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Research Article

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Metabolism of Thyroxine-Binding Globulin in Man

ABNORMAL RATE OF SYNTHESIS IN INHERITED THYROXINE-BINDING GLOBULIN DEFICIENCY AND EXCESS

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ABSTRACT It has been previously suggested that inherited thyroxine-binding globulin (TBG) abnormalities in man may be due to mutations at a single X-chromosome-linked locus controlling TBG synthesis. However, abnormalities in TBG degradation have not been excluded. The availability of purified human TBG and its successful labeling with radioiodide allowed us to examine such possibility.

Human TBG was purified by affinity chromatography, labeled under sterile conditions with ^{131}I or ^{125}I, and mixed with [^{125}I]thyroxine (T_4) or [^{131}I]T_4, respectively, before their intravenous injection. Blood and urine samples were collected over a 10-day period, and the turnover parameters were calculated. In eight normal volunteers mean values ±SD for TBG and T_4 respectively, were as follows: Half time ($t_{\frac{1}{2}}$) 5.3±0.4 and 7.0±0.6 days; distribution space (DS) 7.2±1.0 and 10.8±1.2 liters; and total daily degradation (D) 0.211±0.053 and 0.088±0.011 μmol/day. In all subjects, $t_{\frac{1}{2}}$ of TBG was shorter than that of T_4; and the DS was smaller. 2.4 mol of TBG was degraded for each mole of T_4. In five of six subjects from four families, comprising hemizygous and heterozygous carriers of TBG absence, decrease, and excess, the $t_{\frac{1}{2}}$ and DS for TBG were within the normal range. The D of TBG was proportional to the serum concentration of the protein. Changes in the T_4 kinetics in these patients were compatible with euthyroidism and with the known alterations in the extrathyroidal T_4 pool associated with the changes in serum TBG concentration. A striking decrease in the $t_{\frac{1}{2}}$ of TBG was found only in a patient with acquired diminution in TBG concentration and in patients with thyrotoxicosis or other conditions apparently unrelated to thyroid dysfunction. TBG $t_{\frac{1}{2}}$ was 2.5 days in a patient with multiple myeloma and 3.6 days in two patients with thyrotoxicosis. Decreased TBG $t_{\frac{1}{2}}$ was also observed in three of six patients with nonthyroidal pathology and was associated with an increase in TBG D disproportionate to their level of serum TBG.

These studies indicate that changes in TBG concentration in patients with X-chromosome-linked TBG abnormalities are due to alterations in its rate of synthesis. In other conditions, abnormalities of TBG degradation and/or rate of synthesis may be found.

INTRODUCTION

Thyroxine-binding globulin (TBG),[1] a serum protein migrating electrophoretically, in the inter-α-globulin zone, is the major circulating protein in man involved in thyroid hormone transport. Based on experimental evidence, it has been assumed that this protein plays only a passive role in thyroid hormone homeostasis. Indeed, in the steady state, persons with either inherited or acquired TBG abnormalities, such as with adminis-

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[1] *Abbreviations used in this paper:* D, total daily degradation; DS, distribution space; FTI, free T_4 index; k, fractional turnover rate; NHSA, normal human serum albumin; R-T_4, resin T_4 uptake; T_4, thyroxine; TT_3, serum total triiodothyronine; TT_4, serum total T_4; TBG, thyroxine-binding globulin; TP, serum total protein; TSH, thyroid-stimulating hormone.

tration of estrogens or androgens, are euthyroid and have a normal total daily degradation (D) of thyroxine (T_4) despite gross alterations in the concentration of total serum T_4 (1–4). The observed changes in T_4 kinetics seem to be related to changes in the fractional turnover rate and/or the extrathyroidal T_4 pool associated with the increased or decreased total T_4-binding capacity of TBG in serum (5). Though some reports have indicated that the free T_4 concentration may be altered in the serum of subjects with changes in the T_4 capacity of TBG (6–8), the bulk of evidence and the hormone kinetic studies suggest that the free T_4 level in serum remains within the normal range and that it determines the thyroid hormone-dependent metabolic status of the subjects.

Previous work from this laboratory has shown that in subjects with either increased or decreased concentration of TBG in serum due to X-chromosome-linked abnormalities, TBG is identical to that in normal persons, electrophoretically, immunologically, in its affinity for T_4, and by heat inactivation (3). Other laboratories have confirmed that TBG content measured by immunologic techniques in patients with alterations in the binding capacity to TBG is identical to that quantitated by T_4 saturation techniques (3, 9, 10). Furthermore, no abnormal substances interfering with T_4 binding to TBG have been demonstrated (11, 12). On the basis of such data, it was assumed that the most likely genetic mechanism producing X-linked TBG abnormalities in man is mutation at a single locus controlling TBG synthesis (3). However, the possibility of alterations in the degradation rate of TBG has not been examined. The development of techniques for the purification and iodine labeling of human TBG suitable for intravenous administration provided us with the opportunity to study this possibility. Furthermore, this technique lent itself to an examination of the mechanism of acquired alterations in TBG capacity (13, 14).

This report presents data on the kinetics of TBG and T_4 metabolism derived from the simultaneous intravenous administration of tracer amounts of T_4 and TBG labeled with different iodine isotopes. The results lead to the conclusion that the fractional turnover rate of TBG, but not of T_4, is normal in X-chromosome-linked TBG abnormalities. Acquired changes in the TBG capacity, however, may be due in part to changes in the fractional turnover rate of TBG.

METHODS

Laboratory materials and methods

A single preparation of human TBG purified from pooled blood bank serum by affinity chromatography (15, 16) was used. This preparation was Australia antigen negative. Radioiodination using ^{131}I or ^{125}I was carried out by the chloramine-T method as previously described (17), but under sterile conditions. Sterile Sephadex columns (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) and collecting tubes were used for the separation of the iodo-TBG from unreacted iodide. The first peak fraction of iodo-TBG was at least 95% precipitable with TCA and complexed to an extent of 90% with an excess of specific rabbit anti-human TBG serum (17). Labeled TBG preparations varied in their mean iodine content from 0.8 to 4.8 atoms of iodine per molecule of TBG. An aliquot of the iodo-TBG was removed by sterile capillary and diluted in normal saline containing 5% salt-poor normal human serum albumin (NHSA, Cutter Laboratories, Berkeley, Calif.). The final amount of ^{131}I-TBG injected was 30–35 μCi/2 ml and that of ^{125}I-TBG was 5–6.5 μCi/2 ml. Depending upon the specific radioactivity of the preparation, 20–80 μl of iodo-TBG was needed to achieve this concentration.

