IODINE COMPONENTS OF THE BLOOD. CIRCULATING THYROGLOBULIN IN NORMAL PERSONS AND IN PERSONS WITH THYROID DISEASE

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It is now well established that the amount of iodine circulating in the blood is roughly an index of thyroid activity. Iodine values below 5 gamma per cent are usually found in myxedema, and values over 10 gamma per cent are suggestive of hyperthyroidism. A portion of the blood iodine is inorganic and presumably inert in a hormonal sense; the remainder is organic and probably represents the circulating hormone or its components. Very little is known of the nature of this organic iodine. In the course of experiments on the production of antibodies to human thyroglobulin, it seemed that antiserum potent with respect to thyroglobulin antibodies might be used to detect thyroglobulin in human serum by appropriate immunologic reactions.

METHOD

The technique used to obtain antithyroglobulin serum is similar to that of Hektoen and Schulhof (1), Rosen and Marine (2), and Schulhof (3). Thyroglobulin was prepared from human thyroid glands which had been removed at operation and made relatively free from serum.[1] It was kept in suspension at its isoelectric point. The protein content was usually 1.5 to 2.0 per cent, and the iodine about 5 to 6 mgm. per cent. This material was injected into rabbits intraperitoneally, intravenously, or into subcutaneous nodules according to the method described by Dienes (4). The injections were given for 2 to 3 days in succession, with rest periods of 4 to 6 days. The intraperitoneal injection contained about 75 to 150 mgm. of thyroglobulin, the intravenous injection 15 to 20 mgm. and the intranodular injection 1 to 3 mgm. for each nodule. After a period of 4 to 8 weeks, the serum of such animals usually contained sufficient antibodies to be of value in testing for very small amounts of thyroglobulin. In some instances, animals were injected for several months in succession in order to increase their antibody titer. The rabbit antiserum contained not only antibodies for human thyroglobulin but also small amounts of antibodies for human serum protein. Consequently, before using such serum in immunologic tests it was necessary to absorb the antibodies against human serum. This was done by mixing the antiserum with human serum in the proportion of 1 to 0.25, incubating the mixture overnight, and centrifuging the small precipitate formed. A similar absorption technique was used by Stokinger and Heidelberger (5).

The presence of antibodies was determined by the ring precipitin test, using the undiluted rabbit serum against dilutions of thyroglobulin or human serum. Thyroglobulin was first dissolved in dilute alkali at a pH of 8.0 to 9.0 until it was almost clear and the insoluble portion removed by centrifugation. Normal saline used in making dilutions was also adjusted to the same pH. Otherwise, at the usual pH of normal saline there would be precipitation of thyroglobulin.[2] The antiserum was introduced at the bottom of small tubes and the dilutions of thyroglobulin or human serum were layered on top. The results were read after 1 to 2 hours at room temperature.

In attempting to evaluate the potency of the antiserum, the precipitation test and complement fixation technique were used to confirm the results of the precipitin ring test. However, in detecting thyroglobulin in human serum, only the precipitin test was used.

Blood for tests was obtained from normal people, from patients with myxedema, from thyrotoxic patients before and after iodinization and after operation, and directly from the thyroid veins during operation. In all, about 66 samples of blood were tested. Four were from normal people, 2 from patients with myxedema, 15 from thyrotoxic patients before iodinization, 2 from thyrotoxic patients after iodinization, 22 from the thyroid veins of toxic and non-toxic goiter patients obtained during the course of operation, and 21 from goiter patients after operation. Numerous normal bloods were used as negative controls when the blood from thyroid veins was tested for thyroglobulin. Some of the blood samples were tested with more than one antiserum. In addition, the 24-hour urine specimens of 3 hyperthyroid patients were concentrated according to the method used by Rawson and Starr (6) in concentrating thyrotropic hormones and tested for the presence of thyroglobulin.

[1] I am indebted to Dr. W. T. Salter and Dr. J. M. Muus of the Boston City Hospital for the preparation of thyroglobulin.

