

Study of Four New Kindreds with Inherited Thyroxine-Binding Globulin Abnormalities POSSIBLE MUTATIONS OF A SINGLE GENE LOCUS

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Research Article

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Quantitative variation in the serum concentration of functionally normal TBG was demonstrated by: (a) failure of serum from TBG-deficient subjects to react with anti-TBG antibodies; (b) normal kinetics of T_4 and triiodothyronine-binding to TBG in sera from subjects with low TBG and high TBG capacity; (c) concordance of estimates of TBG concentration by T_4 saturation and by immunological methods; and (d) normal rate of heat inactivation of TBG.

No abnormalities in serum transport of cortisol, testosterone, aldosterone, or thyroxine bound to prealbumin could be detected.

These observations suggest that all the TBG abnormalities thus far observed reflect mutations at a single X-linked locus involved in the control of TBG synthesis.

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Study of Four New Kindreds with Inherited Thyroxine-Binding Globulin Abnormalities

POSSIBLE MUTATIONS OF A SINGLE GENE LOCUS

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ABSTRACT Five families with inherited thyroxine-binding globulin (TBG) abnormalities were studied. On the basis of serum thyroxine (T_4)-binding capacity of TBG in affected males, three family types were identified: TBG deficiency, low TBG, and high TBG capacity. In all families evidence for X-linked inheritance was obtained and in one family all criteria establishing this mode of inheritance were met. Only females were heterozygous, exhibiting values intermediate between affected males and normals. Overlap in heterozygotes was most commonly encountered in families with low TBG.

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These observations suggest that all the TBG abnormalities thus far observed reflect mutations at a single X-linked locus involved in the control of TBG synthesis.

INTRODUCTION

In 1959 Beierwaltes and Robbins described the first family with increased thyroxine-binding globulin

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(TBG)¹ capacity (1). Subsequently, TBG abnormalities have been reported in 20 additional and apparently unrelated families: 15 with diminished (2-14) and 5 with excess (15-19) maximal capacity of thyroxine (T_4)-binding to TBG. In 6 of the 15 families with diminished TBG capacity, males had no demonstrable T_4 -binding to the TBG zone on electrophoresis, and with only a single exception (7), affected females had approximately half normal binding capacity (2-5, 7). The ability to identify the heterozygous ("carrier") state has helped establish X-chromosome-linked inheritance patterns in these families. Of the remaining nine families with diminished TBG, in eight, affected males had low but detectable TBG capacity (8-14), and in some instances "carrier" state in females could not be clearly identified because of overlap in their TBG capacity values with either affected males (8, 9) or normals (13). Because of the presence of TBG in the hemizygous-affected subjects and inability to distinguish the heterozygous state with certainty, in three out of nine families, X-linked mode of inheritance was less obvious (8, 9, 12). Similarly, only in four of the six families with increased T_4 -binding capacity of TBG was X-linked pattern of inheritance recognized (16-19), whereas, in two, an autosomal dominant transmission was suggested (15, 20). On closer scrutiny, it appears that except for two families with unpublished pedigrees (6, 14) no instance of incompatibility with X-linkage could be demonstrated.

¹Abbreviations used in this paper: CBG, cortisol-binding globulin; DF, dialyzable fraction; FT₄, free thyroxine; PBI, protein-bound iodine; R-T₄, resin sponge triiodothyronine uptake; TBG, thyroxine-binding globulin; TBPA, thyroxine-binding prealbumin; TeBG, testosterone-binding globulin; TP, total protein; TT₄, total thyroxine estimated by the competitive-binding assay.

During the past 3 years we studied five families with inherited TBG abnormalities: one with TBG deficiency² (5), three with low TBG, and one with high TBG. This communication presents clinical data on four families not previously described. An attempt was also made to elucidate the nature of these defects using immunologic and biochemical techniques for the characterization of the "abnormal" TBG. From these and previous studies by others and ourselves, a unifying hypothesis is advanced suggesting that mutations of a single gene locus on the X-chromosome may be the mechanism for all types of TBG abnormalities in man so far described.

LABORATORY METHODS

Materials

Sephadex G-100 (medium) was obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N. J. and prepared for column filtration by repeated washing with buffered saline (12.1 mM Na₂HPO₄, 1.28 mM KH₂PO₄, and 145.4 mM NaCl), pH 7.4. Pevikon C-870 (powdered copolymer of polyvinyl chloride and polyvinyl acetate) was obtained from Mercer Chemicals Corporation, New York, and prepared as previously described (21). Agarose (electrophoresis grade) was obtained from General Biochemicals, Chagrin Falls, Ohio, and used as such. Double immunodiffusion plates (Ouchterlony) were obtained from Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif. Complete Freund's adjuvant was obtained from Difco Laboratories, Detroit, Mich.

Radioisotopes. (a) L-Thyroxine-¹²⁵I (T₄-¹²⁵I), SA 40 to 90 μ Ci/ μ g depending on the lot, was obtained from Abbott Laboratories, North Chicago, Ill. On descending paper chromatography in tertiary-amyl alcohol hexane 2 M ammonia (22), T₄-¹²⁵I content of lots used was at least 90% of the total ¹²⁵I activity. The following isotopically labeled compounds were obtained from New England Nuclear, Boston, Mass.: (b) Cortisol-4-¹⁴C (F-¹⁴C) SA 0.143 μ Ci/ μ g (lot 302-267); (c) D-aldosterone-4-¹⁴C (Aldo-¹⁴C) SA 55 mCi/mmol or 0.153 μ Ci/ μ g (lot 467-072); and (d) Testosterone-¹⁴C (Test-¹⁴C) SA 50.6 mCi/mmol or 0.75 μ Ci/ μ g (lot 302-153). All radioisotopes were used without further purification, diluted in ethanol, and evaporated to dryness in polystyrene tubes before addition of serum as previously described (23).

Methods

Electrophoretic techniques. Reverse flow paper electrophoresis was carried out by the Elzinga, Carr, and Beierwaltes modification (24) of the Robbins method (25) in glycine acetate buffer pH 8.6 (26).

Agarose electrophoresis was carried out, according to the method of Laurell and Niléhn (27) on an apparatus of their design, using tris-maleate buffer pH 8.6 (28) as previously described (23).

²From here on the term *TBG deficiency* is reserved for families presenting undetectable T₄-binding to TBG in affected hemizygous subjects. Similarly *low TBG* indicates that hemizygous affected have low but detectable T₄-binding to TBG. The term *high TBG* is self-explanatory.

Antigen-antibody crossed electrophoresis was performed as described by Laurell (29) except that on occasion initial agarose electrophoresis was carried out on a modified apparatus permitting greater separation (30), and barbital buffer was substituted with tris-maleate buffer to permit T₄-¹²⁵I binding to thyroxine-binding prealbumin (TBPA) (28). For radioautography, gels were rapidly dried in a current of warm air without prior fixation of proteins or staining and exposed to Kodak No-screen X-ray film³ as previously described (23). After radioautography, the proteins were fixed with aqueous methanol-acetic acid and stained with amido black.

Pevikon-block electrophoresis was carried out by the method described by Müller-Eberhard (21).

Immuno-electrophoresis was performed using the method described by Scheidegger (31).

Double immunodiffusion (Ouchterlony technique) was carried out on agarose plates at 4°C. For radioautography, the agarose film was washed thoroughly with buffered saline, then transferred on to an agarose-coated glass plate, and dried in a current of warm air. Radioautography was carried out as described above. Precipitation lines were developed by staining with amido-black after removal of the excess protein by washing the agarose plates in buffered saline for 24 hr with frequent buffer changes.

Quantitation of TBG. The maximal T₄-binding capacity of TBG and TBPA were determined by reverse-flow paper electrophoresis (24) in glycine acetate buffer pH 8.6 (26). On some samples from each family TBG was also quantitated by two other methods: the single load ion-exchange resin (32), and by radioimmunoassay (33). The latter was performed by Dr. Richard P. Levy on coded samples.

Partial purification of TBG. 20 ml of serum (TBG capacity 61 μ g T₄/100 ml) from a healthy pregnant donor was enriched with a tracer T₄-¹²⁵I (0.02 μ g/100 ml serum) and subjected to Pevikon-block electrophoresis. Two fractions containing the peak T₄-¹²⁵I activity (67% of the total) were pooled, concentrated by ultrafiltration to a final volume of 0.5 ml, and applied to a Sephadex G-100 column. Three fractions of the highest specific activity were divided in four equal volumes and kept frozen at -20°C until used for immunization.

Antibody preparation. Two adult male New Zealand albino rabbits were immunized with 0.35 ml of the above described material emulsified in 0.7 ml of complete Freund's adjuvant. Injections were given subcutaneously in multiple sites at 2 wk interval. Blood was collected before immunization and at weekly intervals for a month after the last immunization.

Detection and titration of rabbit anti-human TBG antibodies. The presence of antibody to TBG was determined by the capacity of rabbit antiserum to TBG to clear the T₄-¹²⁵I activity of human serum located in the inter- α -globulin zone, on both paper and agarose electrophoreses. For this purpose, normal serum (TBG capacity of 20 μ g T₄/100 ml) was enriched with T₄-¹²⁵I and incubated with rabbit serum for 24-48 hr at 4°C. Uncentrifuged portions were then subjected to electrophoresis and the distribution of the radioisotope examined both qualitatively by radioautography (23) and quantitatively by strip counting (5). Nonimmune rabbit serum obtained from the same animal before immunization was used as control.

Antibody titer was determined from the highest antiserum dilution capable of displacing the T₄-¹²⁵I activity associated with human TBG to the γ -globulin zone on paper and

³Eastman Kodak Co., Rochester, N. Y.

agarose electrophoreses. Alternatively quantitative adsorption of the antibody using different amounts of TBG-containing unlabeled human serum was used. The specificity of a single antiserum possessing the highest antibody titer was examined by immunoelectrophoresis and by the Ouchterlony technique.

Routine tests of thyroid function. Total thyroxine content in serum was estimated from the protein-bound iodine (PBI)⁴ concentration or determined by the competitive-binding assay (TT₄) (34), and the free T₄ (FT₄) calculated from the product of the TT₄ and dialyzable fraction (DF) obtained by a modification (35) of the method of Oppenheimer, Squef, Surks, and Hauer (36). Resin sponge triiodothyronine uptake (R-T₃) was determined using the Triosorb-kit,⁵ and serum total protein (TP) by refractometry.⁶ The FT₄ index was obtained from the product of the PBI and R-T₃ expressed as fraction of a normal control (37), and the TBG index from the ratio of the R-T₃ expressed as per cent of the normal control, and the PBI (2). When the PBI was unavailable the TT₄ was used for the estimation of these indexes (38).

