ASSAY OF BLOOD AND URINE FOR THYREOTROPIC HORMONE IN THYROTOXICOSIS AND MYXEDEMA

SAUL HERTZ AND ERIC G. OASTLER

From the Thyroid Clinic of the Massachusetts General Hospital

BOSTON, MASSACHUSETTS

Reprinted from
ENDOCRINOLOGY:
The Bulletin of the Association for the Study of Internal Secretions, 1930 Wilshire Blvd., Los Angeles, Calif., Vol. 20, No. 4, July, 1936, Pages 520 to 525.

ASSAY OF BLOOD AND URINE FOR THYREOTROPIC HORMONE IN THYROTOXICOSIS AND MYXEDEMA[1]

SAUL HERTZ[2] AND ERIC G. OASTLER[3]
From the Thyroid Clinic of the Massachusetts General Hospital
BOSTON, MASSACHUSETTS

Rogowitch (1) demonstrated hypertrophy of the anterior pituitary body following thyroidectomy in rabbits and dogs as early as 1889. Niepce (2) in 1851 recorded that both man and animals with large parenchymatous goiters had greatly enlarged pituitaries. Boyce and Beadles (3) in 1892 described enlargement of this gland along with colloid in the anterior lobes of the pituitary in cases of human myxedema. This finding was confirmed in 1917 by Hale White; and Wegelin (4) described similar findings in a case of cachexia thyreo priva. Degener (5), Kojima (6) and Kamo confirmed the findings of Rogowitch in animals.

In 1914 Adler (7) demonstrated that destruction of the pituitary in tadpoles depressed metamorphosis and the development of the thyroid gland. Allen (8) and Smith (9) in 1916 independently studied the same relationship in tadpoles. Gudernatch (10) had described the rôle of the thyroid hormone in metamorphosis in 1912 and it was therefore logically suspected that the inhibition of metamorphosis following pituitarectomy was in some way related to secondary diminution in thyroid function. Allen's work (11) in 1921 indicated that pituitary transplants induced premature metamorphosis following thyroidectomy only if some residual tissue was present. Smith and Smith (12) were able to induce metamorphosis in pituitarectomized tadpoles by the intraperitoneal administration of anterior lobe of sheep. Schwartzbach and Uhlenhuth (14) showed that thyroidectomized salamander larvae could not be made to undergo metamorphosis with large doses of pituitary extracts which were readily effective in the presence of the thyroid.

In extending the above concepts to mammals Smith (15) clearly demonstrated that following pituitarectomy in the rat regression could be prevented or repaired by implantation of anterior lobes of rat pituitaries. Aron (16) demonstrated thyreotropic activity of anterior pituitary extracts in the guinea pig. Later studies by Smith and Siebert (17) indicated that extracts of anterior lobe substance increased the metabolic rate in the presence of the thyroid in the guinea pig.

Schockaert (18) reported the production of a syndrome of exophthalmos, thyroid hyperplasia, decreased thyroid iodine and increased metabolic rate in the duck. He noted the tendency of the hyperplastic thyroid to involute in a few of his animals after prolonged treatment with anterior pituitary material. Schockaert drew the analogy between anterior pituitary induced hyperthyroidism and Graves' disease in the human.

Histologic studies by Hertz and Kranes (20) pointed to an exhaustion of the

---

[1] Read before the Association for the Study of Internal Secretions, Atlantic City, June 11, 1935.
This study was aided by a grant from the Proctor Fund of Harvard Medical School.
[2] Henry P. Walcott Fellow in charge of Thyroid Clinic and Metabolism Laboratory, Massachusetts General Hospital.
[3] Rockefeller Research Fellow 1934-1935, Harvard University.

thyroid epithelium after prolonged administration of the thyreotropic factor. The 'natural remission' despite prolonged thyreotropic administration had been noted in the metabolic studies of Friedgood, Loeb and Collip. Friedgood (21) published metabolic data and an account of anterior pituitary-induced exophthalmos and hyperthyroidism in the guinea pig. He also drew attention to the cyclic nature of the thyroid response to stimulation by the thyreotropic factor in a later publication (22).

