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A SENSITIVE *IN VITRO* METHOD FOR THE ASSAY OF THYROTROPHIC HORMONE IN BIOLOGICAL MEDIA

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Since the discovery by Smith & Smith (1922) of thyrotrophic pituitary hormone (TSH), over 90 methods have been described for its assay. Few of these have been found suitable for the measurement of the hormone concentration in human sera. The techniques used up to 1947 have been reviewed by Albert (1949) and those evolved more recently are summarized by Brown (1959) and McKenzie (1960). In general these methods have not been sensitive enough to measure the concentration of TSH in blood in euthyroid states (e.g. Purves & Griesbach, 1949; D'Angelo, Paschkis, Gordon & Cantarow, 1951; Adams & Purves, 1953, 1955; Querido & Lameijer, 1956; McKenzie, 1958), although thyroid-stimulating activity could be demonstrated in serum in certain pathological or experimental conditions. A large amount of information of clinical and experimental interest has been obtained by D'Angelo and his colleagues using a histometric technique and the starved metamorphosing tadpole as an assay animal (D'Angelo, Gordon & Charipper, 1942; D'Angelo & Gordon, 1950; D'Angelo *et al.* 1951; Di George, D'Angelo & Paschkis, 1957). Great accuracy has been claimed for this difficult and laborious technique, and the TSH content of normal rat blood has been measured. It was, however, only occasionally possible to detect the smaller amounts present in human blood. Gilliland & Strudwick (1956) were able to measure the concentration of TSH in human blood, but the method used (^{131}I release in day-old chicks) proved difficult to calibrate. Bates & Cornfield (1957) have been able to obtain satisfactory results with a similar technique, but only after procedures designed to concentrate the hormone in the blood samples. The majority of the methods used up to the present time have been based on the cytological changes in the thyroid gland of a test animal or on the *in vivo* uptake or release by the thyroid of tracer doses of radioactive iodine. Further, the validity of the results obtained with these methods may be subject to criticism from the statistical point of view, in that the criteria

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described by Bliss (1952) and by Gaddum (1953) have not been fully met, particularly that concerning parallelism between standard and unknown.

In vitro techniques have recently been used in an attempt to increase the sensitivity of TSH assay methods. Bottari (1956) evolved a method involving the uptake of ^{131}I in guinea-pig thyroid fragments. Although sensitive, the method has not proved reliable. Bakke, Heideman, Lawrence & Wiberg (1957) used for their parameter the gain in weight of bovine thyroid gland slices following incubation with TSH. This method has been claimed to be very sensitive, although it is unsatisfactory for the assay of whole serum. Recently extracts of serum were successfully assayed by this technique (Heideman, Bakke & Lawrence, 1959).

The present paper describes a technique which offers a number of advantages over the methods previously used from the point of view of acccuracy, sensitivity and statistical validity. In principle, fragments of thyroid tissue are incubated overnight in a nutrient medium containing ^{131}I. Next morning sodium iodide is added to block the further uptake of radioactive iodide, and after 1 hr the ^{131}I content of the medium is measured. By comparison with control samples of medium the uptake of labelled iodine by the thyroid tissue may be determined. TSH is then added and incubation continued for 2 hr, when a second sample of the medium is removed for measurement of its ^{131}I content. The percentage release of labelled iodide from the thyroid tissue may then be calculated. The amount of ^{131}I released from the thyroid bears a linear relation to the logarithm of the concentration of TSH in the medium within certain limits. Preliminary communications on this technique and its applications have been published elsewhere (Bottari & Donovan, 1958; El Kabir, 1960, 1961, 1962, 1963).

METHODS

A standard roller tube technique (without coagulum) is utilized. Rimless hard-glass test tubes (15 × 150 mm), containing the medium and thyroid fragments, are carried in a revolving drum inclined at 10° to the horizontal plane, which turns at 10 rev/hr in an incubator set at 25° C.

