SUMMARY

p-Acetamidobenzaldehyde thiosemicarbazone (T.B.1) has been used in the treatment of leprosy since September 1950.

Experience in 71 patients treated between five months and thirteen months is described. Apart from 1 case of acute agranulocytosis, no serious toxic effects were seen. The treatment was well tolerated, and complications of treatment were few and usually not severe. Clinical and bacteriological response was satisfactory.

T.B.1 treatment is compared with sulphone (D.A.D.P.S.) treatment. The results of the two treatments appear similar. Allergy, shown by drug fever and dermatitis, has been rarer and much milder with T.B.1: complications of treatment ("reaction," eye inflammation, neuritis, &c.) have been fewer and milder than with D.A.D.P.S.

If agranulocytosis is found to be rare, T.B.1 should be a valuable alternative treatment to sulphone. It is much less simple to give, however, and is more expensive. At present its use should be confined to selected inpatients of leprosy institutions, patients in whom the maintenance of sulphone treatment presents difficulties. It seems to be much less widely practicable than sulphone treatment, though it has certain definite advantages.

The work here recorded was carried out by the research unit of the Nigeria Leprosy Service; thanks are due to the Director of Medical Services (Dr. S. L. A. Manuwa) and to the Assistant Director Leprosy Control (Dr. R. H. Bland) for permission to publish.

ADDENDUM

Since this article was written the period of treatment has been extended to seventeen months, and the number of patients is now 126. No further case of agranulocytosis has been seen, and no modification of the views expressed above seems necessary. Earlier references to the use of T.B.1 in leprosy, with good results, have been traced but not seen in the original. Hohenner (*Med. Klin.* 1949, p. 1378), Grosch and Kaliebe (*Zbl. Haut- u. GeschlKr.* 1950, p. 1), and Walter (*Z. Hals- Nas.- u. Ohrenheilk.* 1950, p. 218) each describe 1 case, the last two reports dealing with the same case.

I have treated 4 cases of pulmonary tuberculosis with T.B.1 continuously for the past five months; no serious toxic effects have been seen.

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THE IDENTIFICATION OF 3:5:3′-L-TRIIODOTHYRONINE IN HUMAN PLASMA

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IN a preliminary communication (Gross and Pitt-Rivers 1951), the presence of two iodine-containing compounds other than thyroxine and iodide was reported in human plasma. 3:5:3′-L-triiodothyronine has now been synthesised, and it has been shown that the compound previously named "unknown 1" behaves both on paper chromatograms and on chromatographic columns in exactly the same way as this amino-acid.

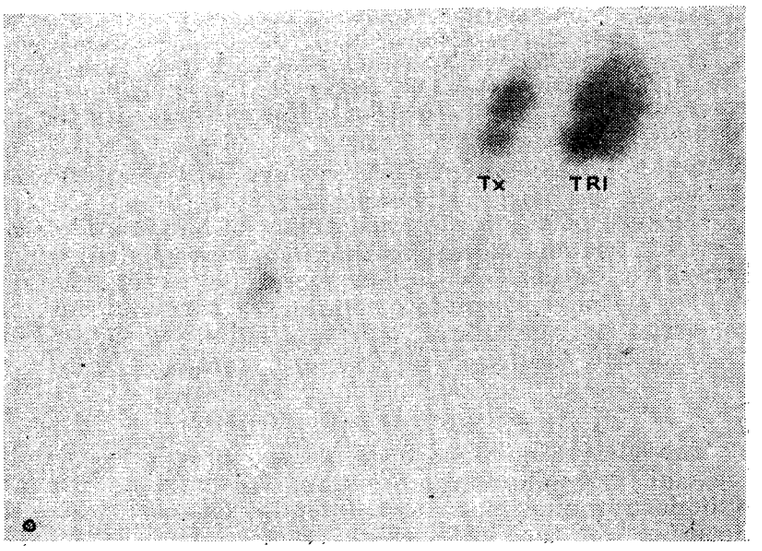

Fig. 1—Two-dimensional paper chromatogram of the plasma extract from case 2 to which 100 μg. triiodothyronine and 50 μg. thyroxine had been added. The origin is shown by the circle in the lower left-hand corner; development was with n-butanol-acetic acid in the vertical direction and with butanol-dioxan-ammonia in the horizontal direction. The thyroxine (TX) and triiodothyronine (TRI) spots corresponded exactly with the positions of radioactive thyroxine and radioactive unknown I (see Gross and Pitt-Rivers 1951) as shown on the corresponding autoradiogram.