CLINICAL MATERIAL

Serum was obtained from members of the following families:

Family P. This family was brought to our attention because the proposita (Mi. P.) was shorter than her peers, had primary amenorrhea, and a PBI of 2.2 $\mu\text{g}/100$ ml which failed to rise with the daily administration of 120 mg of USP thyroid. Further investigation revealed that this patient had Turner's syndrome (XO) and TBG deficiency. There was no known family history of consanguinity, thyroid disease, or other congenital disorders. Both parents were Americans of Irish-Italian extraction. Detailed results from genetic studies of this family, demonstrating unequivocal proof for X-linked inherited TBG deficiency have been published (5).

Family A. The proposita (C. A.), a 23 yr old female was admitted in April 1967 for the investigation and evaluation of irregular menstrual periods and mild facial hirsutism. The parents were from Irish extraction, and there was no known history of consanguinity, goiter, or other endocrine diseases. One paternal aunt (K. A.) and great aunt were said to be unusually hirsute. Physical examination was unremarkable except for mild obesity and minimal facial hirsutism with otherwise normal sexual hair distribution. Gynecologic examination was normal.

The most salient feature of her laboratory tests was a low mean PBI of 3.8 $\mu\text{g}/100$ ml (range of four determinations over the period of 1 yr: 3.6-4.0) and high R-T₃ of 157.0% of control (range 140-167). Thyroidal ¹³¹I uptake was 18%, BMR +6%, and tanned red cell agglutination test for thyroglobulin antibodies was negative. Plasma testosterone level was within normal range for adult females.

The pedigree of this family and pertinent data related to thyroid function appear in Fig. 1 and Table I. None of the members of this family examined had goiter or evidence of thyroid dysfunction.

Family G. The pedigree (Fig. 2) and sera from members of this family were obtained from Dr. Earl M. Chap-

man. No detailed information is available other than all subjects were clinically euthyroid and that TBG abnormality was suspected on the basis of discrepant thyroid function tests. D. G. has been on and off thyroid hormone supplement despite absence of well-documented history of thyroid disease, and R. G. was on 0.3 mg of L-thyroxine (Synthroid) at the time the blood sample was obtained. The family is American-Jewish and there was no known history of consanguinity.

Results of thyroid function tests are listed in Table II.

Family L. The propositus (A. L.) was 17 yr old in June 1966, when he was referred for evaluation of delayed physical and sexual maturation. He was the product of an uneventful pregnancy and delivery. Early childhood development was normal. Because the parents were under the impression that there was a slowing in his growth, four determinations of PBI, cholesterol, and FT₄ were obtained between April 1964 and June 1965. They ranged from 3.0 to 3.6 $\mu\text{g}/100$ ml, 140 to 180 mg/100 ml, and 1.4 to 1.7 ng/100 ml⁷ respectively. The 24 hr thyroidal ¹³¹I uptake was 24%. During the last year, before hospitalization, the appearance of pubic and axillary hair was noted as well as increased hair growth over legs and arms. On physical examination the patient was moderately obese weighing 166 lb. and appeared about 3 yr younger than his chronological age. Height was 66 inches. Teeth were normally erupted. He had minimal facial and axillary hair but more abundant pubic hair. There was no breast enlargement. Penis and testes were normal.

Karyotypic analysis of lymphocytes revealed 44 autosomes and XY sex chromosomal pattern. Thyroidal ¹³¹I uptake was 20, 23, and 24% at 4, 6, and 24 hr, which after a single intramuscular injection of 10 U bovine thyrotropin, rose to 24 and 34% at 4 and 24 hr.

Pedigree and pertinent tests of thyroid function in members of this family are shown in Fig. 3 and Table III. The family is of Polish-Jewish extraction with no known consanguinity. All members examined were euthyroid and had no goiter. The mother (R. L.) had taken thyroid hormone in the past because of tiredness and weight gain. There was however no evidence of hypothyroidism.

As of this writing, the authors have been informed that the propositus (A. L.) has achieved physical and sexual maturity without therapy.

Family N. This family was brought to our attention through the courtesy of Dr. Marvin S. Wool from the Lahey Clinic Foundation, Boston, Mass. The propositus, W. N., a 40 yr old male, presented in January 1969 with the chief complaint of nervousness and epigastric discomfort of 8 months duration, shortly after having been promoted to a higher executive position. There were no other symptoms suggestive of hyperthyroidism and physical examination was within normal limits. The thyroid gland was normal on palpation. Possible TBG abnormality was suspected on the basis of elevated PBI of 18.4 $\mu\text{g}/100$ ml and a normal 24 hr thyroidal ¹³¹I uptake of 11%, in a clinically euthyroid patient. The family is Caucasian, there was no history of consanguinity, and all subjects examined were clinically euthyroid and had no goiter except for N. V., the 60 yr old mother of the propositus. Goiter in N. V. was discovered on routine physical examination, 1½ yr before the initiation of this study. She was postmenopausal and was not taking estrogen-containing drugs. There were no stigmata of hyper- or hypothyroidism except for a 60 lb.

⁷ Performed by Bio-Science Laboratories, Van Nuys, Calif.; normal range 1.6-2.4 ng/100 ml.

⁴ Performed by Boston Medical Laboratories, Boston, Mass.

⁵ Abbott Laboratory, North Chicago, Ill.

⁶ T. C. Refractometer, American Optical Corp., Buffalo, N. Y.

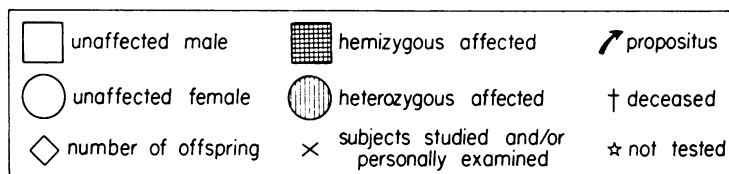
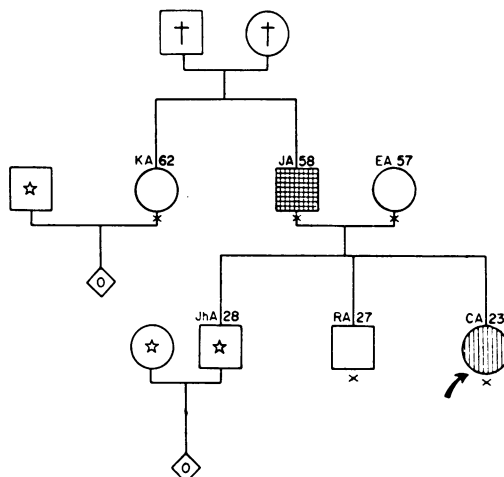


FIGURE 1 Pedigree of a family A. Initials and ages are given above each symbol.

weight loss under strict diet, over the period of 3½ yr. Thyroid gland was approximately three times the normal size, firm and finely nodular.

Pedigree and pertinent data on thyroid hormone transport in serum of members of this family appear in Fig. 4 and Table IV.

RESULTS

Purification of TBG. Results from the first serum fractionation carried on Pevikon-block electrophoresis

are depicted in Fig. 5. 89.4% of the total T_4 - ^{125}I migrated as TBG in the inter- α -globulin zone, and 5.6% with the albumin. The remaining 5% of the total ^{125}I activity was lost in the buffer tank and probably represents iodide- ^{125}I contaminant. Since in the presence of barbital buffer, T_4 does not bind to TBPA (39), no T_4 - ^{125}I was recovered from the prealbumin zone. The specific activity of TBG, in the pooled two peak fractions, increased 17-fold and the recovery was 75%.

TABLE I
Studies of T_4 Transport in Serum of Members from Family A

Subject	Sex	TP	PBI	R- T_4	FT $_4$ index	TBG index	Maximal T_4 -binding capacity	
							TBG	TBPA
		mg/100 ml	μ g/100 ml	% control			μ g T_4 /100 ml	
Normal range		6.4-8.4	3.5-8.0	82-118	2.2-7.1	13-25	17-24	220-350
J. A.	M	7.5	2.3 (3)	167.5 (4)	3.8 (3)	76.6 (3)	2.4 (3)	335 (3)
C. A.	F	7.2	3.8 (4)	157.0 (4)	6.0 (3)	41.4 (3)	2.1-3.1	(317-367)
			(3.6-4.0)	(140-167)	(5.3-6.6)	(37-46)	12.4 (3)	427 (3)
K. A.	F	—	5.2	99.3	5.2	19.1	22.0	—
R. A.	M	—	4.8	110.5	5.3	23.0	18.0	326
E. A.	F	6.7	4.6	110.8	5.1	21.9	19.6	285

For abbreviations used in headings, see text.

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

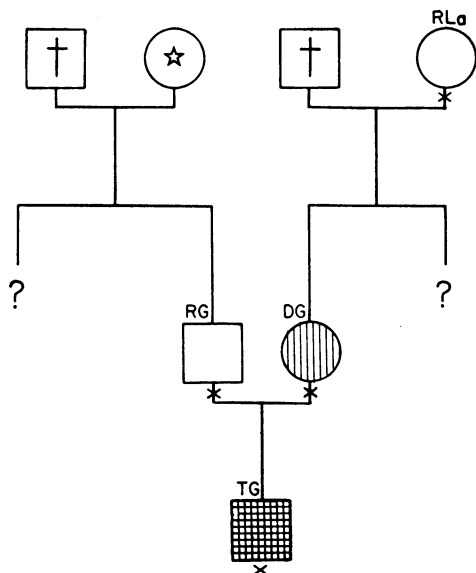


FIGURE 2 Pedigree of family G. For explanation of symbols see Fig. 1.

Further purification of a concentrate of these fractions, on Sephadex G-100 (Fig. 6), resulted in an additional 6.2-fold increase in the specific activity of the three pooled fractions from the peak T_4 - ^{125}I recovered (fractions 10, 11, and 12). The recovery from this step was 50%. Material used for immunization had therefore 105-fold more TBG per milligram of protein than the original serum and the total TBG recovery was 37%. Each injection was estimated to have a TBG capacity of 1.16 $\mu\text{g } T_4$ or 80 μg of TBG, assuming a mol wt of 54,000 and a single binding site for T_4 (40). Accordingly approximately 7.5% of the protein represented TBG.

