CLINICAL AND PHYSIOLOGICAL OBSERVATIONS IN A PATIENT WITH AN IDIOPATHIC DECREASE IN THE THYROXINE- BINDING GLOBULIN OF PLASMA *

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Since its discovery in 1952 (1), the thyroxine-binding globulin of plasma (TBG) has been the subject of extensive investigation designed to elucidate its physicochemical characteristics, alterations in abnormal states, and physiological function. Recent reviews have summarized available knowledge in this area (2, 3).

The present report describes a male patient whose plasma appears to be virtually devoid of thyroxine binding by TBG. This unusual abnormality, fortuitously discovered, afforded a unique opportunity to assess further the role of TBG in regulating the peripheral metabolism of the thyroid hormone. A number of physiological studies was therefore performed in this patient, and the results are reported herein. While these studies were in progress, clinical features of a male patient with a similar thyroxine-binding abnormality were reported by Tanaka and Starr (4). It is likely that the physiological disturbances currently described were also present in the earlier case and that both patients are affected by the same distinct, though rare, disorder in plasma protein metabolism.

CASE HISTORY

History. F.B., a 58 year old unmarried white male, was admitted to the Harvard Medical Service of the Boston City Hospital on September 14, 1958. His chief complaint at that time was increasingly severe exertional dyspnea, orthopnea, and episodes of paroxysmal nocturnal dyspnea of 2 years' duration. He had taken digitalis ir-

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regularly during that period, but had had none during the year prior to entry. The patient had been known to have a heart murmur since childhood. Symptoms of acute rheumatic fever were denied. The patient's only other complaint was weakness of the arms and legs of 2 year's duration. This was especially prominent in the distal musculature and was of moderate, but not incapacitating, severity.

Details of early development were not readily recalled, but pubertal maturation apparently began at about age 14. At age 16 a bilateral inguinal herniorrhaphy was performed. Subsequently, if not immediately thereafter, normal masculine psychosexual development ceased. Libido was minimal throughout the patient's life, although rare erections and nocturnal emissions occurred. Pubic and axillary hair remained scant, and balding did not take place. The patient shaved infrequently.

There were no other symptoms referable to the endocrine organs, including the thyroid, adrenals and pituitary gland, and no symptoms of an intracranial lesion. Review of systems was otherwise negative.

The patient's parents had died when he was a young boy; he had no siblings. He knew of no other close relatives who were alive. The patient was raised by members of a religious order and was living and serving as janitor in their institution at the time he entered the hospital.

Physical examination. T, 98.8°; P, 100; R, 20; BP, 130/70. Salient features of the physical examination were confined to the cardiovascular, endocrine and neuromuscular systems. Examination of the heart revealed cardiomegaly and a prominent precordial heave. Cardiac rhythm was regular. The pulmonic second sound was increased, split, and louder than the aortic second sound. The first sound in the mitral area was booming; there was no opening snap. A grade 4, harsh systolic murmur, radiating widely, was audible over the entire precordium, but was heard best in the fourth left intercostal space in the parasternal area.

Bilateral herniorrhaphy scars and reducible herniae were present. Small, firm testes were felt in the scrotal sac. The phallus was normal in size, the prostate small and firm. Pubic hair was scant, the escutcheon female. Only a few fine strands of axillary hair were present. The skin was fine and smooth, typical of that associated with hypogonadism. Skeletal proportions were not eu-

nuchoid. There was a slight temporal calvities, but no other balding.

There were no peripheral stigmata of hyper- or hypothyroidism. There were no pigmentary abnormalities. The eyes were highly myopic, but there were no cataracts. Visual fields were normal to gross confrontation.

There was moderate weakness of the distal musculature of the arms and legs with atrophy of the interosseous muscles. No myotonia was evident. Deep tendon reflexes and sensory examination were normal.

Laboratory. The urine and blood were within normal limits. Lumbar puncture was normal. Serum chemistries included: urea nitrogen, 15 mg per 100 ml; glucose (fasting), 100 mg per 100 ml; calcium, 10.2 mg per 100 ml; phosphorus, 3.1 mg per 100 ml; cholesterol, 210 mg per 100 ml; sodium, 138 mEq per L; potassium, 4.1 mEq per L; chloride, 104 mEq per L; CO₂, 25.6 mEq per L. Serum iron concentration was 110 µg per 100 ml and iron-binding capacity 320 µg per 100 ml, both within normal limits.

Skull films were normal. A bone survey revealed no abnormalities; the epiphyses of the iliac crests were closed. Visual fields were within normal limits. Twenty-four hour urinary excretion of 17-ketosteroids was 9.9 mg (5), of 17-hydroxycorticoids, 4.3 mg (6).¹ Urinary gonadotropins were positive at 50 and equivocal at 100 mouse units per 24 hours (7).²

Twenty-four hour thyroïdal uptake of I¹³¹ was 28 per cent. BMR's ranged between -5 and +4 per cent. Serum protein-bound iodine (PBI) was 2.0 µg per 100 ml (8).

Course. The patient's cardiac complaints responded well to digitalization and a low salt diet. Mercurial diuretics were not employed. Cardiac catheterization studies were interpreted as consistent with a persistent left vena cava and atrial septal defect, probably of the *secundum* type.

In view of the patient's obvious hypogonadism, a thorough endocrine evaluation was undertaken. The associated subnormal PBI, repeatedly confirmed, strongly raised the possibility of pituitary hypofunction, but this diagnosis seemed clearly excluded by the neurological and X-ray examinations and by other endocrinological findings, especially the increased urinary excretion of gonadotropins. The dissociation between the PBI and other laboratory, as well as clinical, indices of thyroid function raised the possibility of abnormal binding of thyroxine in the patient's plasma. This hypothesis was confirmed by repeated electrophoretic studies of the patient's serum, all of which revealed a virtual absence of TBG.³

¹ Performed at the Boston Medical Laboratory, Boston, Mass. Normal values: 17-ketosteroids, 10 to 24 mg per 24 hours; 17-hydroxycorticoids, 1 to 10 mg per 24 hours.