The nutrient material is composed of modified Gey's solution (70 %) and horse serum (30 %). The Gey's solution contains (g/l.): NaCl 8, KCl 0·38, anhydrous $CaCl_2$ 0·13, $MgCl_26H_2O$ 0·21, $NaHCO_3$ 0·50, KH_2PO_4 0·025, $Na_2HPO_412H_2O$ 0·30, glucose 2. In practice the bicarbonate and glucose are omitted from a five-times-concentrated solution which is submitted to ultrafiltration and stored in the refrigerator. Immediately before use the solution is diluted to the correct concentration with double glass-distilled water and the bicarbonate and glucose are added. Potassium G penicillin (6 mg) and dihydrostreptomycin (5 mg) (both from the Distillers Company Biochemicals Ltd) are further added to the (Gey's solution)–(horse serum) mixture, together with a few crystals of phenol red in order to indicate that the pH remains at 7·4. Radioactive iodine (^{131}I, carrier-free) is employed in the working solution to a final concentration of 0·5 μc/ml.

Male guinea-pigs about 300 g in weight are used. Animals are obtained from a suitable source and are kept at a constant temperature of 72–76° F (21·6–23·8° C). Five days before

assay each guinea-pig is pre-treated with subcutaneous injections of Tapazole (methimazole, Eli Lilly Inc.) 10 mg daily. On the day of assay the guinea-pig is killed by a blow on the head, the thyroid lobes are quickly removed, dissected free of fat and connective tissue in a pool of Gey's solution on an upturned Petri dish, and then separately divided into small fragments with scissors or by means of a tissue chopper. The pieces from each lobe are mixed, then separated into eight groups and the fragments from one lobe paired with those of the other. Thus each animal provides sufficient material for eight tubes, each containing an equal amount of tissue from each thyroid lobe. Care is taken to keep the fragments moist with Gey's solution during manipulation. The portions of thyroid tissue are transferred by means of a needle to test tubes containing 1 ml. of medium, and the tubes placed in the incubator. Control tubes containing 0·1 ml. of the medium but lacking glandular fragments are treated similarly for control purposes.

After 10–12 hr incubation 0·10 ml. of the nutrient medium containing NaI 6 μg/ml. is added to each tube and after 1 hr 0·20 ml. of the fluid is removed. Next 0·10 ml. of the fluid to be assayed or of standard TSH (International Standard Preparation, Mussett & Perry, 1955) dissolved in nutrient medium is pipetted into each tube; the tubes are further incubated and a second sample (0·20 ml.) of the incubation fluid removed 2 hr later.

The portions of the medium removed before and 2 hr after the addition of the sample to be assayed are diluted to 2·0 ml. with distilled water containing potassium iodide approx. 1 g/l., and the radioactivity of each portion is then measured by means of a well-type scintillation counter.

Because of individual variations all assays are carried out on individual guinea-pigs. In early experiments (Bottari & Donovan, 1958) three of six thyroid portions were used to draw a reference curve with known dilutions of TSH and the other three used for the assay of unknown samples. With this procedure all the determinations were made with cross assays, the samples being assayed on two guinea-pigs. Subsequently (El Kabir, 1962, 1963) a 4-point assay design as described by Bliss (1952) and modified by C. Fortier (personal communication) was adopted and has become standard. A single guinea-pig is used. Two doses of the standard preparation within the range of linearity of the assay are prepared and the unknown is diluted to the same log. dose interval as that chosen for the standard doses; each dose is assayed in duplicate. The assay is then tested for significance of slope, sample, and freedom from divergence between standard and unknown. The potency of the unknown is estimated within 95 % fiducial limits.

Calculation of results. The uptake of ^{131}I by the thyroid gland fragments (before adding TSH) may be computed from the difference in radioactivity between the first portion removed from the control tube and that from the tube containing the thyroid tissue. In practice three control tubes are run and the counts averaged. Following the addition of thyrotrophic hormone there is a release of ^{131}I from the glandular fragments which increases the radioactivity of the medium. This increase is related to the initial ^{131}I content of the tissue. The percentage release is calculated after correction for the use of diluted samples, and with allowance for the fact that the first 0·2 ml. sample is taken from 1·1 ml. (1·0 ml. medium + 0·1 ml. NaI solution) and the second from 1·0 ml. (0·9 ml. of medium after removal of the first portion + 0·1 ml. TSH solution).

