26.1 mU/L). These findings suggest that pregnancy has only a transient or minimal influence, if any, on the natural course of Graves' disease and that hyperthyroidism due to Graves' disease subsequently ameliorates to its original status even if it is aggravated after delivery.

481. P72 IS COMPENSATED RADIOIODINE THERAPY OF HYPERTHYROIDISM WORTHWHILE? A.E.Jarløv, [1]L.Hegedüs, L.Ø.Kristensen, B.Nygaard and J.M.Hansen, Department of Internal Medicine and Endocrinology and Ultrasound, Herlev Hospital, DK-2730 Herlev, [1]Department of Internal Medicine and Endocrinology, Odense University Hospital, DK-5000 Odense, Denmark.

Objective: The persistent controversy as to the best approach to radioiodine dose selection in the treatment of hyperthyroidism urged us to perform a study in order to compare a fixed dose regime comprising doses of 185, 370 or 555 MBq (small: < 30 ml, medium: 30-60 ml or large glands: > 60 ml, by palpation, respectively) versus a compensated ^{131}I therapy based on type of thyroid gland and an accurate thyroid volume and 24h radioiodine uptake determinations. **Design:** Prospective randomized study. **Patients:** 221 consecutive hyperthyroid patients referred to ^{131}I treatment. Four patients died of reasons unrelated to hyperthyroidism 7 dropped out, 47 did not receive antithyroid drugs after treatment and thus 163 patients (143 women) were studied. They all received antithyroid drugs prior to ^{131}I treatment and this was resumed 7 days after treatment for a period of 3 weeks. **Measurements:** Thyroid function variables were determined approximately 2 months and 2 weeks before ^{131}I treatment, and again 1, 2, 3, 6, 9 and 12 months after treatment. Prior to ^{131}I therapy the size of the thyroid gland was determined by ultrasound and a 24-hour uptake of ^{131}I was carried out. Thyroid volume was estimated again 12 months after ^{131}I therapy in 78 of the 163 patients. Twelve months after the initial ^{131}I dose the patients could be classified as 1) euthyroid (normal thyroid function without thyroid medication), 2) hyperthyroid (still on antithyroid medication or retreated with a second ^{131}I dose), or 3) hypothyroid (subnormal thyroid function or euthyroid on thyroxine). **Results:** Neither in the total material nor within the 3 subgroups of hyperthyroidism (diffuse, multinodular, toxic nodule) could any significant differences between the two treatment regimes be demonstrated. 31 of 78 patients (40%) in the compensated group and 30 of 85 patients (35%, NS) in the fixed group were classified as hyperthyroid. Eight out of 78 (10%) in the compensated group as opposed to 6 out of 85 (7%, NS) in the fixed group were classified as hypothyroid. Finally, 39 of 78 (50%) in the compensated group compared to 49 of 85 (58%, NS) in the fixed group were euthyroid at 12 months after ^{131}I treatment. Within one year thyroid volume was reduced from 59.3 +/- 9.2 ml (mean +/- SEM) to 36.2 +/- 6.6 ml (39%) in the compensated dose group ($p < 0.001$). This did not differ significantly from the fixed dose group were thyroid volume declined from 61.6 +/- 6.1 ml to 41.7 +/- 4.7 ml (32%, $p < 0.001$).

Conclusions: A semiquantitative approach is probably as good as the more elaborously calculated radioiodine dose for treatment of hyperthyroidism. It is clearly more cost-effective and allows the use of prefabricated standard doses.

482. P73 THE DETECTION OF REVERSE TRANSCRIPTASE IN THYROID GLAND OF GRAVES' DISEASE.

Akira Nakai, Akio Nagasaka, Naohisa Oda, Yoshikuni Sawai, Nobuki Hayakawa, Motoko Kotake, Mitsuyasu Itoh and Shonen Yoshida*.
Dept. of Internal Med. Fujita Health Univ. School of Med., Toyoake, Aichi, Japan. * Res. Inst. Dis. Mech. Cont., Nagoya Univ. Scool of Med., Showa-ku, Nagoya 466, Japan.

Recently retrovirus infections have been reported in several autoimmune diseases. Adult T cell leukemia virus infection was complicated with autoimmune thyroiditis. Some retroviruses characteristically possessing reverse transcriptase (RT) may induce Graves' disease. To confirm retrovirus infection in the thyroid gland of Graves' disease, we measured the RT activities in thyroid tissues and amplified RT like partial cDNA from the mRNA of the thyroid glands. Nuclear proteins were eluted by 0.5M KCl and 1% Triton from pellets obtained by 1000xg centrifugation of thyroid tissue homogenates. RT activities were measured by previously reported methods using MS2 RNA, specific oligo primer and [^{3}H]TTP. The poly A RNA from thyroid tissues was separated by AGPC methods and oligo dT cellulose column. The single strand cDNA was made by AMV RT using random primers. The partial RT like cDNA was amplified by PCR using degenerated oligo primers. The sequences of amplified cDNA were determined. The RT activities in the thyroid gland of Graves' disease were significantly increased, compared with that of the normal thyroid and adenoma. The activities in some carcinoma tissues were also increased. The RT like partial cDNA was amplified from the mRNA of thyroid gland of Graves' disease. Its sequence was highly homologous with RT of MMTV. These findings suggest that retrovirus infection may have some role in the induction of Graves' disease.

483. P74 **GROWTH HORMONE (GH) RESPONSE TO GH-RELEASING PEPTIDE-6 (GHRP-6) IN HYPERTHYROIDISM.** J.C.Ramos-Dias, E.T.Kimura, F.Pimentel Fo, A.F.Reis, L.J.Machado and A.M.J.Lengyel, Division of Endocrinology, Universidade Federal de São Paulo - UNIFESP/EPM, São Paulo, Brazil.

Several abnormalities in the GH response to pharmacological stimuli, including GH-releasing hormone (GHRH), have been described in hyperthyroidism. GHRP-6 is a synthetic hexapeptide that specifically releases GH both *in vitro* and *in vivo*. The mechanism of action of GHRP-6 is unknown. We studied the effects of GHRP-6 in 8 women with hyperthyroidism due to Basedow-Graves Disease (mean age: 32 y) and in 6 control subjects (5M,1F; mean age: 29 y). All hyperthyroid patients had high serum free tiroxine and suppressed serum thyrotropin levels. Patients were free of any medication at the time of the study. Each subject received GHRP-6 (1 μg/Kg, iv), GHRH (100 μg/Kg, iv) and GHRP-6 + GHRH, in random order, in 3 different days. Results are shown as peak GH (μg/L) and area under the curve (AUC, μg/L.120min); mean ± SEM.

	GHRH		GHRP-6		GHRH + GHRP-6	
Group	Peak GH	AUC	Peak GH	AUC	Peak GH	AUC
HYPER	9.5 ± 1.4	855 ± 160	33.5 ± 6.2	1709 ± 367	23.4 ± 3.9	1520 ± 223
CONTROL	25.9 ± 8.3	2022 ± 546	22.1 ± 6.1	1247 ± 431	77.5 ± 22.2	4915 ± 1319
p	< 0.05	< 0.05	NS	NS	< 0.05	< 0.05

GH response to GHRP-6 was similar in hyperthyroid patients and control subjects, while a significant decrease in the responsiveness to GHRH was seen in the former group. The synergistic effect of combined administration of GHRP-6 and GHRH observed in normals was absent in hyperthyroid patients. In conclusion, our data show that impaired GH secretion in hyperthyroidism is a potentially reversible state. Our results also suggest that in hyperthyroidism GHRH transduction pathways are probably altered while those of GHRP-6 are apparently preserved. (Supported by FAPESP)

484. P75 THE EFFECT OF PSYCHOLOGICAL FACTOR ON THE PROGNOSIS OF ANTITHYROID DRUG-TREATED GRAVES' DISEASE PATIENTS. A.Fukao, M.Ito, S.Hayashi, H.Sato. First Department of Medicine, Osaka Medical College, Takatsuki, and Department of Mental Health, School of Hygienic Sciences, Kitasato university, Sagamihara, Japan.

The effect of psychological factor, such as psychiatric personality or stressful life events, on the onset of hyperthyroidism due to Graves' disease has been suggested; however, its role on the prognosis of Graves' disease (remission or non-remission) has not been well investigated, which was the aim of the present study .

Sixty-six patients with Graves' disease who had been an euthyroid state after antithyroid drug (ATD) medication more than one year were classified into the following three groups. Fifty-two patients with Graves' disease who were on ATD medication were classified as Group A, and among them, 36 patients with persistently positive serum anti-TSH receptor antibodies during ATD medication were classified as group A_1, and 16 patients with decreased serum anti-TSH receptor antibodies during therapy as group A_2. Fourteen patients who had been maintaining the remission of hyperthyroidism after cessation of ATD were classified as group B. As the control group, 25 healthy subjects were studied as group C. Psychological state and personality were determined by the Minnesota Multiphasic Personality Inventory (MMPI). Stressful life events were determined by the Natsume's stress inventory, and daily life stress were further determined in 66 subjects by the Hayashi's daily life stress inventory.

The data of MMPI showed that the T-scores of both hypochondriasis scale and depression scale were significantly higher in both groups A_1 and A_2 than those of group C. Both psychasthenia scale and schizophrenia scale had T-scores in the higher order of A_1, A_2, B, and C. Any of the above T-scores of scales were not correlated with either serum thyroid hormone levels nor the duration of illness. The data of stressful life events showed that greater numbers and scores were obtained in group A_1 than the other groups, although the differences were not statistically significant. The data of daily life stress showed that the intensity of stress was significantly greater in the total of groups of A_1, A_2, and B than in that of group C.

In conclusion, patients with Graves' disease frequently have mental disorder even when thyrotoxicosis is ameliorated by ATD treatment. Also, psychiatric abnormality in such patients appear to act as a poor prognostic factor possibly through neuro-immuno-endocrinological triangle.

485. P76 HYPERTHYROIDISM SECONDARY TO PITUITARY TUMOR CO-SECRETING THYROTROPIN AND PROLACTIN--REPORT OF A CASE. Y.M.Song, W.H. Lin. Division of Endocrinology/Metabolism,Taichung Veterans General Hospital,Taiwan,R.O.C.

Thyrotropin(TSH)-secreting pituitary tumor is a rarely recognized clinical entity. We report on a case of pituitary tumor co-secreting TSH and prolactin with clinical hyperthyroidism. A 47-Y/O Chinese female suffering from palpitation and restlessness was found to have non-suppressed TSH in the presence of elevated free thyroxine,T3 and T4. The initial data were: TSH:18.09 uU/ml(0.4-5.0),FT4:2.71 ng/dl (0.7-2.2),T3:259 ng/dl(80-200) and T4:15.98 ug/dl(6.0-12) by RIA. Other anterior pituitary hormones were normal except elevated PRL: 78.71 ng/ml(2.7-26). CT of sella turcica revealed a tumor of 1.5 cm in diameter which was further clearly demonstrated by MRI.Thyrotropin stimulation test was done with the finding of flat TSH response. 131-I thyroid uptake was 85% after 24 hours. Sonogram of thyroid showed multiple small nodules in both lobes which were not readily palpable by hands. Surgery was suggested but refused by patient. Pharmacotherapy was first started with bromocriptin but only with effect on prolactin,which was halved to 40.21 ng/ml after one month' therapy. Due to intolerance to bromocriptin and its limited effect, somatostatin analogue,octreotide,was given. Both TSH and PRL were lowered rapidly to normal range within 24 hours,accompanied by lowering of thyroid hormones. Acute RUQ abdominal pain was the only side effect of this regimen which happened several hours after the first injection. The dose of octreotide was gradually tapered from 100 ug Tid to 50 ug daily with satisfactory result.