Antibodies to TBG. Only one of the two immunized rabbits produced adequate antibody titer. The ability of the antibody to bind TBG was demonstrated on both paper and agarose electrophoreses. When antiserum was incubated with normal human serum enriched with T_4 - ^{125}I , the activity was displaced from its usual location on TBG to the origin and γ -globulin zone (Fig. 7). Reaction between antibody and TBG did not displace the T_4 bound to TBG. As a matter of fact, the same amount of T_4 - ^{125}I was associated with the antibody-TBG complex when T_4 - ^{125}I was added to the serum before or after incubation with the antiserum. The lack of interference of the antibodies with T_4 -binding to TBG suggests that the former bind to a site on the TBG molecule distinct from that responsible for T_4 -binding, and that the tertiary configuration of the antibody-TBG complex left access to the T_4 -binding site on TBG unobstructed.

Untreated rabbit antiserum was not monospecific for TBG. At least six precipitation peaks were observed on stained antigen-antibody crossed electrophoresis. However, antibodies against albumin were not a major contaminant and radioautography of the same plate produced a single peak corresponding to the location of the TBG-antibody complex. Accordingly, rabbit antiserum was adsorbed with serum from a patient with TBG deficiency provided by Dr. T. W. Avruskin. This patient was unrelated to the families reported in this communication. The adsorbed antiserum gave a single arc on immunoelectrophoresis with normal human serum. No precipitin arc was observed with TBG-deficient serum from a member of family P (Mi. P.) (Fig. 8).

Immunologic properties of TBG in sera from subjects with inherited TBG abnormalities. Since our antiserum

TABLE II
Studies of T_4 Transport in Serum of Members from Family G

Subject	Sex	TP	TT ₄	R-T ₄	TF ₄ index	TBG index	Maximal T ₄ -binding capacity	
							TBG	TBPA
		mg/100 ml	$\mu\text{g}/100 \text{ ml}$	% control			$\mu\text{g } T_4/100 \text{ ml}$	
Normal range		6.4-8.4	4.2-9.4	82-118	3.4-9.6	9-20	17-24	220-350
T. G.	M	7.7	1.9	184.4	3.5	97.1	2.5 (3)	304
							(2.4-2.7)	
D. G.	F	7.5	5.3	96.4	5.1	18.2	17.6 (3)	273
							(17.5-18.0)	
R. La.	F	—	7.0	90.9	6.4	12.9	25.4	261
R. G.*	M	—	14.0	183.4	25.6	13.1	19.5	246

See footnote of Table I.

* On 0.3 mg sodium L-thyroxine (Synthroid).

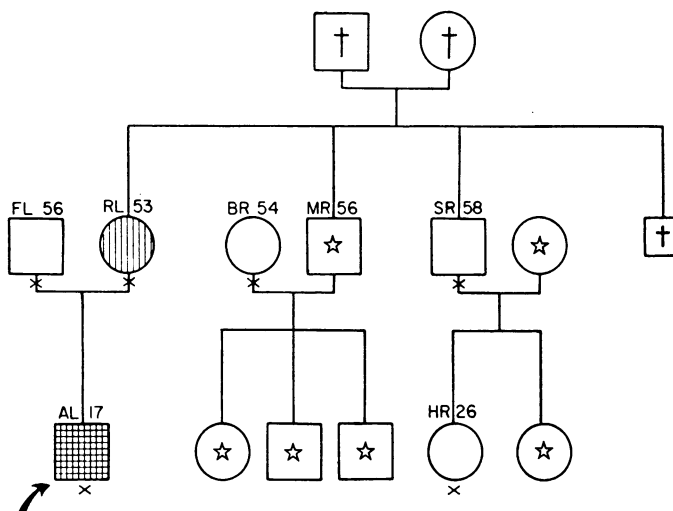


FIGURE 3 Pedigree of family L. For explanation of symbols see Fig. 1.

to TBG reacted with human TBG without displacing or preventing T_4 -binding, tracer T_4 - ^{125}I was used in the assay system.

The presence of immunoreactive TBG in sera from patients with inherited abnormalities of TBG capacity was measured semiquantitatively by its ability to adsorb the antiserum. For this purpose a constant amount of serum from each subject was mixed with an equal amount of diluted antiserum. After 5 days of incubation at 4°C, serial dilutions of the supernatant were used as a source of antibody in a titration assay utilizing a set amount of T_4 - ^{125}I -labeled normal serum. It can be seen in Table V that serum from a TBG-deficient patient (Mi. P.) failed to adsorb the antibody, the titer of which was identical to the nonadsorbed control antiserum. On the other hand, incubation with serum from

J. A., with TBG capacity as low as 3.0 $\mu g T_4/100 ml$, produced a detectable reduction in the antiserum titer. Similarly, reduction in the TBG antibody titer was proportional to the TBG capacity of serum from a normal subject (D. S. R.) and that from a subject with inherited increase in TBG capacity (W. N.).

Adsorbed antiserum, which in the presence of normal serum produced a single arc on immunoelectrophoresis (Fig. 8), was used for the Ouchterlony double diffusion plate. As depicted on Fig. 9, sera from subjects with very low, half normal, normal, and high TBG capacity produced a single precipitin line, on the protein-stained plate. TBG-deficient serum failed to produce visible precipitation. Identical results were obtained with sera from all other hemizygous patients with TBG deficiency. Although quantitative differences in

TABLE III

Studies of T_4 Transport in Serum of Members from Family L

Subject	Sex	TP	PBI	R- T_4	FT $_4$ index	TBG index	Maximal T_4 -binding capacity	
							TBG	TBPA
		<i>mg/100 ml</i>	<i>$\mu g/100 ml$</i>	<i>% control</i>			<i>$\mu g T_4/100 ml$</i>	
Normal range		6.4-8.4	3.5-8.0	82-118	2.2-7.1	13-25	17-24	220-250
A. L.	M	7.8	2.9 (8) (2.0-3.6)	174.8 (6) (165-189)	4.8 (6) (3.3-6.1)	67.4 (6) (51-83)	3.2 (3) (2.4-4.0)	243
R. L.	F	8.7	3.5 (3) (3.0-4.2)	108.2 (3) (107-111)	3.8 (3) (3.2-4.5)	31.9 (3) (26-36)	13.5 (3) (12.5-14.5)	282
S. R.	M	—	4.8	95.8	4.6	20.0	20.2	—
H. R.	F	—	6.6	84.0	5.5	12.7	21.8	252
F. L.	M	8.7	6.0	85.0	5.1	14.2	20.2	276
B. R.	F	—	7.4	114.7	8.5	15.5	21.1	225

See footnote of Table I.

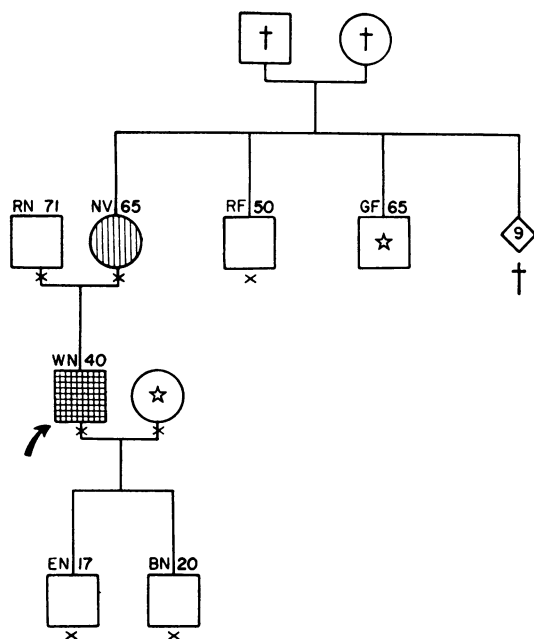


FIGURE 4 Pedigree of family N. For explanation of symbols see Fig. 1.

TBG are not clearly demonstrated on the stained plate, distinct quantitative differences in the TBG content were observed on radioautography.

As Marshall and Pensky have earlier suggested that patients with inherited lack of T_4 -binding to the TBG zone possess a circulating protein immunologically indistinguishable from TBG (40), coded samples of sera from selected members of the five families studied were sent to Dr. Richard Levy (working in collaboration with the above mentioned authors) for TBG determination by radioimmunoassay utilizing ^{125}I -labeled TBG and their own antiserum (33). In addition TBG ca-

capacity was determined by the single load-ion exchange resin method. The quantitation of TBG by these two unrelated methods was compared with that obtained on reverse-flow paper electrophoresis. Results provided in Table VI, show that serum TBG concentrations measured by the three methods are in close agreement and that TBG-deficient patients had no detectable immunoreactive TBG. Similarly patients with high T_4 -binding capacity of TBG had high concentrations of immunoreactive TBG. It is interesting to note that the antiserum used for the radioimmunoassay of human TBG did not cross react with monkey or dog T_4 -binding globulin, although the former possesses a binding globulin electrophoretically identical with human TBG (23).

Physicochemical properties of TBG in sera from subjects with inherited TBG abnormalities. The T_4 association constant of TBG in serum from subjects with decreased, normal, and high TBG capacities was estimated by the competitive binding technique utilized in the TT_4 assay. Similarity in the TBG association constants in these sera is indicated by the slope of the curves (Fig. 10). The shift of the saturation curves is proportional to the maximal T_4 -binding capacity of TBG. Similar results were obtained using triiodothyronine as a ligand.

Finally, the rate of TBG inactivation at 60°C was not statistically different in sera from subjects with high, normal, and low TBG capacities (Fig. 11).

Protein-binding of other hormones. In order to evaluate the possibility of abnormalities in serum protein transport of other hormones, portions of serum from a TBG-deficient patient undergoing treatment with estrogens (Mi. P.), was enriched with labeled T_4 , cortisol, aldosterone, and testosterone. Radioautographs of agarose electrophoretic runs of these sera were compared with those of a normal subject using the same

TABLE IV

Studies of T_4 Transport in Serum of Members from Family N

Subject	Sex	TP	TT ₄	R-T ₄	FT ₄ index	TBG index	Maximal T_4 -binding capacity		DF	FT ₄
							TBG	TBPA		
		mg/100 ml	μg/100 ml	% control			μg T_4 /100 ml		%	ng/100 ml
Normal range		6.4-8.4	4.2-9.4	82-118	3.9-9.6	9-20	17-24	220-350	0.030-0.046	1.7-3.4
W. N.	M	7.8	19.9 (4)	52.0	9.4	2.9	89.2 (4)	301	0.021	3.8
			(18.1-23.7)				(80-102)			
N. V.	F	8.1	12.9 (3)	55.0	7.2	4.3	49.6 (3)	235	0.022	2.8
			(12.0-13.8)				(48-54)			
E. N.	M	—	6.3	110.8	7.0	17.6	20.0	328	0.042	2.7
B. N.	M	—	6.9	111	7.7	16.1	20.2	287	0.045	3.1
R. F.	M	—	6.5	91	5.9	14.0	21.4	332	—	—
R. N.	M	—	6.6	109.9	7.2	16.5	21.1	262	0.041	2.7

See footnote of Table I.

