FAMILIAL INCREASE IN THE THYROXINE-BINDING SITES IN SERUM ALPHA GLOBULIN *

By WILLIAM H. BEIERWALTES AND JACOB ROBBINS

(From the Department of Internal Medicine, Clinical Radioisotope Unit and Thyroid Research Laboratory, University Medical Center, Ann Arbor, Mich., and Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.)

(Submitted for publication February 2, 1959; accepted June 4, 1959)

Thyroxine in human serum migrates during paper electrophoresis at pH 8.6 with at least three protein components: an α globulin intermediate between α_1 and α_2, thyroxine-binding protein (TBP) (1, 2), albumin (1, 2) and a component moving faster than albumin, prealbumin or thyroxine-binding prealbumin (TBPA) (3). Alterations in serum protein-bound iodine (PBI) may occur in certain circumstances without concomitant hyper- or hypothyroidism, but with parallel changes in the thyroxine-binding capacity of TBP. Examples are the elevated PBI and TBP capacity in pregnancy (4, 5) and after estrogen therapy (6), and the depressed PBI and TBP capacity produced by methyl testosterone (7). It seemed logical, therefore, to look for alterations in TBP in patients with abnormal serum PBI levels in whom other evidence of thyroid dysfunction was lacking. We wish to report the occurrence of an elevated PBI and TBP capacity in an otherwise normal adult male and in one of his three children.

CASE REPORT AND METHODS

W.W. was a male 48 years of age when seen on March 17, 1958, because his serum PBI was found to be "elevated" on a routine periodic health examination. He was asymptomatic and gave no history of thyroid disease in himself or in his family. He had never received radiographic contrast media and there was no known ingestion of other organic iodine compounds. He gave no history of diseases known to alter the serum proteins. His libido and potentia were considered to be normal. His physical examination was completely normal. No goiter or evidence of hyper- or hypothyroidism was evident. Hair growth and distribution and genitalia were normal.

His two sons, aged 18 and 12 years, and his daughter, aged 15 years, also presented no abnormal findings by medical history or physical examination except for the history of allergic asthma treated with potassium iodide in the 12 year old boy. The girl gave a normal menstrual history with onset at 12.5 years of age.

Pertinent laboratory findings on the father are presented in Table I. Normal values and references to the published techniques used in obtaining these laboratory data are also presented in this table.

Measurement of the thyroxine-binding capacity of TBP was done as before (8, 9) except that electrophoresis was carried out in 0.1 M ammonium carbonate as well as in barbital buffer. The pH of the solution of U.S.P. grade ammonium carbonate was adjusted to about pH 8.4 by bubbling CO_2 through it for a few minutes. The pH on the paper strip at the end of the run was approximately 8.5 as determined by indicator paper. The thyroxine-binding capacity of TBP determined in ammonium carbonate is the same as when barbital is used (9). Electrophoresis in the former, however, results in a large additional thyroxine peak running ahead of albumin similar to that found with "Tris" maleate buffer (3). In this study, two levels of added I^{131}-labeled L-thyroxine were employed: 2.69 μg. per ml. and 1.34 μg. per ml. The former was done in triplicate and the latter in duplicate.

RESULTS

The findings in the father are given in Table I. The serum PBI and serum butanol extractable iodine (BEI) were elevated to two to three times the mean value found in normal individuals. The I^{131}-labeled triiodothyronine erythrocyte uptake (I^{131} T^3 RBC uptake) which is reported to increase in hyperthyroidism (12, 22) was less than half of the lower limit of published normal values. The basal metabolic rate (BMR) and basal pulse rate were at the low normal to hypothyroid range. The serum cholesterol and I^{131} thyroid uptake were well within the normal range.

The disappearance of intravenously injected I^{131} L-thyroxine from the plasma (expressed in Table I as half time, $T_{1/2}$, and as fraction of the thyroxine pool turned over per day) was slower than the normal mean by almost a factor of two and slower than reported in myxedema (14). The extrathyroidal organic iodine pool (EOI) was

* Expense of this project was defrayed in part by grants from the United States Public Health Service (M-2399) and the Atomic Energy Commission Project No. 15.