[2] Undoubtedly, some of the thyroglobulin is denatured in the process of preparation and on standing, but the amount is seldom more than 10 per cent. The precipitate formed when normal saline is added is probably due to this fraction of thyroglobulin.

RESULTS

The antisera used in these experiments usually were able to detect thyroglobulin in dilutions up to 1:10,000 or 1:20,000 of a 1.5 per cent solution. In other words, the amount of thyroglobulin that could be detected was 0.08 to 0.15 mgm. per 100 cc. of serum or ¼ to ½ gamma per cent of thyroglobulin iodine, an amount practically negligible. Even the less potent sera could detect 1 gamma per cent of thyroglobulin iodine. Table I represents the result of a typical precipitin test for thyroglobulin in the blood of an exophthalmic goiter patient. The unmodified rabbit antiserum gave strongly positive reactions, as indicated in the second horizontal column, but the absorbed serum gave negative reactions, as shown in the last horizontal column. The positive reactions were obviously due to antibodies against human serum. This particular antiserum was able to detect about 0.1 mgm. of thyroglobulin per 100 cc. of blood.

TABLE I

The precipitin test for thyroglobulin in blood of a hyperthyroid patient (J.T. Number 4914) before and after absorption of rabbit antiserum by human serum

	Dilutions of human serum					
	1:2	1:4	1:8	1:16	1:32	1:64
Rabbit Number 2K antiserum...	↓↓+	↓++	↓+	++	++	+
Same antiserum incubated with human serum.....	±	−	−	−	−	−

↓ indicates precipitation. + indicates ring precipitin.

With the precipitin test no detectable amount of thyroglobulin was discovered either in the various normal human sera, in the sera of patients with myxedema, or in the sera of thyrotoxic patients before and during iodinization. The patients with hyperthyroidism were unselected and therefore represent all grades of severity, the metabolic levels ranging from plus 27 to plus 75. Similarly, no thyroglobulin was detected in the urine of 3 thyrotoxic patients.

In spite of the absence of any appreciable amount of thyroglobulin in the blood, there still remained the possibility that thyroglobulin was secreted unchanged directly into the thyroid veins, as reported by Carlson, Hektoen and Schulhof (7) in dogs. Consequently, blood was obtained directly from the thyroid veins during the course of thyroidectomy, attempt being made to get samples both at the beginning and at the end of operation. In most instances, samples were also obtained from the peripheral blood 2 to 4 hours and 24 to 36 hours after operation. In all, 9 cases of exophthalmic goiter and 4 cases of nontoxic goiter were studied in this fashion. In 5 additional cases blood was obtained only in the post-operative period. Where thyroglobulin was present in a blood sample, the attempt was made to determine the quantity by setting up simultaneous precipitin tests with a known solution of thyroglobulin. By comparing the known and unknown tubes, an approximate estimate of the amount present in the unknown was obtained. Table II shows a typical example of precipitin tests for thyroglobulin in blood from thyroid veins and in peripheral blood. Table III summarizes the results of the precipitin tests on all samples of blood taken during and after operation.

In only 2 of the 12 samples of blood taken from the thyroid vein at the beginning of operation was there any evidence of thyroglobulin. This amounted to 0.1 mgm. and 1.6 mgm. per 100 cc., respectively. In 1 of these cases, (C. L.), a second specimen from the opposite thyroid lobe showed a slight increase in thyroglobulin. In the other, (D. K.), the second specimen was missed so that one cannot tell whether there was an increase or not. In this case there was no thyroglobulin detected in the peripheral blood 2 hours after operation. In a third case, (E. Y.), the first thyroid vein sample was obtained during the course of a second hemithyroidectomy. It is therefore listed as a second specimen. Two patients showed traces (±) of thyroglobulin in the first thyroid vein sample, but such evidence is doubtful and must be classified with the negative results.