We conclude that TSH-secreting pituitary tumor must be suspected when TSH is high in a thyrotoxic patient. This entity is diagnosed by TSH stimulation test and image study. Medical treatment with octreotide is satisfactory as long as it is administered. Surgical removal of the tumor is still the treatment of choice.

486. P77 LATE CHANGE OF THYROID FUNCTION AND IMMUNOLOGICAL PARAMETERS AFTER SUBTOTAL THYROIDECTOMY FOR GRAVES' DISEASE. Y.Kasuga, A.Sugenoya, S. Kobayashi, M.Maruyama, Department of Surgery, Shinshu University School of Medicine, Matsumoto, Japan.

The aim of this study is to know whether or not the surgical treatment induces stable endocrinological and immunological remission in each patient with Graves' disease in late course. The postoperative late changes of various thyroid function and immunological parameter remain still unclear. A total of 176 patients with Graves' disease who underwent subtotal thyroidectomy at our hospital between 1970 and 1979 was reviewed. The remnant weight of thyroid gland mainly ranged between 4 and 8 grams. Follow-up surveys were done at both 1984 and 1992. The patients were examined clinically and biochemically to assess precisely the postoperative status of the thyroid gland. Serum levels of free thyroxine, free triiodothyronine and TSH were measured for the biochemical evaluation of thyroid function. Anti-thyroglobulin(TGHA) and antithyroid microsomal(MCHA) antibodies were measured for the immunological parameters. In addition, the lymphocyte subsets were examined with anti-CD3,CD4,CD8 and HLA DR antibodies in 1992. Fifty of 79 patients(63%) with euthyroid state in 1984 still showed euthyroidism in 1992. Twenty nine of 79 patients(37%) reached thyroid dysfunction,i.e. 17 patients(22%) with latent hypo-thyroidism, 7(9%) with hypothyroidism and 5(6%) with hyperthyroidism, respectively. Five of 8 patients(63%) with latent hypothyroidism in 1984 showed same state in 1992. The remaining three patients(37%), however, reached euthyroid state in 1992. Eighty of 87 patients(92%) with positive MCHA in 1984 still showed positive in 1992. Meanwhile, eight of 39 patients(21%) with negative MCHA changed to positive in 1992. Twenty seven of 43 patients(63%) with positive TGHA in 1984 still remained positive in 1992. Out of 83 patients with negative TGHA, 3(4%) showed positive in 1992. With regard to the percentages of CD3,CD4 and CD8-positive cells, there were no significant differences among diverse thyroid function groups of Graves' patients and normal control. However, there found significant difference between Graves' patients with each thyroid function state and controls in Leu HLA-DR-positive cells. These foregoing results suggest that surgical treatment might not completely induce stable endocrinological and immunological remission.

487. P78 **Conversion of Function of TSH-Receptor-Antibodies in a Patient with Myasthenia Gravis**

M.Weissel, 3rd Medical Univ. Clinic, General Hospital Vienna; A-1090 Vienna, Austria.

Three to eight percent of patients with myasthenia gravis (MG) are reported to develop simultaneously hyperthyroid autoimmune disease. We want to present a case with MG with both, the hyper- and the hypothyroid, variants of the disease:

The otherwise healthy female patient was subtotaly thyroidectomized because of euthyroid goiter at the age of 22 yrs. She developed diplopia together with fatigue when she was 46. MG was treated with oral pyridostygmine for several years. Eight years after the onset of MG hyperthyroidism was diagnosed and treated with methimazole. At the age of 56 the patient underwent thymectomy, which resulted in drug free complete remission of MG. Six months after thymectomy also methimazole could be successfully withdrawn. 11years later she presented clinically in a classical hypothyroid state. Her thyroid gland was sonographically small without nodules. Her TSH was 43.2 mU/l,her thyroglobulin antibody concentrations were normal with 22IU/ml her microsomal antibodies were pathologically increased with >3000 IU/ml as well as her TSH-receptor antibodies (TRAB) , that were repeatedly between 312 and 365 U/l (Henning assay). Substitution therapy with l-T4 was started.

Because of these high concentrations of TRAB we tried to determine their functional activity: The TSH dependant adenylate cyclase activation of TSH-deprived FRTL-5 cells was measured after pre-incubation of FRTL-5 cells with the patient's serum. The cAMP synthesis of cell supernatants was measured by a protein-binding assay. The serum of the patient showed only weak inhibiting, but no stimulating activity. Additional testing for Yersinia-enterocolitica virulence proteins (YOP) revealed a typical YOP-antibody-profile of subacute yersinosis, which has been shown to be associated with autoimmune thyroid disease.

In summary we believe that this patient with MG developed TRAB, that elicited hyperthyroidism before her thymectomy and that changed their functional activity resulting in hypothyroidism a decade later.

488. P79 DIFFERENTIAL DIAGNOSIS FOR A THYROTOXIC RELAPSE IN THE POSTPARTUM PERIOD IN GRAVES' DISEASE USING ANTITHYROID ANTIBODY MEASUREMENT BY RADIOIMMUNOASSAY. T. Shimizu, Y. Daimon, M. Kitano, Y. Yamazaki, Y. Umezu, Y. Arakawa and Y. Shishiba, Toranomon Hospital, Tokyo, Japan.

In the course of Graves' disease, there are episodes of thyrotoxic relapse; one is due to true relapse of hyperthyroidism and the other due to destructive painless thyroiditis. We examined the possibility that we can differentiate the thyrotoxic relapse by examining anti-thyroglobulin and anti-TPO antibodies by highly sensitive radioimmunoassay (ATG-RIA and ATPO-RIA, respectively). Five cases of Graves' disease who had remained euthyroid during pregnancy and experienced thyrotoxic relapse 2-4 months after delivery were examined prospectively. Two of the five experienced true relapse of hyperthyroidism and three had destructive painless thyroiditis. Sera were obtained monthly and analyzed for ATG by hemagglutination (ATG-HA) or RIA, and for ATPO by HA and RIA. The results were shown in Table.

	1st trimester				just before delivery				post-partum relapse			
	ATGHA	ATGRIA	ATPOHA	ATPORIA	ATGHA	ATGRIA	ATPOHA	ATPORIA	ATGHA	ATGRIA	ATPOHA	ATPORIA
Graves (2)	negative	0.351	negative	0.127	negative	0.210	negative	0.101	negative	0.296	negative	0.101
	negative	0.210	negative	0.101	negative	0.210	negative	0.101	negative	0.526	negative	0.101
painless (3)	1:24	6.360	1:240	57.4	1:24	2.63	1:240	7.881	1:240	15.27	1:2400	163.9
	1:240	20.556	1:24	0.596	1:24	4.62	1:24	0.101	1:240	14.88	1:24	0.298
	1:24	0.689	1:2400	157.1	1:24	0.521	1:240	18.75	1:24	0.75	1:2400	56.5

The nature of thyrotoxic relapse in postpartum period can be predicted from the antibody at 1st trimester: high titer of either ATG or ATPO predicts the occurrence of destructive painless thyroiditis, while the negative or low titer predicts relapse of Graves' disease. The simultaneous rise of ATG and ATPO at postpartum relapse tends to indicate the former, while the rise of ATG alone indicates the latter.

489. P80 W. Reinhardt, E. Freygang*, G. Kummer, M. Gosselink, F. Jockenhövel, D. Reinwein, K. Mann
Medical Clinic, Division of Endocrinology, University of Essen, D-45122 Essen and
*Nordseeklinik Borkum, D-26757 Borkum

Significant changes in thyroid function before and after a 4 week vacation period

Numerous studies have been carried out concerning the influence of various acute or chronic diseases on thyroid function. However, so far data evaluating the effects of vacation- and recovery periods on thyroid hormone economy are missing. Therefore, thyroid function was evaluated in 108 male patients before and after a 4 week stay in a health spa on the island Borkum in the North Sea. Most of the patients had mild to moderate chronic obstructive lung disease and/or allergic dermatological diseases. All were clinically euthyroid and no patient received drugs, known to influence thyroid function (oral corticosteroids, beta receptor blocking agents etc). Blood- and urine samples were collected in the morning of the second day and after 4 weeks for determination of T4, T3, fT4, TSH and urinary iodine excretion.
We observed a significant fall of Total T4 from 95,5 ± 17,6 nmol/l to 88,1 ± 17,0 (mean ± standard deviation) ($p < 0,001$; paired t-test) and total T3 from 2,20 ± 0,30 to 2,11 ± 0,31 nmol/l ($p < 0,001$; paired t-test). FT4 decreased from 16 to 15 pmol/l ($p < 0,005$; Wilcoxon-Test) and TSH increased from a median of 1,20 to 1,55 mU/l ($p < 0,001$; Wilcoxon-Test). No change in the urinary iodine excretion (median: 69 ug J/g creatinine versus 68 ug J/ g creatinine) was observed, demonstrating no increased exposure to iodine at the North Sea within 4 weeks.
In conclusion, we observed a subtle but significant fall of thyroid hormone concentrations with a concomitant rise in TSH levels. Possibly a decreased requirement for thyroid hormones during a vacation period may exist.

490. P81 THERMOGENIC EFFECTS OF TRIAC IN CULTURED BROWN ADIPOCYTES. M.-J. Obregón, A. Hernández. Inst. Investigaciones Biomédicas (CSIC). Madrid. Spain.

The main function of brown adipose tissue (BAT) is to produce heat. This is acomplished by the BAT specific mitochondrial uncoupling protein (UCP), that uncouples oxidative phosphorylation, leading to generation of heat. Cold exposure and diet stimulates BAT activity by norepinephrine (NE) release from the sympathetic nervous system. Maintenance of the energy balance is closely related to BAT activity. Thermogenic inactivation of BAT is linked to development of obesity in transgenic mice and animal obesity models.

Euthyroid status is essential for a full thermogenic activity in BAT. We have previously found that T3 strongly potentiates the adrenergic stimulation of both UCP mRNA levels and type II 5'-deiodinase (5'D) activity in brown adipocytes differentiated in culture. Triiodothyroacetic acid (Triac), a physiological product of T3 metabolism, has similar or higher affinity than T3 for thyroid hormone receptors. Due to its thyromimetic potency and to its *in vivo* short half-life, Triac has been used as inhibitor of TSH secretion. Triac has been also used in anti-obesity treatments.

The **aim** of this work is to compare T3 and Triac potencies on the adrenergic stimulation of 5'D activity and UCP mRNA levels in brown adipocytes. We have also compared T3 and Triac effects on the inner ring deiodinase (5 D) activity.

