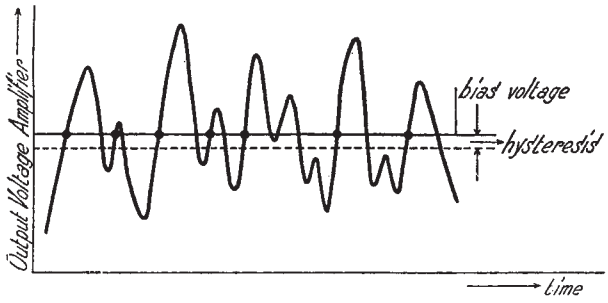

Fig. 2. Diagram of standard signals produced by the discriminator. Times at which a standard signal originates are indicated by circles

In the counter apparatus a so-called Schmitt discriminator is used. If the voltage on the first grid of the discriminator exceeds a certain bias voltage, the discriminator gives a standard signal which is counted by the counter; the counter contains one 'scale-of-two' stage and one power stage, and drives a mechanical counter register. If the voltage on the first grid decreases by more than the amount of the so-called hysteresis under the bias (in order to reset the discriminator) and after that exceeds it again, a new standard signal originates (Fig. 2).

The bias can be adjusted, so that with the same apparatus, fluctuations caused by one single sperm cell can be counted, as well as those caused at the same moment by a number of cells. To measure the motility it is necessary to count a number of fluctuations in a given time with a suitable diaphragm aperture, bias voltage, and amplification factor; together with the measurement of concentration, these results may give a good measure of the motility.

The concentration is determined in the following way. With a large aperture in the screen and a low magnification of the microscope, the light scattered by a large number of sperm cells falls on the photomultiplier; then the output voltage of the amplifier is a measure of the concentration of a sperm sample.

The whole microscope is maintained at 37° C., because the immersion oil between the dark field condenser and the slide is responsible for considerable heat transport from the slide to the condenser.

In discussing our method, it can be said at once that there is no doubt about its objectivity. It is possible to control the sensitivity of the apparatus by placing a frosted glass on the microscope stage; then the amplifier should give a standard output voltage.

As fluctuations caused by a concentrated preparation as well as those originating from one single sperm cell can be measured, it is evident that the method is applicable to concentrated as well as to diluted samples of semen.

When we were building up the apparatus it was very desirable and also possible to see what happened in the preparation by projection on the screen, and to compare the observations with the reactions of the electronic apparatus.

Some objections to this method can be made. (1) Dead sperm masses might affect the measurement of motility; but in our apparatus only moving particles are counted. (2) The apparatus is not able to distinguish abnormal movements, such as circular movements or the movements of two or more sperm cells swimming together forward or backward. This is true; but then the investigator has to decide about the character of the movements. (3) The photomultiplier cannot distinguish whether it is the same or another sperm cell which passes the opening. This objection can, of course, be made against all methods of determining the motility of sperm cells.

Details of our experiments will be published elsewhere.

This research took place under the auspices of the Unit for Research on Animal Husbandry, T.N.O. The hospitality of Prof. J. M. W. Milatz made it possible to work in the Physical Laboratory, Utrecht. We are also indebted to Prof. J. T. Randall for reading the manuscript.

[1] Rothschild, Lord, *Nature*, **163**, 358 (1949); *J. Exp. Biol.*, **25**, 219 (1948); *J. Agric. Sci.*, **40**, 82 (1950).
[2] v. Zijl, W. J., *Ned. Tijdschr. Diergeneesk. Dl.*, **74**, 553 (1949).

NATURE OF THE CIRCULATING THYROID HORMONE - PLASMA PROTEIN COMPLEX

By A. H. GORDON, J. GROSS*, D. O'CONNOR and ROSALIND PITT-RIVERS

National Institute for Medical Research, Mill Hill, London, N.W.7

It is now generally agreed that the biologically active fraction of the iodine in plasma is thyroxine itself[1–3], possibly together with another unidentified iodine-containing compound which has been demonstrated in the plasma of animals[4] and man[5]. Iodine determinations on plasma protein fractions[2,6,7] have indicated that the albumin fraction contains most of the iodine, although some is found in the α- and β-globulin fractions in high concentration. Experiments[8] on the binding of radioactive thyroxine by similar plasma protein fractions have indicated that the α-globulin present as a contaminant in the albumin and β-globulin fractions might be the actual binding protein. In order to obtain more definite information as to the nature of the binding protein, the following experiments were attempted.