Attempts to extend the anterior pituitary hypothesis to the pathological mechanism operative in human Graves' disease have been made. Marine (24) in a recent review on thyroid physiology stressed the resemblance between the hyperthyroidism produced in animals by injection with thyreotropic hormone and exophthalmic goiter in man and infers that there is an over-production of thyreotropic principal in Graves' disease. He suggests as an alternative possibility that exophthalmic goiter may occur in man when the capacity to produce an antithyreotropic material is impaired. He also states that a decreased production of thyreotropic hormone is probably the immediate cause of myxedema.

Aron (25) tried to obtain evidence of increased thyreotropic activity of the pituitary in human cases of Graves' disease. He used young guinea pigs as test animals and depended upon minor histologic changes such as vacuolization of the colloid as criteria in reading the test. It was with a similar view that the studies of Krogh and Okkels (26) were made. In their early experiments they attempted to repeat the technique of Aron. They agreed that there was no evidence of increased thyreotropic material in the urine of patients with Graves' disease, but added that the histologic assay method was not reliable. By using metabolic rate determinations in their assay technique these workers could find no further evidence of thyreotropic hormone in the urine of thyrotoxic patients, even after its concentration.

Castillo and Magdalena tried to verify Aron's work and used his method of assay. Their results forced them to the conclusion that guinea pigs were unsuitable for assay of thyreotropic action of sera. They found that of 10 hypophysectomized dogs, 3 had sera which gave a positive reaction by Aron's technique and criteria. Obviously the results of Aron must be regarded in the light of this nonspecificity of his assay method.

We therefore devised our own method of assay for thyreotropic factor using the pituitarectomized rat as test object. In so doing we followed the work of Collip and his co-workers in their attempts to purify thyreotropic hormone from pituitary tissue. This method can be regarded as specific for thyreotropic factor since it depends upon a principle similar to that underlying the substitutional transplant method used by Smith in his original contributions to the thyreotropic field.

METHOD AND RESULTS OF ASSAY

The method of assay which we finally developed and consider suitable for assay of biologic fluids for thyreotropic activity is as follows. White rats of Wistar strain were used throughout our experiments. Males weighing between 150 and 200 grams were selected. They were pituitarectomized by a modified Selye* technique. Five days were allowed postoperatively for recovery. Intramuscular injections of urine or serum were then instituted and carried on

* We wish to express our thanks to Professor J. B. Collip and Dr. H. Selye working in his laboratory for their many kindnesses extended to us at the inception of this study, and to Professor J. H. Means for his review of our material and stimulating suggestions.

over a period of 5 days. On the 10th or 11th day the animals were sacrificed and their thyroid glands obtained for histological examination. In our experience 2 injections daily of 5 cc. of untreated fresh urine and of 1 or 2 cc. of blood serum were well tolerated by pituitarectomized animals in good condition.