The mean count of each tube is calculated, background is subtracted and the count corrected for the decay of ^{131}I during the period of counting. Then if

x = mean count of the 0·2 ml. portion of the control sample,

y = the decay-corrected mean count of the first portion of one tube containing thyroid fragments, and

z = the decay-corrected mean count of the second portion of the same tube,

$5{\cdot}5x$ = total radioactivity in each tube;

$5{\cdot}5x - 5{\cdot}5y$ = uptake of ^{131}I in the tube containing thyroid fragments;

$5z - 4{\cdot}5y$ = total amount of ^{131}I released from the same fragments after the addition of the standard or unknown; and

$$\frac{5z - 4{\cdot}5y}{5{\cdot}5x - 5{\cdot}5y} \times 100 = \text{release of } ^{131}\text{I (\%)}.$$

Statistical treatment. A four-point (2+2) assay design has been adopted, and the results subjected to an analysis of variance and factorial analysis according to the method described by Bliss (1952). The slope, sample and divergence are tested for significance, the lambda is calculated and the potency of the unknown in terms of the standard is determined with its 95 % fiducial limits.

Treatment of blood samples. In general plasma has been used although serum is equally suitable, though less convenient. Blood was withdrawn from the marginal ear vein of chinchilla rabbits with a heparinized syringe. It was centrifuged and the plasma stored at − 10° C until required. Separate portions of a plasma sample were taken and stored individually when two or more assays were performed on one unknown. The serum samples were thawed immediately before use and then discarded.

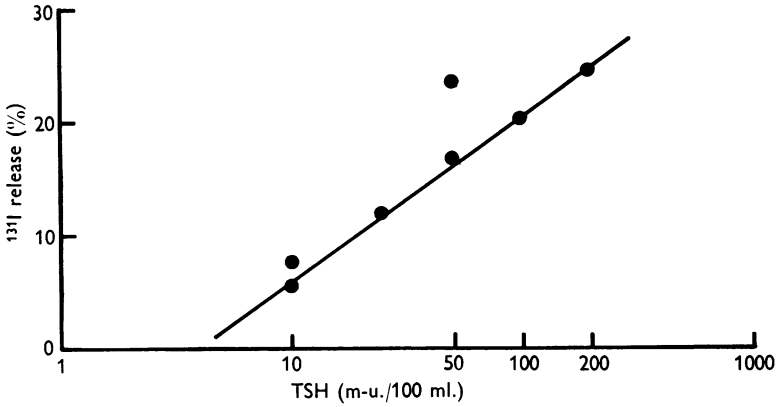

Fig. 1. To show the linearity of the ^{131}I release response of the thyroid fragments to the addition of a range of concentrations of TSH (El Kabir, 1961), semi-log-scale

RESULTS

Sensitivity. A linear log–dose response curve was obtained for the release of ^{131}I from thyroid tissue by TSH (Fig. 1). The curve obtained from serial dilutions of a standard preparation of TSH spans a range of sensitivity between 0·05 and 1·5 m-u. International Standard/ml. The assay is thus capable of detecting 0·005 m-u. of TSH. However, sensitivity may vary from one guinea-pig to another.

Accuracy. The accuracy of the technique was tested by estimating the potency of solutions of International Standard TSH of unknown concentration, and calculating the estimated potency with 95 % fiducial limits, and determining the statistical significance of the differences in potency between standard and unknown at the 5 % level of significance. The results of ten experiments are shown in Table 1.

Precision. The precision of the assay method has been tested by: (*a*) observing the effect of known dilutions of International Standard TSH on the thyroid tissue of individual guinea-pigs as well as on different animals; and (*b*) the assay of separate portions of the same serum sample

on the same and on different thyroid glands when reproducible results were obtained (Table 2).

Specificity of the response to TSH. In order to test the specificity of the response to TSH various other products, including pituitary trophic hormones, were assayed. A solution of adrenocorticotrophic hormone (Crookes Laboratories Ltd.) containing 25 u./ml. was prepared, Pitressin

TABLE 1. Actual and estimated values of antilog M (potency ratio). Four-point assays. Both unknown and standard consisted of solutions of International Standard thyrotrophic hormone. The log. dose interval was 0·699. 5 % point of the F distribution = 7·71

	λ	M (actual)	M (estimated)	95 % fiducial limits	F-ratio slope	F-ratio sample
1	0·104	2·5	2·33	1·36–6·84	40·58*	11·15+
2	0·267	2·5	2·68	—	6·1	2·3
3	0·074	2·0	2·12	1·28–4·10	79·80§	17·34+
4	0·101	2·0	2·15	1·01–5·93	42·45*	9·69+
5	0·144	2·0	2·26	0·92–8·06	21·19	5·44
6	0·204	2·0	1·62	0·38–10·13	10·37+	0·94
7	0·078	1·5	2·01	1·07–4·88	50·0*	9·56+
8	0·109	1·5	1·36	0·63–3·42	36·98*	1·32
9	0·079	1·0	0·9	0·55–1·56	70·82*	0·29
10	0·095	1·0	1·13	0·58–2·32	48·91*	0·29

+, $P < 0·05$; *, $P < 0·01$; §, $P < 0·001$.