TABLE I

Familial increase in thyroxine-binding sites in serum alpha globulin: Pertinent laboratory findings in the father

Laboratory tests			Time in months (1958)					
Name	Reference to method	Normal values	Mar.	Apr.	May	June	Nov.	Dec.
PBI (μg. %)	(10)	4.0–7.4	13.9	16.0			11.8	14.7
Inorganic iodine (μg. %)		0.5–3.0	0.9					
BEI (μg. %)	(11)	3.4–6.8		13.0				12.3
I^{131} T^3 RBC (% uptake @ 2 hrs.)	(12)	10.0–17.0			4.8			
I^{131} thyroxine plasma $T_{\frac{1}{2}}$ (days)	(13, 14)	6.6 ± 0.7						12.2
Thyroxine turnover rate (%/day)	(13, 14)	10.5 ± 1.1						5.7
Extrathyroidal organic iodine pool (μg.)	(13, 14)	548 ± 107						1,099.0
Thyroxine degradation rate (μg./day)	(13, 14)	57 ± 11						63.0
BMR (%)		−15–10	−20					
Basal pulse rate		60–90	60					
Serum cholesterol (mg./100 ml.)	(15)	125–325	212					
I^{131} thyroid uptake (% of dose)	(16)	10.0–36.0 (24 hrs.)	1 hr. = 4.2 2 hr. = 6.6 6 hr. = 10.0 24 hr. = 21.6					
Total serum proteins (Gm. %)	(17)	6.5–8.5				7.6		
Serum albumin (Gm. %)		4.0–5.5				4.8		
Serum globulin (Gm. %)		1.5–3.5				2.8		
BSP (% retention at 45 min.)	(18)	0–6.0				2.3		
17-Ketosteroids (mg./24 hrs.)	(19)	6.0–20.0			19.5			
Urinary estrogens (rat units/24 hrs.)	(20)	0–4.0			10.0	5.0		
Follicle stimulating hormone (mouse units/24 hrs.)	(21)	6.0–48.0			6.0	Neg. at 3 M.U.		

about twice as large as the normal mean and comparable to that in thyrotoxicosis (14). The degradation rate of thyroxine in micrograms per day was, however, within the normal range.

Normal values were found for serum proteins, bromsulfalein retention, and urinary 17-ketosteroid excretion. The slightly elevated values for 24 hour urinary estrogen excretion were of questionable significance, as was the low value for urinary follicle stimulating hormone.

TABLE II

Familial increase in the thyroxine-binding sites in serum alpha globulin: Laboratory findings in children of propositus

Child	Age	Sex	PBI	Inorganic iodine	BEI	I^{131} T^3 RBC uptake	BMR
	yrs.		*μg. %*	*μg. %*	*μg. %*	*per cent*	
1	18	M	5.8	<10	5.4	10.7	−6
2	12	M	>25	500	6.2	8.4	
3	15	F	10.1	<10	9.1	4.5	−6

TABLE III

*Thyroxine-binding capacity**

Subject	Thyroxine-binding capacity of TBP		Thyroxine in prealbumin
	Added T_4 = 1.34 μg./ml.	Overall average†	Added T_4 = 2.69 μg./ml.
	μg. T_4/ml.	*μg. T_4/ml.*	*μg. T_4/ml.*
Normal A	.22	.24	1.2
Normal B	.16	.21	1.2
Father (Nov.)	.47	.45	1.3
Child 1	.14	.14	1.5
Child 2	.23	.20	1.1
Child 3	.36	.29	1.5

* Determinations made by electrophoresis in 0.1 M ammonium carbonate.

† Overall average of five determinations, two at 2.69 μg. per ml. T_4 added and three at 1.34 μg. per ml. T_4 added.

Table II summarizes a few pertinent laboratory findings on the children of the propositus.