[^{125}I]T_4 in 50% propylene glycol was obtained from Abbott Laboratories, North Chicago, Ill., and [^{131}I]T_4 from Industrial Nuclear Co., Inc., St. Louis, Mo. The specific activity in both preparations varied from 60 to 100 μCi/μg and at least 90% of the isotopic content was in the form of labeled T_4.

40–100 μl of radioiodinated T_4 was added to the iodo-TBG solution diluted in the saline solution containing 5% NHSA. The final amount of labeled T_4 injected was 30–35 μCi/2 ml for [^{131}I]T_4 and 5–6.5 μCi/2 ml for [^{125}I]T_4. [^{131}I]T_4 was mixed with ^{125}I-TBG and [^{125}I]T_4 with ^{131}I-TBG. The mixture of diluted labeled T_4 and TBG was passed through a Millipore filter (Millipore Corp., Bedford, Mass.) into a sterile vial and was used without further treatment for intravenous injection. Cultures containing this material showed no bacterial growth.

To ascertain further the safety of the iodo-TBG preparation for human use, the first turnover study was performed in a single volunteer (E. H.) utilizing ^{131}I-TBG only. Blood was obtained before injection, and thereafter at weekly intervals for 6 wk, and at monthly intervals for 6 mo for the determination of total proteins, albumin, total bilirubin, lactic dehydrogenase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, creatinine phosphokinase, alkaline phosphatase, Ca, P, Na, K, Cl, CO_2, glucose, creatinine, blood urea nitrogen, cholesterol, total T_4, free T_4 index, thyroid-stimulating hormone, TBG capacity, prothrombin time, and complete blood count including differential, platelets, and reticulocytes. Urine samples were also obtained for routine analyses. The subject was kept under observation in the Clinical Research Center although he carried out his usual activities as laboratory technician during the day. Vital signs, including temperature, were obtained twice daily and periodic physical examinations were performed. There was no evidence of untoward effects by physical examination. Vital signs and blood chemistries remained within normal limits except during a bout of viral pharyngitis associated with sore throat, cervical lymphadenopathy, temperature of up to 38.1°C, tachycardia, and lymphocytosis which lasted for 3 days and occurred during the 7th wk after administration of the ^{131}I-TBG. Iodo-TBG was not given to other patients until 8 mo after this initial study had elapsed.

Iodine-labeled TBG and T_4 turnover studies. A mixture containing 30–35 μCi of ^{131}I-TBG and 5–6.5 μCi of [^{125}I]T_4 or 5–6.5 μCi of ^{125}I-TBG and 30–35 μCi of [^{131}I]T_4 in 2 ml of normal saline containing 5% NHSA prepared as described above, was injected through the antecubital vein. Recirculation of released radioiodide through the thyroid was blocked with five drops of a saturated solution of KI given orally 2 h before the injection and twice daily

through the study period. Blood samples were obtained from the contralateral arm before the injection and at 5 min and at daily intervals for 10–11 days after the injection. All samples were aliquoted into counting vials and were kept frozen at −20°C until termination of the sample collection. The remaining serum from each sample was used for the measurement of total protein, total T_4, free T_4 index, and thyroid-stimulating hormone. 2 ml of serum from each sample was counted along with appropriate standards for 10 min in a Nuclear-Chicago 3-inch crystal dual-channel, well-type scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.), both before and after TCA precipitation. Corrections were made for the contribution of ^{131}I to the reading in the ^{125}I channel. Error due to decay of the isotopes during the counting interval was eliminated by repeat counting of all samples and standards in reverse order. A 2-ml aliquot of serum obtained before administration of the isotope was used for determination of the background and another 2-ml aliquot, after addition of 0.1% of the injected dose, as a standard.

In some patients 24-h urine samples were also obtained. Urine samples were placed in plastic bottles and kept at 4°C during the 24-h collection. The volume was measured, and 30-ml aliquots were sealed in counting vials and frozen at −20°C. At the termination of the turnover study, all vials were counted together, along with appropriate standards prepared in identical volume and vials. To ascertain the completeness of each 24-h urine collection, the creatinine content was determined.

Data from the serum disappearance of TCA-precipitable ^{131}I and ^{125}I activity was used to calculate the $t_{\frac{1}{2}}$, fractional turnover rates (k), distribution spaces (DS), and total daily degradations (D) of TBG and T_4. All data were corrected for diurnal changes in serum protein concentration as previously described (18), and analyzed by the method of single compartment kinetics. Slopes of the curves and intercepts were calculated by the least-squares method, utilizing an Olivetti P-602 microcomputer (Olivetti Underwood Corp., N. Y.). Previous studies have shown that, at all times, more than 95% of the serum radioactivity derived from an intravenous injection of labeled TBG to rabbits was both antibody and TCA precipitable and migrated electrophoretically as authentic iodo-TBG (17). Similarly, the percent radioactivity precipitable with TCA and with a specific TBG antiserum, or migrating electrophoretically as intact iodo-TBG was comparable when labeled TBG was incubated for up to 9 days at 37°C in the presence of sterile human serum.

In some instances, the k of labeled TBG and T_4 were also calculated from data on the urinary excretion of the isotopes. The percent of the dose cleared each day was derived from the ratio of the percent dose excreted on that day and the percent dose retained. The latter was obtained by subtracting the cumulative percent dose excreted for each 24-h period. The k's calculated for each day, beginning with day 3 after injection, were then averaged. More than 95% of the isotopic content in urine was TCA soluble.