Brown preadipocytes were isolated from 20 days-old rats, and cultured in DMEM+10 mM Hepes+15 µM ascorbic acid+3 nM insulin+10% newborn calf serum. Differentiated cells were used (8 days after seeding). T3 or Triac were added in hypothyroid medium. UCP mRNA was measured by Northern blots. 5'D specific activity (1U= 1fmol/h/mg protein) was determined using ^{125}I-T4, 2 nM T4+1µM T3, 50 mM DTT, 1mM PTU, pH=7. For 5D activity (same units) we used inner ring labelled T3 as tracer (provided by Drs Rokos & Thoma, Henning, Berlin), 25 nM T3, 20 mM DTT, 1mM PTU at pH=7.5.

Results: Both T3 and Triac potentiate the adrenergic induction of UCP mRNA levels (up to 50-fold). In the presence of NE (1µM, 12h), exposition of cells to 0.025, 0.05 and 0.2 nM of T3 or Triac for 24 h, results in a UCP mRNA levels of 218, 259 and 484 units when using T3 and 1012, 3695 and 5247 units when using Triac (>10 times more potent than T3).

T3 and Triac also potentiate the adrenergic induction of 5'D activity in brown adipocytes (up to 40-fold). In the presence of NE (3 µM, 8h), cells exposed 20 h, to gradual doses of T3 (from 0.025 to 10 nM) showed a gradual increase in 5'D activity from 6 to 333 U. Triac at 0.2 nM (20 h) was as potent as 10 nM T3 potentiating 5'D activity (336 U) and the effect of 0.05 nM Triac comparable to 1 nM T3 (115 U). Higher doses of Triac (1, 5 and 25 nM) decreased 5'D activity.

T3 and Triac both increase 5D activity in the presence or absence of NE stimulation. Exposition of cells in serum-free medium to 2 nM T3 (20 h) increased 5D activity from 339 to 564 U, while cells exposed to 0.2 nM Triac (20 h) showed 830 U. In the presence of NE (3 µM, 8h), cells exposed to T3 doses from 0.025 to 10 nM (20 h) increased 5D activity from 835 to 1872 U, while cells exposed to 0.05 nM Triac (20 h) had the same effect as 1 nM T3 on 5D activity (1189 U).

Comparison of T3 and Triac uptake in brown adipocytes *in situ* showed that Triac cellular uptake is lower than that of T3, with a 50% higher nuclear to cell ratio for Triac at short times (up to 6 hours).

In summary, Triac is at least 10 times more potent than T3 in the potentiation of the adrenergic response of UCP mRNA, and 50-times more potent in the potentiation of the adrenergic response of 5'D activity. The higher thermogenic potency of Triac should be taken in account when used in anti-obesity treatments. *Grants from PGC 92/0061 and FISS 94/0277*

491. P82 PLASMA T3 AND T4 ARE MORE HIGHLY CORRELATED WITH TISSUE T3 CONCENTRATIONS THAN PLASMA TSH LEVELS, IN THYROID HORMONE-INFUSED THYROIDECTOMIZED RATS. H.F. Escobar-Morreale, F. Escobar del Rey and G. Morreale de Escobar. Instituto de Investigaciones Biomédicas, CSIC & UAM, Madrid, SPAIN.

Plasma TSH is considered to be an accurate marker of tissue thyroid hormone status in clinical practice. Moreover, the determination of plasma TSH levels is the single most important analytical procedure in the diagnosis and management of primary hypothyroidism, and the normalization of plasma TSH is the final endpoint of thyroxine replacement therapy. However, up to date tissue concentrations of T4 and T3 have not been measured in humans, and the statements described above are based in the correlation between plasma TSH levels and several plasma markers of thyroid hormone action on different target organs (Gow et al, J Clin Endocrinol Metab. 1987, 84:364-370), which may not be excessively accurate. We have studied the correlation and the concordance between tissue levels of T3, and circulating levels of T3, T4 and TSH, in normal rats, thyroidectomized rats infused with placebo, thyroidectomized rats infused with T4 or T3, or different combinations of T4+T3.

Young adult female Wistar rats were surgically thyroidectomized and injected 100 µCi ^{131}I within a week. After one month the rats with complete stasis of body weight were divided into groups of 6 rats each, and implanted sc with osmotic minipumps delivering either placebo, or T4 (0.2 to 8.0 µg/100 g BW / day), or T3 (0.25, 0.5, 0.75, 1.0, 2.0 µg/100 g BW / day), or a combination of fixed doses of T4 (0.8 and 0.9 µg/100 g BW / day) with different doses of T3 (0.10, 0.15 and 0.20 µg/100 g BW/day). After 12-13 days of infusion the animals were killed and perfused. Plasma and 12 tissues were assayed for T3 and T4 (Morreale de Escobar et al., Endocrinology 117: 1890, 1985). Plasma TSH was measured. The correlation between plasma TSH (after logarithmic transformation) and tissue T3 was studied by correlation analysis. These correlations were also studied by partial correlation analysis, using plasma T3 as control variable, to detect spurious correlations derived of the relationship between plasma TSH (log) and plasma T3. Finally, multiple regression analysis was performed, including tissue T3 as dependent variable, and plasma T3, T4 and TSH (log) as independent variables.

Analysis was performed including data from all the rats, to reproduce all the possible situations present on the clinical practice, from hypothyroidism to overt hyperthyroidism due to excess of T4 or T3. Plasma TSH correlated with plasma T3 (r=-0.62, P<0.001). Plasma TSH also correlated with tissue T3 (r= -0.34 to -0.68, P<0.001). These correlations where not present, or even changed its sign, when plasma T3 was introduced as the control variable. Multiple regression analysis showed that, in all the tissues studied, the changes in plasma T3 were the major determinant of the changes observed in the T3 concentrations of all tissues ($R2$= 0.22 to 0.89, P<0.001). In cerebral cortex and brown adipose tissue, changes in plasma T3, T4 and TSH only explained 48% ($R2$=0.48) and 22% ($R2$=0.22) of the changes in tissue T3; a consequence of the autorregulatory mechanisms present in these tissues.

Our results show that, at least in the rat, plasma T3 is the major determinant, and the best serum marker, of tissue T3 concentrations. On the contrary, plasma TSH did not correlate accurately with tissue T3. These results cast doubt upon the hypothesis that, in humans, plasma TSH is the best marker of tissue euthyroidism.

Grants: FISS fellowship 93/5168 and 94/5082, Grant FISS 92/0888, and financial aid by Henning Berlin (GhmB).

492. P83 3,5,3'-TRIIODOTHYRONINE SULFATE (T3S) CONCENTRATIONS IN PREMATURE AND FULL-TERM NEWBORNS. F. Santini, P. Lapi, *P. Ghirri, E. Fiore, G. Bendinelli, D. Taddei, M. Ciampi, R. Centoni, F. Lippi, **I.J. Chopra and L. Chiovato. Institute of Endocrinology and *Department of Neonatology, University of Pisa, Italy; **UCLA, Los Angeles, CA.

Sulfation is a metabolic pathway that accelerates deiodination of iodothyronines by type I-iodothyronine monodeiodinase (type I-MD). T3S may also represent a reservoir of T3 for selected tissues equipped with sulfatases which can reconvert T3S to T3. An increase in serum T3S levels has been found in human fetus and newborn in spite of very low T3 levels, but the mechanisms responsible for this phenomenon are poorly understood. Aim of this study was to analyze serum T3S concentrations in 16 premature (26-37 weeks) and 31 full-term newborns, and to relate T3S levels with other parameters of thyroid function. Serum T3S was measured by RIA using a specific rabbit antiserum. As assessed in cord blood, serum T3S values increased with gestational age reaching a plateau (30±9.1ng/dl) at 34-36 weeks, and then declined until week 41 (19±4.5 ng/dl). In newborns delivered at 35 to 41 weeks of gestation, a significant ($p<0.005$) negative correlation was found between cord blood T3S levels and duration of pregnancy. When premature and full-term newborns were considered together, T3S values were negatively correlated with total T3 levels ($p<0.05$) and positively correlated with rT3 values ($p<0.001$), while no correlation was found between T3S and total T4, TSH, or TBG. As compared to cord blood concentrations (21 ± 3.6 ng/dl), serum T3S levels in full-term newborns significantly increased during the first 3-4 days of life (33±9.4 ng/dl; +55%, $p<0.001$) reaching values that were 8 to 15 times greater than in normal adults (3.3±1 ng/dl). Serum T3S levels declined thereafter, but they were still 5 to 10 times higher than in normal adults at 2 weeks of life. A similar T3S peak was observed in premature newborns (44±15 ng/dl at 3-4 days of life vs. 29±4.1 in cord blood; +61%, $p<0.001$), and the decline in T3S values was even slower than in full-term neonates.

In conclusion: i) serum T3S levels increase during gestation reaching a maximum at 34-36 weeks, and then decline until birth; ii) during the last trimester of pregnancy changes in serum T3S concentrations are mainly dependent on the activity of type I-MD; iii) a T3S peak occurs early after birth, which may result either from the concomitant peak of T3 or from interruption of transplacental passage of fetal T3S to the maternal circulation.

493. P84 1.4-DIHYDROPYRIDINES AS T3-ANTAGONISTS - FURTHER EVIDENCE FOR "MOLECULAR MIMICS"? G.H.Scholz*, S.Vieweg*, M.Uhlig§, S.Kistner*, H.-J.Hofmann§, S.Goldmann#, D.K.Chalmers.$, S.L.A. Munro$, D.J.Topliss$, J.R.Stockigt$, *Dept. Int. Med. III, §Dept. Biosciences, Univ. Leipzig, #Bayer AG Leverkusen, Germany, $Vic. Coll. Pharmacy (Monash Univ.) and Downie Metabolic Unit, Alfred Hosp., Melbourne, Australia

The uptake of L-triiodothyronine (L-T3) into somatic cells is an essential step in thyroid hormone action. Inhibitors of L-T3 uptake may be useful agents for the rapid treatment of thyroid hormone excess. The mechanism of L-T3 incorporation is not clear. An active receptor mediated process may be involved. Bicyclic aromatic compounds such as 4-phenyl-1.4-dihydropyridines (DHP) are potent inhibitors of L-T3 uptake in H4 rat hepatoma cells. Strongest is nitrendipine with an IC_{50}-value of 1.13×10^{-6} M. Inhibition of uptake is independent of action as calcium channel antagonists. The inhibitory potency of these compounds and newly synthesized DHPs was estimated in human Hep G2 hepatoma cells. Analysis of quantitative structure-activity relationships (QSAR) and quantum chemical *ab initio* calculations demonstrate that in comparison to L-T3 the DHPs have similar stereo- and physicochemical properties. Essential seems to be the perpendicular orientation of the ring systems of the DHPs to each other simulating the stereochemical arrangement of the L-T3 molecule. Rotation barriers of the DHP-derivatives were calculated to be 3.74-6.74 kcal. The molecular volumes of the basic structures of L-T3 (242.6Å^3) and the DHPs (258,50Å^3) are comparable. The intersection volume is 180.88 Å^3. Differences were obtained in respect to their electrostatic potentials. In contrast to L-T3 with a charge range of -24.42 - +17.94 the DHPs have a distinct negative surface potential caused by free electrons of ester group oxygen (nitrendipine: -106 to -90). Kind and position of substituents at the phenyl ring of the DHP-structure also influence the inhibitory potency. We investigated the interaction of a representative series of DHP-derivatives with a defined L-T3 binding site by computer-assisted substrate fitting to the X-ray structure of thyroxine binding globuline (TBG). Using molecular modeling strategies in conjunctions with systematic drug screening we developed a model for structural comparison of L-T3 uptake inhibitors and propose a pharmacophore for ligand interaction with the postulated L-T3 transport site.