² Performed at the Gynecological-Endocrinological Laboratory, Peter Bent Brigham Hospital, Boston, Mass.

³ In the present report, for purposes of brevity, a change in the thyroxine-binding activity of TBG will be

Studies of the peripheral turnover of I¹³¹-labeled thyroxine were then performed. Several days after these were complete, the left inguinal hernia became incarcerated and emergency repair was performed. At this time the testis was seen to be grossly atrophic and a testicular biopsy was taken. In view of the muscular weakness, a biopsy of the anterior tibial muscle and overlying skin was also made. The latter revealed no abnormality. Histologically, the testis was fibrotic, with atrophy and fibrous replacement of tubular elements and decrease in interstitial cells, consistent with chronic restriction of blood supply. Genetic sex was masculine, as assessed in both testicular and cutaneous biopsies.

When the patient's convalescence was complete, oral administration of diethylstilbestrol, 30 mg daily, was begun. This was continued for 7 weeks. During the latter portion of this period, the patient developed increasing dyspnea, pretibial edema, marked areolar pigmentation, and pronounced tender gynecomastia. Urinary excretion of gonadotropin declined to less than 5 mouse U per 24 hours. Electrophoretic studies of hormonal binding in the patient's serum were performed at weekly intervals during the administration of diethylstilbestrol. During the last 10 days of this regimen, studies of the peripheral metabolism of labeled thyroxine were repeated.

Two months later, after clinical evidence of estrogenic effect had disappeared, a course of adrenocortical suppressive therapy was begun. The patient was given 10.0 mg prednisolone⁴ by mouth, each day for a period of 6 weeks. Under this treatment urinary 17-ketosteroids declined to 1.1 mg daily. Bloods were drawn weekly for electrophoretic analysis of thyroxine binding.

After gradual withdrawal of corticoid therapy, a 6-week course of sodium-L-thyroxine, 0.2 mg by mouth daily, was instituted. The patient experienced no symptoms from this medication; physical examination, PBI, and electrophoretic findings with regard to thyroxine binding remained unaltered. Twenty-four hour thyroïdal uptake of I¹³¹ was suppressed from its control value of 28 per cent to 6 per cent, however.

After withdrawal of exogenous thyroid hormone, the patient remained well and has continued thus, save for one episode of congestive heart failure that followed discontinuance of digitalis, which quickly responded to re-digitalization. Bloods have subsequently been obtained at intervals for electrophoretic analysis; these have continued to show a virtual absence of TBG.

MATERIALS AND METHODS

Thyroxine-binding capacities of individual serum proteins were determined by zonal electrophoresis in filter paper of serum enriched with variable quantities of I¹³¹-labeled and stable L-thyroxine.

referred to as a change in TBG; no implications concerning the absolute concentration of the protein are intended.

⁴ 11β,17α,21-trihydroxy-4-pregnadiene-3,20-dione.

I^{131} -labeled hormones (L-thyroxine, T_4 ; 3,5,3'-L-triiodo-thyronine, T_3 ; 3,5,3'-triiodothyroacetic acid, triac; 3,5,3,5'-tetraiodothyroacetic acid, tetrac) obtained from a commercial source,⁵ were 90 to 95 per cent pure, as assessed chromatographically, and were diluted with 1.0 per cent human serum albumin to a final concentration of 160 μ c per ml immediately upon arrival. This procedure reduced both the degradation of labeled compounds and their adsorption to glassware. Nevertheless, labeled materials were not employed for studies after 1 week of storage in this form.

The procedure employed in preparing samples of serum for the electrophoretic estimation of thyroxine binding by TBG and thyroxine-binding prealbumin (TBPA) was as follows:

To 6.2 ml of serum, 75 μ l of I^{131} -labeled T_4 in human serum albumin was added, yielding a final concentration of approximately 2 μ c per ml (Solution I). A 3.0-ml aliquot of Solution I was pipetted into a separate tube, and to this was added 100 μ l of solution of 1.0 per cent human serum albumin containing 14.0 μ g of stable T_4 . This yielded a solution containing 451 μ g of added stable T_4 per 100 ml (Solution G). Aliquots of Solution I, Solution G, and mixtures of the two were employed to prepare samples containing 87, 112.5, 130, 340, 395, and 451 μ g of added stable T_4 per 100 ml. The three lower concentrations were employed to calculate the binding capacity of TBG and the three higher concentrations, the binding capacity of TBPA.⁶ Two additional samples of serum containing I^{131} -labeled but no stable T_4 were prepared. The first, Solution A, is a mixture of equal parts of Solution I with fresh serum (1 μ c I^{131} per ml) and the second (Solution H) a 1:10 dilution of Solution A with fresh serum (0.1 μ c I^{131} per ml). In general, the specific activity of the I^{131} -labeled T_4 is such that these two samples contain approximately 4 μ g and 0.4 μ g of added stable T_4 per 100 ml, respectively. Electrophoresis was performed in sheets of Whatman no. 3 filter paper, 22.5 $\times$ 12 inches, large enough to accommodate the 8 separate specimens. After being moistened in buffer medium and blotted, filter paper sheets were placed upon a carefully ground, siliconized glass plate,⁷ 15 inches long, 13 inches wide, and 1 inch thick. Aliquots of serum (20 μ l) were then applied in thin lines, equidistant from the ends of the sheet, to areas carefully predetermined from a template. Usually, duplicate aliquots of serum were applied to a second filter-paper sheet carefully placed directly upon the first sheet. A minute quantity of bromphenol blue (BPB) was applied to each serum band to serve as a marker for the electrophoretic migration of albumin. Sheets were then covered by a second glass plate, similar