Tests of thyroid function. Total T_4 (TT_4) concentration in serum was determined by competitive-binding assay, and the resin T_4 uptake (R-T_4), and free T_4 index (FTI) as previously described (19). Serum total triiodothyronine (TT_3) and thyroid-stimulating hormone (TSH) were measured by radioimmunoassay (20, 21). TBG was measured by the single T_4 load ion-exchange resin method (14) and results are expressed either as the maximal T_4-binding capacity in μg T_4/100 ml or as micromolar concentration of TBG. The TBG index was calculated from the ratio of the R-T_4, expressed as percent of normal control, and the TT_4 (22). Serum total proteins (TP) were measured using a T.C. refractometer (American Optical Corp., Buffalo, N. Y.).

Clinical material

Normal subjects. Eight normal volunteers, six males and two females, 19–44-yr old, in good health, and with no past or family history of thyroid illness were studied.

Subjects with inherited TBG abnormalities. Three were subjects from a family with TBG absence previously described (11). One of them, (Mi. P.) a 21-yr-old phenotypic female with XO Turner's syndrome, was taking estrogens (Enovid, 5 mg/day) but had no detectable T_4 binding in the inter-α-globulin region. Two TBG-T_4 turnover studies were performed approximately 4 mo apart. In one study ^{131}I-TBG was used and in the other ^{125}I-TBG. The two other members of the family were her mother (L. P.) and sister (P. R.), both heterozygous with decreased serum TBG concentration (3, 11). There was no evidence of thyroid dysfunction or goiter in any member of this family.

A male (Or. W.) with decreased serum TBG concentration, thyrotoxicosis, and mild diabetes mellitus from an unrelated family was also studied. Sera from 17 members of the family were analyzed for TT_4, FTI, and TBG capacity. Of 15 blood relatives tested, 8 (3 males and 5 females) had decreased TBG capacity and 7 had normal TBG capacity. Five sons of three affected fathers were normal, and all five daughters of three affected males had a reduction in the TBG capacity. The TBG capacity of the four affected males ranged from 6.4 to 8.0 μg T_4/100 ml, and that of the five affected females ranged from 10.5 to 12.8 μg T_4/100 ml. The data satisfy all criteria for an X-chromosome-linked mode of inheritance. Details, including a pedigree of this family has been presented (23), and will be published elsewhere. While thyrotoxic, the propositus Or. W. had a TBG capacity of 6.8 μg T_4/100 ml, a TT_4 of 5.7 μg/100 ml, and FTI of 14.1.[2] 6 mo after successful treatment with ^{131}I, the TBG capacity remained in the pretreatment range of 7.1 μg T_4/100 ml while the TT_4 was 3.3 μg/100 ml, and the FTI was 6.1.

Two unrelated male patients with inherited TBG excess were also studied. One (W. N.) was the propositus of "family N" previously reported and compatible with X-chromosome-linked mode of inheritance (3). The other patient was D. No., and propositus of "family No," not previously reported. Blood samples could be obtained from five additional members of this family, four blood relatives, and one by marriage. All four blood relatives including the mother, a sister, an aunt, and a niece were most likely affected heterozygotes as evidenced by the less marked elevation in the TBG capacities and TBG indexes when compared to the hemizygous affected propositus (Table I and Fig. 1). Although there were no available male offspring of affected males to prove X-chromosome linkage, the data available are not incompatible with such a mode of inheritance. The FTI was normal in all members of the family tested, and there was no evidence of thyroid disease in blood relatives.

Acquired reduction in TBG capacity. M. K. had multiple myeloma of the IgG κ-type, diagnosed 5 yr before this study. Her course was complicated by hyperviscosity requiring repeated plasmaphoreses, multiple osteolytic lesions

[2] Normal values, TBG, 16–24 μg T_4/100 ml; TT_4, 4.2–9.4 μg/100 ml; FTI, 3.6–9.3.

TABLE I

Studies on T_4 Transport in Serum of Members from Family No

Subject	Sex	TT_4	R-T_4	FTI	TBG capacity	TBG (index)
		μg/100 ml	*% control*		*μg T_4/100 ml*	
Normal range		4.2–9.4	80–120	3.6–9.3	16–24	8–22
D. No.	M	17.0 (5) (16.0–18.4)	39 (3) (36–44)	6.5 (3) (5.9–7.4)	63.5 (3) (62.2–68.0)	2.2 (3) (1.9–2.6)
R. H.	F	16.3 (2) (14.3–18.2)	37.0 (2) (36–38)	6.0 (2) (5.4–6.6)	57.3 (2) (54.6–60.0)	2.3 (2) (2.0–2.6)
E. N.	F	10.5	41	4.3	56.0	3.9
H. L.	F	12.4	61	7.6	40.0	4.9
P. No.	F	16.1	41	6.6	59.6	2.6
J. No.	F	5.1	108	5.5	13.8	21.2

When more than one determination was obtained on samples drawn at different times over a period of few months or few years, the number of determinations is given in parentheses beside and the range below the calculated mean value.

with progressive skeletal destruction resulting in pathological fractures, and coronary heart disease with angina pectoris. Additional medical history included thyroidectomy at the age of 34, apparently for euthyroid multinodular goiter. Since then, she had been taking 1½ grain of desiccated thyroid. She was clinically euthyroid, but for at least 3 yr she has been known to have a TBG capacity of from 1 to 4 μg T_4/100 ml in the presence of normal FTIs, both during and after discontinuation of Halotestin for a 4-mo period. TP was 13.7 g/100 ml and albumin was 3.4 g/100 ml. Blood urea nitrogen and creatinine were within normal limits. Serum iron was 60 μg/100 ml and total iron-binding capacity was 156 μg/100 ml. Medications at the time of the study included digoxin, 0.125 mg/day; quinidine, 200 mg every 6 h; Synthroid, 0.125 mg daily; Halotestin, 5 mg three times daily; Keflex, 500 mg four times daily, allopurinol, 100 mg three times daily; Colace, 100 mg twice daily; and Talwin, 50 mg every 4 h as needed. The possibility of inherited TBG abnormality was excluded by the finding of normal TBG capacity in sera of eight blood relatives (three daughters, four sons, and one grandson).