The serum PBI and BEI were definitely elevated in the daughter although not so high as in the father. The low I^{131} T^3 RBC uptake in the daughter was approximately the same as in the father. The daughter's normal BMR value agreed with the clinical impression that she also was euthyroid. The serum of the second son was contaminated by relatively large quantities of inorganic iodine which apparently resulted in elevation of the serum PBI. The spurious nature of this elevation was borne out by the normal BEI. The slight depression of the I^{131} T^3 RBC uptake value in the second son must be ascribed to contamination with iodide (22) until proved otherwise.

Values obtained with ammonium carbonate for the thyroxine-binding capacity of TBP in the members of the family, as well as in two normal adults who were studied simultaneously, are listed in Table III. In addition to the overall averages for five determinations, the averages for duplicate

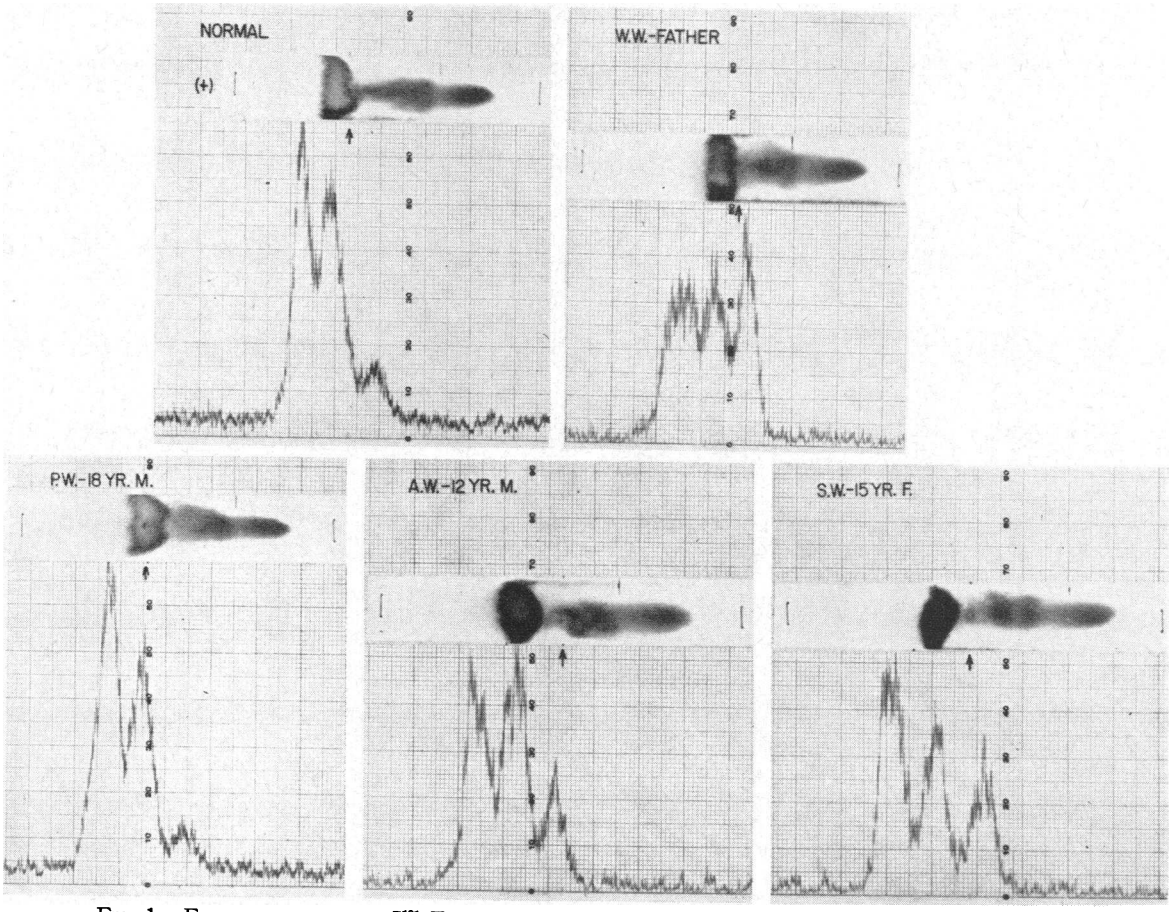

FIG. 1. ELECTROPHORESIS OF I^{131} THYROXINE-SERUM MIXTURES IN 0.1 M AMMONIUM CARBONATE

Each serum mixture contained 1.34 μg. of added L-thyroxine per ml. The paper strips, stained with bromphenol blue, are mounted above their radioactivity records. The arrows indicate the points of application. Electrophoresis was by the reverse-flow technique.