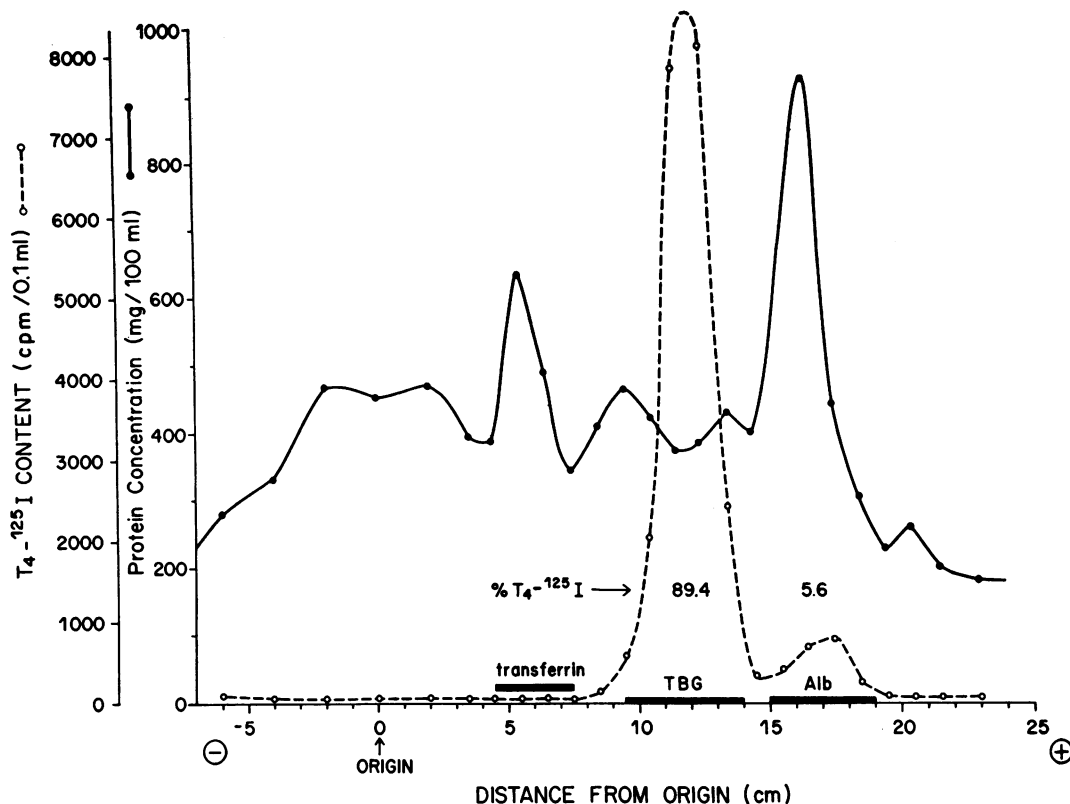


FIGURE 5 Purification of TBG on Pevikon-block electrophoresis (barbital buffer, pH 8.6). 10 ml of serum from a healthy pregnant female at term (TBG capacity $61 \mu\text{g T}_4/100 \text{ ml}$), enriched with a tracer $\text{T}_4\text{-}^{125}\text{I}$ (2 ng) was applied to the origin. Protein concentration and ^{125}I activity were measured on eluates from sections of supporting medium. Per cent of the total ^{125}I activity recovered in the TBG and albumin zones is indicated.

concentration of the labeled hormones. It can be seen in Fig. 12, that although estrogen treatment failed to demonstrate T_4 -binding in the inter- α -globulin zone of the TBG-deficient serum, binding to cortisol-binding globulin (CBG) and testosterone-binding globulin (TeBG) was increased when compared with the normal control. No obvious differences were noted in aldosterone-binding to serum, using this technique.

Effect of T_4 administration. A hemizygous subject (P. R.) from family P was given T_4 in incremental doses (0.1, 0.2, and 0.3 mg daily), changing the dosage every 2 wk. TBG and TBPA capacities remained unchanged. As in subjects with normal TBG capacity, stepwise suppression of thyroid gland activity was observed. During the control period thyroidal ^{131}I uptake was 30% dropping to 14, 7, and 0.6% on the final dose of 0.3 mg of T_4 daily. The TT_4 rose from 2.4 to $5.0 \mu\text{g}/100 \text{ ml}$. In the absence of changes in the TBG capacity, TT_4 elevation was interpreted as being the result of endogenous T_3 suppression.

Studies of TBG gene location on the X-chromosome. Information on genetic linkage was sought in order to

help mapping the locus of TBG gene on the X-chromosome. Two X-linked genetic characters, red-green color vision, and the Xg^a red-cell antigen were examined. All studied members from families A, G, L, and N had normal color vision and were Xg^a positive. Results from a similar study in subjects from family P have been previously reported (5).

DISCUSSION

Of the five families with inherited TBG abnormalities studied, the TBG-deficient "family P" satisfied all criteria supporting X-linkage (5), namely: (a) full expression of the defect is manifested only in hemizygous subjects (possessing a single X) usually males; (b) heterozygous affected have two X-chromosomes and are therefore females; (c) all female offspring of affected males are heterozygous for the trait; (d) all male offspring of affected males are normal; (e) affected males are offspring of heterozygous females; and (f) heterozygous females are offspring of affected males or females. In addition, (g) heterozygous females may be distinguished from hemizygous affected or nor-

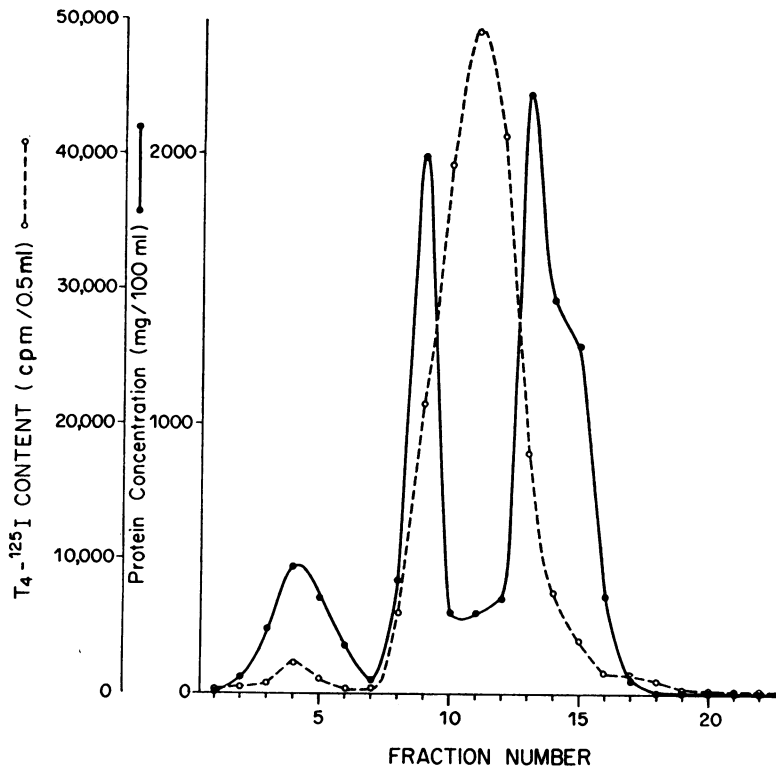


FIGURE 6 Sephadex G-100 filtration of TBG fractions purified on Pevikon-block electrophoresis. The 1×20 -cm columns were equilibrated overnight with buffered saline, pH 7.4 and eluted using the same buffer at a flow rate of approximately 0.2 ml per min. Protein concentration and ^{125}I activity was measured on portions from each 0.5 ml fraction.

mals by partial expression of the trait. This observation offers no proof for but is compatible with the single-active-X hypothesis (Lyon's hypothesis) which proposes random and permanent inactivation of all X-chromosomes in excess of one in somatic cells during early fetal life (41-44). It is rationalized that this inactivation provides a mechanism of dosage compensation for X-chromosome-linked traits.

In the three families with low TBG (families A, G, and L), males were always more profoundly affected than females, permitting distinction between the affected hemizygous and heterozygous states, thus satisfying criteria (a), (b), and (g). Most importantly, all available hemizygous offspring of affected males were normal and female offspring heterozygous. Criterion (g) was not completely satisfied in family G as the affected

TABLE V
Adsorption of TBG Antibody by Human Sera

Adsorbent serum		Antibody dilution						
Type	TBG capacity	1:4	1:8	1:16	1:32	1:64	1:128	1:256
	$\mu\text{g}/100 \text{ ml}$							
None (control)		+	+	+	+	+	+	-
TBG deficient (Mi. P.)	0	+	+	+	+	+	+	-
Low TBG (J. A.)	3.0	+	+	+	+	±	-	-
Normal (D. S. R.)	19.8	+	+	+	+	-	-	-
High TBG (W. N.)	88.0	+	±	-	-	-	-	-

+, binding of $\text{T}_4\text{-}^{125}\text{I}$ to the γ -globulin zone on agarose electrophoresis; -, no binding of $\text{T}_4\text{-}^{125}\text{I}$ to γ -globulin zone.

female (D. G.) could only be clearly distinguished by TBG capacity from the affected male (A. L.) but not from normals. Nevertheless, her TBG capacity although within normal limits, was lower than any of the unaffected members of the same family. This phenomenon is not unique. As in other X-linked inherited abnormalities (42-44) TBG values in heterozygous females cover a wider middle range between hemizygous affected and normals (3, 4, 7, 13). Thus interpretation should be made by analysis of the pedigree with assumption of X-chromosome linkage and subsequent testing by analysis of the progeny. Also, in several instances when measurement of the TBG capacity alone was not sufficient to detect the heterozygotes (4, 7), examination of the PBI, TT_4 , R-T₄, and calculation of the TBG index as proposed by Marshall, Levy, and Steinberg (2) have facilitated the identification of the heterozygotes. Rigid adherence to a predetermined TBG capacity level for the selection of the affected females