in dimension to the first. When, as is often the case, it is desired to analyze an additional 8 samples upon the same machine, a thin glass plate (13 $\times$ 15 $\times$ $\frac{1}{8}$ inch) is placed upon the first pair of duplicate sheets. A second pair of sheets containing the additional samples can then be placed upon the thin plate and the entire "sandwich" covered by the second thick glass plate. To minimize evaporation, the hiatus between the top and bottom glass plates at the front and rear of the apparatus was sealed with cellophane tape. At the lateral margins of the plates, filter paper sheets largely filled this hiatus and were allowed to hang into buffer baths. Gravitational flow of buffer across the filter paper was eliminated by means of a horizontal leveling device in the buffer reservoirs.

Buffer systems employed in the present studies were either 0.05 M veronal or a (hydroxymethyl) amino-methane (Tris)-maleate buffer, both at pH 8.6.⁸ Through platinum electrodes immersed in the buffer reservoirs, a potential of 110 was applied across the filter paper for a period sufficient to produce approximately a 4-inch migration of the serum albumin. This usually required about 18 hours at normal room temperature. No attempts were made to cool the apparatus, as heating is only slight under these conditions.

When migration of proteins was sufficient, filter paper sheets were dried. One sheet was set aside for radio-autography and subsequent staining with BPB, making possible the localization of major protein components. The second sheet was cut into 8 strips and, except in the case of Sample H, radioactive scanning of individual electrophoretograms was performed by means of a continuous scanner and dual-channel recorder. Areas under the individual peaks were determined with an electronic integrator whose output is recorded synchronously with the scan itself. The distribution of labeled T_4 was calculated from the recorded integral as the proportion of added hormone bound by each of the demonstrated binding proteins. The total quantity of labeled T_4 bound by each protein per unit volume of serum was calculated as the product of the proportion bound and the estimated total concentration of T_4 in the specimen. The latter estimate is derived from the total concentration of added T_4 (I^{131} -labeled plus stable) plus an estimate of endogenous T_4 , assuming the endogenous PBI to be comprised entirely of this hormone. Sample H was divided into segments 0.5 cm wide; these were counted individually in a well-type scintillation counter.

In view of the decreased thyroxine-binding capacity of TBG in the present patient's serum, samples were also assayed which had been enriched with both labeled and stable T_4 in a number of concentrations below the usual lowest enrichment with 87 μ g of stable T_4 per 100 ml.

The concentration of the major electrophoretically distinguishable groups of plasma proteins was assessed by densitometric scanning of electrophoretic strips stained with BPB.

⁸ Preparation of the Tris-maleate buffer has been described in an earlier communication (2). The final solution is 0.073 M with respect to both Tris and maleate.

⁵ Abbott Laboratories, Oak Ridge, Tenn.

⁶ Evidence that thyroxine-binding sites of TBG and TBPA in normal serum are saturated at the concentrations indicated has been presented briefly in a previous report (2) and will be presented in detail in a later communication.

⁷ Available from the Pittsburgh Plate Glass Co., Boston, Mass.

Thyroxine turnover studies. Analyses of the peripheral turnover of I^{131} -labeled T_4 *in vivo* were performed according to methods described in detail elsewhere (9). Serial samples of serum and complete 24-hour urinary collections were obtained daily for 8 to 10 days after the intravenous administration of a tracer dose of I^{131} -labeled T_4 . These were analyzed for their concentration of I^{131} . Aliquots of urine were also subjected to a butanol extraction procedure, similar to that described for serum (10), and the proportion of total urinary I^{131} present as butanol-extractable I^{131} was determined. The several functions calculated and their derivations are described in the Appendix.

Red blood cell uptakes of I^{131} -labeled hormones. Blood was drawn from a single normal donor (Type O) into a heparinized syringe. After centrifugation, plasma was removed and the erythrocytes were washed 4 times in 3 volumes of normal saline. After the final wash, sufficient normal saline was added to bring the red blood cell suspension to a standard hematocrit. A sufficient volume of the suspension was pipetted into a series of 13×100 mm counting tubes to provide 0.5 ml of packed red blood cells in each. Samples of fresh plasma (oxalate anticoagulant) from the patient and from a group of normal control subjects were enriched with $0.02 \mu\text{C}$ per ml of I^{131} -labeled compounds; 0.5-ml aliquots of plasma were pipetted into tubes containing the packed red blood cells. Tubes were stoppered and agitated at 37°C for 1 hour. The radioactivity in each tube was then assessed directly in a well-type scintillation counter. Tubes were centrifuged, the plasma removed by suction and the red blood cells were then washed 5 times with 3 volumes of physiological saline solution at room temperature. After the last wash, 0.5 ml of saline was added to the packed red blood cells to bring the final volume to 1 ml. The cells were resuspended, and the radioactivity in the tube was again measured.

Red blood cell uptakes of labeled hormones were calculated as the ratio of final to initial counting rates, expressed as a per cent. All analyses were performed in duplicate. Electrophoretic analyses of thyroxine-protein-binding interactions in the sera studied were performed in all instances.

RESULTS

Electrophoretic analyses (Figures 1, 2; Table I). Repeated electrophoretic analyses of the patient's serum failed to reveal more than a trace of T_4 in the inter- α -globulin (TBG) area, during electrophoresis of serum in either the Tris-maleate or the veronal buffer systems.⁹ Radioactivity in the TBG zone was most frequently found when electrophoretograms of serum enriched with only

⁹ Values described for thyroxine-binding by TBG and TBPA represent the means of those found during electrophoretic analysis of at least 3 specimens obtained during each treatment period.