determinations at an added thyroxine concentration of 1.34 μg. per ml. are given. The latter are considered to be more accurate since the area under the TBP peak at the higher thyroxine level (2.69 μg. per ml.) was very small and its measurement was subject to greater error. The two averages agree closely except in Normal B and Child 3.

The values for the normals were in the range previously reported for normal adults (8). The results in Child 1 and Child 2 were also normal, but TBP in sera from the father and Child 3 had greater than normal capacity to bind thyroxine. The highest value was found in the father. Thyroxine-binding capacities determined with barbital buffer gave similar results. Representative electrophoretic patterns are presented in Figure 1 and demonstrated no abnormality other than the quantitative difference already noted.

Table III also gives values for the quantity of thyroxine associated with TBPA at the higher thyroxine level. In the case of Normal A, thyroxine in TBPA was determined at a series of thyroxine concentrations. The results indicated that, although a plateau was approached at the 2.69 μg. per ml. level, it had not been reached. The results in Table III are probably lower, therefore, than the capacity of TBPA for thyroxine, but they permit comparison between the normals and the subjects of this study. The significance of the rather small differences observed is uncertain, but it is noteworthy that the value in the father did not differ from that in the normals.

DISCUSSION

This report describes the occurrence of an elevation of the thyroxine-binding capacity of TBP in an appraently euthyroid man with an otherwise unexplained elevation of serum PBI and BEI. No cause for this alteration was apparent. The finding of a similar abnormality in one of his children, however, suggested that it might have a genetic basis. The evidence presented does not distinguish between an increase in the concentration of TBP itself or in available sites on the TBP molecule as a cause for the increased binding capacity. There appeared to be no alteration in the binding of thyroxine by TBPA in the affected subjects or of any qualitative alteration in the electrophoretic distribution of thyroxine. Chemical identification of the serum PBI was not done, but in the presence of an elevated thyroxine-binding capacity it is reasonable to assume that it was thyroxine.

If, as seems likely, the increase in TBP capacity was the primary alteration, it is of interest to speculate on how the other observed abnormalities in thyroid physiology might have come about. If it is assumed that thyroxine (or triiodothyronine) combined with serum protein is unavailable to the tissues and that the metabolic action of the hormones is exerted only by the diffusible free thyroxine, which is in equilibrium with the bound, then a higher total thyroxine level in serum would be required for maintenance of euthyroidism (2). A normal thyroid-pituitary system would result, therefore, in an elevated serum PBI together with an expanded extrathyroidal thyroxine pool, as observed in the case presented. In the euthyroid steady state, however, the quantity of thyroxine degraded per day would be normal, so that the fraction of the total thyroxine pool turning over per day would be decreased. In this steady state the function of the thyroid gland would be no different from normal. The findings presented are compatible with such an hypothesis.

The depression in I^{131} T^3 RBC uptake may also be explained by the increase in TBP capacity for thyroxine since triiodothyronine and thyroxine interact with the same sites on this protein (23). From the expression for an equilibrium reaction,

$$k\ (\text{free thyroxine}) = \frac{(\text{thyroxine bound to TBP})}{(\text{unoccupied TBP sites})}.$$

If free thyroxine remains constant in the presence of an increase in total TBP sites and total serum thyroxine, then the concentration of unoccupied TBP sites must increase. The addition of a small increment in labeled thyroxine (or triiodothyronine) to such an altered serum would result in a greater normal proportion of the labeled substance being bound to TBP. There would then be less free *labeled* hormone available for uptake by the red blood cells even though the total quantity of hormone taken up might be normal. In the absence of other alterations affecting RBC uptake, the changes in TBP and PBI in the patient would, therefore, explain the lowered I^{131} T^3 RBC uptake.