TABLE 2. The results of assay of separate portions of the same sample of rabbit serum in duplicate, and on different thyroid glands

Rabbit	Serum TSH concentration (m-u./100 ml.) First assay	Second assay	Third assay
1	35, 37	34	
2	23, 25·5	20·5, 25·5	23, 28
3	38	38, 41·5	38, 43
4	34, 40	38, 38	36, 40

(vasopressin; Parke, Davis & Company) was used at a concentration of 10 u./ml. and gonadotrophin (Gestyl, Serum Gonadotrophin B.P., Organon Laboratories Ltd) tested at a concentration of 200 u./ml. All dilutions were made with the (Gey's solution)–(horse serum) medium. It was found that 1 ml. of each solution contained less than 2 m-u. TSH. Heparin was also tested, in view of its possible use as an anticoagulant in the collection of blood samples, but failed to display any TSH-like activity. Other samples of TSH, such as Ambinon (Organon Laboratories Ltd), Thyrotropar (Armour Laboratories) and Actyron (Ferring A-B) have been tested and all gave log-dose response curves.

Nature of the iodine released. The nature of the radioactive material released from the incubated thyroid slices has been examined chromatographically by analysing the incubation medium. It was shown that 88·5 % of the ^{131}I released following the addition of TSH to the medium was

in the form of inorganic iodide and that there was no detectable radioactive thyroxine in the medium.

Statistical treatment. In nineteen consecutive assays of samples of serum and of pituitary extracts, the mean λ was 0·117 with a range between 0·033 and 0·258. There was no significant divergence between International Standard Thyrotrophic Hormone and human blood, or between the TSH

TABLE 3. Statistical analysis of blind assay of rat blood. Four-point assay. Dose of standard (S_2) was 50 m-u./100 ml. International Standard Thyrotrophic hormone. 5 % point of the F distribution = 7·71

	df	F
Slope	1	218 (P = 0·001)
Sample	1	2·74 (N.S.)
Divergence	1	1·4 (N.S.)
Within dose = 0·045	4	
Potency of unknown	42 m-u./100 ml.	
5 % fiducial limits	30–56 m-u./100 ml.	

Responses	S_1	S_2	U_1	U_2
	16·4	50·4	13·3	45·6
	16·5	46·6	17·7	40·0

Log. dose interval 0·7

activity of the blood of thyroidectomized rabbits and International Standard TSH. A full analysis of the results of one assay of a sample of rat blood is shown in Table 3.

The assay of TSH in blood

It has been shown (El Kabir, 1960, 1961, 1962, 1963) that in valid two-dose assays there is complete parallelism between the slopes of the International Standard and those of thyroid-stimulating hormone circulating in human blood, in thyroidectomized rabbit blood, and in normal rat blood. This is taken as presumption of the identical biological actions of the material assayed in blood to International Standard thyrotrophic hormone. Figure 2 illustrates the parallelism between the circulating TSH in the plasma of the thyroidectomized rabbit and International standard TSH.

The TSH content of rabbit blood. The concentration of TSH in the serum of normal female chinchilla rabbits has been measured in a series of ten animals. The body weights ranged between 2·96 and 4·08 kg with a mean of 3·36 kg. The animals were fed on MRC 18 pellet diet (Bruce & Parkes, 1947) and kept at a temperature of between 72 and 78° F. The assays were performed during the summer months, and all were carried out blindly. The resting values obtained ranged between 3 and 14 m-u. TSH/100 ml. serum, with an average of 6 m-u./100 ml. serum.

Blind assays were performed on blood samples from seven rabbits obtained at different intervals of time after thyroidectomy. The results for

each individual animal are shown in Table 4. It would appear that following surgical thyroidectomy the TSH concentration in blood rises slowly to reach a peak some 3–4 weeks after operation and then slowly declines.