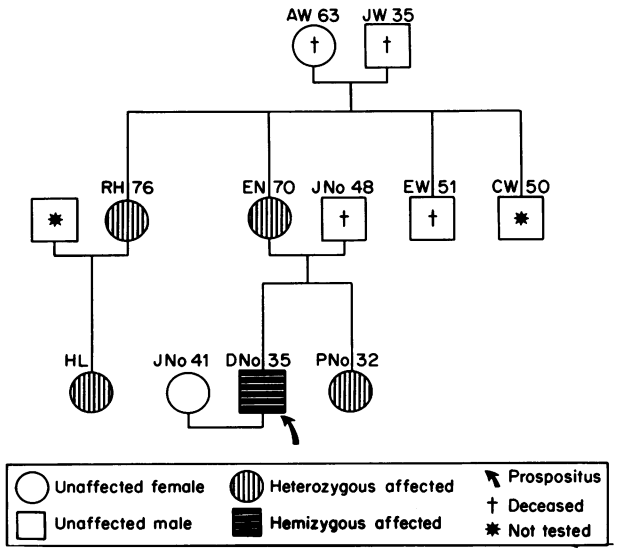

FIGURE 1 Pedigree of family No. Initials and ages are given above each symbol.

Thyrotoxic patients. Two female patients (A. F. and G. W.) were studied in addition to the thyrotoxic male with inherited TBG deficiency described above. Both had classical history and findings of thyrotoxicosis due to Graves' disaese.

Hypothyroid patients. Three patients (one male and two females) with serum tests compatible with hypothyroidism were studied. K. W. had secondary hypothyroidism and acromegaly; G. N. had asymptomatic hypothyroidism 4 mo after treatment with ^{131}I for Graves' thyrotoxicosis; and J. A. had previously undergone total thyroidectomy for thyroid carcinoma. She also had asymptomatic reduction of the FTI 2½ wk after the deliberate discontinuation of T_4 replacement therapy for the purpose of evaluating the possible usefulness of ^{131}I therapy. This particular patient was not given KI which would have otherwise precluded the planned scanning procedures with ^{131}I.

Patients with other than thyroid pathology. S. W. had diabetes mellitus and gout; V. U., exogenous obesity; and C. G., Stein-Leventhal syndrome. A. M. had disseminated cystadenocarcinoma of the ovary; and A. McG. and P. D. had metastatic breast carcinoma. These latter three had extensive metastases with pleural effusion and/or ascites. P. D. and A. M. were taking conjugated estrogens (Premarin) 0.625 mg/day.

An informed written consent was obtained from each subject before initiation of the study. It was also ascertained that no subject was pregnant. The study protocol was approved by both the Clinical Investigation Committee and the Committee on the Human Use of Isotopes.

RESULTS

TBG and T_4 metabolism in normal subjects. Table II lists data on the kinetics of TBG and T_4 metabolism in normal adults. Without exception the k of TBG was

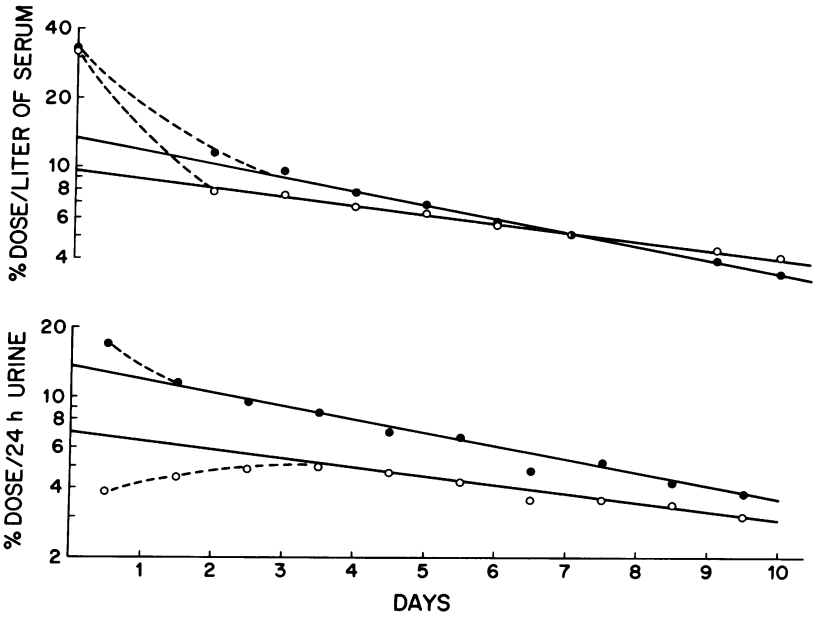

FIGURE 2 Serum disappearance and urinary excretion of isotopes given intravenously as [^{125}I]-TBG (●) and [^{131}I]T_4 (○) to a normal subject (E. E.). The plot represents TCA-precipitable activity in serum expressed as percent dose per liter and total activity in 24-h urine sample expressed as percent dose.

faster than that of T_4 ($P < 0.0001$). This was true when calculated from either the plasma disappearance of the TCA-precipitable activity or the urinary excretion of the isotopes (Fig. 2). Also, the DS of TBG was smaller than that of T_4 ($P < 0.0001$). In average the D of TBG was 2.4-fold greater (mole per mole) than that of T_4 ($P < 0.0001$).

One normal subject, E. H., was studied twice 8 mo apart. A variation of 24% in both the $t_{\frac{1}{2}}$ and DS was observed between the two studies. The reason for such a discrepancy is unclear and does not seem to be the rule since in another patient (Mi. P.) the differences in the TBG $t_{\frac{1}{2}}$ and DS, between two studies performed 4 mo apart, were only 4 and 2%, respectively. Furthermore, no discrepancies were found in the TBG kinetic data of E. H. when ^{125}I-TBG and ^{131}I-TBG, labeled with an average of 0.8 and 4.8 atoms I/molecule, respectively, were administered simultaneously (Table II). Similar results have been obtained in rabbits (17).