494. P85 REGULATION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR BY THYROID HORMONE AND AMIODARONE IN HEPG2 CELLS.

F.Hudig and O.Bakker - Dept. of Endocrinology, Academic Medical Centre, University of Amsterdam, The Netherlands.

Amiodarone (AM), a potent antiarrhythmic drug, decreases plasma and tissue T_3 and increases plasma LDL cholesterol resembling the changes seen during hypothyroidism. The increase of LDL cholesterol during amiodarone medication is associated with a 50% decrease of the mRNA encoding for the LDL receptor (LDL-R) and a 70% decrease of the LDL-R protein in rat liver. In order to understand the molecular mechanism by which amiodarone and thyroid hormones regulate LDL-R gene expression, effects of T_3 and AM were studied in HepG2 cells. We selected this human hepatoma cell line because it expresses both T_3 receptors (low abundance) and the LDL-R. Methods HepG2 cells were cultured in 6 well plates in DMEM supplemented with 10% FCS, stripped of T_3 and T_4. Two types of experiments were conducted: a) cells were treated with 1 μM T_3 alone or in combination with 1 μM AM or 1 μM desethylamiodarone (DEA, the major metabolite of AM) for 48 hours whereafter mRNA for LDL-R was determined by northern blotting, b)transfection studies with pLDLrCAT-6500 (6.5 kB upstream region of the human LDL-R gene in front of a CAT reporter gene). Luciferase was used as internal standard. Cells were treated with several concentrations of T_3 with or without AM. Results HepG2 cells treated with 1 μM T_3 showed a 2 fold increase of LDL-R mRNA compared to nontreated cells. AM and DEA decrease LDL-R mRNA approximately 50% compared to cells treated with T_3 alone. Transfection studies with the 6.5 kB upstream region of the LDL-R showed an increased CAT expression in a dose-dependent manner (10^{-9}M, 2.5-fold increase; 10^{-6}M, 7.4-fold increase). 10^{-6}M AM combined with 10^{-7}M T_3 increased CAT expression 30-fold. Conclusions T_3 stimulates LDL-R mRNA expression. AM and DEA decreased this T_3 induced LDL-R mRNA in HepG2 cells. It is presently unknown if this decreased gene expression is the result of the recently described competition of the binding of T_3 to the T_3-receptor by DEA. Our experimental data suggest that the T_3 induced increase of LDL-R mRNA is mediated through a putative thyroid hormone response element present at the upstream region of the LDL-R.

495. P86 DEMONSTRATION OF THYROMIMETIC EFFECTS OF 3,5,3'- TRIIODOTHYRONINE SULFATE (T3S) IN EUTHYROID RATS.

INDER J. CHOPRA AND DAVIS NGUYEN, DEPARTMENT OF MEDICINE, UCLA SCHOOL OF MEDICINE, LOS ANGELES, CA 90024

We have previously demonstrated that T3S exhibits thyromimetic effects in hypothyroid rat and that, on a molar basis, its activity approximates 20% that of T3 (Endocrinology 133: 105, 1993). Since T3S is avidly deiodinated by 5'-deiodinase, type I (5'-DI) and 5'-DI activity is markedly reduced in hypothyroidism, it seemed possible that T3S is active only in hypothyroidism and not in euthyroid state wherein normal tissue 5'-DI activity will rapidly degrade T3S and little T3S will be left for metabolism (desulfation) to biologically active T3. This study was undertaken to test this possibility. We studied the effect of T3S (3.5, 10.5 or 31.5 µg/day x 7,i.p.) and T3 (0.6, 2.0, 6.0 µg/day x 7, i.p.) in groups of male Sprague-Dawley rats (5-6/group); control group was treated with saline i.p. The following parameters of thyroid hormone effects were examined: A=body weight, % change from baseline; B=serum TSH, ng/ml; C=serum T4, µg/dl; D=serum T3, ng/dl; E=hepatic 5'-DI, % of control; F=renal 5'-DI, % of control. Results are expressed as mean ± SEM.

		Parameter Under Study				
#	Treatment	A	B	C	D	E	F
I.	Control (saline)	8.3±2.7	4.8±1.3	2.3 ± 0.48	145±9.1	100±14	100±30
II.	T3S 3.5	6.5±2.2	2.4±0.54*	1.4±0.29	119±28	211±61	105±17
III.	T3S 10.5	10±1.4[a]	1.2±0.24*	0.40±0.10*	110±22	272±72*	101±21
IV.	T3S 31.5	20±4.6[b]	0.49±0.12*	<0.15 ±0*	149±42	286±67*	226±29*
V.	T3 0.6	4.0±2.5	0.95±0.28*	0.48±0.13*	61±11*	83±22	74±26
VI.	T3 2.0	0.92±3.0	0.94±0.25*	0.65±0.31*	126±27	378±172	138±35
VII.	T3 6.0	7.3±1.7	0.44±0.04*	<0.15±0*	68±22*	353±72*	266±53*

(*Cf. control, P<0.05; a Cf. group VI., P<0.05; [b]Cf group VII P<0.05))

Conclusions: T3S exhibits thyromimetic effects in euthyroid rats; just as in hypothyroidism, on a molar basis, biological potency of T3S approximates 1/5th that of T3. Biological effects of T3S may be due to T3 generated in tissues by desulfation of T3S.

496. P87 SOURCE OF TRIIODOTHYRONINE SULFATE (T_3S) IN MAN. JS LoPresti, SJ Eng, JT Nicoloff, Dept of Med, USC School of Med, Los Angeles, CA, USA

T_3S production has been estimated to represent up to 50% of T_3 disposal in man. Increased serum levels of T_3S have been measured in various conditions associated with the low T_3 state, including illness, fasting, and fetal life. Studies in the rat have shown that T_3S is likely produced from T_3 in the liver. However, the circulating source of T_3S has not been established. To address this issue, 3 euthyroid subjects received an IV bolus of $^{125}IT_3$ and ^{131}I Na with and without the concurrent PTU administration. Sequential, timed urinary $^{125}I/^{131}I$ ratios were determined to evaluate the deiodination patterns of T_3. Serum and urine $^{125}IT_3$ metabolite patterns were evaluated by HPLC analysis. Unexpectedly, $^{125}IT_3$ underwent minimal rapid deiodination as shown by the modest rise in the urinary $^{125}I/^{131}I$ appearance and was uninfluenced by PTU administration. In contrast, late deiodination reflecting extrahepatic metabolism was inhibited by PTU. HPLC analysis showed that T_3S could not be detected in the serum until 90 min and gradually increased for 1440 min after injection (T_3S/T_3 1.3 @ 90 min, 6.9 @ 1440 min) and that PTU did not change this pattern. Serum T_3S/T_3 ratio plateaued by 12 hrs. Urinary T_3 metabolites included T_2S, 3,3'-T_2 and diac. PTU administration allowed the detection of T_3S, which gradually increased with time (T_3S/T_3, 0-2 hr 3.3, 24-48 hr, 19.2). In addition, triac only could be identified in urine after PTU. Conclusions: 1) PTU influences only late deiodination suggesting that T_3 must first undergo extrahepatic metabolism to make it a better substrate for deiodination; 2) T_3S is likely produced from T_3 in extrahepatic tissues; 3) the early deiodination of T_3 is not mediated by PTU-inhibitable hepatic 5'-deiodinases; 4) presumably, the elevated T_3S levels associated with low T_3 states are secondary to inhibition of the hepatic 5'-deiodinase and the resultant slowing in clearance.

497. P88 DIFFERENTIAL EFFECTS OF HORMONES OF THE ADRENAL AXIS ON THYROID FUNCTION IN EMBRYONIC CHICKENS. V.M. Darras, S. Kotanen, K.L. Geris and E.R. Kühn, Zoological Institute, Catholic University of Leuven, Belgium.

In mammals glucocorticoids are thought to decrease thyroid function by decreasing thyroidal hormone release and/or by decreasing peripheral thyroxine (T_4) to triiodothyronine (T_3) conversion. In posthatch chickens too, glucocorticoid treatment seems to decrease circulating T_3 levels, but some reports on a stimulatory action in chicken embryos are existing as well. We have been studying the acute effects of glucocorticoids, namely corticosterone (CORT) and dexamethasone (DEX), but also of corticotropin (ACTH) and corticotropin releasing hormone (CRH) on peripheral thyroid hormone metabolism in 18-day-old embryonic chickens and compared it with the effect of thyrotropin (TSH).The treatment consisted of a single intravenous injection and the parameters followed were plasma T_4 and T_3 as well as *in vitro* outer ring deiodinating type I (ORD-I) and inner ring deiodinating type III (IRD-III) activity in hepatic and renal microsomes. In a first experiment DEX was found to decrease plasma T_4 after 4 and 24 hrs but not after 48 hrs, while an increase in plasma T_3 was present at each time point. Hepatic ORD-I activity was increased after 24 and 48 hrs while renal ORD-I activity was not significantly influenced. IRD-III activity was strongly depressed after 4 and 24 hrs in the liver and after 24 hrs in the kidney. When comparing the effect of different hormones 1, 4 and 24 hrs after injection, it was found that ACTH and DEX seemed to act in the same direction, with a decrease of plasma T_4 after 4 hrs and an increase in plasma T_3 after 1 and 4 hrs. Only the long acting DEX increased plasma T_3 after 24 hrs also. Both ACTH and DEX increased hepatic ORD-I after 24 hrs and decreased hepatic IRD-III after 1 and 4 hrs, while for DEX this decrease was still present after 24 hrs. The influence of CORT injection was less clear, maybe due to rapid degradation of the hormone, but there were similarities with the effects of DEX: decreased plasma T_4, increased plasma T_3, increased hepatic ORD-I and decreased hepatic and renal IRD-III. CRH, on the other hand, did not lower plasma T_4 at any time point but, like TSH, highly increased T_4 after 1 hr. The increase in plasma T_3 after 1 and 4 hrs was consistent with both TSH and ACTH action. The CRH effects on deiodination activity partly resembled the ACTH effects, including increase in hepatic ORD-I after 24 hrs and decrease in hepatic IRD-III after 4 hrs. We can conclude that in embryonic chickens glucocorticoids increase plasma T_3 by acutely decreasing peripheral IRD-III activity and increasing ORD-I activity somewhat later. The ACTH effects on peripheral deiodination seem to be mediated through its corticosterone releasing activity, while CRH seems to act also as a thyrotropic factor, probably by increasing TSH-induced thyroidal T_4 release.

498. P89 EFFECTS OF CYCLIC NUCLEOTIDES AND PHOSPHODIESTERASE INHIBITOR ON TYPE II IODOTHYRONINE 5'-DEIODINASE ACTIVITY IN CULTURED RAT BRAIN MIXED GLIAL CELLS. A.Gondou, Y.Ogawa and M. Yoshimura. Second Department of Internal Medicine, Kansai Medical University, Moriguchi, Osaka 570, Japan.