$0.4 \mu\text{g}$ of T_4 per 100 ml were cut into strips and counted in a well counter. Even at this concentration, however, no more than an approximate 5 per cent of total radioactivity was localized in the TBG zone. Often, both before and during administration of diethylstilbestrol, no TBG could be demonstrated. In sera enriched with approximately $4.0 \mu\text{g}$ of T_4 per 100 ml, a small upward convexity in the radioactive scan was occasionally seen in the TBG zone. This was evident on radioautographs as a faint zone of darkening. Direct well counting of such strips indicated that no more than 2 to 3 per cent of added radioactivity was localized with TBG. In sera enriched with higher concentrations of T_4 , no band in the TBG zone could be discovered. It thus appears likely that this patient's serum was either not completely or not always devoid of TBG. The binding capacity of TBG, when demonstrable, could not be assessed with any accuracy but clearly seemed no more than a small fraction of $1 \mu\text{g}$ of T_4 per 100 ml of serum.

Thyroxine-binding by TBPA was evident in all specimens subjected to electrophoresis in the Tris-maleate buffer system. The thyroxine-binding capacity of TBPA was usually near the normal mean of approximately $120 \mu\text{g}$ of T_4 per 100 ml of serum

FIG. 1. THE EFFECT OF INGUINAL HERNIORRHAPHY AND OF DIETHYLSTILBESTROL ON THE BINDING OF THYROIDINE IN THE SERUM OF A PATIENT WITH DECREASED THYROIDINE-BINDING GLOBULIN (TBG). Postoperative serum obtained 2 days after surgery. Serum obtained during therapy (obtained after 5 weeks of treatment with diethylstilbestrol, 30 mg daily, p.o.). Curves shown are scans of I^{131} -labeled thyroxine added in the indicated concentrations to sera subjected to electrophoresis in Tris-maleate buffer, pH 8.6. The small peak seen in the TBG zone during estrogenic therapy was not evident in other samples obtained during the same treatment period.

(2), but varied with the patient's clinical state, declining to subnormal values during exacerbations of congestive heart failure or in the period following emergency inguinal herniorrhaphy. A zone of radioactivity corresponding in electrophoretic migration to that of serum albumin was the only other area in which added I^{131} -labeled T_4 was localized.

As previously reported (11, 12), I^{131} -labeled triac and tetrac were bound primarily to TBPA and secondarily to albumin during electrophoresis in either Tris-maleate or veronal buffers. At standard concentrations of added labeled compounds, the percentile distribution of the derivatives between these two proteins in the patient's serum, obtained during the control state, was not appreciably different from normal.

During the administration of estrogen, prednisolone, or sodium-L-thyroxine, appreciable or consistent differences in thyroxine binding by TBG, as compared with those found during the control state, could not be discerned; the small radioactive peak seen in Figure 2 was not found in other sam-

ples obtained during the same treatment period. The thyroxine-binding capacity of TBPA was slightly reduced during administration of diethylstilbestrol, but was unaffected by treatment with prednisolone and L-thyroxine.

Red blood cell uptakes of labeled hormones (Table I). *In vitro* uptakes of labeled T_4 and T_3 from the patient's plasma by red blood cells were greatly increased in the basal state and were not appreciably altered during the several treatment periods. The red blood cell uptake of labeled triac was normal during both the basal state and the administration of diethylstilbestrol, but underwent a pronounced increase during the immediate postoperative period.

Thyroxine metabolism in vivo (Table I). The peripheral metabolism of I^{131} -labeled T_4 , measured while the patient was in the basal state, was grossly abnormal (9, 13). Fractional rate of turnover of administered hormone was rapid, 23.1 per cent per day. The T_4 distribution space was moderately increased when related to total body weight (0.22 L per kg), and hormonal clearance

FIG. 2. THE EFFECT OF DIETHYLSTILBESTROL ON THE PROTEIN BINDING AND PERIPHERAL TURNOVER OF THYROXINE IN A PATIENT WITH DECREASED TBG. Thyroxine turnover studies during estrogenic therapy begun on Day 41 of treatment with 30 mg of diethylstilbestrol daily, p.o. Distribution of I^{131} -labeled thyroxine (0.4 μ g per 100 ml) in sera obtained during each thyroxine turnover study and subjected to electrophoresis in Tris-maleate buffer, pH 8.6. The small peak of radioactivity seen in the TBG zone during estrogenic therapy was not evident in other samples obtained during the same treatment period.

TABLE I

*Values for several aspects of thyroid hormone economy in normal subjects and in a patient with idiopathic decrease in thyroxine-binding globulin **

Function	Normal values†	Patient F.B.				
		Control	Diethylstilbestrol	Prednisolone	Postoperative	L-thyroxine
Thyroid I ¹³¹ uptake (% dose)	20-50	28	26			6
Basal metabolic rate	-15-+15	+3	+6	-1		+4
Serum cholesterol (mg %)	150-270	210	204			198
Protein-bound iodine (μg %)	4.0-8.0	2.0	2.1	2.1	1.8	2.3
Thyroxine turnover studies (9)						
Thyroxine space (TDS; L)	9.4 ± 2.0	13.2	15.7			
Turnover rate (k; %/day)	10.6 ± 0.9	23.1	19.9			
Clearance rate (C; L/day)	1.0 ± 0.3	3.05	3.11			
Organic iodine pool (ETT; μg I)	508 ± 146	264	329			
Daily hormone disposal (D; μg I/day)	53.6 ± 16.5	61.0	65.3			
Fecal fraction (F _{max} ; % dose)	26.9 ± 9.2	25.7	27.2			
Urinary fraction (U _{max} ; % dose)	73.1 ± 9.3	74.3	72.8			
Fecal clearance (L/day)	0.27 ± 0.11	0.78	0.85			
Degradative clearance (L/day)	0.73 ± 0.21	2.27	2.26			
Fecal excretion (μg I/day)	14.6 ± 6.8	15.6	17.8			
Degradation rate (μg I/day)	39.0 ± 12.7	45.3	47.5			
Hormonal binding studies (2, 36)						
TBG capacity (μg thyroxine/100 ml)	18-25	±‡	±	±	±	±
TBPA capacity (μg thyroxine/100 ml)	90-160	136	85	120	18	121
RBC thyroxine uptake (%)	0.6-0.9	1.5	1.5		1.7	
RBC triiodothyronine uptake (%)	5.0-8.5	13.2	12.8		14.4	
RBC triiodothyroacetic acid uptake (%)	1.2-2.1	1.5	1.9		2.9	