TBG and T_4 metabolism in X-linked inherited TBG abnormalities. Studies were performed in six subjects from four families with TBG abnormalities, exhibiting in the hemizygous affected state total absence, decrease, or excess in serum TBG. Heterozygotes for the trait were also studied. Data presented in Table II indicate that with a single exception (L. P.), the k of TBG in the subjects studied was within 2 SD of normal. The DS of TBG was also within 2 SD of the normal mean except in Mi. P., a patient of short stature, in whom a DS of 56% the normal mean was proportional to her smaller body weight of 52% the normal mean. The D of TBG, however, varied strikingly from subject to subject but remained proportional to the serum TBG concentration (Fig. 3). Thus the defect is compatible with an abnormality in TBG synthesis. With the exception of the thyrotoxic patient, Or. W., T_4 kinetics were compatible with changes in the extrathyroidal T_4 pool size. As previously shown, an inverse correlation exists between the DS of T_4 and the TBG concentration due to a shift in the relative distribution of the extrathyroidal T_4 between the vascular and extravascular compartments. This finding has been previously summarized (3, 5). Furthermore, as expected, the D of the hormone in the euthyroid patients was normal as was the FTI.

TBG and T_4 metabolism in acquired TBG abnormality. A single patient with profound decrease in the TBG capacity (M. K.), suffering from multiple myeloma, was studied. Investigation of the famly (see Clinical Material) failed to reveal inherited TBG abnormality. As shown in Table II, contrary to patients with inherited reduction in serum TBG concentration, this patient showed a rapid k (212% the mean normal), and a larger DS (306% the mean normal) for TBG. Nevertheless, the D of TBG was within 2 SD of normal, suggesting a defect in TBG degradation. The striking diminution in serum TBG concentration could not be

TABLE II

Metabolism of T_4 and TBG in Normal Subjects, in Subjects with

Subject	Diagnosis	Age and sex	Height	Weight	TT_3	FTI	TSH
		yr	*cm*	*kg*	*ng/100 ml*		*μU/ml*
Normal subjects							
E. H.		21 M	182	66.5	114.2	6.3	1.5
E. H.		22 M	182	67.2	—	6.3	3.7
A. L.		42 F	169	56.8	92.5	7.1	9.1
B. P.		29 M	175	71.8	118.8	8.0	<0.1
E. E.		44 M	175	63.6	102.6	7.6	4.8
J. H.		23 M	173	77.3	121.3	6.8	10.2
J. C.		22 M	178	70.5	89.3	8.4	5.2
R. M.		21 M	175	70.5	132.0	7.9	2.7
Y. H.		19 F	179	69.0	140.8	6.4	4.0
Mean		27.0	176	68.1	113.9	7.30	4.6
SD		9.5	4	5.7	18.2	0.80	3.3
Inherited TBG Abnormalities							
TBG absence family P							
Mi. P.	XO-Turner's hemizygous deficient	21 F	140 140	35.8 36.0	60.3	9.5 9.2	— 9.8
L. P.	Heterozygous carrier	51 F	171	79.4	66.2	8.4	—
P. R.	Heterozygous carrier	27 F	171	70.3	70.8	8.5	6.8
Decreased TBG family W							
Or. W.	Hemizygous low TBG, thyrotoxicosis, diabetes	43 M	180	61.5	—	14.1	<0.1
High TBG							
W. N.	Hemizygous affected (family N)	46 M	175	83.9	252.4	8.2	2.8
D. No.	Hemizygous affected (family No)	38 M	187	83.1	223.5	8.4	5.1
Acquired TBG abnormalities							
M. K.	Multiple myeloma	77 F	157	49.0	—	7.8	—
Thyrotoxicosis							
G. W.	Graves' disease	52 F	165	57.8	—	18.2	4.4
A. F.	Graves' disease	74 F	152	47.6	275.0	13.8	—
Hypothyroidism							
K. W.	Secondary hypothyroidism, acromegaly	68 M	158	92.0	—	0.8	2.0
G. N.	Post-^{131}I therapy for Graves' thyrotoxicosis	30 F	160	67.6	—	2.9	25
J. A.	Post-total thyroidectomy; off hormone for 2½ wk	25 F	160	54.2	51.2	3.8	84.4
Other than thyroid pathology							
S. W.	Diabetes mellitus, gout	58 F	167	87.8	—	8.1	—
V. U.	Exogenous obesity	51 F	160	101	—	7.4	—
C. G.	Stein-Leventhal syndrome	19 F	165	47.6	—	7.3	—
A. M.	Metastatic ovarian cancer; pleural effusion and ascites; on estrogens	59 F	163	67.3	—	6.1	—
A. McG.	Metastatic breast cancer; pleural effusion	38 F	160	89.5	—	6.6	2.1
P. D.	Metastatic breast cancer; pleural effusion and ascites; on estrogens	70 F	165	54.0	—	5.9	—

k values calculated from urinary excretion data are in parentheses.
E. H. had two TBG turnover studies 8 mo apart; one was done with ^{131}I-TBG, and the second with simultaneous injection of ^{131}I-TBG and ^{125}I-TBG.
Mi. P. had two TBG-T_4 turnover studies 4 mo apart. The isotopes used in the second study were reversed in comparison to those used in the initial study.
Isotope used for labeling of T_4 and TBG appears as asterisk next to the k value. *, ^{131}I; ‡, ^{125}I.