Of the 8 thyroid vein samples obtained during or at the end of the second stage of subtotal thyroidectomy, 7 showed appreciable amounts of thyroglobulin, varying from 0.2 to 13.0 mgm. per 100 cc. In 5 of these, the first thyroid vein sample taken at the beginning of operation was negative. In only one subtotal thyroidectomy,

TABLE II

The precipitin test for thyroglobulin in blood of a hyperthyroid patient (C.L. Number 218911) compared to a known thyroglobulin solution

	Dilutions of human thyroglobulin					
	1 : 1,000	1 : 2,000	1 : 4,000	1 : 8,000	1 : 16,000	1 : 32,000
Rabbit antiserum incubated with human serum	↓+	++	+ or ++	+	+	±

	Dilutions of patient's sera					
	1 : 1	1 : 2	1 : 4	1 : 8	1 : 16	Blood sample number
Rabbit antiserum incubated with human serum	+	±	−	−	−	1. From right middle thyroid vein, beginning of operation.
	++	+	±	−	−	2. From left inferior thyroid vein, end of operation.
	+ or ++	+	−	−	−	3. Peripheral blood 3 hours post-operative.
	−	−	−	−	−	4. Peripheral blood 24 hours post-operative.

TABLE III

Results of precipitin tests for thyroglobulin in bloods taken from thyroid veins during operation, and from peripheral blood after operation (absorbed serum used)

Case	Right middle thyroid vein	Left inferior thyroid vein	2–4 hours post-operative	24–36 hours post-operative	Postoperative reaction
HYPERTHYROID PATIENTS					
M.L.	0	0			mild
A.C.	0	2			mild
R.B.	±	16		1 or ±	severe
M.LaF.*	±	±	0	0	moderate
D.K.	16		0	0	moderate
E.Y.†		8	±		moderate
C.L.	1	2	2	0	mild
I.C.	0	128	2	2	mild
I.R.	0	4	2		moderate
A.T.			0	0	mild
R.LeG.			0	0	mild
M.M.				0	mild
E.C.				0	mild
B.P.				0	thyroid "crisis"
NON-TOXIC GOITER PATIENTS					
A.B.	0				mild
E.M.	0	8		0‡	mild
E.G.*	0	0		0‡	mild
S.Y.*	0		0		mild

The values indicate the highest dilution of human serum which gives a positive precipitin test; ± represents an amount less than 1.

* Hemithyroidectomy.

† Second hemithyroidectomy.

‡ 12 hours postoperative.

(M. L.), were both thyroid vein samples negative for thyroglobulin. In 2 other cases, (M. LaF. and F. G.), the operative procedure was that of initial hemithyroidectomy. Here, the second thyroid vein sample was obtained at the end of hemithyroidectomy, but from the unoperated side. The latter received very little manipulation before the blood was drawn. In both, the blood was free of thyroglobulin at the beginning and at the end of operation. These results suggest that the operative procedure of cutting and manipulating the gland caused the extrusion of thyroglobulin into the blood. As indicated before, the blood of 2 thyrotoxic patients was tested for the first time postoperatively at 4 hours and at 24 hours, and was found to be free of detectable thyroglobulin. The blood of 2 others was negative 24 hours after operation. The blood of a fifth patient was examined 36 hours after operation during a thyrotoxic crisis and no thyroglobulin was found. Thus, of the 9 samples of blood examined 2 to 4 hours after operation, 3 showed small amounts of thyroglobulin; of the 12 samples examined 12 to 36 hours postoperatively, 1 showed a small amount and another showed a possible trace of thyroglobulin. In general, the thyroglobulin that gets into the blood stream during operation tends to diminish or to disappear altogether.

It might be supposed that the amount of thyroglobulin present in the blood during and after operation would condition the severity of the postoperative reaction. This was not the case. As indicated in the next to the last column of Table III, the severity of the postoperative reaction had no relation to the amount of thyroglobulin found in the thyroid veins during operation or in the peripheral blood after operation. The patient, (I. C.), who had the most thyroglobulin in the thyroid veins during operation had a mild postoperative reaction. This patient was 1 of the 2 patients who still showed thyroglobulin in the blood after 24 hours. Moreover, the 1 patient with a "thyrotoxic crisis" did not have any thyroglobulin during this episode. Unfortunately, blood from the thyroid vein was not obtained in this case. The only other severe reaction was shown by a patient, (R. B.), whose thyroid veins contained a moderate amount of thyroglobulin. However, this patient's gland was friable and bled easily so that the technical procedure was difficult and of more than average duration.