The findings suggest that some of the instances of elevated serum PBI levels without accompany-

ing hyperthyroidism, which are found sporadically and are usually attributed to contamination with nonhormonal iodine (24), may be the result of an increase in the thyroxine-binding capacity of serum TBP. Tanaka and Starr (25) described 10 such patients, all of whom were women. The present report is, to our knowledge, the first indication that such an abnormality may be familial. The frequency of this abnormality is unknown. It is not unlikely that the more frequent application of the test of I^{131} T^3 RBC uptake, even though subject to alteration by factors unrelated to TBP, may serve to bring such cases to attention. Measurement of TBP capacity in suspected cases would, of course, be a more direct approach.

It is also possible that alterations in TBP capacity can explain other paradoxical deviations in the PBI level. Tanaka and Starr (25) apparently detected instances of euthyroidism with low PBI and low TBP capacity, although they grouped these patients with subjects usually considered to be in the normal range of PBI. It might be reasoned, also, that an elevated or depressed TBP capacity could explain the occurrence of a normal PBI in clinical hypothyroidism or hyperthyroidism, respectively. We know of no examples of this.

It should be emphasized that TBP is not the sole controlling factor in extrathyroidal distribution and metabolism of thyroid hormone. Alterations analagous to those described could result from changes in thyroxine binding by serum albumin or prealbumin and by factors influencing the peripheral degradative or metabolic processes. Further study is required to distinguish between these possibilities.

SUMMARY

Because the serum protein-bound iodine (PBI) has been found elevated in euthyroid states associated with an increase of thyroxine-binding capacity of plasma thyroxine-binding α globulin (TBP) due to known causes, a search was made for individuals with unexplained elevation of the serum PBI. A euthyroid man was found with serum PBI and butanol extractable iodine (BEI) levels ranging between two and three times normal and maintained over an observation period of nine months. A subnormal I^{131} triiodothyronine erythrocyte uptake (I^{131} T^3 RBC uptake) suggested that the serum PBI elevation was related to increased affinity of the plasma proteins for thyroid hormone. This suspicion was confirmed by the finding of an increase in thyroxine-binding capacity of TBP by reverse-flow serum electrophoresis. No abnormality was detected in thyroxine binding to albumin or prealbumin. Although the extrathyroidal organic iodine pool was expanded to roughly the same size as that found in patients with untreated thyrotoxicosis, the degradation rate in micrograms of thyroxine per day was within the normal range. The finding that one of this man's three children also had elevated serum PBI and BEI levels, a subnormal I^{131} T^3 RBC uptake and an elevated TBP capacity constitutes evidence that the abnormality is familial and could conceivably be hereditary. No other cause for this abnormality was detected. The finding of such markedly increased levels of thyroid hormone in the serum of euthyroid individuals supports the contention that the total serum thyroxine concentration does not alone govern the metabolic status of the individual.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Miss Norma Spafford, Mrs. Sharon Owings, Miss Dorothea Jermany and Mr. William Merrit for their valuable technical assistance. Dr. Walter Wilde's suggestions and encouragement were most helpful.

REFERENCES

1. Gordon, A. H., Gross, J., O'Connor, D., and Pitt-Rivers, R. Nature of the circulating thyroid hormone-plasma protein complex. Nature (Lond.) **1952, 169**, 19.
2. Robbins, J., and Rall, J. E. The interaction of thyroid hormones and protein in biological fluids. Recent Progr. Hormone Res. **1957, 13, 161**.
3. Ingbar, S. H. Pre-albumin: A thyroxine-binding protein of human plasma. Endocrinology 1958, **63**, 256.
4. Dowling, J. T., Freinkel, N., and Ingbar, S. H. Thyroxine-binding by sera of pregnant women, newborn infants, and women with spontaneous abortion. J. clin. Invest. **1956, 35**, 1263.
5. Robbins, J., and Nelson, J. H. Thyroxine-binding by serum protein in pregnancy and in the newborn. J. clin. Invest. 1958, **37**, 153.
6. Dowling, J. T., Freinkel, N., and Ingbar, S. H. Effect of diethylstilbestrol on the binding of thyroxine in serum. J. clin. Endocr. 1956, **16**, 1941.
7. Federman, D. D., Robbins, J., and Rall, J. E. Effects of methyl testosterone on thyroid function,