T_4 Metabolism						TBG Metabolism					
TT_4	k (fraction of day)	$t_{\frac{1}{2}}$	DS	D	r	TBG	k (fraction of day)	$t_{\frac{1}{2}}$	DS	D	r
μg/100 ml		*days*	*liters*	*μmol/day*		*μM*		*days*	*liters*	*μmol/day*	
5.3		—	—	—	—	0.210	0.147*	4.71	10.38	0.320	0.997
5.4		—	—	—	—	0.216	0.116*	5.99	8.07	0.202	0.995
							0.115‡	6.04	8.19	0.203	0.994
4.7	0.105* (0.102)	6.63	12.60	0.0797	0.998	0.196	0.111‡ (0.110)	6.25	6.53	0.142	0.996
6.3	0.100*	6.95	11.81	0.0956	0.952	0.192	0.137‡	5.07	7.12	0.187	0.998
7.2	0.089* (0.083)	7.75	10.43	0.0913	0.930	0.252	0.136‡ (0.127)	5.09	7.43	0.247	0.996
7.0	0.112* (0.103)	6.21	10.65	0.1071	0.997	0.277	0.139‡ (0.129)	5.01	7.77	0.297	0.996
7.3	0.088* (0.081)	7.85	8.91	0.0739	0.996	0.207	0.135‡ (0.128)	5.13	5.81	0.162	0.998
6.5	0.103* (0.095)	6.74	9.86	0.0849	0.996	0.201	0.140‡ (0.148)	4.95	6.72	0.189	0.996
6.0	0.098* (0.097)	7.04	11.29	0.0855	0.991	0.221	0.128‡ (0.131)	5.43	7.19	0.203	0.903
6.30	0.0993 (0.0942)	7.02	10.79	0.0883	—	0.219	0.1323 (0.1288)	5.29	7.23	0.211	—
0.91	0.0086 (0.0094)	0.59	1.23	0.0109	—	0.029	0.0094 (0.0121)	0.42	1.02	0.053	—
2.6	0.186*	3.74	13.38	0.0833	0.995	0	0.157‡	4.41	4.03	0	0.995
2.5	0.211‡	3.29	12.84	0.0871	0.990	0	0.151‡	4.58	4.10	0	0.997
3.2	0.176*	3.94	12.74	0.0924	0.997	0.064	0.164‡	4.23	8.02	0.085	1.000
3.3	0.162‡	4.27	13.51	0.0932	0.987	0.053	0.133*	5.23	6.23	0.044	0.998
6.7	0.126‡	5.5	15.2	0.1647	0.999	0.088	0.136*	5.10	7.30	0.087	0.996
21.8	0.049*	14.13	7.09	0.0977	0.961	1.093	1.134‡	5.16	8.33	1.223	0.947
16.8	0.059‡	11.7	8.51	0.1086	0.998	0.824	0.137*	5.06	7.75	0.875	0.996
1.1	0.256*	2.71	20.38	0.0739	1.000	0.021	0.280‡	2.47	22.15	0.128	0.993
13.0	0.121‡ (0.115)	5.74	10.12	0.2044	0.996	0.196	0.194* (0.181)	3.58	6.35	0.239	0.999
12.6	0.151‡	4.59	5.92	0.1449	0.997	0.224	0.195*	3.55	4.35	0.191	0.995
0.9	0.088‡	7.87	12.30	0.0126	0.994	0.251	0.112*	6.17	10.15	0.286	0.995
2.7	0.104*	6.68	9.23	0.0332	0.999	0.206	0.116‡	5.95	8.06	0.202	0.997
4.0	0.054*	12.82	7.59	0.0357	0.975	0.264	0.144‡	4.81	4.02	0.121	0.972
9.0	0.089‡	7.80	9.20	0.0947	0.989	0.319	0.141*	4.92	10.41	0.469	0.999
7.9	0.079* (0.072)	8.73	9.86	0.0777	0.998	0.300	0.190‡ (0.192)	3.65	7.25	0.413	0.998
6.9	0.114*	6.07	8.24	0.0835	0.994	0.247	0.117‡	5.95	8.24	0.237	0.998
7.8	0.102*	6.78	9.29	0.0954	0.997	0.367	0.180‡	3.84	11.15	0.736	0.992
6.5	0.224*	3.10	5.53	0.1036	1.000	0.259	0.252‡	2.75	10.99	0.718	0.993
7.9	0.083*	8.40	8.66	0.0726	0.986	0.396	0.154‡	4.49	5.64	0.345	0.995

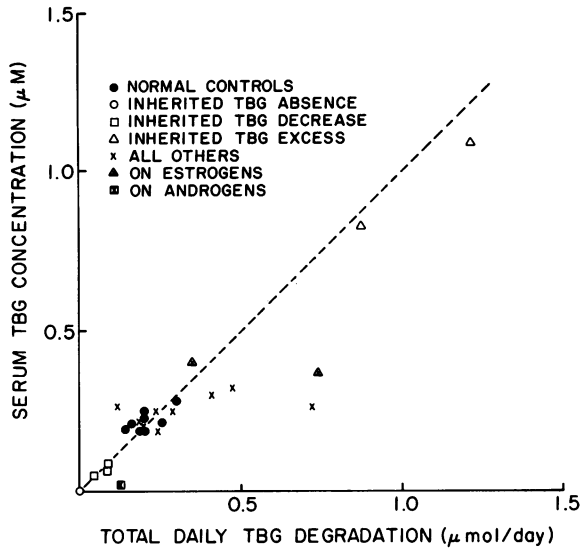

FIGURE 3 Correlation of serum concentration and D of TBG in normal volunteers, in patients with inherited TBG abnormalities, and in patients with a variety of unrelated pathologic states. The dashed line corresponds to the expected correlation between serum concentration and D of TBG in the absence of abnormalities in TBG degradation. It was derived from data obtained from the normal controls.

ascribed to treatment with Halotestin since no significant changes were observed after discontinuation of the hormone (see Clinical Material).

TBG and T_4 metabolism in thyrotoxicosis and hypothyroidism. Three subjects with serum tests compatible with thyrotoxicosis and three with hypothyroidism were studied (Table II). One (Or. W.) had associated inherited decrease in serum TBG concentration which did not change significantly after achievement of euthyroidism (see Clinical Material). Thus, with the exception of Or. W., the two thyrotoxic patients had a TBG $t_{\frac{1}{2}}$ below 2 SD of normal. One of them (A. F.) also had reduced TBG, and T_4 DS and body weight. Only one hypothyroid patient (K. W.) had a prolonged TBG $t_{\frac{1}{2}}$ as well as increased TBG DS. In another (J. A.) only the DS of TBG was reduced. It should be noted, however, that in both thyrotoxic and hypothyroid patients the serum concentration and D of TBG remained within the range found in normal persons. Furthermore, two of the hypothyroid patients were asymptomatic and had a normal $t_{\frac{1}{2}}$ of T_4, possibly owing to the short duration of the hormonal deficiency. The D of T_4 was compatible with the patients' diagnoses.