The TSH content of rat blood. Blood samples were obtained from four male Wistar rats of body weights 250–350 g. The animals were fed on MRC diet 41 B and were kept at a constant temperature of 70° F (21·1° C). They were kept in artificial light for 17½ hr and in darkness for 6½ hr daily. The results obtained ranged from 43 to 55 m-u./100 ml. serum. A further

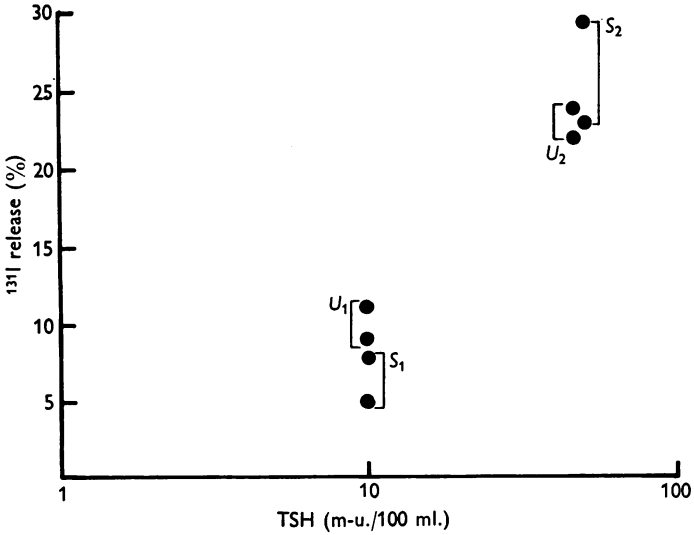

Fig. 2. Log. dose–response curve of two-point assay of plasma obtained from thyroidectomized rabbit. Potency = 46·5 m-u./100 ml., λ = 0·064, F-ratio for the slope 69·90 ($P < 0·01$). F-ratio for the divergence not significant. S_1, S_2, U_1, U_2 see Table 3.

TABLE 4. Concentration of TSH (m-u./100 ml. plasma) after thyroidectomy

	Rabbit no.						
Days	1	2	3	4	5	6	7
0	3	5	3	14	—	—	—
2	11	15	—	—	—	—	—
3	—	—	12	10	—	—	—
10	20	45	—	—	—	—	—
21	—	—	96	42	—	—	—
28	56	135	—	—	—	—	—
42	—	—	52	72	—	—	—
49	111	66	—	—	—	—	—
63	—	—	58	—	—	—	—
70	108	—	—	—	—	—	—
200	—	—	—	—	46	—	—
300	—	—	—	—	—	—	72
530	—	—	—	—	—	62	—

sample from a male Lister rat, weight 340 g, was also estimated blindly. The TSH content was estimated to be 43 m-u./100 ml. (λ = 0·034).

In order to test the specificity of the technique the effect of thyroxine administration on thyrotrophic hormone levels in the blood of the rat was investigated. One group consisting of six male rats, of average weight 340 g, were anaesthetized, blood was collected from the external jugular vein of each, pooled and the plasma stored at − 18° C. After collecting the blood each animal received a subcutaneous injection of 50 μg L-thyroxine (Glaxo) daily for 3 days and a final injection of 100 μg on the fourth day. Twelve hours after the final injection the animals were killed, blood was collected from each and the specimens were pooled. Blood was also obtained from a further group of three normal male rats and from a group of six rats treated with thyroxine as described above. Code numbers were given to the samples, which were then assayed blindly. Values of 25·5 m-u./100 ml. (λ = 0·104) and of 38·8 m-u./100 ml. (λ = 0·066) were obtained for the plasma TSH levels of the two groups of normal rats. No TSH was detectable in the first group of animals treated with thyroxine and only 7 m-u./100 ml. was detected in the second group so treated.

DISCUSSION

The basis of the present technique rests on the assumption that although the amount of living, normal thyroid tissue in the different tubes is unknown, it is proportional to the uptake of ^{131}I from the medium. It has been observed that uptake is greatly reduced or abolished if tissue fragments are damaged by drying or by heat. Weighing the fragments in each tube is therefore avoided; in any case the weight of the fragments would not indicate the actual amount of functional tissue they contain. Thus the amount of radioactivity released by TSH represents a proportion of the amount of radioactivity taken up by functional tissue of the thyroid fragments.