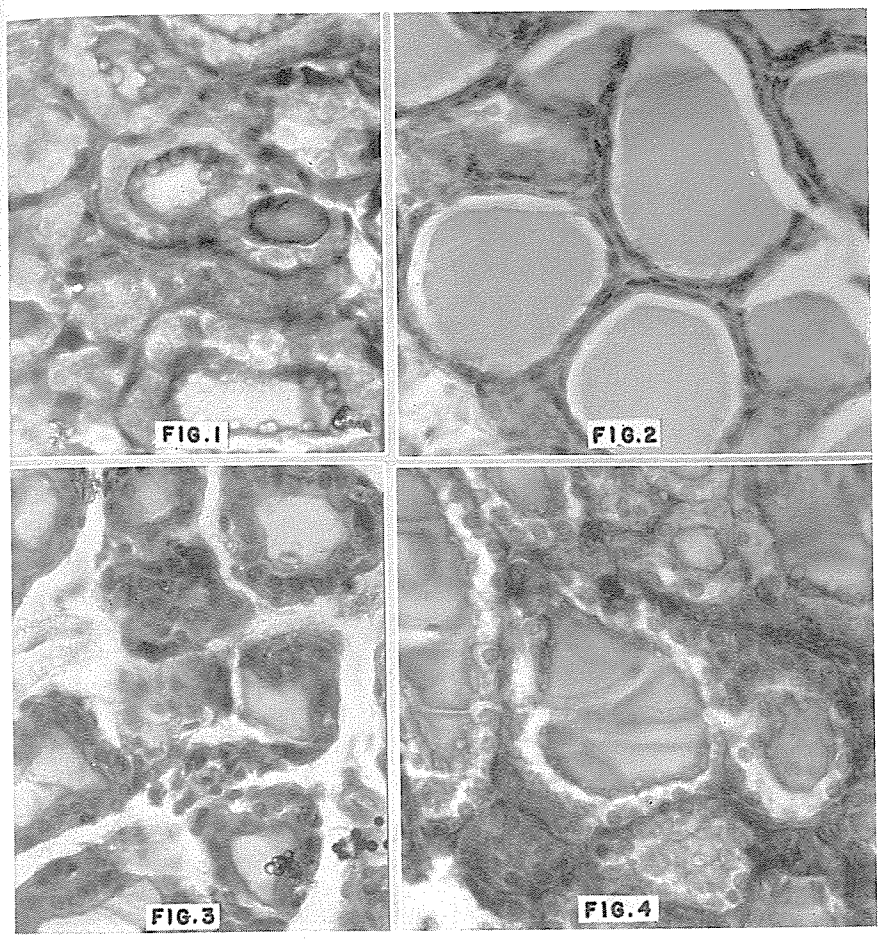
An ideal assay should include the following requirements. Three or 4 animals should be used in the assay of the biologic fluid in question. The totality of the pituitarectomy should be checked by gross palpation of the testes before starting the injections. (By the fifth postoperative day the testes should have shown a decrease in size and softening; the scrotum should have lost its normal turgescence and one or both of the testes should have ascended into the abdomen). At postmortem examination the pituitary fossa should be examined and sections made of any suspected material at the site of operation. Microscopic examination of the condition of the other endocrine glands such as the testis and adrenal should show atrophy. It is important to kill the animals at the end of the period of injections and to discard any which have died in order to obtain the microscopic material free of postmortem changes. These occur in the thyroid within 2 to 4 hours after death.

Selection of patients for assay. In this study only untreated and typical cases of thyrotoxicosis and myxedema were selected. Serum was obtained from venous blood drawn before any therapy was instituted. Urines were obtained as soon after voiding as possible and morning specimens were used in most instances. In addition to fluids obtained from 7 thyrotoxic patients and 9 myxedematous patients assays were carried out on the sera of 5 normal individuals and on the urines of 8 normal subjects.

Results of assays. The results of the assays can best be reported by reference to photomicrographs of histologic sections taken from typical examples of the assay material. Figure 1 shows a section of the thyroid gland of a normal (non-pituitarectomized) rat of our colony. The epithelium is cuboidal or low columnar. The colloid is abundant in amount, takes the stain uniformly and is slightly vacuolated at the edges. Figure 2 shows a section of a normal rat's thyroid 10 days after pituitarectomy. This rat received no injections and served as a control. This section shows the typical regression of the thyroid epithelium which takes place following pituitarectomy in the absence of any potent substitution therapy. Notable features of the section are the marked decrease in the height and number of epithelial cells, the large accumulation of colloid and the absence of even the normal degree of peripheral vacuolization of the uniformly stained acinar contents. Appearances entirely similar to these were obtained in the thyroid glands of totally pituitarectomized rats which had been treated with the bloods and urines of all of our normal individuals. Such assays will henceforth be referred to as 'negative for thyreotropic effect'. All the assays on bloods and urines of 8 cases of thyrotoxicosis gave similar negative findings.