METHODS

Samples of plasma were obtained from the series of patients shown in the accompanying table at various time-intervals after the administration of the radioactive iodine. The plasmas were acidified, and extracted with butanol, and the butanol extracts were evaporated as described previously. It should be noted that in the course of evaporation most of the *iodide* present in the sample is lost (fig. 2*d*).

The samples from patients 1 and 2 were placed on paper, together with 100 μg. of synthetic triiodothyronine and thyroxine, and developed two-dimensionally with two solvent pairs—butanol-dioxan-ammonia against butanol-acetic acid (fig. 1), and butanol-dioxan-ammonia against collidine-ammonia. The latter solvent was prepared according to Taurog et al. (1950), and by itself did not separate triiodothyronine from thyroxine.

The remaining extracts were analysed on a modified form of the kieselguhr column suggested by Grönkvist and Hellberg (1951).

The column was prepared from 5 g. of acid-washed kieselguhr to which has been added 4 ml. of 0·5*N* NaOH; this was suspended in butanol containing 20% of chloroform and packed into a glass tube with an internal diameter of 13 mm.; 3 mg. of triiodothyronine (together with 3 mg. of thyroxine in some cases) was added to the plasma extract which was then placed on top of the column and chromatographed with the butanol-chloroform solution. The effluent was passed through a liquid-flow Geiger counter at the rate of 1 ml. per 5 minutes and then fractionated in 1·5 or 3·0 ml. lots by an automatic fraction collector. The radioactivity was measured by a count-rate meter and recorded on the paper chart of a recording milliammeter. The individual fractions were recorded on the same chart by a separate pen

THE PATIENTS AND THE DOSES OF RADIO-IODINE

Patient	Dose of radio-iodine	Diagnosis
1	5 mc.	Hyperthyroidism
2	1 mc.	,,
3	4 mc.	,,
4	5 mc.	Thyroid carcinoma; euthyroid
5	100 μc.	Hyperthyroidism
6	100 mc.	Carcinoma of the thyroid functioning metastases, hypothyroid

connected to contacts in the fraction collector. The added triiodothyronine or thyroxine were measured in the fractions by the photometric estimation of the colour formed when they were treated with nitrous acid and then with alkali; the distribution of these substances in the effluent was then compared with the distribution of the radioactivity (figs. 2*a* and *b*).

Complete details of this procedure will be published later.

Because of the low radioactivity of the sample from patient 5, four successive fractions were pooled, evaporated to dryness on shallow metal plates and counted under a thin end-window counter. After counting, the material on the plates was washed off with aqueous alcohol and estimated for triiodothyronine.

RESULTS AND DISCUSSION

The relative locations of thyroxine and triiodothyronine on the paper chromatograms are shown in fig. 1. The triiodothyronine spot corresponds exactly with the location of the radioactive spot previously labelled unknown 1.

The third peak of radioactivity occurring in the eluate from the column (fig. 2*a*) corresponds with the location of synthetic triiodothyronine; this close correspondence is shown in fig. 2*b* and was found in all the samples examined. The second peak in fig. 2*a* corresponds with thyroxine.

The possibility that triiodothyronine might be an artefact of the extraction procedure was eliminated by adding tracer amounts of synthetic radioactive thyroxine to plasma and analysing the mixture on the column; fig. 2*c* shows that no triiodothyronine is formed under these conditions. Gordon et al. (1952) have shown that radioactive thyroxine added in vitro to plasma is indistinguishable electrophoretically from the naturally occurring thyroxine plasma-protein complex.