Paper electrophoresis was carried out on nine samples of serum (or plasma) from patients who had received radioactive iodine. Of these, three were clinically euthyroid and had received 100 mC. of iodine-131 for the treatment of thyroid carcinoma, while the fourth was hyperthyroid and had received a 5-mC. dose of radioiodine. The serum was collected at intervals from 2 hr. to 4–5 days after administration of iodine-131. The electrophoretic procedure was a modification of the technique described by Durrum[9]. A strip of a special Munktell 20 paper (cf. Cremer and Tiselius[10]), 20 mm. × 500 mm., was dipped in $M/20$ veronal buffer at pH 8·5 and blotted. Twenty microlitres of serum was applied as a spot to the damp paper, which was then suspended on two glass rods fixed over the two beakers containing buffer, so that the serum spot was located between the rods about one inch from that rod over the cathode (see Fig. 1). The whole assembly was covered with a large glass jar and after half an hour a potential of 5 volts/cm. of paper was applied for 18 hr. The strip was then allowed to dry at room temperature and placed on an automatic counter assembly which counted successive 4·5-mm. bands of the paper.

* Merck Fellow in the Natural Sciences of the National Research Council of Canada.

Those samples which contained sufficient activity were then autographed by being placed against a sheet of X-ray film with an intervening sheet of 'Cellophane'. After these procedures, the protein bands were made visible with bromphenol blue (Fig. 2), again according to the method of Durrum, and their positions compared with that of the radioactivity.

In all the sera examined, the maximum concentration of radioactivity was found in a band immediately following the albumin band (Fig. 2*a* and *b*), with a much smaller amount associated with the albumin fraction. Paper chromatographic analysis of the radioactive material present in these samples showed that most of the iodine-131 was present as iodide or as thyroxine[5]. Further experiments were therefore carried out in which synthetic radio-thyroxine of high activity and radioactive sodium iodide were added to normal human serum, and the positions of the radioactivities were again determined. Trace amounts of sodium iodide-131 alone migrated more rapidly than any of the protein bands and, when mixed with serum, about 5 per cent of the radioactivity added remained associated with the albumin band. Radio-thyroxine in the absence of serum proteins migrated as shown in Fig. 2*e*. When, however, radio-thyroxine (0·15 γ, 0·5 μC./ml. of serum) was added to serum *in vitro*, most of the radioactivity was detectable as a band (Fig. 2*c* and *d*) occurring in the same position as that due to the circulating radioactivity in the experimental sera (Fig. 2*a* and *b*). The serum used in the *in vitro* experiments was then subjected to electrophoresis with veronal buffer at *p*H 8·5, in the Tiselius apparatus. The pattern obtained (Fig. 2*f*) was enlarged photographically so that the centres of the albumin and γ-globulin peaks would correspond with the centres of the same protein bands on the paper; it can be seen that all the protein peaks then corresponded with the equivalent bands on the paper.

By comparing Figs. 2*c*, *d* and *f*, it is evident that radio-thyroxine is associated with one or more serum constituents having mobilities at *p*H 8·5 close to that of α_1-globulin. It may be relevant that a lipoprotein has been found with a very similar mobility[11].

These results suggest that the thyroid hormone secreted from the gland may well be largely associated with a single plasma protein constituent. In this case the results of previous investigators who found concentrations of iodine[2,6,7] and thyroxine-binding capacity[8] in several fractions obtained by Cohn's procedure[12] are probably due to incomplete separation of the plasma constituents.

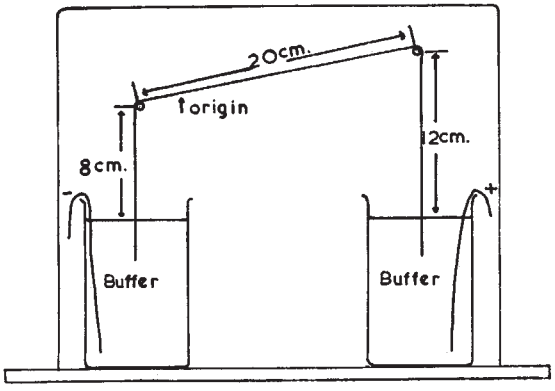

Fig. 1. Schematic diagram of the electrophoresis apparatus, drawn to scale. The arrow marked 'origin' indicates the position of the serum at the beginning of the electrophoretic run