The nature of the ^{131}I released was shown to be largely inorganic iodide. It would thus appear that the assay is based upon the release of unbound iodide from the thyroid fragments. The significance of the release of inorganic iodide induced by thyrotrophic hormone from the thyroid gland is not fully understood, but there is now evidence that this is possibly a physiological mechanism. Purves (1957) observed that TSH releases not only hormone but also iodide from the guinea-pig thyroid. Halmi, Granner, Doughman, Peters & Müller (1960) reported that TSH produces a prompt decrease in the thyroid:serum-iodide ratio in both normal and hypophysectomized rats. Rosenberg, Athans & Behar (1960) described the release of inorganic iodide from the thyroid gland of the dog following the

injection of TSH. Similar results were reported in the rat and in the human by Rosenberg, Ahn & Chalfen (1961) and in the dog by Nagataki, Shizume & Okinaka (1961). It would appear from these experiments that the thyroid gland responds to TSH by a prompt discharge of inorganic iodide before the discharge of thyroid hormone. The hypothesis that the iodide so discharged may derive from de-iodination reactions within the gland has been advanced by Rosenberg and his associates (Rosenberg *et al.* 1960; Rosenberg *et al.* 1961). Although the physiological significance of the parameter used in the assay may thus require further clarification, its use is justified on the grounds of the reliability and the statistical significance of the assay method.

The arrangement of the design of the assay is a formal four-point assay design, as first described by Gaddum (1933), with duplicate estimations of standard and unknown at two dose levels, the same logarithmic interval being used for dilution of both standard and unknown. The advantages of using this type of design have been reviewed by Gaddum (1953). The significance of the slope, the potency of the unknown and the significance of its difference from the potency of the standard, and the significance of any departure from parallelism between the log. dose-response curves of the standard and of the unknown may be calculated. In addition, the use of the λ enables comparisons of the precision of different assays to be readily assessed. As the λ is independent of the design of the assay, comparisons with other assay methods may also be made from the point of view of precision.

Before the adoption of this design a line of best fit was drawn for the responses at three dose levels and the unknowns estimated in duplicate from the regression line (Bottari & Donovan, 1958). In addition to lacking the convenience of a statistical analysis, this design has occasionally proved unreliable, and has been abandoned (El Kabir, 1962).

Great differences in the responses of assay animals from different sources have been experienced. Initially guinea-pigs were obtained from dealers, but variability was found in different batches of animals. Only one source of guinea-pigs from a hospital animal house has been found to give consistently satisfactory responses. Similar experiences have been reported in Quebec by Fortier (personal communication) using the present method. That strains of animals may vary greatly within a species has been indicated by Bates & Condliffe (1960) in the case of chicks used for the assay of TSH, by the differing experiences of Albert (1956) and Loraine & Brown (1954, 1956) with the bioassay of pituitary gonadotrophins using immature mice, and also by the experiences of J. M. McKenzie (personal communication) and of D. S. Munro (personal communication) in using different strains of mice for the measurement of TSH. It has been found that the precision

of assays is improved by pre-treating the assay animal with Tapazole (methimazole, Eli Lilly) for 5 days before the experiment.

The sensitivity of the technique is such that 0·005 m-u. of TSH can readily be detected. Occasionally smaller quantities of hormone have been measured. This compares favourably with other methods of TSH assay. Of the more sensitive methods, that of D'Angelo & Gordon (1950) has a sensitivity of 0·5 m-u. of TSH, that of Adams & Purves (1955) approximately 0·1 m-u., and that of McKenzie (1960) 0·025 m-u. Thus the present technique would appear to be five to ten times more sensitive than the methods previously described. It would appear to be possible to obtain greater sensitivity by this technique by the use of volumes larger than the 0·1 ml. at present used.

From the studies on the accuracy of the present technique, it can be seen that it is possible to estimate the concentration of known quantities of TSH to good approximation. It can also be seen that the technique is capable of discriminating a twofold, and even a one-and-a-half-times increase in TSH at the 5 % level of significance. This accuracy, however, can be seen to depend on the slope and the standard deviation of each individual assay, as reflected by its λ. D'Angelo (personal communication) is capable of distinguishing a variation of TSH concentration of the order of 1 : 1·3. Such discrimination has not, however, been subjected to statistical analysis.