DISCUSSION

The absence of any appreciable amount of thyroglobulin in normal human blood is in agreement with the findings of Hektoen, Carlson and Schulhof (8) in goitrous dogs. One might have expected that in hyperthyroidism, which is characterized by a tremendous outpouring of thyroid hormone and an increase in blood iodine, there would be detectable amounts of thyroglobulin in the circulation. Hektoen and Schulhof (9) failed to find thyroglobulin in the blood of a small number of exophthalmic goiter patients. The absence of thyroglobulin in the peripheral blood in hyperthyroidism at various stages of the disease, as indicated above, is confirmatory. It proves that the excess organic iodine in hyperthyroid blood is not due to thyroglobulin. What it is due to I am unable to say on the basis of the present experiments.

The finding of appreciable amounts of thyroglobulin in thyroid vein blood and in thyroid lymph of dogs by Carlson, Hektoen and Schulhof (7) would lead one to believe that most of the thyroid hormone is secreted directly into the blood capillaries and lymphatics as thyroglobulin. The absence of thyroglobulin in the general circulation of these dogs may be due either to rapid dilution by the blood, rapid absorption of thyroglobulin by the tissues or rapid digestion of the protein molecule into smaller components. However, it would be difficult to explain their inability to find thyroglobulin in the thoracic duct. The question naturally arises, could their finding of thyroglobulin in the thyroid veins and lymphatics be explained on the basis of trauma? These investigators themselves were unable to exclude possible injury to the thyroid cells.

In humans, Hektoen and Schulhof (9) report the finding of thyroglobulin in 5 of "a considerable number" of thyroid vein blood samples. Unfortunately, no information is available regarding the stage of operation at which these bloods were obtained. In goiter operations on humans it is almost impossible to avoid some degree of trauma in exposing the blood supply of the gland, whether the goiter is toxic or non-toxic. Consequently, the results of Hektoen and Schulhof and the above reported absence of detectable amounts of thyroglobulin in 10 of the 12 thyroid vein samples at the beginning of operation are significant. They immediately suggest that the positive results are due to artificial factors, namely trauma. The finding of thyroglobulin in 7 of 8 thyroid vein samples taken in the later stages of subtotal thyroidectomy supports this viewpoint. Between the first and second thyroid vein samples considerable manipulation and cutting of the gland had taken place. In short, colloid was squeezed into the circulation but not secreted into it. It must therefore be concluded that neither in the normal gland nor in the hyperplastic gland does thyroglobulin enter the blood from the follicle as such, but must be broken down into smaller components.

The anatomic mechanism for release of thyroid has recently been studied by Williams (10). He observed living thyroid follicles in chambers implanted in rabbits' ears. He describes a process by which a droplet of colloid is pinched off and comes to lie within the follicular wall. The droplet slowly decreases in size until it disappears. It is never extruded into the interfollicular spaces. In other follicles a whole section of wall may be compressed for a short time. It

is supposed that colloid diffuses through this thin area in the follicle. Williams maintains that the follicular wall remains intact. If this is the case, one may logically assume that the thin portion of the follicle acts as a living semi-permeable membrane and that a large molecule like thyroglobulin could hardly diffuse through it. It would seem more likely that thyroglobulin is first digested and that the resultant small fragments then diffuse through the follicle wall. Thus the uncommon occurrence of thyroglobulin in thyroid veins is consistent with the anatomic observation of Williams.

It is of interest to speculate on the rôle of thyroglobulin in metabolic processes. Is thyroglobulin merely the protein storehouse for smaller and hormonically active components? The failure of the thyroid follicles to release thyroglobulin as such and the circulation in the blood of fragments of thyroglobulin rather than the parent substance would suggest that thyroglobulin is not the metabolic hormone. Thyroxine or thyroxine-like substances must be the true accelerators of oxidative processes. However, the possibility remains that these fragments are resynthesized into thyroglobulin when they reach the tissues and that hormonic action is after all dependent upon the formation of thyroglobulin.