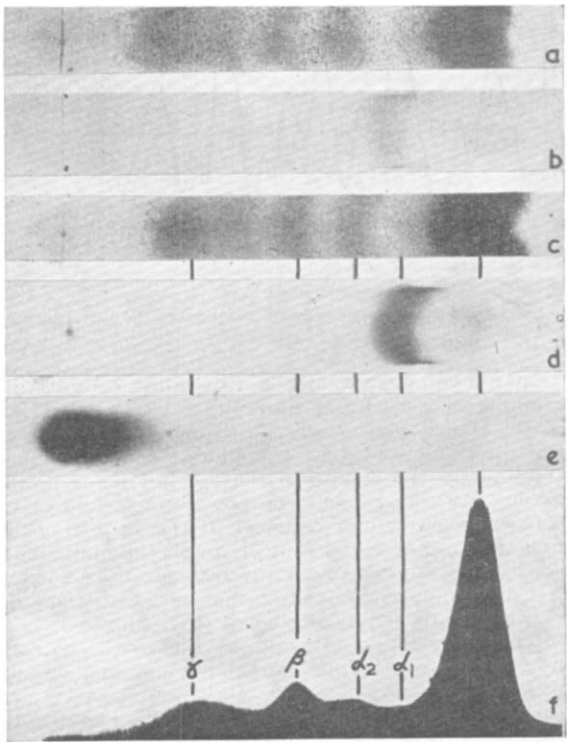

Fig. 2. (*a*) Distribution of serum proteins after the electrophoresis on paper of 0·02 cm.³ of serum from a euthyroid subject five days after the administration of 100 mC. of sodium iodide-131 for the treatment of thyroid carcinoma.

(*b*) Autoradiograph obtained by placing strip (*a*) in contact with X-ray film. The position of the serum radioactivity is indicated by the darkening of the film and is mainly concentrated in a band immediately following the albumin area. A faint darkening corresponding to the albumin area itself is visible on the original film, although not clearly apparent in photographic reproduction.

(*c*) Electrophoretic paper diagram of 0·02 cm.³ of serum, from an untreated euthyroid subject, to which radioactive thyroxine had been added prior to electrophoresis.

(*d*) Autoradiograph of strip (*c*) showing a similar distribution of radioactivity as in (*b*).

(*e*) Autoradiograph of a paper electrophoresis of the radioactive thyroxine alone.

(*f*) Electrophoretic pattern obtained in the Tiselius apparatus of the serum shown in (*c*).

We are indebted to Dr. É. E. Pochin and Dr. N. B. Myant for the blood samples, to Mrs. A. Dovey for carrying out the electrophoresis of the serum in the Tiselius apparatus and to Mr. C. Sutton and Mr. E. Hitchcock for the preparation of the autoradiographs and photographs. [Sept. 24.

[1] Trevorrow, V., *J. Biol. Chem.*, **127**, 737 (1939).
[2] Taurog, A., and Chaikoff, I. L., *J. Biol. Chem.*, **176**, 639 (1948).
[3] Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H., *Science*, **111**, 605 (1950).
[4] Gross, J., and Leblond, C. P., *Endocrin.*, **48**, 714 (1951).
[5] Gross, J., and Pitt-Rivers, R., *Lancet*, **2**, 766 (1951).
[6] Salter, W. T., *Ann. New York Acad. Sci.*, **50**, 358 (1949).
[7] Silver, S., and Reiner, M., *Bull. New York Acad. Med.*, **26**, 277 (1950).
[8] Gross, J., Franklin, A. E., Leblond, C. P., and Quastel, J. H. (unpublished).
[9] Durrum, E. L., *J. Amer. Chem. Soc.*, **72**, 2943 (1950).
[10] Cremer, H. D., and Tiselius, A., *Biochem. Z.*, **320**, 273 (1950).
[11] Quoted in Oncley, J. L., Gurd, F. R. N., and Melin, M., *J. Amer. Chem. Soc.*, **72**, 458 (1950).
[12] Cohn, E. J., Strong, L. E., Hughes, W. L., jun., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., *J. Amer. Chem. Soc.*, **68**, 459 (1946).