TBG and T_4 metabolism in patients with other than thyroid pathology. Six patients with a variety of unrelated conditions were studied and their data are shown in Table II. The TBG $t_{\frac{1}{2}}$ was decreased in three patients (V. U., A. M., and A. McG.), and was normal in the remaining three. The TBG DS was increased in two of the patients with decreased TBG $t_{\frac{1}{2}}$ (A. M. and A. McG) and in another patient S. W. It should be noted, however, that the D of TBG was increased in all patients except one, (C. G.), in whom all parameters of TBG metabolism were normal. The augmented D of TBG is due to a combination of abnormalities including increased serum TBG concentration, increased TBG DS, and accelerated TBG $t_{\frac{1}{2}}$. Only in two patients (A. M. and A. McG.), in addition to M. K., the accelerated D of TBG was disproportionate to the serum TBG concentration (Fig. 3), thus suggesting an abnormality in TBG degradation in addition to synthesis. Only one patient (A. M.) was taking estrogens. The other patient (P. D.), treated with an identical dose of estrogens, had a proportional increase in both D and serum concentration of TBG (Fig. 3).

Because extravascular sequestration of serum proteins may have affected the metabolism of TBG, data on TP, TT_4, TBG capacity, and FTI in pleural fluid obtained by thoracentesis from A. M. simultaneously with a serum sample is shown in Table III. Although deviations in the $t_{\frac{1}{2}}$ and/or DS of T_4, greater than 2 SD of normal, were observed in two patients (V. U. and A. McG.), the D of T_4 was normal in all patients of this group, compatible with their euthyroid status.

DISCUSSION

Simultaneous administration of ^{131}I- and ^{125}I-labeled TBG of different specific activities to a single man revealed no differences in the kinetic parameters when calculated from data obtained from either isotope (E. H., Table

TABLE III

Simultaneous Analysis of Pleural Fluid and Serum in a Patient (A. M.) with Metastatic Ovarian Cancer

	TP	TT_4	TBG capacity	FTI
	g/100 ml	*μg/100 ml*	*μg T_4/100 ml*	
Pleural fluid	5.7	7.5	23.5	7.5
Serum	7.6	6.2	28.7	6.2
Pleural fluid/serum ratio	0.75	1.21	0.82	1.21

II). These results indicate that as in the rabbit (17), the level of TBG iodination in man does not alter its metabolism in vivo, and thus labeled iodo-TBG is suitable for the study of TBG turnover in man. TBG metabolism was studied on two consecutive occasions a few months apart in two subjects. In one subject (E. H.) variations of 24% in the $t_{\frac{1}{2}}$ and DS of TBG was observed while in the other (Mi. P.), differences were only 4 and 2%. No adequate explanation for this discrepancy can be offered. However, studies by Braverman et al. (24), on the metabolism of T_4-binding prealbumin, showed variations in the DS of this protein, in the same subjects studied twice. They were as great as 30% in the absence of significant changes in the mean DS of the entire group before and after treatment with norethandrolone.

Simultaneous determination of TBG and T_4 metabolism in normal subjects showed that the k and D of TBG was significantly greater than that of T_4. In fact, an average of 2.4 moles of TBG was degraded for each mole of T_4. The significance of this observation and its influence on the T_4 metabolism accompanying acute changes in serum TBG concentration need further studies. The DS of TBG (7.2±1.0 liters) is smaller than that of T_4 (10.8±1.2 liters) and identical to that of albumin (7.3±0.9 liters) (25). It is also probably similar to that of T_4-binding prealbumin since normal mean values of 6.0±0.5 (26), 9.4±1.6 (27), and 9.1±3.1 liters (24) have been reported. Thus, as other serum proteins, TBG is mainly confined to the intravascular space while some T_4, unassociated with serum-binding proteins, is present in the extra-albumin or intracellular space.

Studies on 34 families with inherited TBG abnormalities have been reported so far. We have herein described two additional families. According to the concentration of TBG in serum in affected males, the families could be subdivided into three categories: absence of TBG (13 families), low TBG (16 families), and high TBG (7 families). We have previously analyzed published data on 24 families from which it was concluded that although only in one-third of the families was X-chromosome-linked transmission suggested by the authors, the remaining kindreds showed no evidence of incompatibility with such mode of inheritance (3). With a single exception (12), a similar conclusion can be drawn from the analyses of 10 pedigrees with TBG abnormalities published since our previous review (7, 8, 12, 28–33) and from the two new families presented. The family in question included an XX female, brother, and son with undetectable serum TBG, reduced TBG in a daughter, and normal TBG in the parents (12). Though various speculations to explain this observation were offered by the authors, a convincing explanation is not forthcoming.

In this study the metabolism of TBG and T_4 was evaluated in six members from four unrelated families with absent, low, and high serum TBG concentration, compatible with X-chromosome-linked inheritance. The k of TBG in a TBG-deficient hemizygous subject and her heterozygous sister, with low TBG, showed no significant deviation from that in normal subjects. The k of TBG in the heterozygous mother was slightly below 2 SD of the mean normal, but not as low as in some other patients with various nonthyroidal abnormalities. The DS of TBG was normal in all three, after correction for the strikingly decreased body size of Mi. P. The k and DS of TBG was also normal in an unrelated hemizygous male with low TBG and in two unrelated hemizygous males with high TBG. The D was proportional to the serum concentration of TBG (Fig. 3). These data indicate, that at least in the four families studied, there were no abnormalities in TBG degradation to explain the observed three types of inherited TBG variations (absence, diminution, and excess). TBG concentration has been previously measured in the serum of three of the subjects studied (Mi. P., P. R., and W. N.) by immunological and competitive-binding techniques, both of which gave identical results (3, 9). The two methods for TBG quantitation in serum identify different sites on the TBG molecule since the TBG-antibody complex does not interfere with subsequent T_4 binding to TBG (3). The affinity of T_4 and T_3 for TBG from a heterozygous-affected subject, member of a family with TBG deficiency (P. R.), and a hemizygous-affected male with TBG excess, was indistinguishable from normal. During heat inactivation, TBG from P. R. and W. N. exhibited a $t_{\frac{1}{2}}$ identical to normal persons (3). Finally, serum from TBG-deficient patients does not interfere with T_4 binding to TBG from normal persons (10, 11). Therefore, the data on TBG metabolism, together with the information summarized above, lend further support to our contention that inherited TBG abnormalities in man are due to mutations in a single gene locus controlling TBG synthesis. The total T_4 and T_3 concentrations in serum, but not the FTI, vary according to the serum concentration of TBG. However, the D of T_4 remains normal (Table II and Fig. 4).