In the brain, intracellular T_3 which is converted from T_4 by type II iodothyronine 5'-deiodinase (5'D-II) plays a key role of thyroid hormone action. To analyse the regulatory factors that influence 5'D-II activity in the central nervous system, we have investigated the effects of dibutyryl cyclic AMP (DBC), 8-bromo-cyclic GMP (8-Br cGMP) and isobutylmethylxantine (IBMX) on 5'D-II activity. We used neonatal rat whole brain mixed glial cells cultured for 9 days. The homogenate of cultured cells had the activity of $^{125}I^-$ releases from [^{125}I] rT_3, which depended on temperature and amounts of protein. Basal 5'D-II activity of mixed glial cells was 1.9±0.2 (SE) fmol of I^- released/mg protein/h. When we performed the cell culture for 4 to 12 hours in the presence of 10^{-4} to 10^{-2}M DBC, it significantly augmented 5'D-II activity. The maximal effect was observed at 8 hour-incubation by 10^{-3}M DBC, the value being 20-folds of the basal level. 4 hour-incubation with 10^{-3}M 8-Br cGMP stimulated 5'D-II activity, being 9-folds of the basal level. 5'D-II activity was increased about 10-folds of the basal level by the 4 hour-stimulation of 10^{-3} M IBMX, which inhibits dissolution of cyclic nucleotides. When cultured for 4 hours with 10^{-4}M DBC and 10^{-4}M 8-Br cGMP, 5'D-II activity significantly increased about 4-folds of the basal level, but the additive effect was not observed. The incremental amount of 5'D-II activity by simultaneous addition of DBC, 8-Br cGMP and 10^{-3}M IBMX was more than that by the addition of DBC and 8-Br cGMP but was less than that by the addition of 10^{-3}M IBMX only. It is supposed that cGMP induces the increase of phosphodiesterase activity and that the phosphodiesterase inhibits the effect of cAMP for 5'D-II. These findings suggest that 5'D-II may influence brain function with the interaction of cyclic nucleotides and phosphodiesterase.

499. P90 CHANGES IN PROTEIN LEVEL AND ENZYME ACTIVITY OF PROTEIN DISULFIDE ISOMERASE/MEMBRANE-ASSOCIATED THYROID HORMONE BINDING PROTEIN. T.Mori, R.Gemma, H.Natsume, R.Horiuchi*, T.Yoshimi. Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; Gumma University School of Medicine*, Maebashi, Gumma, Japan.

Protein disulfide isomerase(PDI) is a microsomal multifunctional enzyme. It participates in post-translational modifications of secretory proteins by catalyzing the protein disulfide bond formation. It is also known that PDI is the membrane-associated thyroid hormone-binding protein, the function of which is still unknown. In addition, PDI acts as the subunit of prolyl-4-hydroxylase and of triglyceride transfer complex. Northern blot analysis and immunohistochemical study showed the wide distribution of PDI in various tissues. Since quantitative analysis of the PDI protein has not been reported, we first measured PDI protein contents in several rat tissues. Western blotting using the specific anti-PDI antiserum (Eur J Biochem 183:529,1989), but not with the control serum, detected the 55kD protein band. Among several tissues, the liver contained the largest amount of PDI protein, followed by the kidney where 1/3 to 1/5 of hepatic PDI protein existed. The PDI protein band was also detected in the heart, muscle and fat tissue, but not in the brain. Since fasting is known to affect some thyroid hormone binding proteins such as nuclear T3 receptor, we studied whether fasting would influence the PDI protein content and whether the change in the protein level, if any, would correlate with the isomerase activity in rat liver and kidney. Starvation for 3 days, but not one day, significantly decreased the PDI protein in the liver to 60% of the control. After re-feeding for 3 days, the PDI protein recovered to the control level. The same change was observed in the kidney also. On the other hand, the change in the PDI activity, measured by the method using "scrambled" ribonuclease as the PDI substrate, was not parallel to that of the PDI protein level. The isomerase activity decreased to 50-60% of the control during fasting in both liver and kidney, but different from the protein level, re-feeding did not recover the enzyme activity in either tissue. The results showed that the PDI protein level dose not always reflect the PDI enzyme activity. Since PDI is the multifunctional protein, the isomerase activity may be influenced by other PDI's function as subunits. Alternatively, the change in some co-factor(s) may be involved in the regulation of the enzyme activity.

500. P91 THYROID HORMONE CONCENTRATIONS AND SERUM T_4 BINDING CHARACTERISTICS IN THE KOALA (PHASCOLARCTOS CINEREUS)

Veronica Lawson, *Andrea J. Curtis, Frank Carrick, *John W. Barlow
Department of Zoology, University of Queensland, St. Lucia, Qld. Australia, 4072, and *Ewen Downie Metabolic Unit and Monash University Department of Medicine, Alfred Hospital, Melbourne, Vic. Australia, 3181.

We have previously shown that the thyroid gland of the marsupial koala (P. cinereus) is large in comparison to whole body weight. Electron micrographs revealed a moderate number of microvilli, large intercellular spaces, a moderate number of mitochondria, distended ergastoplasmic sacs and enlarged Golgi vesicles. Together with the low BMR of koalas, these features suggest glands of low activity in this species. In this study, we examined total and free T_3 and T_4 levels in sera of coastal and inland koalas and determined the serum T_4 binding characteristics.

Results: Mean total T_4 levels were 3.2 ± 2.1 nM, a value which is low in comparison to other marsupial and mammalian species. Levels were significantly higher in inland-dwelling females (4.6 ± 2.1 nM) than in coastal-dwelling males (2.0 ± 1.4 nM, $p=0.03$). Free T_4, 3.3 ± 2.1 pM was also low as were total T_3, 0.4 ± 0.2 nM and free T_3, 1.4 ± 0.9 pM. Ethanol extraction and re-assay confirmed the low measurements. No cross-reactivity of koala serum was found in a human TSH assay. Electrophoresis of [^{125}I]-T_4-labelled serum revealed two proteins of electrophoretic mobility the same as human transthyretin (TTR) and albumin. Scatchard analysis of [^{125}I]-T_4 binding, determined after overnight incubation at 4C in serum diluted 1:100 with a charcoal separation, gave a curvilinear plot which resolved into two binding sites with affinities identical to those of TTR (8.8×10^7 M^{-1}) and albumin (2.5×10^5 M^{-1}) but concentrations of 30 mg/L and 4 g/L respectively, which are low compared with other mammalian species. No higher affinity binding was detected.

Conclusions: Histologic features suggesting low thyroid gland activity in koalas are associated with low concentrations of free and total thyroid hormones and low concentrations of TTR and albumin. The mechanism of maintenance of euthyroidism in this species remains to be determined.

501. P92 EARLY EFFECTS OF NEONATAL DIABETES ON THYROID HORMONE DEPENDENT PARAMETERS IN THE RAT. D. van der Heide, J. P. Schroder-van der Elst*, P.M. Versloot, T.C. Viets, M.E. van Bakel, G.J.E.J. Hooiveld and I. H.C. Vos. Human and Animal Physiology, Agricultural University, Wageningen * and Endocrinology, University Hospital, Leiden, The Netherlands.

In earlier studies we showed that in streptozocin-induced diabetes mellitus (type I) in adult rats iodide uptake, thyroidal T_4 secretion and peripheral T_3 production are strongly decreased, leading to lowered thyroid hormone plasma and tissue levels. There is a shift in the metabolic pathways of thyroid hormones in liver and brain comparable to hypothyroidism.

In this study we investigated the early effect of neonatal diabetes. We measured the iodide uptake in vivo and thyroid hormone dependent parameters in rats made diabetic by an iv. injection with streptozocin (45 mg/kg bwt) at the day of birth. Female and male rats were used and compared with age- and gender matched controls, which received saline. After 40-70 days the iodide uptake in vivo was measured. Rats were injected iv with Na^{125}I (10 uCi) and the radioactivity in the thyroid was monitored continuously during 4 hrs with a gamma-probe located directly above the neck. ^{125}I activity in blood was measured and the disappearance curve was fitted in a mathematical model. Other rats were bled and perfused; organs were removed, homogenized, and subcellular fractions were prepared. The activities of malic enzyme (ME), alpha-glycerophosphate dehydrogenase (α GPD), deiodinase type I, II (ID-I, ID-II) and T_4-uridine-diphosphate glucuronyl transferase (UGT-T_4) were measured. In pituitary homogenates TSH, GH and prolactin were determined. In plasma T_4, T_3, rT_3, TSH and insulin levels were measured by specific rat RIA's. There is a normal increase in bodyweight. Clear differences in thyroid hormone parameters between female and male are present. The iodide uptake is lower in the female than in male rat. Plasma T_4 is lower and ID-I activity in liver is 50% lower in the female rat. In contrast the ID-I activity in kidney was the same. During DM iodide uptake as % dose ^{125}I at 4 hrs increased with 40%. Plasma T_4 decreased with DM-time, as did UGT-T_4 (liver) and ID-I (kidney) activities in males. In the liver of female rats αGPD and ID-I activities also decreased. The other parameters did not change significantly during this period of 40-70 days.

Most effects of neonatal DM could be detected after 55 days of treatment with streptozocin. The changes found were different from those found earlier in adult diabetic rats.

502. P93 **UPTAKE OF TRIIODOTHYRONINE(T_3) IN THE RAT KIDNEY IS INHIBITED BY MALEIC ACID.**

E.J. Rolleman, H.F. Bernard and M. de Jong. Depts. Internal Med. III and Nuclear Medicine, Erasmus University Medical School Rotterdam, The Netherlands.

Active transport of thyroid hormones (TH) is not only important for the intracellular action of these hormones, but is also rate limiting in T_4 to T_3 conversion. In many cell types active, stereospecific, ATP dependent, transport of TH has been identified. In kidney cells, which significantly contribute to T_4 to T_3 conversion, metabolism has been studied extensively, but less attention has been paid to TH transport. We reported carrier-mediated transport of TH into kidney cell lines[1]. After glomerular filtration, T_3 is reabsorbed in the proximal tubule, and therefore, in contrast to cells of other organs, tubule cells have two sites for uptake of T_3: the luminal and the peritubular site. We studied the uptake of T_3 in the rat kidney in vivo and the influence of maleic acid on this process. Maleic acid inhibits temporarily amino acid reabsorption in the proximal tubule by inhibiting the Krebs cycle, resulting in a lower ATP concentration. This lowered ATP concentration may also decrease energy-dependent T_3 uptake from the peritubular site. These effects of maleic acid are only present in tubular cells.

Methods: Adult male Wistar rats were placed in metabolic cages at time= -24 h. At time = 0 rats were injected with 125I-T_3(pM dose), with or without a single dose maleic acid (MA;400 mg/kg). After 0.5, 4 and 20 hours rats were sacrificed with ether and organs were isolated. Tissue distibution of the radioactive T_3 was studied by measurement of radioactivity and data were expressed as %injected dose (ID)/g tissue, mean ± SD. N≥2.