That triiodothyronine may be a radiation artefact is unlikely for two reasons: (1) it was detectable in the plasma of patient 5 who had received only 100 μc. of radio-iodine; and (2) it has been isolated from the thyroid tissue of animals which had not received any radioactive iodine at all (unpublished).

The radio-iodine which appears in the first peak (fig. 2*a*) is largely an artefact of the procedure. This is demonstrated in fig. 2*d* which shows the curve obtained when radioactive iodide is added to normal plasma and processed in the usual manner. At the same time thyroxine may, by destruction, contribute to this first peak (see fig. 2*c*); the nature of the compounds it contains is being investigated further.

SUMMARY

An iodine-containing substance present in the plasma of patients given radioactive iodine has been shown to behave in a manner identical with that of 3:5:3′-L-

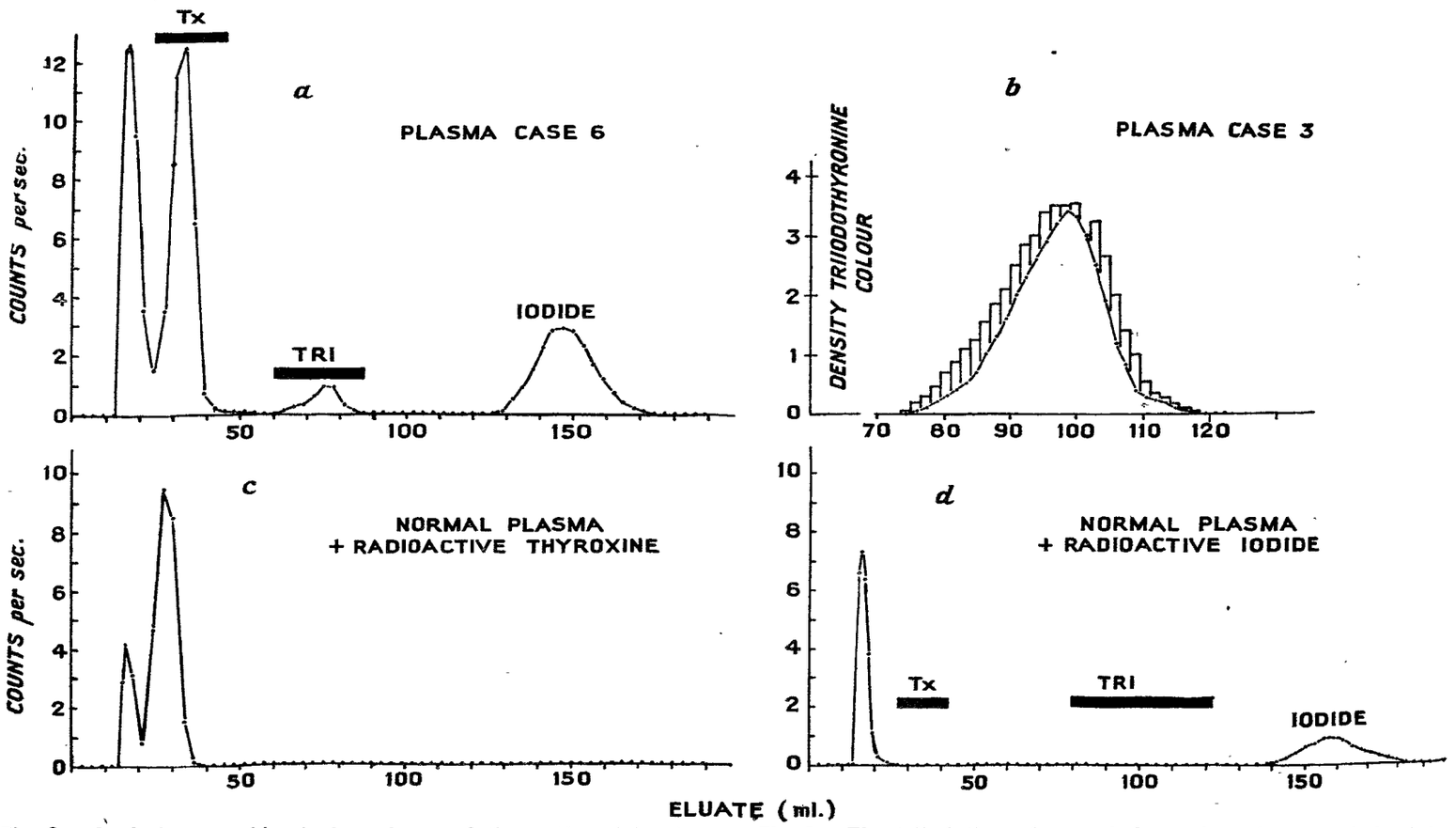

Fig. 2*a*—Analysis on a kieselguhr column of the radioactivity in a butanol extract of plasma from case 6 (24 hours after administration of radio-iodine), together with 3 mg. each of thyroxine and triiodothyronine. The curves represent the distribution of radioactivity in successive fractions of eluate from the column. The horizontal black bars indicate the positions of the added thyroxine (TX) and triiodothyronine (TRI) determined colorimetrically; the nature of the iodine in the first peak (13-20 ml. of eluate) is unknown (see fig. 2*d*).

Fig. 2*b*—The triiodothyronine peak from the column analysis of the plasma extract with 3 mg. of triiodothyronine added, from case 3, 24 hours after the dose of radio-iodine. The dots indicate the concentration of radioactivity and the columns represent the concentrations of triiodothyronine in the corresponding fractions of eluate as indicated by the density of the colour reaction.