SUMMARY AND CONCLUSION

Rabbits injected with human thyroglobulin produced antiserum which was able to detect by precipitin reaction minute amounts of thyroglobulin in solution, namely 0.08 to 0.15 mgm. per 100 cc. By means of this reaction no detectable amounts of thyroglobulin were discovered in the blood of numerous normal patients, 2 myxedematous patients, 15 thyrotoxic patients before iodinization, and 2 thyrotoxic patients after iodinization. No thyroglobulin was detected in the urine of 3 hyperthyroid patients.

It is therefore concluded that the excess iodine usually present in the blood of hyperthyroid patients is not due to circulating thyroglobulin.

The precipitin reaction was applied to venous blood from the thyroid obtained at various stages of thyroidectomy in 9 cases of exophthalmic goiter and in 4 cases of non-toxic goiter. Ten of the 12 thyroid vein samples obtained at the beginning of operation were negative; 2 thyroid vein samples obtained at the end of hemithyroidectomy, but from the unoperated side, were also negative. On the other hand, 7 of 8 thyroid vein samples obtained during or at the end of the second stage of subtotal thyroidectomy showed appreciable amounts of thyroglobulin. The thyroid vein blood of 5 of these cases was negative at the beginning of operation. These results suggest that the presence of thyroglobulin in the blood during and after operation is due to the extrusion of colloid into the circulation by trauma to the gland, and that under ordinary conditions thyroglobulin does not leave the follicles, normal or hyperplastic, as such. This deduction is consistent with the observations of Williams on the release of colloid from living thyroid follicles.

The thyroglobulin that gets into the circulation during operation is either rapidly destroyed or fixed by the tissues. Of the 12 blood samples obtained 12 to 36 hours after operation, 10 showed no thyroglobulin, 1 showed 0.2 mgm. per cent, a decrease from 13.0 mgm. per cent, and 1 showed a possible trace, a decrease from 1.6 mgm. per cent.

The presence or absence, or the amount of thyroglobulin in the blood during and after operation, does not correlate with the degree of postoperative reaction. This fact does not exclude the possibility that absorption of hydrolyzed products of thyroglobulin during or immediately after operation plays an important rôle in the development of thyrotoxic "crises."

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BIBLIOGRAPHY

1. Hektoen, L., and Schulhof, K., The precipitin reaction of thyroglobulin. J. A. M. A., 1923, **80**, 386.
2. Rosen, S. H., and Marine, D., Immunity to iodothyroglobulin does not affect its physiological action. Am. J. Physiol., 1937, **120**, 121.
3. Schulhof, K., The effect of antithyroglobulin serum on the physiological action of thyroglobulin. Am. J. Physiol., 1930, **93**, 175.

4. Dienes, L., The immunological significance of the tuberculous tissue. J. Immunol., 1928, **15**, 141.
5. Stokinger, H. E., and Heidelberger, M., A quantitative theory of the precipitin reaction. VI. The reaction between mammalian thyroglobulins and antibodies to homologous and heterologous preparations. J. Exper. Med., 1937, **66**, 251.
6. Rawson, R. W., and Starr, P., Direct measurement of height of thyroid epithelium. Method of assay of thyrotropic substance; clinical application. Arch. Int. Med., 1938, **61**, 726.
7. Carlson, A. J., Hektoen, L., and Schulhof, K., Attempts to produce experimental increase in the rate of output of thyroglobulin by the thyroid gland. Am. J. Physiol., 1924–5, **71**, 548.
8. Hektoen, L., Carlson, A. J., and Schulhof, K., The precipitin reaction of thyroglobulin. Presence of thyroglobulin in the thyroid lymph of goitrous dogs. J. A. M. A., 1923, **81**, 86.
9. Hektoen, L., and Schulhof, K., The precipitin reaction of thyroglobulin. Proc. Nat. Acad. Sc., 1925, **11**, 481.
10. Williams, R. G., Microscopic studies of living thyroid follicles implanted in transparent chambers installed in the rabbit's ear. Am. J. Anat., 1937, **62**, 1.