Table

	CONTROL %ID/g kidney	MALEIC ACID %ID/g kidney
0.5 hr	3.200 ± 0.113	2.160 ± 0.141 *
4 hr	1.223 ± 0.233	0.803 ± 0.057 #
20 hr	0.341 ± 0.059	0.274 ± 0.043

#: p<0.05; *: p<0.001 versus control

Results: At 0.5 and 4 hrs but not at 20 hrs a significant lower %ID/g in kidneys of MA treated rats was seen. (See Table). In all other organs (blood, liver, spleen, pancreas, thymus and thyroid) there was a time dependent decrease of %ID/g, but no significant differences were detectable between CON and MA rats. These results provide additional evidence for ATP dependent active TH uptake in the kidney in vivo. The effects of MA are temporary, indicating that the ATP concentration was normalized.

1) J.Endocrinol.Invest. 17(S):21;1994

503. P94 SIGNIFICANCE OF LIVER VISUALIZATION ON 131 I TOTAL BODY SCANS IN FOLLOW-UP OF THYROID CARCINOMA.

M. L. Maayan, G. C. Schussler, S. D. Sarkar, E. M. Lopez, and M. S. Axelrod, Veterans Affairs Medical Center and SUNY Health Science Center, Brooklyn, New York.

Partial thyroidectomy followed by large ablative doses of 131-I are sometimes used in the treatment of localized thyroid carcinoma. Smaller, but still fairly large, doses of 131 I are used for whole body scanning at intervals to detect functional metastases. We present three patients sumbitted to partial thyroid-ectomy for non iodide concentrating carcinomas in whom the liver was clearly visualized after administration of 131 I, either for treatment or for follow-up total body scintigraphy. Aside from uptake in the thyroid remnants there was no other localization of activity in the whole body scan. Liver metastases were ruled out by complementary planar and SPECT liver scintigraphy with 99m Tc Sulfur Colloid, Abdominal CT, and MRI. Liver function tests were normal. At the time of scintigraphy almost all of the 131 I activity was protein bound and chromatographically identical to thyroxine.

The ability of the liver to retain a major portion of the extrathyroidal thyroxine is well known. However, unless the correspondence of the diffuse hepatic uptake of T4 with the dimensions of the liver is recognized, it could be mistaken for uptake by metastases. Since 131 I - iodide is not concentrated by the liver, hepatic visualization during the whole body 131 I scan implies synthesis of organic iodide, predominantly T4, and therefore the existence of a thyroid remnant and functioning metastases.

It is of physiological interest that, in what is essentially a steady state distribution, the only visualized accumulation of 131 I T4 at levels above background is in the liver. This is in contrast to recent studies demonstrating predominant localization of T4 in the intestine of the rat. Although hepatic accumulation of T4 seems appropriate to the critical role of the liver in T4 metabolism, the physiological basis of this accumulation, particular subcellular localization, and the relative importance of active vs. passive accumulation, is uncertain.

504. P95 LACK OF EFFICACY OF REVERSE T3 FOR DIFFERENTIATING BETWEEN HYPOTHYROIDISM AND EUTHYROIDISM IN ILLNESS. L. A. Burmeister. University of Pittsburgh School of Medicine, Pittsburgh, PA.

To assess the efficacy of reverse T3 for differentiating between the hypothyroid and euthyroid state in the setting of illness a retrospective review of all reverse T3 determinations obtained between 1991-December, 1994 in a University teaching hospital system was analyzed. A total of 262 reverse T3 determinations (Nichol's Institute Reference Laboratory) was made in 246 patients. Thyroid function tests, bilirubin, albumin and creatinine within one day of reverse T3 were analyzed to determine thyroidal state, appropriateness of reverse T3 determination and determinants of reverse T3. An inverse linear relationship between the log TSH and reverse T3 was demonstrated. Patients with hypothyroidism plus illness may have a normal reverse T3 and patients with euthyroidism plus illness may have a low reverse T3. Reverse T3 is linearly related to bilirubin up to a bilirubin of approximately 171 μM (10 mg/dL). Sixty percent of the reverse T3 determinations were obtained for seemingly inappropriate indications. In association with a low free T4 index and or T4, an unmeasurable reverse T3 did not lead to institution of thyroid hormone treatment in over 65% of cases. Conclusions: Although reverse T3 may be elevated in the setting of nonthyroidal illness, it is not reliable in distinguishing between the ill hypothyroid and the ill euthyroid patient. This is probably because of differential drug and disease effects on thyroid hormone metabolism in euthyroid sick patients as well as the presence of sufficient T4 substrate for conversion to reverse T3 in many hypothyroid sick patients.

505. P96 RECOMBINANT CHICKEN PROLACTIN DECREASES PLASMA CONCENTRATIONS OF THYROXINE AND INCREASES HEPATIC INNER RING DEIONATING TYPE III ACTIVITY IN THE CHICK EMBRYO. E.R. Kühn[1], K. Shimada[2], L.M. Vleurick[1], L.R. Berghman[1] and V.M. Darras[1], [1] Zoological Institute, Catholic University Leuven, Belgium and [2] Faculty of Agriculture, Nagoya University, Japan.

In the chicken hepatic growth hormone receptors do not distinguish between chicken growth hormone (cGH) and ovine prolactin (oPRL) (1). In order to study the biological effects of chicken prolactin (cPRL), it was therefore essential to rule out any possible binding of this hormone to the GH receptors. Displacement studies on hepatic microsomes of both embryonic and adult chickens confirmed that parallel inhibition curves can be obtained with native cGH, recombinant cGH, native oGH and native oPRL. Recombinant cPRL (rcPRL), on the contrary, was not able to compete with radiolabeled cGH for receptor binding. In a series of experiments rcPRL and immuno-affinity purified cGH were injected intravenously in 18-day-old chicken embryos and their influence on plasma concentrations of triiodothyronine (T_3) and thyroxine (T_4) as well as on *in vitro* hepatic and renal outer ring type I (ORD-I) and inner ring type III (IRD-III) deiodination was studied. cGH increased plasma T_3 by inhibiting hepatic and eventually also renal IRD-III activity and consequently T_3 degradation, whereas ORD-I activity was not influenced, confirming previous results. The observed decrease in plasma T_4 may be explained by a negative feedback exerted by T_3 on pituitary thyrotropin secretion. These effects were dose dependent, the lowest dose used being 0.4 µg cGH, whereas a maximal response was obtained with 2 and 10 µg cGH. The rcPRL preparation (2 and 10 µg) did not influence plasma T_3, but depressed plasma T_4. At the same time hepatic, but not renal IRD-III activity was increased. No influence on hepatic or renal ORD-I activity could be found. These results indicate that prolactin may have a role, together with GH, in controling peripheral thyroid hormone metabolism. (1) Leung *et al.* (1984), Gen. Comp. Endocrinol. 56: 389-400.

506. P97 **MARKERS OF BONE METABOLISM IN PATIENTS WITH GRAVES' HYPERTHYROIDISM AND THE EFFECTS OF ANTI-THYROID DRUG THERAPY.** S-W Kuo*, W-K Liao and S-L Su. Division of Endocrinology and Metabolism, Tri-Service General Hospital, Taipei, Taiwan 100.

Hyperthyroidism is a well-known cause of altered bone metabolism, characterized by an increase in both osteoclastic and osteoblastic activities with a predominance of bone resorption and resulting in decreased bone mass. In this study, we used several biochemical markers of bone turnover to investigate the bone disorders associated with thyroid disease. Markers of bone formation (serum osteocalcin [OC] and carboxyterminal propeptide of type I procollagen [PICP]) and bone resorption (cross-linked carboxyterminal telopeptide of type I collagen [ICTP] and the urinary excretion of deoxypyridinoline [D-Pyr]) were measured in 25 patients with Graves' hyperthyroidism (M/F = 10/15, age 25.2 $\pm$ 7.3 years) before and after anti-thyroid drug therapy and in 15 age-matched controls. Hyperthyroid patients had higher levels ($p < 0.001$) of all 4 markers compared with control values: OC - 2.4 $\pm$ 1.2 vs 0.9 $\pm$ 0.2 ng/ml; PICP - 196 $\pm$ 87 vs 89 $\pm$ 42 *u*g/L; ICTP - 9.7 $\pm$ 2.9 vs 6.1 $\pm$ 2.7 *u*g/L; D-Pyr - 326 $\pm$ 135 vs 46 $\pm$ 36 nmol/mmol creatinine. The markers of bone resorption were significantly decreased ($p < 0.001$) after anti-thyroid drug therapy, but the markers of bone formation showed variable response with a small but insignificant decrease after anti-thyroid therapy. Serum ICTP and urine D-Pyr had good correlation ($r = 0.720$, $p < 0.001$); both markers correlated well with serum FT_4 levels ($p < 0.001$, respectively). In addition, serum OC correlated well with PICP ($p < 0.05$), but neither correlated with serum FT_4 levels. In conclusion, serum ICTP and urine D-Pyr are highly sensitive markers for altered bone metabolism in Graves' hyperthyroidism.

507. P98 EFFECTS OF THYROID HORMONE ON CARBONIC ANHYDRASE I CONCENTRATION IN HUMAN ERYTHROLEUKENIC CELLS AND ERYTHROID BURST FORMING UNIT DERIVED CELLS. Y. Kiso, K. Yoshida, N. Sayama, H. Fukazawa, K. Kikuchi, Y. Aizawa, H. Hori, K.Abe. The Second Department of Internal Medicine and Department of Clinical Biology and Hormonal Regulation, Tohoku University School of Medicine, Sendai, Japan

Red blood cell carbonic anhydrase I (CAI) concentrations are decreased in patients with hyperthyroidism, although the mechanism responsible for this effect is not known. We have recently shown that individuals with hyperthyroidism have red blood cell CAI concentrations which reflect the integrated serum thyroid hormone concentration over the preceding few months, and that T3, at a physiological free concentration, decreased CAI concentrations in human erythroleukemic cell line (YN-1). In the present study, we studied the effect of T3 on CAI concentrations in other erythroleukemic cells and burst forming unit-erythroid (BFU-E)-derived cells obtained by culturing peripheral blood mononuclear cells (PBMCs). Erythroleukemic cells, YN-1, Y1K, HEL and KU-812, were cultured in Iscove's modified Dulbecco's medium (IMDM) with 10% FCS in the presence of T3 (10^{-10}-10^{-7} mol/l) for 4 days. FCS was pretreated with 0.1% dextran and 1% charcoal. After the treatment, T3 concentration of FCS was less than the sensitivity of the assay (0.23 nmol/l). Heparinized venous blood was drawn from 5 normal volunteers. PBMCs were isolated on a Ficoll gradient and cultured in IMDM containing 30% FCS, 0.8% methylcellulose, and various cytokines including erythropoietin for 14 days. Hemoglobin synthesis was assessed by benzidine. The CAI concentration was measured by RIA. Mean CAI concentrations of YN-1, Y1K, HEL and KU-812, cultured in T3 free medium, were 1.3, 1.3, 2.3 and 5.1 ng/10^4 cells, respectively. $3x10^{-9}$ mol/l or more of T3 significantly decreased the CAI concentration in YN-1 cells, as previous reported. However, T3 had no effect on CAI concentrations in other erythroleukemic cells. BFU-E-derived cells contained a high concentration of CAI (10.3 ng/10^4 cells). T3 at a concentration of 10^{-8} mol/l significantly decreased the number of BFU-E-derived cells, and at a concentration of $3x10^{-10}$ mol/l significantly decreased CAI concentration. T3 decreased CAI concentration in a concentration-dependent manner (29, 41 and 55% decreases at $3x10^{-10}$, $1x10^{-9}$ and $3x10^{-9}$ mol/l). The free T3 concentration in medium containing 30% FCS and $1x10^{-9}$ mol/l of T3 was $9.4x10^{-12}$ mol/l. These results suggest that YN-1 and BFU-E-derived cells may be used to study the effect of T3 on human red blood cell CAI. This BFU-E system may prove useful in the tissue diagnosis of resistance to thyroid hormone.