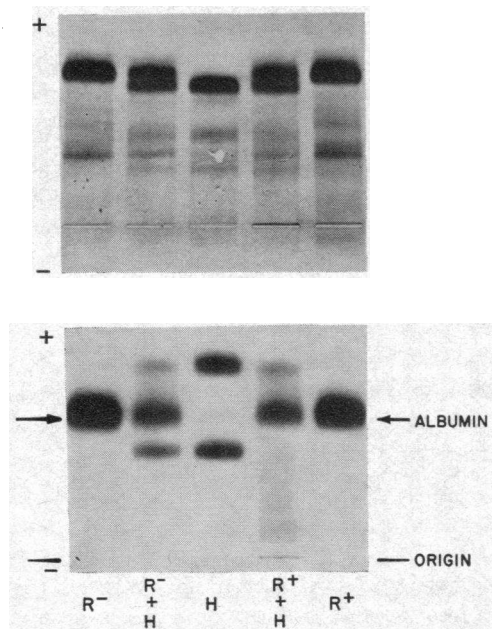


FIGURE 7 Identification of rabbit anti-human TBG antibodies. T_4 - ^{125}I was added to nonimmune rabbit serum (R^-), immune rabbit serum (R^+), and normal human serum (H). Mixtures of equal amounts of R^- with H, and R^+ with H were incubated overnight at $4^\circ C$, and electrophoresed on agarose. Amido-black stain (top) and corresponding radioautographic pattern (bottom) are printed on scale. The distribution of T_4 - ^{125}I in R^- and R^+ is identical. In presence of TBG antiserum the activity associated with the human TBG band migrated more cathodally, corresponding to the γ -globulin zone ($R^+ + H$). No displacement of the human TBG band occurred in the control containing $R^- + H$. Note the difference in electrophoretic mobility of human and rabbit albumins, creating a "bisalbuminimic like" pattern on the protein stain of serum mixtures.

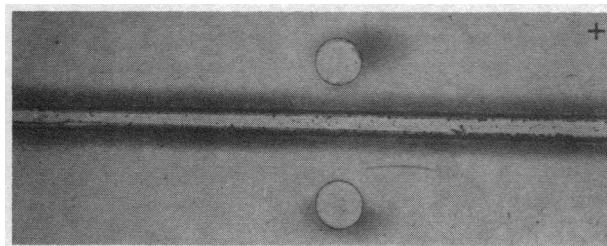


FIGURE 8 Immunoelectrophoretic pattern of TBG-deficient serum from Mi. P. (top antigen well), and normal human serum (bottom antigen well) developed with rabbit anti-human TBG serum adsorbed with serum from an unrelated patient with TBG deficiency.

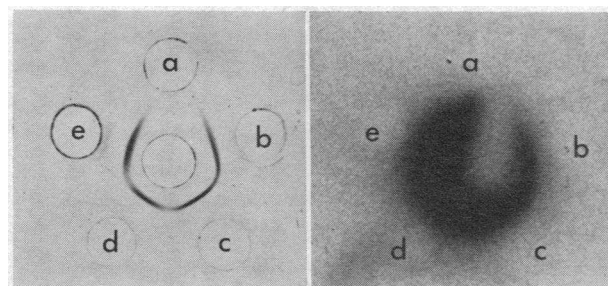


FIGURE 9 Ouchterlony double immunodiffusion plate. Amido-black protein stain is on the left and radioautograph on the right. The central well contained rabbit anti-human TBG serum adsorbed with serum from a TBG-deficient patient. Peripheral wells contained sera from: (a) TBG-deficient male (R. R.), member of family P; (b) male with low TBG capacity (A. L.), member of family L; (c) heterozygous female with decreased TBG capacity (P. R.), member of family P; (d) normal serum pool (pool 4); and (e) male with high TBG capacity (W. N.), member of family N.

was undoubtedly responsible for overlooking a typical X-linked inheritance pattern in at least two families (13, 20).

The pedigree of "family N" with high TBG satisfied criteria (a), (b), (e), (g), and most importantly (d). Data for (c) and (e) were unavailable. Mean TBG capacity of the heterozygous female (N. V.) was 49.6 compared with 89.2 μg T_4 /100 ml in the affected male (W. N.). Assuming that the heterozygote has a cellular mosaic with respect to paternal and maternal X-chromosomes, and that random inactivation resulted in an even number of both cell types, the expected TBG capacity for N. V. should be approximately 55 μg T_4 /100 ml, which is similar to the observed value of 49.6.

Since evidence for X-linked inheritance was presented in two-thirds of families with TBG abnormalities so far described, and it has been repeatedly suggested that in the remaining one-third some families possess many features compatible with an identical mode of inheritance (3, 5, 11), we revised all previously published data. When individual values were available,

TABLE VI

Measurement of TBG Concentration in Sera from Patients with Inherited TBG Abnormalities, Utilizing Three Different Assay Systems

Description	Genotype	Method A	Method B	Method C
		$\mu\text{g T}_4/100 \text{ ml}$	$\mu\text{g T}_4/100 \text{ ml}$	$\text{mg}/100 \text{ ml}$
Normal controls				
Serum pool 3	nXX + nXY	20.0	19.6	3.6
Serum pool 4	nXX + nXY	20.0	20.8	3.5
Pregnant female	nXX	61.0	58.0	8.1
TBG deficient				
Family P				
Mi. P.	aXO	0	0	0
R. R.	aXY	0	0	0
P. R.	cXX	6.0	7.1	1.0
D. L.	cXX	8.0	9.8	1.4
Low TBG				
Family A				
J. A.	aXY	3.0	3.1	0.6
C. A.*	cXX	20.0	21.0	4.1
E. A.	nXX	20.0	20.8	3.6
Family G				
T. G.	aXY	2.6	2.5	0.8
D. G.	cXX	17.5	17.5	3.8
Family L				
A. L.	aXY	5.0	2.4	0.6
R. L.	cXX	17.5	12.5	2.8
F. L.	nXY	25.0	20.2	4.1
High TBG				
Family N				
N. V.	cXX	54.0	48.0	9.9
Animal sera				
Pregnant rhesus monkey	—	33.0	—	0
Dog	—	3.5	—	0

Method A, single load-ion exchange resin; method B, reverse-flow paper electrophoresis; method C, radioimmunoassay; a, hemizygous affected; c, heterozygous affected ("carrier"); n, normal; *, on oral contraceptives.

mean values for PBI, T_4 , and TBG capacity were calculated (Tables VII, VIII, and IX).⁸ Subjects from each family were divided in four groups: hemizygous affected (males), heterozygous (females), blood relatives, and relatives by marriage. According to the type of TBG abnormality exhibited in the affected males, families were identified as TBG-deficient (D), low TBG (L), and high TBG (H), and numbered in the order of publication.

Data from six families with TBG deficiency (D) are listed in Table VII. One family with presumed TBG

⁸ Complete data including information on ethnic background, consanguinity, number of generations involved, and presence of other abnormalities could be obtained from the National Auxiliary Publication Service.

deficiency reported by Kraemer and Wiswell (6) was not included because values for TBG capacity were too high for the technique used (47). Such technical difficulties are due to oversaturation of TBG by T_4 and staining of the electrophoretic strips before counting.⁹ Two additional families with inherited TBG deficiency are mentioned by the authors of families D-5 and D-6 but no published data are available. In all families listed in Table VII, mean PBI levels in affected hemizygous subjects are consistently lower than in heterozygous females, the latter having mean values intermediary between affected hemizygotes and normals, with overlap of individual values in only few cases.

⁹ Personal observation.

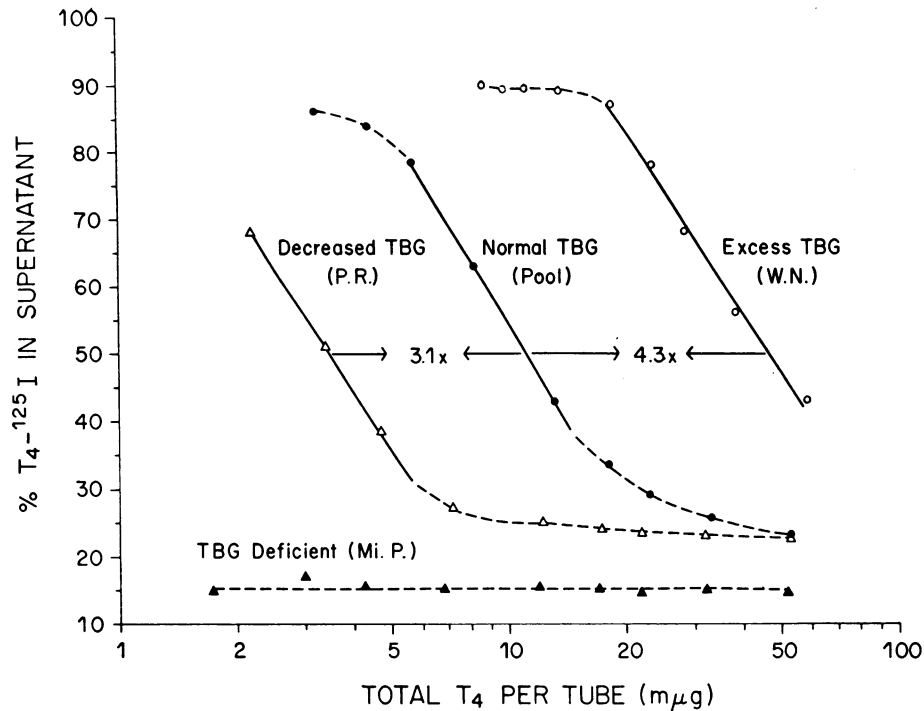


FIGURE 10 Competitive binding analysis of sera from subjects with various inherited TBG abnormalities. Serum was diluted 1:40 with barbital buffer pH 8.6. Each tube contained 25 μ l of undiluted serum. The slope is proportional to the T_4 association constant and the shift to the left of each curve, to the T_4 -binding capacity of TBG.