Acquired abnormalities in TBG capacity have also been described. They are occasionally encountered in association with nephrosis, hepatitis, lymphoma, hyper- and hypothyroidism, and many other unrelated diseases (13, 14, 34). In this study we have shown that in one patient with severe acquired decrease in TBG associated with multiple myeloma (M. K.), the $t_{\frac{1}{2}}$ was considerably shortened and the DS was strikingly increased in the presence of normal D of TBG. These findings are in contrast with those in patients with inherited decrease of TBG concentration of the same

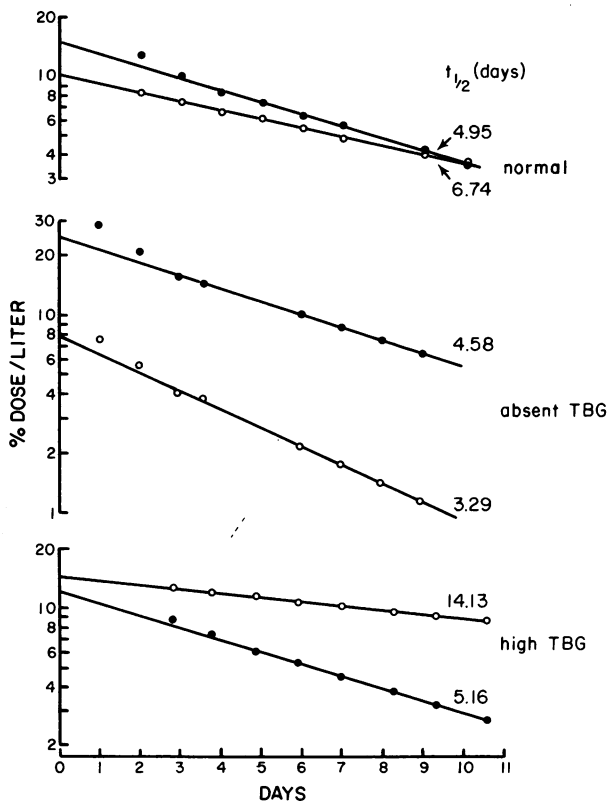

FIGURE 4 Disappearance of simultaneously administered labeled TBG and T_4 in a normal patient, a patient with absent serum TBG, and a patient with TBG excess. Note the similarity in the $t_{\frac{1}{2}}$ of TBG (●) as compared to the wide variation in $t_{\frac{1}{2}}$ of T_4 (○).

magnitude and indicate an abnormality of TBG degradation in the presence of a normal rate of synthesis. This cannot be attributed to treatment with androgens since the magnitude of decrease in serum TBG concentration was much greater than that observed in normal patients receiving androgens (1), and because cessation of hormonal therapy in our patient failed to affect the serum concentration of TBG. The augmented DS of TBG may be related to an increase in vascular permeability related to the underlying pathology. The T_4 kinetics are compatible with the low serum TBG and euthyroidism.

The two thyrotoxic patients without inherited TBG abnormality had TBG $t_{\frac{1}{2}}$ below the normal range. TBG $t_{\frac{1}{2}}$ was elevated in two of the three hypothyroid patients. Yet all thyrotoxic and hypothyroid patients had normal D and serum concentration of TBG. Thus, these limited studies failed to correlate previously reported acquired changes in TBG capacity with changes in TBG metabolism. This is not surprising since diminution in TBG concentration in thyrotoxicosis and elevation in hypothyroidism is minimal and has been demonstrated only when the mean TBG levels in larger groups of patients were compared (13, 14).

More striking are the findings in a heterogenous group of patients without thyroid pathology. Some of the patients were quite ill, others presented with conditions which did not affect their general state of well being. With the exception of a patient with Stein-Leventhal syndrome, the other five showed a significant elevation in the D of TBG. Yet only in three patients (S. W., A. M., and P. D.) was serum TBG concentration significantly increased. Furthermore, though two patients were taking estrogens (A. M. and P. D.) and had comparable increases in the serum concentration of TBG, only in one (A. M.) was a striking discrepancy between the serum concentration and D of TBG observed (Fig. 3). All patients in this group were euthyroid as determined by the lack of clinical stigmata and normal FTI and D of T_4. A tentative interpretation would suggest an abnormal TBG synthesis and/or degradation in a variety of unrelated diseases. Similar findings of increased synthesis and/or degradation of T_4-binding prealbumin have been reported in nonthyroidal illness (26) and in acute stress (27).

Our results on the TBG metabolism in man are in complete agreement with those of Cavalieri (35) which were published after the submission of our work. His mean±SD TBG $t_{\frac{1}{2}}$ and DS for six normal controls were 5.01±1.21 days and 6.68±1.31 liters, respectively, compared to our mean±SD values of 5.29±0.42 days and 7.23±1.02 liters for the same parameters. Furthermore, data on the TBG metabolism in Cavalieri's hypothyroid patients overlapped that obtained in the normal controls. The observed increase in the mean TBG $t_{\frac{1}{2}}$, DS, and D in his small group of hypothyroid patients did not achieve statistical significance.

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