508. P99 FUNCTIONAL PROPERTIES OF MUTANT T3 RECEPTOR(R338W) IDENTIFIED IN PATIENTS WITH PITUITARY RESISTANCE TO THYROID HORMONE. H.Nakamura, S.Sasaki*, S.Andoh, T.Tagami*, K.Nishiyama, R.Kitahara, T.Yoshimi. Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu; Kyoto University School of Medicine*, Kyoto, Japan.

Resistance to thyroid hormone(RTH) is clinically classified into generalized (GRTH) and pituitary (PRTH) types. Since similar mutations have been identified in T3 receptor(TR)β gene in GRTH and PRTH, they are now considered to be a continuous spectrum with the same genetic defect. R338W identified in a PRTH-patient in our laboratory is interesting, since this mutant has been found in several other patients with PRTH. To clarify how R338W induces clinical features presenting PRTH, we investigated the functional properties of this mutation.

The function of R338W was compared with that of a GRTH-mutant(m)TR, K443E. The levels of thyroid hormones and inappropriately elevated TSH (SITSH) were similar between subjects with K443E and with R338W. When reporter gene containing TRE-pal2, DR4 or MHCα was used, the transcriptional activity of R338W was higher than that of K443E on any TREs. The dominant negative effects on wild TRβ1 or wild TRα1 were significantly milder in R338W than in K443E. Dominant negative activity on TRE-TSHα subunit was also milder in R338W than in K443E. T3-binding activities of mTRs expressed in the cells were remarkably reduced with no difference. Interestingly, gel shift assay revealed poor homodimer formation of R338W on DR4-TRE. Heterodimerization with RXRα was similar among R338W, K443E and wild TRβ1. We next introduced a R338W-mutation into mTR-K443E and also mTR-F451X. F451X is a truncated mTR with 11 amino acid deletion from the extreme C-terminus and has very strong dominant negative activity. Both double-mutant mTRs, R338W/K443E and R338W/F451X, showed negligible T3 binding and transcriptional activities. Introducing a R338W-mutation, however, changed the dominant negative activity milder; the inhibitory activities of R338W/K443E and R338W/F451X were significantly weaker than those of K443E and F451X, respectively.

In summery, under the present assay conditions, dominant negative activity of a PRTH-mTR was mild. This may be related with its weak homodimer formation. Introducing a R338W-mutation into a mTR changed the dominant negative activity significantly weaker. Mild dominant negative activity may be one of the factors necessary for presenting PRTH-phenotype.

509. P100 TISSUE DISTRIBUTION OF HUMAN T_3 INDUCIBLE GENE (I-4) PRODUCTS AND THE EFFECT OF THYROID AND GLUCOCORTICOID HORMONES ON ITS EXPRESSION.
T. Miyazaki, Y. Murata, S. Refetoff*, and H. Seo. Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-01, Japan and The University of Chicago*, Chicago, IL. 60637, U.S.A.

In order to study thyroid hormone action in human tissues, it is important to identify T_3 responsive genes. Using differential display of mRNAs, we have recently cloned a T_3 inducible gene (I-4) in cultured human skin fibroblasts. A nucleotide sequence of I-4 cDNA fragment did not show any homology with known cDNAs. To study the tissue distribution of this gene product, two microgram poly (A) RNA from human tissues, lung, liver, brain, heart, kidney, pancreas, skeletal muscles and placenta were subjected for Northern blot analysis with high stringency using I-4 cDNA as a probe. I-4 mRNA was detected in brain, skeletal muscle, heart and liver. The size of the transcript varied among the tissues. In skeletal muscle, dominant band was about 3 kb in size and bands of 1.4 and 0.8 kb were also observed but were less abundant. The mRNAs of 1.4 and 0.8 kb were also expressed in heart and liver, but the mRNA of 3 kb was absent. In brain, only the band of 1.4 kb was observed. None of these bands were detected in lung, pancreas, kidney and placenta. We next examined how thyroid and glucocorticoid hormones regulate I-4 mRNA in human skin fibroblasts. Confluent cultures of fibroblasts from normal subjects were incubated in the medium supplemented with 10% thyroidectomized bovine serum for 3 days in the absence or presence of various concentrations of T_3, 10^{-8}M dexamethasone (DEX) or both together. The content of the 3 kb I-4 mRNA increased with physiological concentration of T_3 (10^{-10}M to 10^{-9}M for this culture condition). Maximal induction by T_3 was approximately 5- to 7-fold. On the other hand, DEX suppressed the expression of I-4 mRNA to almost one half of the control level. DEX also attenuated the induction of I-4 mRNA by T_3 when DEX and T_3 were added together. In order to examine whether the increase in I-4 mRNA by T_3 is the result of enhanced transcription or the increased stability of the mRNA, actinomycin D was added to the culture pretreated with 10^{-7} M T_3 for 3 days and further incubated for 24 hours. T_3 had no effect on I-4 mRNA stability and thus T_3 likely increases I-4 mRNA by enhancing its transcriptional activity. Results of the present study indicate that I-4, a newly identified T_3 inducible human gene is expressed in various tissues and the expression is regulated by both T_3 and DEX in opposite directions. Elucidation of the structure and the function of I-4 gene product could reveal important and unrecognized T_3 and glucocorticoid functions in the target tissues.

510. P101 POST-TRANSCRIPTIONAL REGULATION OF EGF mRNA: AU-BINDING PROTEINS AND OTHER TRANSACTING FACTORS INTERACT WITH THE 3' UTR OF EGF TRANSCRIPTS. Lowell G. Sheflin, Elizabeth M. Brooks, and Stephen W. Spaulding. Buffalo VAMC and SUNY at Buffalo, Buffalo NY 14215

Our recent studies on the hormonal regulation of the diverse transcripts of EGF present in the salivary and thyroid glands of male mice have established that several polyadenylation sites are used, that there are tissue-specific differences in the length of the poly-A tail attached at the most distal polyadenylation site in the 3' untranslated region (3' UTR), and that prolonged thyroid hormone treatment increases the stability of the form with the longer tail, which is only found in the salivary gland and not the thyroid. Another possible regulatory feature is also present in the 3' UTR, namely multiple AUUUA sequences, which are found between the two major polyadenylation sites in EGF. Such AUUUA elements are involved in regulating the stability for a number of other cytokines and growth factor RNAs, and can involve several different cytoplasic AU-binding factors (AUBFs), which have been detected by RNA gel-shift assays and/or UV cross-linking in different cells. The conservation of these AUUUA elements in the 3' UTR of the mouse, pig, rat and human EGF genes suggests that this sequence may be involved in a common regulatory mechanism, so we have begun to study whether AUBFs play a role in regulating EGF transcripts via AUUUA elements.

We prepared various radiolabeled 3' UTR RNA probes, ranging from an upstream probe that lacks the AUUUA elements, to a probe encoding only the final polyadenylation signal sequence up to the beginning of the poly-A tail, as well a probe encompassing the entire 3' UTR. We then preincubated the probes with various concentrations of cytosol and performed RNA gels-shift assays using 4% PAGE. This RNA gel-shift assays show that the RNA probes which contained the AUUUA elements displayed the greatest retardation when bound to cytoplasmic extracts from the salivary gland. Cytoplasmic extracts run on 8% denaturing PAGE hybridized with downstream probes containing the AUUUA elements revealed a 35 kD band which was 5 times more prominent in cytosolic than in polyribosomal preparations (per mg protein), and was not detectable in nuclear extracts. With the entire 3' UTR as probe, two higher molecular weight binding proteins were also prominent (about 60 and 75kD) along with three distinct bands of 35 to 38 kD. The most 3' probe detected a unique band of about 45 kD. Since the bands around 35-38 kD have the size and subcellular distribution of AU-binding proteins reported in several other tissues, this suggests that AUBF are involved in binding to the 3' UTR of EGF mRNA. Thus, in addition to the regulation of poly-A tail length by thyroid hormone, AUBF and possibly other transacting factors may also interact with cis-acting elements in the 3' UTR of EGF transcripts. These AUBFs may affect transcript stability, but may also play a role in determining polyadenylation site selection.

511. P102 A CARBOXY TERMINAL PEPTIDE FROM THE THYROID HORMONE RECEPTOR β1 BLOCKS T3 BINDING TO THYROID HORMONE RECEPTORS AND INDUCES CONFORMATIONAL AND DNA BINDING CHANGES SIMILAR TO T3. L.R. Goldberg, M. Abboud, L.D. Madison, Northwestern University Medical School, Chicago, IL

The COOH terminus of the thyroid hormone receptors (TR) is involved in critical aspects of receptor function. Its sequence suggests that it has an amphipathic α helix structure with hydrophobic residues opposite acidic residues. The chicken oncogene, v-erbA, has a deletion of nine COOH-terminal amino acids, and is unable to bind T3 or transactivate. The COOH terminus of the β isoform is one of two "hot spots" for mutations in the syndrome of resistance to thyroid hormone. The mutations in the COOH terminus have only a mild to moderate effect on T3 affinity but create strong dominant negative activity in the mutant receptors. This suggests that the COOH terminal amino acids are not directly involved in ligand interactions, but rather stabilize a receptor conformation induced by ligand binding elsewhere in the TR. A synthetic peptide consisting of ELFPPLFLEVFED derived from the 13 COOH terminal amino acids of TRβ1 was synthesized. The effect of the peptide on T3 binding affinity was studied using a filter binding assay, ^{125}I-T3, and TRβ produced in *E. coli* . The peptide reduced ^{125}I-T3 binding with an IC_{50} of between 1 and 10 μM. Scatchard analyses suggested that the peptide was a non-competitive antagonist, reducing apparent receptor number but not affinity. Two control peptides, TRβ 380-400, and a peptide with the identical composition as the inhibitory COOH peptide but in random order, did not affect ^{125}I-T3 binding. The peptide also inhibited ^{125}I-T3 binding to the α isoform of the TR produced in *E. coli*, to *in vitro* translated TRα and TRβ, and to a mixed receptor pool found in GH4 cell nuclear extracts. ^{125}I-T3 binding to an anti-T3 antibody was not affected by the COOH peptide suggesting that; 1) there was not an interaction between the peptide and T3 and 2) the inhibitory effect of the peptide was receptor specific. In order to determine the consequences of peptide interaction with the receptor, limited proteolysis and gel mobility shift studies were done. Limited proteolysis revealed that the peptide produced a conformational change in the receptor similar to that seen with T3. In a gel-shift assay, using an inverted repeat of AGGTCA (LAP), the peptide disrupted TR homodimer formation but minimally affected heterodimer formation with RXR, similar to changes caused by T3. These results indicate that the COOH peptide interacts with other critical domains in the TR, that the presence of excess COOH peptide induces a receptor conformation similar to that induced by T3 alone, and that the receptor conformational change precludes subsequent ligand binding.