Reduced, but detectable TBG capacity in the affected hemizygotes (L) have been described in 11 families including the 3 families described in this communication. Table VIII summarizes pertinent data published. In family L-2 the four males from the second generation (offspring of an affected father) were considered normal, as values for both PBI and TBG capacity were within normal limits and higher than all values from other affected subjects either male or female. Thus the only serious objection for X-linked mode of inheritance was eliminated. Also, in family L-6 a male (No. 15), with TBG capacity of 18.2 $\mu g T_4/100$ ml and normal $R-T_3/PBI$ ("TBG index") of 19.3 was removed from the "possibly affected group". For the same reason, a female (No. 16) with $R-T_3/PBI$ of 23.2, which is the highest value in the "normal group", consisting of 11 patients, was considered as carrier of the trait.

Seven families with high TBG (H), including family N, have been reported. Data on T_4 transport in serum appear in Table IX. Although an autosomal dominant mode of inheritance was initially suggested for families H-1 and H-2 (20, 15), Nikolai and Seal have brought forth valid arguments in favor of an X-linked inheritance pattern in these families (3). Accordingly, in family H-1, case 70 (D. B.) with a TBG capacity of 28 $\mu g T_4/100$ ml and a normal PBI of 6.2 $\mu g/100$ ml

was considered as normal. With only this modification the trait would be derived from M. H. (maternal grandmother of the propositus W. W.), and the pedigree fulfills all criteria for X-linkage. Consistency with X-chromosome-linked inheritance is also apparent in family H-2, with TBG capacity in the single affected male higher than any of the three affected females. In all families, there is a remarkable consistency in the degree of TBG elevation in affected males (mean TBG elevation 2.8- to 4.3-fold normal) as compared with affected females (mean TBG elevation 2.0- to 2.4-fold normal). Absolute TBG capacities reported vary over a wider range according to methodological differences.

As the most crucial criterion supporting X-linked inheritance is lack of male-to-male transmission, the progeny of all affected males was analyzed (Table X). All sons were normal and most daughters could be identified as heterozygous. The probability that this distribution would have occurred by chance if inherited as autosomal dominant is less than 1:2,000 for D, less than 1:4,000 for L, and less than 1:2,000,000 for H.

TBPA concentration was normal in our families and in others (5, 8, 10, 11, 14, 15, 20). In only one family (L-6) TBPA levels appeared to have an inverse correlation with the TBG capacity (13). Similarly, other serum proteins including CBG (3, 8, 16, 18), hapt-

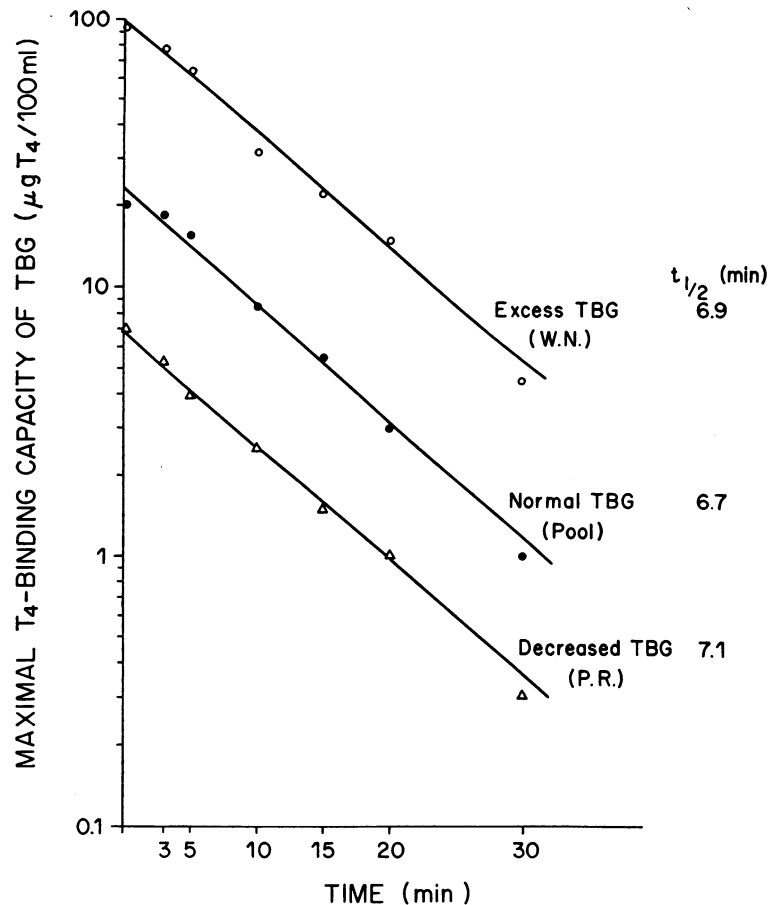


FIGURE 11 Rate of TBG inactivation at 60°C. Portions of serum from each subject were heated in a constant temperature bath, for variable periods of time, as indicated on the abscissa. The serum was then immediately cooled in an ice bath and the TBG capacity determined.

globin (3, 14), iron-binding globulin (3, 8), and ceruloplasmin (3) were present in normal concentrations. The turnover rate of serum albumin was normal in one of our patients with TBG deficiency (5). CBG responds normally to the administration of estrogens or to pregnancy (3, 5, 16). Although associated abnormalities such as hypothyroidism (3, 11), toxic nodular goiter (18), asthma (1, 20), pernicious anemia (16), cataracts (16), herpes zoster (16), XO Turner's syndrome (5) and XYY/XY/XO with ambiguous genitalia (11) have been described, these associations are believed to be fortuitous. On the other hand, Shanë, Seal, and Jones described a family with increased TBG and high incidence of goiter (12 of the 14 affected subjects) (19). Hereditary ectodermal dysplasia of the anhidrotic type, also often manifesting as an X-chromosome-linked disorder (49) was associated with inherited TBG elevation (17, 48). A male presenting with this full blown syndrome had also the highest TBG capacity.

His mother and maternal grandmother with intermediary TBG capacity elevations had very thin hair and abnormalities in teeth eruption also suggestive of heterozygosity for ectodermal dysplasia. As the authors do not mention whether two additional members of this family, with high TBG, exhibited stigmata of ectodermal dysplasia, the possibility of proximity for the two loci on the X-chromosome is uncertain.

Localization of the TBG locus on the X-chromosome was sought from information of genetic studies using two known X-linked traits namely color vision, and the Xg^a red-cell antigen. None of the subjects tested had red-green color blindness and all were Xg^a positive except for three members from family P. Although data from Xg^a-typing in this family were useful in the identification of the maternal origin of the single X in a patient with Turner's syndrome, no conclusions can be drawn as to its proximity to the TBG locus (5). Similar attempts of linkage tests in families with TBG de-

iciency (2, 7), one with low (11), and one with high TBG (16) have failed. More extensive study of the Xg^a red-cell antigen in a family with high TBG indicates that it is very unlikely that the two loci are closely linked (18).

The mechanisms for production of these inherited TBG abnormalities remain obscure. Hypotheses such as production of an abnormal protein with altered binding affinity and/or capacity (4, 5, 11, 12, 15, 16, 18, 20), presence of an abnormal substance interfering with T₄-binding to TBG (5) or absence of normally present extraneous inhibitor of T₄-protein interaction (20), abnormality in the mechanism controlling TBG synthesis and possible degradation resulting in quantitative variations in TBG concentration (5, 12, 15, 18, 20), and presence of two types of TBG controlled by different genes (5), have been considered. Based on the model of genetic control of protein synthesis in bacteria, structural gene mutations (4, 5, 11, 16, 18, 20), mutations at the control sites (repressor) or operator (5, 16, 18), and structural gene duplication resulting in double quantity of dose of a gene (16, 18) have been also considered. Unfortunately, evidence for any particular mechanism is lacking and is at best, only partial.

Serum of all hemizygous members from our family with TBG deficiency ("family P") and that of another affected male from an unrelated family lacked immunoreactive TBG when assayed by two different laboratories, utilizing two different antisera for the Ouchterlony technique, radioimmunoassay and titration by antibody adsorption. Recently absence of immunoreactive TBG was reported in two affected males from family D-1 (33). The same authors question the validity of earlier results which suggested presence of a defective TBG in these patients (40). TBG was also quantitated by two electrophoretic methods, a method utilizing resin as competitor of T₄-binding and by radioimmunoassay giving similar results for all patients covering the spectrum of TBG abnormalities.²⁰ TBGs in both hemizygous and heterozygous members of families with low or high TBG, as well as heterozygotes from the family with TBG deficiency, have similar affinity for either T₄ or T₃ and do not differ in their behavior to heat inactivation. Other authors have shown no differences in the electrophoretic mobility on polyacrylamide (3, 11, 12), cellulose acetate (7), and 2 dimension paper-starch (20) electrophoreses using a variety of buffer systems. Similarity in the shape of the TBG stabilization curve of normal sera and sera from patients with inherited TBG elevation has been demonstrated (15). Also we have previously demonstrated that TBG-deficient subjects do not possess an abnormal substance capable of interfer-

²⁰ These assays are capable of detecting 1/200 of normal serum TBG level.

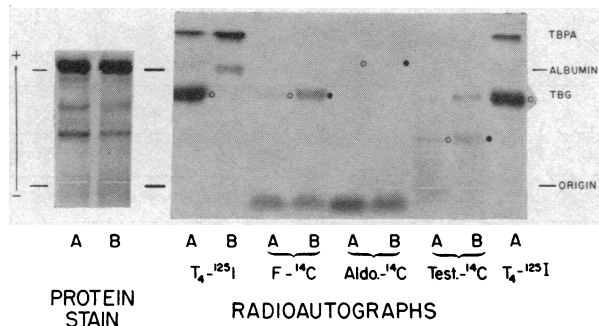


FIGURE 12 Study of serum protein-binding of T₄, cortisol (F), aldosterone (aldo), and testosterone (Test) on agarose electrophoresis. A, normal serum; B, serum from a patient with TBG deficiency receiving estrogens (Mi. P.). Location of the major serum protein-binding site for each hormone is indicated with an open circle (for normal), and closed circle (for TBG-deficient serum), on the right of each pattern. The position of the anode, cathode, TBPA, albumin, TBG, and origin are marked. Free F-¹⁴C and Aldo-¹⁴C migrate cathodally to the origin. Because of the low SA of Test-¹⁴C, necessitating addition of approximately 130 μg of Test/100 ml serum, the primary binding site in the β-globulin position has been oversaturated. The excess of Test-¹⁴C appears bound to the inter α-globulin zone (most probably CBG).

ing with T₄-binding to TBG. It is therefore apparent that data so far available, fail to demonstrate the presence of structural alterations of TBG in patients covering the entire spectrum of inherited TBG abnormalities.