Fig. 2*c*—Column analysis of the extract from 10 ml. of normal plasma to which 0·30 μg. of radioactive thyroxine had been added before extraction. Note that no radioactivity is detectable at the usual site of the triiodothyronine peak (60-120 ml. of eluate) although some radioactivity occurs in the first peak.

Fig. 2*d*—Column analysis of the extract from 3 ml. of normal plasma to which 5 μc. of tracer Na I^{131} had been added before extraction, plus 2 mg. each of thyroxine (TX) and triiodothyronine (TRI). Only about 1% of the added radioactivity has remained in the extract after evaporation to dryness; of this, 45% appears as iodide and 55% in the first peak. This indicates that most of the radioactivity in the first peak in fig. 2*a* is an artefact derived from iodide.

triiodothyronine on two-dimensional paper chromatograms and on a kieselguhr column.*

Evidence has been given that this compound is not an artefact of the analytical procedure or of the destructive effects of radiation.

It is concluded that 3:5:3′-L-triiodothyronine is a normal constituent of the organic iodine fraction of the plasma, since it has been found in the plasmas of both euthyroid and hyperthyroid individuals.

We are indebted to Dr. A. J. P. Martin for much help throughout this work. We also wish to thank Dr. E. E. Pochin and Dr. N. B. Myant for providing the plasma samples, Mr. P. Bovill for help with the analytical procedure, and Mr. C. Sutton and Mr. E. Hitchcock for the autoradiograms and photographs.

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MERCURY AND PINK DISEASE

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AN association between mercury and pink disease (infantile acrodynia) was suggested by the work of Warkany and Hubbard (1948, 1951), Fanconi et al. (1947), and Fanconi and Botsztejn (1948). In 38 of 41 investigated cases Warkany and Hubbard found an abnormal quantity of mercury excreted in the urine. Other workers (Bivings and Lewis 1948, Elmore 1948, van Crefeld and Paulssen 1949, Lefebvre 1949, Loebenstein 1949, Watkins 1950, Kromann 1950) have supported Warkany's and Fanconi's claims.