Figure 3 is a photomicrograph of a section of thyroid gland from a pituitarectomized animal which received Collip's thyreotropic hormone from the fifth postoperative day for 5 days before sacrifice (0.25 cc. twice daily). This

section shows the characteristic 'support' of the thyroid histology by an adequate substitution therapy with thyreotropic material. This failure of regression of the thyroid epithelium following pituitarectomy will be referred to as evidence of a 'positive thyreotropic action' on the part of any material which produced it. The notable features of the section are the maintenance of the height and number of epithelial cells and the mild vacuolization of the colloid



which is not increased in amount and which shows a normal staining reaction.

Figure 4 shows a section of thyroid gland of an animal which was totally pituitarectomized 11 days before sacrifice and which received 5 cc. of urine from a completely myxedematous patient twice daily after the fifth postoperative day. The section shows an undoubtedly positive thyreotropic reaction. This is indicated by the presence of a mild degree of hyperplasia, columnar cells which are increased in number and show a slight tendency to papillary infolding, associated with marked vacuolization of the colloid which is decreased in amount and rather lightly stained. Entirely similar results were obtained with the urines of 8 and the blood sera of 9 myxedematous individ-

uals. A concentrate of 1 myxedematous patient's urine (1/5), made by vacuum distillation at room temperature gave a positive thyreotropic result whereas the unconcentrated urine gave a negative test. The assay on the serum of this patient was positive.

DISCUSSION

A comparison of figure 4 with figure 3 reveals the striking potency of myxedematous body fluids in supporting the thyroid histology following pituitarectomy as compared with the effect of the best available thyreotropic substance derived from pituitary tissue up to the time of these experiments. The appearance of slight hyperplasia in figure 4 is in sharp contrast to the regressive stage of the untreated pituitarectomized control animal's thyroid illustrated in figure 2. In contrast we believe we have demonstrated by the methods described above that the body fluids of neither normal nor thyrotoxic patients contain appreciable amounts of thyreotropic hormone in the quantities used in our experiments.

The fact that positive reactions were obtained with the body fluids of myxedematous patients indicates that the method is valid for the demonstration of the specific thyreotropic factor. The negative results obtained in normal and thyrotoxic patients serve as control data for the specificity of the reaction since neither the urinary contents nor the foreign serum protein of the negative samples had any effects on the thyroid histology of the pituitarectomized rat.

While this report confirms Aron's finding of 'thyreostimuline' in myxedema fluids we cannot agree that normal urine, even in amounts up to 90 cc. per rat, has any thyroid stimulating substance. We interpret Aron's positive results with normal urine as a reflection of the non-specificity of the guinea pig reaction since our substitutional assay method failed to confirm this. It is entirely conceivable, however, that Aron was able to detect the difference in titer of thyreotropic material which exists between myxedema and thyrotoxic fluids as shown by our results despite the non-specificity of his test method.

With the above data in mind we find it difficult to accept the anterior pituitary hypothesis in its present form as an explanation of the pathological mechanism of human Grave's disease despite the close analogy that has been drawn between the experimental and clinical syndromes. That the hyperthyroidism associated with anterior pituitary acidophilic tumors is on a pituitary thyreotropic basis seems likely. Cushing in 1927 found that of 72 cases of acromegaly more than half had a B.M.R. greater than + 10 per cent, the highest being + 61 per cent. Removal of a chromophil adenoma in his cases was followed by a "fall in B.M.R. as uniform and as striking as that which occurs following subtotal thyroidectomy in Grave's disease". However, it would appear from our data that there is no definite reason to suppose that the ordinary variety of patient with Grave's disease (not associated with acromegaly) is suffering from 'anterior pituitary hyperthyreotropism'.