The effect of estrogen administration on the TBG capacity of both hemizygous and heterozygous subjects with different inherited TBG abnormalities is listed in Table XI. In all patients with TBG deficiency, administration of estrogens failed to result in detectable T₄-binding to the TBG zone. However, heterozygotes responded with elevation in the TBG capacity. Other estrogen-dependent serum proteins responded normally. In one patient with TBG deficiency (Mi. P.), evidence for increase in the CBG and TeBG capacity was obtained by semiquantitative analysis on agarose electrophoresis (Fig. 12). Estrogen had no apparent effect on aldosterone-binding to serum proteins. Estrogens had a less uniform effect on the T₄-binding capacity of TBG in subjects from families with low and high TBG. TBG capacity remained unchanged in one (11), but increased in another (14) hemizygous affected subject with low TBG. Similarly, evidence for increase in TBG capacity was not obtained in all patients with high TBG. Thus conclusions on the genetic mechanism of inherited TBG abnormalities based on such data (16) should be made with caution. Nevertheless estrogens have been useful in assessing the rate of TBG degradation. In two heterozygous patients, one from a family with TBG deficiency (P. R.) and one from a family with low TBG

TABLE VII
Summary of Data from Families with TBG Deficiency

Family No. and reference	Tests	Mean \pm SD and (range)				Suggested mode of inheritance	Consistent with X-linkage*	Criteria for X-linkage† and remarks
		Affected		Normal relatives				
		Hemizygous	Heterozygous	Blood	By marriage			
D-1 (2)	N	6	8	17	7	X-linked	yes	(a), (b), (d), (e), (f), (g).
	n	6	8	16	7			
	PBI	2.1 \pm 0.4	3.7 \pm 0.4	5.4 \pm 0.9	5.1 \pm 0.9			
D-2 (3, 45)	TBG	—	—	—	—	X-linked	yes	(a), (b), (c), (e), (f), (g).
	N	10	8	23	10			
	n	10	4	23	9			
D-3 (4, 45, 46)	PBI	2.1 \pm 0.8	4.7 \pm 0.8	6.2 \pm 0.7	5.9 \pm 1.1	X-linked	yes	(a), (b), (c), (d), (e), (f), (g).
	TBG	0	6.8 \pm 3.8	19.4 \pm 3.0	15.8 \pm 3.0			
	N	8	13	25	9			
D-4 (5)	n	8	12	21	9	X-linked	yes	(a), (b), (c), (d), (e), (f), (g).
	PBI	2.5 \pm 0.3	4.5 \pm 0.9	6.7 \pm 1.3	5.7 \pm 1.0			
	TBG	0	10.9 \pm 3.9	22.5 \pm 4.2	19.8 \pm 4.8			
D-5 (7)	N	3	3	3	3	X-linked	yes	(a), (b), (c), (d), (e), (f), (g).
	n	3	3	3	3			
	PBI	1.9 \pm 0.4	3.1 \pm 0.1	5.3 \pm 0.5	5.7 \pm 0.8			
D-6 (7)	TBG	0	8.4 \pm 2.8	19.2 \pm 1.0	20.4 \pm 2.5	X-linked	yes	(a), (b), (c), (d), (e).
	N	4	3	32	32			
	n	4	3	(4.4-7.3)	(4.6-11.8)			
D-6 (7)	PBI	2.3 \pm 0.9	4.7 \pm 0.2	(4.4-7.3)	(4.6-11.8)	X-linked	yes	(a), (b), (c), (d), (e), (f), (g).
	TT ₄	1.7 \pm 1.1	6.9 \pm 1.5	(4.6-11.8)	(18-26)			
	TBG	0	21.7 \pm 1.5	(18-26)	(18-26)			
D-6 (7)	N	3	5	1	0	X-linked	yes	(a), (b), (c), (d), (e), (f), (g).
	n	3	3	1	—			
	PBI	2.3 \pm 0.1	3.3 \pm 1.5	6.5	—			
D-6 (7)	TT ₄	1.4 \pm 1.0	3.7 \pm 2.4	9.3	—	X-linked	yes	(a), (b), (c), (d), (e), (f), (g).
	TBG	0	12.7 \pm 0.6	21	—			

All affected hemizygous have a single X-chromosome, (males or XO-Turner's syndrome) and all affected heterozygous are females. Patients on drugs capable of altering the TBG capacity, pregnant during the study, or taking thyroid hormone are excluded. When mean value could not be calculated, range is given in parenthesis.

N, Total number of subjects; n, number of subjects used for calculation of mean values.

* Yes: on review of published pedigree, there is no indication for incompatibility with X-linked inheritance.

† For details see text.

(C. A.), a 2- to 2½-fold elevation in the TBG capacity could be achieved after treatment with estrogens for 3-4 months. Serial determinations of the TBG capacity after discontinuation of therapy revealed a slow decline with return to pretreatment values after 6-8 wk, which is similar to that observed in normal controls (33). It is therefore fair to conclude that the abnormal TBG concentration in serum from these patients is not due to altered rate of TBG degradation.

Examples of abnormalities in the enzymatic activity and in rates of synthesis of enzymes due to structural gene mutations have been described in man. Deficiency with discrepancy between the quantitation of the protein enzyme by immunologic and enzymatic assays in the case of the "silent gene" for pseudocholinesterase (51), and increase in both enzymatic and immunologic activities in the case of both Hektoen variant of glucose-6-phosphate dehydrogenase (G6PD) (52) and pseudocholinesterase-E Cynthiana (53) have been noted.

A single amino acid substitution in the G6PD Hektoen was demonstrated (54), and although no differences were found by comparing the immunologic and enzymatic activities or by studies of the kinetics of thermal stability, structural abnormality was suspected on the basis of differences in electrophoretic mobility (52, 54). Similarly, E Cynthiana could be distinguished from the common enzyme both electrophoretically and by the association of the subunits.

No electrophoretic abnormalities of TBG could be demonstrated using four different supporting media and five buffers. Furthermore, the properties of the TBG antiserum used in our study is unique in that biological activity (T₄-binding to TBG) was not inhibited after the formation of the antigen-antibody complex. Thus, a fundamental difference exists between our studies and studies of G6PD and pseudocholinesterase variants in which addition of antisera produced proportional loss of the biologic activity. In this instance, quantitation of

TABLE VIII
Summary of Data from Families with Low (Nonzero) TBG

Family No. and reference	Tests	Mean \pm SD and (range)				Suggested mode of inheritance	Consistent with X-linkage*	Criteria for X-linkage† and remarks
		Affected		Normal relatives				
		Hemizygous	Heterozygous	Blood	By marriage			
L-1 (8)	N	3	3	6	1	Autosomal- dominant	yes	(a), (b), (d), (e), (f).
	n	3	3	6	0			
	PBI	2.7 \pm 0.7	3.0 \pm 0.2	4.9 \pm 0.4	—			
	TBG	4.3 \pm 0.6	6.0 \pm 2.2	19.8 \pm 1.3	—			
L-2 (9)	N	3	2	8	0	Autosomal- dominant	yes	(a), (b), (c), (d).
	n	3	2	6	0			
	PBI	2.5 \pm 0.6	3.0 \pm 0	4.4 \pm 1.1	—			
	TBG	5.3 \pm 0.6	6.9 \pm 2.0	17.3 \pm 4.6	—			
L-3 (10)	N	3	4	5	1	X-linked or autosomal dominant	yes	(a), (b), (e), (f), (g).
	n	3	4	5	1			
	PBI	1.9 \pm 0.3	2.6 \pm 0.6	5.7 \pm 0.6	5.1			
	TBG	6.8 \pm 1.1	12.1 \pm 3.0	22.2 \pm 1.9	21.0			
L-4 (11)	N	3	2	3	3	X-linked	yes	(a), (b), (e), (g).
	n	3	2	3	3			
	T ₄ c	2.1 \pm 1.0	3.3 \pm 1.3	5.4 \pm 2.3	4.9 \pm 0.9			
	TBG	6.0 \pm 1.0	11.0 \pm 2.8	22.0 \pm 5.6	22.3 \pm 4.0			
L-5 (12)	N	1	2	7	2	Unknown	yes	(a), (b), (c), (d), (f), (g).
	n	1	2	5	1			
	PBI	2.2	4.4 \pm 1.1	5.6 \pm 1.1	7.4			
	TBG	10	12.0 \pm 1.4	24.2 \pm 4.7	21.0			
L-6 (13)	N	2	7	12	0	X-linked or autosomal- dominant	yes	(a), (b), (e), (f), (g).
	n	2	7	10	—			
	PBI	3.3 \pm 0.4	4.5 \pm 0.5	5.9 \pm 0.5	—			
	TBG	11.1 \pm 3.8	17.6 \pm 2.3	23.2 \pm 3.5	—			
L-7 (14)	N	3	3	—	91	Probably X-linked	?	(a) incomplete pedigree not published).
	n	—	—	—	—			
	PBI	3.0	2.0	—	—			
	TBG	3.6 (3.1–4.2)	10.7 (8.4–13.4)	—	—			
L-8 (14)	N	3	1	—	18	Autosomal- dominant	?	Unknown (pedi- gree not pub- lished).
	n	3	1	—	—			
	PBI	—	—	—	—			
	TBG	12.9	13.6	—	—			
L-9 (This report)	N	1	1	2	1	Probably X-linked	yes	(a), (b), (c), (?d), (f), (g).
	n	1	1	2	1			
	PBI	2.3	3.8	5.0 \pm 0.3	4.6			
	TBG	2.4	12.4	21.1 \pm 2.6	19.6			
L-10 (This report)	N	1	1	1	1	Probably X-linked	yes	(a), (b), (e), (g).
	n	1	1	1	0			
	TT ₄	1.9	5.3	7.0	—			
	TBG	2.5	17.6	25.4	—			
L-11 (This report)	N	1	1	2	2	Probably X-linked	yes	(a), (b), (e), (g).
	n	1	1	2	2			
	PBI	2.9	3.5	5.7 \pm 1.3	6.7 \pm 1.0			
	TBG	3.2	13.5	21.0 \pm 1.1	20.7 \pm 0.6			

For abbreviations and symbols see footnotes of Table VII.
T₄c, serum total T₄ iodine by column.

the TBG concentration by its capacity to bind T₄ and immunologically, measures two distinct sites on the TBG molecule. Nevertheless, the possibility of structural gene mutation giving rise to a minimal alteration of the TBG molecule, undetectable by all methods so far used, and resulting in either decreased or increased

rate of synthesis, cannot be excluded. Such alteration however could not be located at the biologically active or immunologically reactive sites of the TBG molecule.