thyroxine metabolism and thyroxine-binding protein. J. clin. Invest. 1958, **37**, 1024.
8. Robbins, J., and Rall, J. E. Thyroxine-binding capacity of serum in normal man. J. clin. Invest. 1955, **34**, 1324.
9. Robbins, J. Unpublished data.
10. Barker, S. B., Humphrey, M. J., and Soley, M. H. The clinical determination of protein-bound iodine. J. clin. Invest. 1951, **30**, 55.
11. Man, E. B., Kydd, D. M., and Peters, J. P. Butanol-extractable iodine of serum. J. clin. Invest. 1951, **30**, 531.
12. Hamolsky, M. W., Stein, M., and Freedberg, A. S. The thyroid hormone-plasma protein complex in man. II. A new *in vitro* method for study of "uptake" of labeled hormonal components by human erythrocytes. J. clin. Endocr. 1957, **17**, 33.
13. Sterling, K. Radiothyroxine turnover studies in thyroid disease after therapy. J. clin. Invest. 1958, **37**, 1348.
14. Sterling, K., and Chodos, R. B. Radiothyroxine turnover studies in myxedema, thyrotoxicosis, and hypermetabolism without endocrine disease. J. clin. Invest. 1956, **35**, 806.
15. Sperry, W. M., and Webb, M. A revision of the Schoenheimer-Sperry method for cholesterol determination. J. biol. Chem. 1950, **187**, 97.
16. Beierwaltes, W. H., Johnson, P. C., and Solari, A. J. Clinical Use of Radioisotopes. Philadelphia, W. B. Saunders Co., 1957, pp. 91–92.
17. Howe, P. E. The determination of proteins in blood—a micro method. J. biol. Chem. 1921, **49**, 109.
18. Gaebler, O. H. Determination of bromsulphalein in normal, turbid, hemolyzed, or icteric serums. Amer. J. clin. Path. 1945, **15**, 452.
19. Robbie, W. A., and Gibson, R. B. Rapid clinical determination of urinary 17-ketosteroids. J. clin. Endocr. 1943, **3**, 200.
20. Gallagher, T. F., Peterson, D. H., Dorfman, R. I., Kenyon, A. T., and Koch, F. C. The daily urinary excretion of estrogenic and androgenic substances by normal men and women. J. clin. Invest. 1937, **16**, 695.
21. Klinefelter, H. F., Jr., Albright, F., and Griswold, G. C. Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis. J. clin. Endocr. 1943, **3**, 529.
22. Hamolsky, M. W., Golodetz, A., and Freedberg, A. S. The plasma protein-thyroid hormone complex in man. III. Further studies on the use of the *in vitro* red blood cell uptake of I^{131}-L-triiodothyronine as a diagnostic test of thyroid function. J. clin. Endocr. 1959, **19**, 103.
23. Deiss, W. P., Albright, E. C., and Larson, F. C. Comparison of *in vitro* serum protein binding of thyroxine and triiodothyronine. Proc. Soc. exp. Biol. (N. Y.) 1953, **84**, 513.
24. Beierwaltes, W. H. The value of radioactive iodine uptake and protein-bound iodine estimations in the diagnosis of thyrotoxicosis. Ann. intern. Med. 1956, **44**, 40.
25. Tanaka, S., and Starr, P. Clinical observations on serum globulin thyroxine-binding capacity, using a simplified technique. J. clin. Endocr. 1959, **19**, 84.