* For a description of functions and abbreviations shown under thyroxine turnover studies, see Appendix. For details regarding the several treatment periods, see text.

† Values presented are normal range or mean ± standard deviation. Normal values are those presented in previous studies or calculated from data contained therein, and were obtained by methods similar to those employed in Patient F.B. Numbers in parentheses provide references to these sources.

‡ ± Indicates traces of TBG inconsistently seen, and, when demonstrable, present in concentrations too low to permit calculation of thyroxine-binding capacity.

rate was greatly augmented (3.05 L per day). Because of the subnormal PBI, however, total hormonal disposal, 61.0 μg of iodine per day, was within normal limits. The extrathyroidal organic iodine pool was greatly reduced.

Radioiodine appeared in the urine more rapidly than normal after administration of labeled thyroxine. Butanol extractions of urine on the third, fourth, and fifth daily collections revealed that 3.3, 4.6, and 4.0 per cent, respectively, of urinary I¹³¹ was soluble in butanol and relatively insoluble in aqueous alkali; these values were similar to those found in normal individuals (14). Calculated values for the ultimate urinary and fecal excretion of I¹³¹ were 74.3 and 25.7 per cent of the administered dose, respectively. Both fecal clearance (0.78 L per day) and degradative clearance of T₄ (2.27 L per day) were greatly increased, but the daily fecal excretion rate and daily degradation rate were within normal limits.

Neither the peripheral metabolism of T₄ nor the concentration of hormonal iodine in the plasma was significantly altered by the prolonged administration of large doses of diethylstilbestrol. Neither prednisolone nor thyroid-suppressing doses of sodium-L-thyroxine increased the PBI.

DISCUSSION

There is general agreement that TBG serves as a rate-regulating factor in the peripheral metabolism of T₄. TBG, like other proteins with which T₄ is associated, is thought to limit the concentration of free or unbound hormone, thereby retarding its cellular penetration and decreasing the proportion of extrathyroidal hormone that is affixed to the cell. If, then, a constant proportion of the hormone within cellular confines is metabolized per unit time, the fractional rate of disposal of the entire peripheral pool of hormone will vary inversely with the binding activity of TBG (2, 3,

14). This hypothesis is largely based on inferential evidence, consisting of a series of correlations between changes in extracellular thyroxine-TBG interactions and changes in the metabolism of the hormone, either *in vitro* or *in vivo*. Thus, the uptake of T_4 by cellular systems *in vitro* varies inversely with the concentration of TBG and directly with the concentration of hormone in the suspending medium (15). *In vivo* correlations have mainly required the use of pharmacological agents to alter the thyroxine-binding capacity of TBG, associated effects on the peripheral metabolism of labeled T_4 being then determined. Diethylstilbestrol and natural estrogens, for example, increase the binding capacity of TBG (13, 16) and decrease the fractional rate of turnover of the hormone (13, 17), while methyltestosterone produces converse effects (18). These *in vivo* observations, while consistent with the major hypothesis, have not been entirely conclusive because of the possibility that the agents employed might directly affect cellular mechanisms for the disposal of hormone. This seemed especially possible in the case of diethylstilbestrol, in view of reports that this compound inhibits the deiodination of T_4 both by intact cellular preparations and by a partially purified thyroxine-deiodinase (19, 20).

More compelling evidence in favor of the postulated function of TBG has been afforded by the recently described patient in whose plasma there was found an apparently idiopathic increase in TBG (21). This abnormality was accompanied by changes in the peripheral metabolism of T_4 similar to those found in association with the increased TBG induced by estrogens (13). No gross evidence of endogenous excess of estrogens was evident in the male patient described. However, values for urinary estrogens were rather high and those for urinary gonadotropin rather low. These findings, together with the familial nature of the disorder, raised the possibility that some inherited disturbance in the secretion or metabolism of estrogens may have produced the increase in TBG which this patient manifested.

In the present patient, the apparently coincidental association of testicular hypofunction with virtual absence of TBG in the plasma afforded an unusual opportunity to complement existing data concerning the function of TBG. In view of the theoretical objections to the *in vivo* studies de-

scribed above, it appeared desirable to obtain conclusive evidence that the abnormality in TBG displayed by this patient was not due to disturbances in known hormonal or metabolic factors which might, in themselves, influence the metabolism of T_4 .

This patient's distinct hypogonadism excluded excessive secretion of normal androgens as the cause of decreased TBG. It appeared possible, however, that the patient's abnormal testes were secreting an unusual, nonandrogenic steroid capable of decreasing TBG. Apparently the capacity of certain steroids to decrease TBG is not a function of their androgenic potency, since norethandrolone,¹⁰ a steroid with relatively more anabolic than androgenic activity (22), has proved capable of inducing pronounced reductions in TBG within a period as short as 1 week (23). During this time fractional peripheral turnover of T_4 was accelerated, but androgenic effects were not evident, even in hypogonadal patients (23). The large doses of diethylstilbestrol administered to the present patient produced gynecomastia and edema, suppressed gonadotropin production, and should have been adequate to suppress any lingering function that his testes may have retained. Nevertheless, diethylstilbestrol failed to restore the binding capacity of TBG to normal, and, indeed, any increase in TBG that may have been induced by this agent was too small to be recognized consistently during electrophoretic analyses.