That the urine and serum of cases of myxedema contain thyreotropic factor in considerable quantities deserves emphasis for several reasons. We believe this fact answers the question raised in the literature by Krogh and

Okkels as to whether thyreotropic material is excreted by the kidney. Furthermore the chemist will find in myxedema urine an excellent source of thyreotropic factor for purposes of purification and chemical identification.

SUMMARY

A method of substitutional assay is described as applicable to body fluids such as blood and urine for the detection of thyreotropic material. The method makes use of the pituitarectomized rat as test object and histologic examination of the thyroid glands of the assay animals is used in reading the test. Support of thyroid histology is considered as evidence of a positive test. Failure of injected material to prevent the regression of the thyroid epithelium following pituitarectomy is considered evidence of a negative test.

It was found by this method that blood serum and urine from myxedematous patients contain appreciable quantities of thyreotropic material, whereas none of the thyroid stimulating factor could be demonstrated in the body fluids of either thyrotoxic or normal individuals in the dosages used.

In discussion the current hypotheses of the mechanism of human Graves' disease are considered, and the significance to the chemist of the occurrence of thyreotropic factor in myxedema fluids is stressed. The specificity of the substitutional method of assay for the thyreotropic factor used in this study is held to be greater than that of other methods which have so far been used in attacking the problem.

REFERENCES

1. Rogowitch, N.: Beitr. z. path. Anat. u. zallg. Path. **4:** 453. 1888-9.
2. Nièpce, P.: cited in Nice—med. **3:** 81. 1878-9.
3. Boyce, R. and C. F. Beadles: J. Path. & Bact. **1:** 223. 1892.
4. Wegelin, C.: Wien. klin. Wchnschr. **38:** 5. 1925.
5. Degener, Lyda M.: Quart. J. Exper. Physiol. **6:** 111. 1913.
6. Kojima, M.: Quart. J. Exper. Physiol. **11:** 255. 1917.
7. Adler, L.: Roux's Arch. **39:** 21. 1914.
8. Allen, B. M.: Science **44:** 755. 1916.
9. Smith, P. E.: Science **44:** 280. 1916.
10. Gudernatch, J. F.: Zentralbl. f. Physiol. **26:** 323. 1912.
11. Allen, B. M.: Anat. Rec. **20:** 192. 1921.
12. Smith, P. E. and J. P. Smith: Proc. Soc. Exper. Biol. & Med. **20:** 51. 1922.
13. Hogben, L. S.: Proc. Roy. Soc. London, S.B. **94:** 204. 1922.
14. Schwartzbach, S. S. and E. Uhlenhuth: Anat. Rec. **24:** 119. 1926.
15. Smith, P. E.: J.A.M.A. **88:** 158. 1927.
16. Aron, Max: Compt. rend. Soc. de biol. **102:** 682. 1929.
17. Siebert, W. J. and R. S. Smith: Proc. Soc. Exper. Biol. & Med. **27:** 622. 1930.
18. Schockaert, J. A.: Proc. Soc. Exper. Biol. & Med. **29:** 306. 1931.
19. Marine, D. and S. H. Rosen: Proc. Soc. Exper. Biol. & Med. **30:** 901. 1933.
20. Hertz, S. and A. Kranes: Endocrinology **18:** 415. 1934.
21. Friedgood, H. B.: Bull. Johns Hopkins Hosp. **54:** 48. 1934.
22. Friedgood, H. B.: Arch. Int. Med. **56:** 833. 1935.
23. Schockaert, J. A. and G. L. Foster: J. Biol. Chem. **95:** 89. 1932.
24. Marine, D.: J.A.M.A. **104:** 2250. 1935.
25. Aron, M., C. VanCaulaert and J. Stahl: Compt. rend. Soc. de biol. **107:** 64. 1931.
26. Krogh, M. and H. Okkels: Compt. rend. Soc. de biol. **113:** 635. 1933.