Attempts have been made to assess the biological significance of TBG abnormalities. Other authors, as well as we, have tested the integrity of the thyroptui-

TABLE IX
Summary of Data from Families with High TBG

Family No. and reference	Tests	Mean \pm SD and (range)				Suggested mode of inheritance	Consistent with X-linkage*	Criteria for X-linkage† and remarks
		Affected		Normal relatives				
		Hemizygous	Heterozygous	Blood	By marriage			
H-1 (1, 20)	N	5	2	17	5	Autosomal-dominant	yes	(a), (b), (c), (d), (e), (f), (g).
	n	5	2	17	5			
	PBI	14.4 \pm 1.2	9.9 \pm 1.4		5.4			
	TBG	62.2 \pm 10.4	40.5 \pm 3.5	15.4 \pm 2.7	17 \pm 6.3			
H-2 (15)	N	1	3	3	1	Autosomal-dominant	yes	(a), (b), (f), (g).
	n	1	3	3	1			
	PBI	14.8	12.1 \pm 0.9	6.1 \pm 1.9	8.6			
	TBG	62	39.3 \pm 7.5	19.7 \pm 5.5	21			
H-3 (16)	N	2	7	24	5	X-linked codominant	yes	(a), (b), (c), (d), (e), (f), (g).
	n	2	7	19	5			
	PBI	14.9 \pm 0.9	12.6 \pm 3.4	6.4 \pm 1.0	6.4 \pm 1.6			
	TBG	59.0 \pm 7.1	48.4 \pm 12.4	22.0 \pm 4.5	20.2 \pm 3.6			
H-4 (17, 48)	N	2	3		12	Autosomal or X-linked	yes	(a), (b), (e), (f), (g).
	n	2	3		11			
	PBI	13.7 \pm 1.2	10.8 \pm 1.2		5.6 \pm 0.9			
	TT ₄	23.8 \pm 1.1	18.7 \pm 1.4		11.2 \pm 2.0			
	TBG	106.0 \pm 33.9	72.0 \pm 7.2		37.9 \pm 4.7			
H-5 (18)	N	4	6	35	15	Probably X-linked	yes	(a), (b), (c), (d), (e), (f), (g).
	n	4	6	34	15			
	PBI	—	—	—	—			
	TBG	63.8 \pm 2.5	53.3 \pm 9.6	23.9 \pm 3.8	24.6 \pm 3.0			
H-6 (19)	N		19		22	X-linked codominant	?	(c), (d). Incomplete (pedigree not published).
	n		—		—			
	PBI		15.7		—			
	TBG		(44-91)		(10-30)			
H-7 (This report)	N	1	1	3	1	X-linked	yes	(a), (b), (d), (e), (g).
	n	1	1	3	1			
	TT ₄	19.9	12.9	6.6 \pm 0.3	6.6			
	TBG	89.2	49.6	20.5 \pm 0.8	21.1			

For abbreviations and footnotes, see Table VII.

tary axis by T₄ suppression (7, 15, 50), and TSH stimulation tests (3). No abnormalities could be detected. Similarly, the BMR (5, 7, 8, 20, 39), cholesterol (3, 5, 7, 13, 16, 17, 20, 39, 50, 55, 56), and thyroidal ¹³¹I uptake (3, 7, 10, 15-17, 20, 39, 50, 55-57) were within normal limits. No evidence for thyroid autoimmunity could be obtained from measurement of circulation

thyroglobulin antibodies (5, 11). In this study and others (1, 5, 15), no adrenal or pituitary abnormalities could be detected. The clinical impression of euthyroidism in these patients is supported by the presence of a normal level of FT₄ (5, 14, 16, 17) and normal daily degradation of T₄ (Table XII). The latter is achieved at the expense of an altered turnover rate compensating

TABLE X
Analysis of the Progeny of Affected Males

Type of TBG abnormality	Affected fathers	Progeny					
		Sons			Daughters		
		Affected	Heterozygous	Normal	Affected	Heterozygous	Normal
D	12	0	0	10	0	20	2
L	7	0	0	11	0	7	2
H	14	0	0	20	0	19	0

TABLE XI
Effect of Estrogens on the Serum TBG in Patients with Inherited TBG Abnormalities

Family No. (Reference)	Type of patient (Identification)	Preparation	Daily dose	Period of administration	TBG capacity	
					Before	After
D-2 (3)	Affected male (R. F.)	Diethylstilbestrol	5	2 wk	0	0*
D-3 (4)	2 Affected males	"	5	3 wk	0	0†
	Affected female	"	"	?	13	20
D-4 (5)	Affected XO Turner's (Mi. P.)	Enovid	5	2 months and 2 yr	0	0‡
D-5 (this study)	Affected female (P. R.)	"	"	4 months	7.3	18.1
(47)	Male (F. B.)				1	1
L-4 (11)	Affected XYY/XY/YO (III-4)	Premarine	5	6 wk	6	6
L-8 (14)	Affected male	Diethylstilbestrol	?	?	9	20
L-9 (this study)	Affected female (C. A.)	Enovid	5	3 months	13	21
H-3 (16)	Affected male (A. S.)	Diethylstilbestrol	5	6 days	54	97
	Affected female (J. M.)	"	1.5	"	41	57
	Affected female (D. S.)	Oral estrogens	?	?	35¶	44
H-6 (19)	2 Affected females	Estrogens	?	?	?	60%**
(50)	Female (F. H.)	Diethylstilbestrol	30	3 wk	52	63
	"	"	"	"	56	60

* Normal increase in CBG (from 40 to 122 mg/liter) and ceruloplasmin (from 0.4 to 0.88 U).

† Normal 2.5-fold increase in CBG.

‡ Plasma cortisol increased from 8.6 to 29.0 µg/100 ml.

|| Increase in PBI and CBG also noted.

¶ Value from 6 wk after discontinuation of estrogens.

** Per cent increase from base line.

TABLE XII
T₄ Metabolism in Patients with Inherited TBG Abnormalities

Type of TBG abnormality	Family	Subject	Age and sex	TBG	PBI	K	t _{1/2}	DS	ETT	D	Reference
				capacity							
			yr	µg T ₄ /100 ml	µg/100 ml	fr./day	days	liters	µg T ₄ -I	µg T ₄ -I/day	
Congenital familial TBG-deficient	D-3	R. F.	38 M	0	1.9	0.18	3.8	17.2	326	59	Nikolai et al. (4)
	"	H. Wa.	35 F	13	3.2	0.20	3.4	8.1	259	52	"
	D-4	Mi. P	14 F (XO)	0	2.2	0.195	3.55	13.75	303.8	59.0	Refetoff et al. (5)
Low TBG	L-1	D. P.	30 M	5.0	2.9	0.198	3.5	12.8	374.0	75.0	Nicoloff et al. (8)
	L-3	G. F.	32 M	5.6	2.0	0.182	3.8	13.9	278	50.7	Bayley et al. (10)
	L-8	—	M	9	—	0.16	4.3*	—	—	normal‡	Barbosa et al. (14)
High TBG	H-1	W. W.	48 M	56	14.7	0.057	12.2	75*	1099.0	63.0	Beierwaltes et al. (1, 20)
	H-2	T. W.	59 M	62	14.8	0.051*	13.6	8.2*	1213*	62.0	Florsheim et al. (15)
	H-3	O. S.	53 M	64	15.5‡	0.05	14.2	6.9	1131	55.2	Jones et al. (16)
	"	J. M.	73 F	41	11.2‡	0.07	9.6	7.3	694	50.0	"
	H-6	—	F	—	—	0.64*	10.8	—	—	51	Shane et al. (19)
Sporadic, probably genetic TBG-deficient		F. B.	58 M	0 to <1	2.0	0.231	3.1	13.2	264.0	61.0	Ingbar (47)
	"	"	after estrogen	"	2.10	0.199	3.5	15.7	329	65.3	"
	"	—	35 M	0	3.00	0.185	3.75	11.2	336	62.2	Beisel et al. (56)
	"	J. G.	43 M	<1	2.1	0.158*	4.4	21.5	452	71	Cavalieri et al. (57)
	"	T. R.	76 M	<1	1.5	0.289*	2.4	16.7	250	72	"

* Calculated from author's data.

‡ Reported as normal; numerical value not given.

§ Value used for the calculation of ETT and D was not given by the authors.

|| Calculated from author's data; original values reported as 326 µg/1.73 m² and 54 µg/1.73 m² per day.

K, fractional turnover rate; t_{1/2}, half-life; DS, distribution space; ETT, extrathyroidal T₄-I; D, daily T₄-I degradation.

for the wide variation in the extrathyroidal thyroxine pool associated with both high and low TBG capacities. When estrogens or pregnancy (3, 7) give rise to detectable changes of T₄-binding capacity to TBG, the absolute values attained are different than those obtained in normal subjects. There is no indication, however, that pregnancy and fetal development are affected by abnormalities in either maternal or fetal TBG capacity. Finally, the dose of exogenous thyroid hormone required for substitution and thyroid gland suppression is not different from that normally used in patients with normal TBG.

TBG abnormality seems to be an isolated and specific defect of thyroid hormone transport in blood. Each family presents a characteristic pattern. From our studies and those of others (1-20), we suggest that all TBG abnormalities in man so far described are probably X-chromosome-linked and manifested by quantitative variations in the serum concentration of an apparently structurally unaltered TBG. Mutations at a single locus controlling TBG synthesis could then explain the entire spectrum of genetic TBG abnormalities in man.

Addendum: Since the submission of this paper, complete data related to family H-6 (19) has been published (1971. *J. Clin. Endocrinol. Metab.* 32: 587.) and is compatible with X-chromosome-linked inheritance. Nusynowitz, Clark, Strader, Eserin, and Seal have published three families with TBG deficiency and suggested X-linked inheritance pattern (1971. *Amer. J. Med.* 50: 458.). We are in the process of studying four additional families; two with elevated TBG and two with low TBG. So far a large number of subjects were tested in only one family which clearly demonstrates X-chromosome inheritance pattern.

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