Finally, it seems unlikely that an androgen-like steroid of adrenocortical origin was responsible for the decrease in TBG, since prolonged adrenocortical suppression failed to influence the abnormality in hormonal binding. The patient with decreased TBG reported by Tanaka and Starr had a history of hepatitis and displayed abnormal cephalin flocculation and thymol turbidity tests. In the present patient there was no clinical or laboratory evidence of hepatic or renal disease. Therefore, currently recognized causes of decreased TBG appear to have been excluded in the present case.

The abnormalities in the peripheral metabolism of T_4 which accompanied the decrease in TBG in this patient were consonant with the postulated function of TBG. The greatly increased red

¹⁰ 17 α -ethyl-17-hydroxynorandrostenone.

blood cell uptakes from the patient's plasma of T_4 and T_3 , hormones bound primarily by TBG (14), provided evidence that an abnormally high proportion of these hormones was unbound or "free." As would therefore be expected, the T_4 distribution space was moderately increased *in vivo* and the fractional turnover of hormone was greatly accelerated. Accordingly, total hormonal clearance rate was markedly increased. However, the PBI was abnormally low, with the result that total hormonal disposal was well within the normal range.

Studies of urinary I^{131} and estimates of fecal excretion made possible an assessment of the general metabolic pathways by which the increased fractional rate of turnover was effected. Although fecal clearance of T_4 was increased several-fold, a finding in accord with earlier evidence that protein binding of T_4 limits its gastrointestinal excretion (24, 25), this increase accounted for only a small fraction of the increase in total T_4 clearance which the patient displayed. In the presence of an increased proportion of unbound hormone in the plasma, an increased renal clearance of T_4 would also be expected (14). Normally, however, this route of disposal contributes so little to total hormonal clearance that an enormous increase in this function would have been required to contribute significantly to the abnormality in hormonal turnover (26, 27). Measurements of the butanol-extractable I^{131} in the patient's urine confirmed the relative unimportance of urinary excretion of T_4 as a factor in the urinary excretion of I^{131} and in total hormonal disposal. Thus, increased hormonal turnover was due, in greatest part, to increased disposal of T_4 by those metabolic pathways which ultimately lead to hormonal deiodination.

It seemed important to exclude the possibility that this patient's subnormal PBI was not due to the abnormality in hormonal binding, but rather reflected the secretion of some thyroid hormone, like T_3 , which, in amounts sufficient to maintain a normal metabolic state, does not contribute greatly to the PBI (28). If this were the case, then the metabolism of labeled T_4 would not have accurately reflected the metabolism of endogenous hormone. However, if T_3 were indeed the major hormone in the patient's plasma, then doses of L-thyroxine adequate to suppress endogenous thy-

roid function should have restored the PBI to normal. Since they did not, it seems likely in the present patient, as is normally the case (29), that T_4 constituted the major secretory product of the thyroid gland and the major constituent of the PBI. Calculated values for hormonal disposal were therefore validated.

The present association of normal thyroid function and metabolic status with normal values for daily T_4 disposal and deiodination, despite a subnormal PBI, supplements previous evidence, reviewed elsewhere (30), that both the secretion of thyrotropin and the metabolic state of the patient are more closely related to the quantity of hormone available to the tissues than to the total concentration at which they are delivered. The concentration of unbound hormone in the plasma, could it be readily determined, would likely constitute a better index of metabolic state than does the total PBI.

The failure of diethylstilbestrol to alter the kinetics of T_4 metabolism in this patient indicates that, in man, in contradistinction to certain cellular preparations *in vitro*, this agent apparently does not directly affect the cellular degradation of T_4 . It therefore follows that the changes in T_4 metabolism that accompany the administration of estrogen to patients with normal TBG are indeed due to the resulting increases in TBG. Furthermore, the present findings support the belief that the changes in T_4 metabolism induced by androgenic hormones are not due to a direct cellular effect of the androgens, but rather to the associated decrease in TBG. Thus, the data lend considerable weight, both directly and indirectly, to the postulated function of TBG, described above. They do not, however, conclusively prove the hypothesis. Alternative interpretations of the function of TBG in the light of the present and previous observations concerning the metabolism of T_4 may also be envisioned (2). Furthermore, one cannot logically exclude the possibility that the abnormalities in T_4 metabolism which this patient displayed arose, not from the decreased TBG, but from unrelated abnormalities in the cellular metabolism of the hormone.

In the absence of significant hormonal binding by TBG, an unusually large share of the transport of T_4 must devolve upon other proteins, notably TBPA (31). However, a consideration of the

possible physiological role of TBPA in the present patient, or indeed, in general, is beyond the scope of this report. While, in a number of circumstances, correlated changes in thyroxine binding by TBPA and in T_4 metabolism indicate that this protein contributes significantly to hormonal binding *in vivo* (2, 12), other evidence suggests that it may not (32, 33). Nevertheless, hormonal binding by TBPA seems adequate to explain several phenomena observed in the present studies. Thus, the normal values for thyroxine binding by TBPA found in the patient's serum when he was in a basal state correlated well with normal values for the red blood cell uptake of triac, since this derivative is primarily bound to TBPA, and not to TBG (11, 12). Similarly, the increased red blood cell uptake of triac obtained when the patient was in the post-operative period is probably explained by the reduced hormonal binding by TBPA which occurred at that time, a change similar to that found previously in sera from a large number of patients with other nonthyroidal illnesses (34). Subsequent studies in a large number of patients have indicated that the *in vitro* uptake of deaminated derivatives of T_4 and T_3 by an adsorptive particulate system varies inversely with, and is a convenient means of estimating, the thyroxine-binding capacity of TBPA (35).