In Manchester pink disease is relatively common. In the three Manchester children's hospitals during 1950 the numbers of cases given this diagnosis were :

Royal Manchester Children's Hospital	37
Booth Hall Hospital	7
Duchess of York Hospital for Babies	17
Total	61

These figures probably give a reliable picture of the incidence of pink disease, because it is fair to assume that most of the affected infants come to hospital at some stage of their illness.

The use of powders to prevent or to cure teething difficulties is widespread. Such powders are variously labelled as "teething" or "cooling" powders, and some of them contain mercury in relatively large quantities. Their content of calomel varies from 16 to 33%.

INVESTIGATION AND RESULTS

We have approached the problem of mercury in pink disease from various angles.

(1) We followed up many cases in the hope of finding some other form of clinical hypersensitivity to mercury or an association with other allergic disorders in the patients or their families.

(2) We tried to find the incidence of the ingestion of mercurial teething powders among a healthy infant population and its regional and numerical correlation with pink disease.

(3) The urinary excretion of mercury was studied.

(4) The response to dimercaprol (BAL) was investigated.

Follow-up

The children followed up were those in whom pink disease had been diagnosed at the Duchess of York Hospital for Babies in 1930–50. The case-notes of 213 patients were available. Of these 37 had died, the cause of death, so far as could be ascertained, being as follows :

Mortality in 213 cases

Pink disease	16
Bronchopneumonia	12
Sudden death	4
Empyema + fibrinous pericarditis	1
Gastro-enteritis	1
Bronchiectasis	1
Bronchiolitis	1
Septicæmia	1
Total	37 (17%)

The main single complication leading to a fatal outcome was bronchopneumonia. In 4 cases death took place suddenly. In 16 cases no definite cause of death was found and death was *attributed* to the primary illness.

Of the remaining 176 patients 110 were re-examined, and a detailed history was obtained from the parents, particularly with regard to exposure to mercury, sequelæ of the disease, and allergic disorders. In 80 (73%) development had been normal, mentally and physically ; the other 30 (27%) were described as nervous or highly strung, some of them having temper tantrums, nocturnal enuresis, nail-biting, exaggerated fears, and stammer. One girl still keeps rubbing her hands, and the mother maintains that this dates back to her acrodynia.

In only 10 patients could a history of allergic disorders be elicited. In most of them these were urticarial rashes, and only 1 had asthma. In 7 other cases a family history of allergy was obtained. Thus 15% of the patients had some relationship to the allergic diathesis. This figure comes well within the suggested range of allergic disorders among the general population.

No instance of recrudescence or second illness was reported.

5 children with severe pink disease showed simultaneous recovery from an intercurrent infection and the acrodynia. The intervening illnesses were measles in 3 cases, chickenpox, and tonsillitis.

Mercurial Teething Powders

In 97 cases a clear answer was given to the question of exposure to mercury : 49 children (50%) had received mercurial teething powders. In 29 cases these had been given before the onset of their illness, while in the other 20 they had been given to alleviate its manifestations. Thus almost 30% had certainly ingested mercury, often for many months, before the beginning of the pink disease. In 5 cases the child had had mercurial teething powders throughout the illness and for years afterwards without ill effect.

A relatively high incidence of ingestion of mercury by affected infants having been found, it seemed of desirable import to ascertain how frequently teething powders are given to the healthy infant population. The subjects of this inquiry were 1561 infants; from four months to two years of age, attending the welfare centres of Manchester and Salford public-health authorities. Of these, 619 (39·6%) had received teething or cooling powders of various kinds, and in 109 (6·9%) these were mercurial.

As the habit of giving babies teething powders may vary considerably from region to region, we then chose another area where we knew that the incidence of pink disease was low—i.e., the county of Warwick, where there are 5–10 cases a year in a population about half that of Manchester and Salford. The parents of 1588

* We have recently been informed by Dr. William E. White from the Veterans Administration Hospital, San Francisco, that he too has detected, on paper chromatograms of human plasma, an unknown iodinated compound, which he suspects to be triiodothyronine.