A comparison of available data on the indices of precision (λ) of various assay methods would place the present technique among the most satisfactory from this point of view. Thus Bates & Cornfield (1957) quote a precision index of between 0·2 and 0·25, Gilliland & Strudwick (1956) had a much less satisfactory λ, varying between 0·4 and 0·5. McKenzie (1958) obtained a mean of 0·24, while the average λ for the method of Bakke *et al.* (1957) is 0·28. No figures for λ have been published by D'Angelo and his co-workers.

The values of TSH obtained in the blood of normal rabbits by the present technique are lower than those described by Bottari (1957) using an *in vitro* ^{131}I uptake method, and those described by Bottari & Donovan (1958). These differences may be ascribed to the increased accuracy and more reliable design of the present technique. The values obtained for the normal rat agree with those obtained by D'Angelo (1955) and by Adams & Purves (1955). Bates (1963) has obtained values of approximately 10 m-u./100 ml. for the rat after concentrating the serum by percolation. The loss of potency through this procedure has been estimated at approximately 25–50 %. Similar discrepancies have been observed in the results obtained in the assay of normal human blood. With the Bottari & Donovan (1958) assay technique the mean TSH concentration in human blood was found

to be 22 m-u./100 ml. (Bottari, 1960; El Kabir, 1960, 1961). This agrees with the findings of D'Angelo (personal communication), of McKenzie (1958), of Bates & Condliffe (1960) and of Gilliland & Strudwick (1956), but not with the subsequent findings of Bates (1962) or those of Purves & Adams (1960) or of Bakke (1963). However, all the latter workers used serum subjected to concentration procedures. Purves & Adams (1960) record a range of 1·2–10 m-u./100 ml. for normal human sera. The first figure refers, however, to pooled material which was not freshly obtained. Bates (1963) has revised his previous estimate of the normal TSH level in man to the same figures. No explanation other than loss of potency through handling and concentration of serum can as yet be offered for these diverging results.

The effect of thyroidectomy on TSH secretion has not been systematically studied in the rabbit. The present study would show a remarkably consistent sequence of changes in TSH secretion in each of the four animals studied, a peak increase in TSH occurring approximately 3–4 weeks after thyroidectomy. Bottari (1958) describes an increase in the circulating TSH of the rabbit to more than 1000 m-u./100 ml. 5 days after thyroidectomy. This does not accord either in the timing or in the magnitude of the response with the results presented in this paper. Increases in TSH concentration in the blood following thyroidectomy have been reported in the rat by Gordon, Goldsmith & Charipper (1945) and by Adams & Purves (1955).

The essential requirements in hormone assay procedures have been reviewed by many workers. Loraine (1958) has summarized these criteria in postulating that the efficiency of a given method depends on its reliability and its practicability. The reliability of a technique is in turn dependent on criteria of accuracy, precision, specificity and sensitivity. The accuracy of a technique is defined as 'the nearness with which a given analytical result approaches the "true" result' (Loraine). The precision of an assay method may be assessed by carrying out multiple determinations on the same sample. The present technique would seem to fulfil these criteria, although the limited number of observations possible within its experimental design is reflected in the width of the fiducial limits of the results. The practicability of the method is more open to question. It is speedy, in that a complete assay may be completed in just over one day, and is also fairly simple in operation; however, considerable skill is necessary both in handling the fragments and in pipetting the samples of media, and the number of unknowns which it is possible for one operator to estimate in any one day may not exceed two; to these disadvantages must be added the problem of strain variability. It is felt, however, that these disadvantages are compensated for by the positive value of the assay in measuring thyrotrophic hormone in blood.

SUMMARY

1. A new method is described for the assay of thyrotrophic hormone (TSH) based on the release of ^{131}I-labelled iodine from guinea-pig thyroid tissue fragments.

2. The thyroid tissue is incubated for 10–12 hr at 25° C in a nutrient medium containing ^{131}I. Next morning sodium iodide is added and after 1 hr the ^{131}I determined. Following the addition of TSH to be assayed, incubation is continued for 2 hr and the amount of radioactivity released from the thyroid is measured. The release of ^{131}I is, within limits, proportional to the logarithm of the concentration of TSH in the medium.

3. The sensitivity of the method is such that a significant release of ^{131}I may be obtained with 0·005 m-u. International Standard TSH, while the normal range of the method covers concentrations from 0·05 to 1·5 m-u./ml.

4. The TSH content of serum of normal human subjects, rats and rabbits may be determined on unconcentrated serum and values for normal rats and rabbits subjected to various experimental procedures are presented.

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