As was the case in the patient reported by Tanaka and Starr (4), the defect in TBG in the present patient was associated with no gross abnormalities in the concentration of the major electrophoretically distinguishable groups of plasma proteins. Of the many transport functions of the plasma proteins only the transport of iron was assessed, and this proved to be normal. Thus, it is uncertain whether the abnormality described here is an isolated defect. In neither patient with this disorder has it been possible to ascertain whether the decrease in TBG was congenital or acquired, or whether it represented an inherited abnormality. The male patient with absent TBG described by Tanaka and Starr had no evidence of cardiac disease (4). Consequently, it is uncertain whether the congenital cardiac lesion in the present patient with decreased TBG represents a related abnormality or merely a fortuitous association.

Although the defect in the present patient has been referred to as a decrease in TBG, the concen-

tration of the protein itself was not measured. Conceivably, decreased hormonal binding could have resulted from the presence of a highly effective inhibitor of thyroxine binding or from a minor structural abnormality in the protein that impaired the attachment of T_4 . More likely, however, the abnormality described in this paper represents, as in other dysproteinemias (36), a decrease in the concentration of the protein itself. Whether, if this be true, the virtual absence of TBG from this patient's plasma is due to excessive destruction or defective protein synthesis, as is the case in agammaglobulinemia and analbuminemia (36), can not be determined at present. Resolution of these and other intriguing questions concerning the function and metabolism of TBG await ample supplies of the purified protein.

SUMMARY

1. Clinical features have been described of a euthyroid male patient with postoperative primary hypogonadism and a congenital atrial septal defect, whose plasma was virtually devoid of thyroxine binding by the thyroxine-binding globulin (TBG).
2. Prolonged administration of large doses of synthetic estrogen or adrenal corticosteroid failed to alter significantly the binding activity of TBG, suggesting that the abnormality in this protein was not produced by an androgen of testicular or adrenocortical origin.
3. This seemingly idiopathic decrease in TBG was associated with pronounced abnormalities in the peripheral metabolism of thyroxine. Both the volume of distribution and fractional rate of turnover of I^{131} -labeled thyroxine were increased; hormonal clearance rate was markedly augmented. However, the serum protein-bound iodine was subnormal, with the result that daily disposal of hormone by both excretory and degradative routes was within normal limits.
4. In contrast to its action in normal individuals, in whom it induces an increased binding activity of TBG, estrogenic therapy had no significant effect upon the concentration or peripheral turnover of thyroxine in this patient.
5. The several findings in this patient are viewed as consistent with the hypothesis that TBG regulates the peripheral metabolism of thyroxine by

limiting its cellular penetration and hence its peripheral metabolism and action.

APPENDIX

In this manuscript and Table I the following terms have been employed. These were described in detail in an earlier communication (9).

TDS, thyroxine distribution space; the virtual volume of body fluids through which exchangeable thyroxine would be distributed were it present throughout at the same concentration at which it exists in the plasma.

PBI, concentration of hormonal iodine in the plasma, and, by definition, the geometrical mean concentration of hormonal iodine in the TDS; assumed to represent solely iodine in thyroxine.

ETT, extrathyroidal thyroxine or the organic iodine pool; the total quantity of thyroxine within the TDS, in terms of its content of iodine.

k, fractional rate of turnover of thyroxine; the proportion of ETT removed from the TDS per unit time.

$T_{1/2}$, thyroxine half-time; the time required for one-half of the ETT to be removed from the TDS.

C, thyroxine clearance rate; the volume of plasma containing an amount of thyroxine equal to that removed from the TDS per unit time. Defined as "volume turnover, V" in an earlier communication (9).

D, hormonal disposal rate; the quantity of hormone removed from the TDS per unit time, in terms of its content of iodine.

F_{max} , the proportion of D accounted for by fecal excretion of hormone; U_{max} , the proportion of D accounted for by urinary excretion of organic iodine and iodide released during hormonal deiodination; fecal clearance, the volume of plasma that contains a quantity of hormone equal to that lost in the feces per unit time; degradative clearance, the volume of plasma containing a quantity of hormone equal to that removed by nonexcretory pathways per unit time. As calculated, degradative clearance includes the renal clearance of thyroxine, but the latter comprises a negligible proportion of the total; fecal excretion, the quantity of hormone excreted in the feces per unit time; degradation rate, the quantity of hormone removed by nonexcretory pathways per unit time.

$ETT = PBI \times TDS$. $C = TDS \times k$. $D = C \times PBI = ETT \times k$. $T_{1/2} = 0.693/k$. Fecal clearance = $C \times F_{max}$. Degradative clearance = $C - \text{fecal clearance} = C \times U_{max}$. Fecal excretion = fecal clearance $\times PBI = D \times F_{max}$. Degradation rate = degradative clearance $\times PBI = D - \text{fecal excretion}$.