512. P103 EXPRESSION OF THYROID HORMONE RECEPTOR SUBTYPES α1, α2 AND β1 IN THE LIVER OF THE FASTING RAT. O. Bakker, H. Razaki, C. Ris-Stalpers*, Departments of Endocrinology and *Pediatric Endocrinology, Academic Medical Centre, Meibergdreef 9 1105 AZ Amsterdam, The Netherlands.

A phenomenon accompanying most systemic illnesses and fasting is non-thyroidal illness (NTI), characterised mainly by low T_3, high rT_3 serum levels. There is also a reduction in the expression of some T_3-dependent genes that cannot always be overcome by increasing serum T_3 levels to normal with exogenous T_3. So other mechanisms must be involved. Our hypothesis is that the dominant negative variant of the thyroid hormone receptor (T_3R), $T_3R\alpha 2$, is (partly) responsible for this persistent downregulation during NTI. The aim of this study is to measure the mRNA levels of the T_3R subtypes in the liver of rats that are fasted for 0, 8, 12, 24 and 48 hours. At these times livers were excised and processed to prepare polyA+-RNA. The mRNA was reverse transcribed to obtain cDNA. The method we use to look at the specific mRNA levels in the livers of these rats (n=3), is a competitive PCR technique in which we compare the levels in a semi-quantitative manner by looking at the change in the ratio of the mRNA and competitive fragment in time. We constructed the competive fragments such that they were either shorter (α1 and α2 by 82 bp) or longer (β1 by 47 bp) then the product arising from the mRNA (α1, 345 bp; α2, 240 bp; β1, 175 bp). RNA input was corrected for using β-actin primers. Fragments were separated on 6% polyacrylamide gels, the gels stained with ethidium bromide and then photographed. The negatives were scanned to obtain the data with which we could calculate the ratio of competitor fragment to mRNA fragment. The results we obtained were that the α2 mRNA level increases gradually over time during fasting to about a 5-10 fold increase at 48 hours. A less than 2-fold downward change is seen in the β1 mRNA level. No change was observed in the α1 mRNA level. In conclusion, fasting is associated with a marked increase in $T_3R\alpha 2$ and a slight decrease in $T_3R\beta 1$ mRNA in rat liver.

513. P104 CHANGES IN PORCINE HEPATIC THYROID RECEPTOR RNA DURING EXTENDED EXPOSURE TO THERMAL EXTREMES

H.L. Reed, J. Anderson, Y.Y. Djuh, F. Carr. Departments of Clinical Investigation and Medicine, Walter Reed Army Medical Center, Washington D.C. 20367-5001.

Swine exposed to cold (4°C) air for 25 days (d) have an increased triiodothyronine (T_3) plasma appearance rate (PAR) and total body T_3 hormone pool (Q) when compared to controls (22°C) [Reed et al. *Am J Physiol* 266:E786,1994]. To better understand the tissue responses of these kinetic changes, we studied 6 six-month old boars living 50 d at 4°C and contrasted them with 6 control (22°C) and 4 heat-exposed (36°C) animals. As we previously reported for the cold-exposed and control animals [Reed et al. *FASEB J* 6:A1830, 1992] noncompartmental (NC) $^{125}I[T_3]$ kinetics were measured following bolus injection in the heat-exposed animals. In the present report we extend our findings to the heat-exposed animals and the characterization of RNA for thyroid hormone sensitive hepatic proteins. We used frozen liver tissue from each of the animals as well as a control T_3 treated rat to extract total RNA. cDNA probes from rat, for malic enzyme (ME), and both α (TRα) and β (TRβ) thyroid receptor forms were random primer labeled with ^{32}P dCTP and hybridized with purified total RNA (25-100μg) using both Northern and Dot blot techniques. The T_3 treated rodent controls were used to confirm swine and rodent RNA homology for these probes. *Results:* The cold-exposed swine showed a doubling of the NC T_3 PAR over controls ($p<0.006$) while heat-exposed animals tended to have ~20% decrease in PAR compared to controls. NC T_3 Q was doubled ($P<0.005$) in cold exposed animals while heat-exposed animals were not different than controls. Serum T_3 was not different between cold-exposed and control but the heat-exposed animals showed ~25% decrease below control values ($p<0.02$). Hepatic ME RNA levels were similar in heat-exposed and control swine. With cold-exposure ME RNA increased ~30% but did not achieve statistical significance. TRα and TRβ were not altered by heat-exposure. In contrast, TRβ RNA decreased by 27% ($p<0.02$) in cold-exposed animals without a change in TRα. *Conclusion:* Thyroid hormone responsive hepatic proteins appear more affected by cold than heat exposure and this is in general agreement with *in vivo* kinetic data for total hormone stores and production rates.

514. P105 **Human granulosa cells and oocytes express multiple thyroid hormone receptor isoform mRNAs.** **S.S. Zhang, A.J. Carrillo, D.S. Darling, Dept. of Biological and Biophysical Science, and Dept. of OB/GYN, University of Louisville, and Alliant Health System, Louisville, KY, USA.**

Both T3 and T4 are present in human follicular fluid, raising the question of whether the granulosa cells, or oocytes, are responsive to thyroid hormone. We investigated expression of four TR isoform mRNAs in mural granulosa cells (primarily from the follicle wall), cumulus granulosa cells (adjacent to the oocyte), and failed-fertilized oocytes by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). All the procedures followed the guidelines of the Human Study Committee. Mural granulosa cells were obtained at the time of oocyte retrieval from consenting patients undergoing in vitro fertilization at Alliant Health System, Louisville, KY. Cumulus cells were physically separated from oocytes after in vitro fertilization. Oocytes that did not develop visible pronuclei within 40 hours after insemination were classified as "failed-fertilized" and were used for RT-PCR. Total mRNA was extracted with phosphate cell lysis buffer containing 0.02% DEPC, followed by reverse transcription to cDNA. PCR reactions were performed with cDNA from 5,000 granulosa cells, or 1 to 2 oocytes, for every 50 µl reaction. PCR amplification was conducted for 45 cycles, which included denaturation at 94 C for 30", annealing at 55 or 59 C for 45" and extension at 72 C for 45". The specific PCR primers, and probes for southern blots, were designed with Oligo Primer Analysis Software. A pair of oligonucleotides for amplification of human β actin served as a positive control. Identification of the amplified products was based on observing the predicted size by agarose gel electrophoresis, and on southern blot hybridization. RT-PCR and southern blot results demonstrate that human mural and cumulus granulosa cells express TRα1, TRβ1, TRβ2, and erbAα-2 isoform mRNAs. There was no significant difference indicated by image density analysis, when comparing the amount of TR mRNA expressed by mural and cumulus cells from the same patient. Failed-fertilized oocytes express both TRα1 and erbAα-2. No amplified products from granulosa cells or oocytes were detected in controls with single oligos, or by PCR without prior reverse transcription. Conclusion: Both oocytes and granulosa cells express mRNA for multiple TR isoforms.

515. P106 DEMONSTRATION OF INHIBITION OF THYROXINE BINDING TO BOTH THYROXINE BINDING GLOBULIN AND FDH-ALBUMIN IN A PATIENT WITH FAMILIAL DYSALBUMINEMIC HYPERTHYROXINEMIA (FDH). B.N. Premachandra, J. Wortsman and I.K. Williams. VA Medical Center and Washington Univ., St. Louis, MO. and SIU School of Medicine, Springfield, IL.

A hypothyroid female with familial dysalbuminemic hyperthyroxinemia (FDH) displayed unusual free thyroxine (FT_4) responses to moderate thyroxine (T_4) replacement therapy. Her initial thyroid function tests were compatible with hypothyroidism and FDH [T_4 = 78 nmol/l (normal range = 58-154), triiodothyronine (T_3) = 1.08 nmol/l (1.23-3.08), FT_4 = 11.6 pmol/l (11.6-34.7), thyroid stimulating hormone (TSH) = 45 mU/l (0.3-5.0)]. When she was initially treated with T_4 (0.088 - 0.112 mg/day) there was an increase in percent dialyzable (free) T_4 resulting in FT_4 elevation to hyperthyroid levels accompanied by TSH inhibition (FT_4 = 51 pmol/l, TSH = < 0.03 mU/l); the patient also complained of intolerance and nervousness and T_4 treatment was discontinued. When T_4 therapy was later resumed, at a dosage of .075 mg/day she again showed a marked increase in percent dialyzable T_4 (0.02; pretreatment 0.015, $p < .05$). The elevation in percent dialyzable T_4 during moderate T_4 replacement in a patient with FDH is unusual. FDH albumin normally acts to buffer increase in serum FT_4 level by dampening the increase in percent dialyzable T_4, i.e. despite increase in circulating T_4 levels, FT_4/T_4 ratio remains constant prior to half-maximal saturation of FDH albumin which is not attained during conventional T_4 therapy [this has been demonstrated in our previous studies in the patient's son (who also had FDH) where T_4 treatment with 0.6 mg T_4/day failed to alter percent dialyzable T_4 (0.013)]. Thyroxine binding globulin (16.9 mg/l) and thyroxine binding prealbumin (280 mg/l) levels were normal. The presence of an inhibitor which reduced T_4 binding to both TBG and FDH albumin probably explains the elevation in percent dialyzable T_4 during T_4 treatment. This inference is made on the basis that if the inhibitor prevented T_4 binding to only one protein (TBG), the binding of T_4 to the other protein (FDH albumin would have suppressed the increase in percent dialyzable T_4, and this clearly was not evident. This FDH patient represents the first case of a putative inhibitor of T_4 binding to both TBG and FDH albumin. The inhibition of T_4 binding by these disparate proteins suggests that the inhibitor effect is mediated nonspecifically.

516. P107 CHANGES IN GLUT2 IN THE LIVER OF HYPERTHYROID RAT (STUDY ON GLUCOSE TRANSPORTER IN SECONDARY DIABETES.)

Takehiko Mokuno, Keiko Shimazaki, Yasutoshi Itoh, Naohisa Oda, Yuko Nishida, Kiyosi Asano, Nobuki Hayakawa, Michiko Hamada, Rumi Masunaga, Seiya Kato and Tetsuya Kawabe.
Dept. of Internal Med. Fujita Health Univ. School of Med., Toyoake, Aichi, Japan.

Impaired glucose tolerance is frequently observed in hyperthyroidism. An increase in liver glyconeogenesis is considered to be one of the causes of impaired glucose tolerance. Glucose transporter 2(GLUT2),the localization of which is known in the liver and pancreas, may be involved in impaired glucose metabolism. Thus we examined the changes of GLUT2 in the liver of hyperthyroid and hypothyroid rats using the GLUT2 antibody. The rats received subcutaneous injections of 200μg of l-thyroxine (T4), or 1mg of 1-methyl-2-mercaptoimidazole (MMI) every day for one month. Plasma membranes (PM) were isolated from the homogenized liver tissues by density gradient centrifugation. PM was electrophoresed in SDS-PAGE and GLUT2 in PM was estimated by Western blotting. Liver GLUT2 was increased in hyperthyroid rats, but was not significantly changed in hypothyroid rats. These findings suggest that the increase of liver GLUT2 may partly causes secondary diabetes mellitus induced by hyperthyroidism.