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REFERENCES

- 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.
- Ingbar, S. H., and Freinkel, N. Regulation of the peripheral metabolism of the thyroid hormones. *Recent Progr. Hormone Res.* 1960, 16, 353.
- Robbins, J., and Rall, J. E. Proteins associated with the thyroid hormones. *Physiol. Rev.* 1960, 40, 415.
- Tanaka, S., and Starr, P. A euthyroid man without thyroxine-binding globulin. *J. clin. Endocr.* 1959, 19, 485.
- Drekter, I. J., Heisler, A., Scism, G. R., Stern, S., Pearson, S., and McGavack, T. H. The determination of urinary steroids. I. The preparation of pigment-free extracts and a simplified procedure for the estimation of total 17-ketosteroids. *J. clin. Endocr.* 1952, 12, 55.
- Reddy, W. J. Modification of the Reddy-Jenkins-Thorn method for the estimation of 17-hydroxycorticoids in urine. *Metabolism* 1954, 3, 489.
- 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.
- Zak, B., Willard, H. H., Myers, G. B., and Boyle, A. J. Chloric acid method for determination of protein-bound iodine. *Analyt. Chem.* 1952, 24, 1345.
- Ingbar, S. H., and Freinkel, N. Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis. *J. clin. Invest.* 1955, 34, 808.
- Ingbar, S. H., Freinkel, N., Hoeprich, P. D., and Athens, J. W. The concentration and significance of the butanol-extractable I^{131} of serum in patients with diverse states of thyroidal function. *J. clin. Invest.* 1954, 33, 388.
- Tanaka, S., and Starr, P. The binding of thyroxine analogues by human serum proteins. *Acta endocr. (Kbh.)* 1959, 31, 161.
- Ingbar, S. H. The interaction of the thyroid hormones with the proteins of human plasma. *Ann. N. Y. Acad. Sci.* 1960, 86, 440.
- Dowling, J. T., Freinkel, N., and Ingbar, S. H. The effects of estrogens upon the peripheral metabolism of thyroxine. *J. clin. Invest.* 1960, 39, 1119.
- Robbins, J., and Rall, J. E. The interaction of thyroid hormones and protein in biological fluids. *Recent Progr. Hormone Res.* 1957, 13, 161.
- Freinkel, N., Ingbar, S. H., and Dowling, J. T. The influence of extracellular thyroxine-binding protein upon the accumulation of thyroxine by tissue slices. *J. clin. Invest.* 1957, 36, 25.
- Dowling, J. T., Freinkel, N., and Ingbar, S. H. Effect of diethylstilbestrol on binding of thyroxine in serum. *J. clin. Endocr.* 1956, 16, 1491.
- Engbring, N. H., and Engstrom, W. W. Effects of estrogen and testosterone on circulating thyroid hormone. *J. clin. Endocr.* 1959, 19, 783.
- Federman, D. D., Robbins, J., and Rall, J. E. Effects of methyl testosterone on thyroid function, thy-

- roxine metabolism, and thyroxine-binding protein. J. clin. Invest. 1958, 37, 1024.
19. Yamazaki, E., and Slingerland, D. W. The *in vitro* metabolism of thyroxine, triiodothyronine and their acetic and propionic acid analogues. Endocrinology 1959, 64, 126.
 20. Lissitzky, S., Roques, M., and Benevent, M. T. Desiodation enzymatique de la thyroxine et de ses derives. I. Purification et proprietes de la thyroxine-desiodase de muscle de lapin. Bull. Soc. Chim. Biol. In press.
 21. Beierwaltes, W. H., and Robbins, J. Familial increase in the thyroxine-binding sites in serum alpha globulin. J. clin. Invest. 1959, 38, 1683.
 22. Saunders, F. J., and Drill, V. A. The myotrophic and androgenic effects of 17-ethyl-19-mortestosterone and related compounds. Endocrinology 1956, 58, 567.
 23. Ingbar, S. H. Unpublished observations.
 24. Rasmussen, H. Thyroxine metabolism in the nephrotic syndrome. J. clin. Invest. 1956, 35, 792.
 25. Myant, N. B. Relation between the biliary clearance rate of thyroxine and the binding of thyroxine by the serum proteins. J. Physiol. (Lond.) 1957, 135, 426.
 26. Myant, N. B., and Pochin, E. E. The metabolism of radiothyroxine in man. Clin. Sci. 1950, 9, 421.
 27. Rall, J. E. Iodine compounds in the blood and urine of man. J. clin. Endocr. 1950, 10, 996.
 28. Blackburn, C. M., McConahey, W. M., Keating, F. R., Jr., and Albert, A. Calorigenic effects of single intravenous doses of 1-triiodothyronine and 1-thyroxine in myxedematous persons. J. clin. Invest. 1954, 33, 819.
 29. Ingbar, S. H. and Freinkel, N. Thyroid hormones in Hormones in Human Plasma, H. N. Antoniades, Ed. Boston, Little, Brown, 1960, p. 515.
 30. Ingbar, S. H. Clinical and physiologic implications of thyroxine turnover in man in Clinical Endocrinology, E. B. Astwood, Ed. New York, Grune & Stratton, 1960, vol. I, p. 91.
 31. Ingbar, S. H. Pre-albumin: A thyroxine-binding protein of human plasma. Endocrinology 1958, 63, 256.
 32. Hamolsky, M. W., Stein, M., Fischer, D. B., and Freedberg, A. S. Further studies of factors affecting the plasma protein-thyroid hormone complex. Endocrinology 1961, 68, 662.
 33. Christensen, L. K., and Litonjua, A. D. Is thyroxine binding by pre-albumin of physiologic importance? J. clin. Endocr. 1961, 21, 104.
 34. Richards, J. B., Dowling, J. T., and Ingbar, S. H. Alterations in the plasma transport of thyroxine in sick patients and their relation to the abnormality in Graves' disease (abstract). J. clin. Invest. 1959, 38, 1035.
 35. Braverman, L., and Ingbar, S. H. A rapid method for individually assessing hormonal binding by thyroxine-binding proteins. Clin. Res. 1961, 9, 176.
 36. Gordon, R. S., Jr. Hereditary hypoproteinemias and other plasma protein abnormalities in The Metabolic Basis of Inherited Disease, J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson, Eds. New York, McGraw-Hill, 1960, p. 1379.