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Research Article

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Lack of Significant Binding of L-Triiodothyronine by Thyroxine-binding Globulin *In Vivo* as Demonstrated by Acute Disappearance of ^{131}I-labeled Triiodothyronine *

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Since Gordon and his colleagues' (1) description of the association of thyroxine (T_4) with an α-globulin [later termed "thyroxine-binding globulin" (TBG)] in 1952, thyroid hormone transport has been the subject of intense study. The demonstration of "thyroxine-binding prealbumin" (TBPA) by Ingbar (2) in 1958 provided further understanding of the binding of T_4 by the plasma proteins. It has also been shown that the exogenous administration of estrogens (3) or increased endogenous production as in pregnancy (4, 5) is accompanied by an elevation of the thyroxine binding capacity of TBG. Other substances (e.g., androgens) have been found to decrease the binding capacity of TBG (6) or to interfere with it through a competitive action [e.g., diphenylhydantoin (DPH)] (7, 8). Of the previous studies, most have related to thyroxine specifically rather than to triiodothyronine.

The available data on the binding of 3,5,3'-L-triiodothyronine (T_3) in human plasma are derived from *in vitro* studies, performed under unphysiological conditions of pH, ionic strength, and temperature, which may alter the binding pattern. Most studies agree, however, that T_3 is bound mainly by TBG and secondarily by albumin, with no binding by TBPA (9–16). About 99% of T_3 in normal serum is in the bound form in *in vitro* measurements (17). The *in vitro* uptake of T_3 by red cells or resin, which has become a valuable test for thyroid disorders, is another evidence of the *in vitro* binding of T_3 by the plasma proteins. It is evident that the erythrocyte uptake of T_3-^{131}I is unphysiological in respect to lack of blood flow, the presence of an anticoagulant, and the rise of blood pH even after a short period of standing. The strong avidity of the T_4-TBG interaction has previously been emphasized (18, 19), whereas that of T_3 for TBG has been variously estimated as being one-third that of T_4 (19) and fifteen times weaker than T_4 (20). It is of interest that T_3 and T_4 have equal biological potency in the chicken; this has been ascribed to the lack of TBG in this species (21).

The interaction between T_4 and the thyroxine-binding proteins (TBP) can be demonstrated *in vitro* by electrophoresis and *in vivo* by turnover rate determinations, respectively. Agreement between *in vitro* and *in vivo* results has been shown before and after the binding capacity of TBG (22) and of TBPA (23) has been altered by drug therapy. Little has been done, however, to determine by *in vivo* studies whether T_3 circulates bound mostly to TBG, as suggested by *in vitro* techniques. Furthermore, it has not been previously ascertained whether changes of the binding capacity of TBG are accompanied by concomitant variations of the rate of disappearance of T_3 from the blood.

The purpose of the present study is to assess the effect of alterations of the TBG binding capacity on the acute disappearance of T_3 and T_4 from the circulation within 50 minutes of injection

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of tracer amounts of the labeled hormone. This early phase of hormone disappearance has previously been studied (24, 25) and has been chosen since it precedes the phase of significant cellular metabolism. Alterations in the binding capacity of TBG have been shown to profoundly affect this phase of T_4 disappearance from the circulation (25). The subjects studied included a control group of euthyroid persons, patients with thyroid and liver disease, patients given either estrogens or DPH, and a patient with idiopathic lack of TBG. The current results are correlated with *in vitro* observations of electrophoretic binding and of the T_3 resin uptake test in serum from the same patients.

Methods

The euthyroid group consisted of healthy volunteers from hospital personnel and a few hospitalized patients without thyroid or liver disease or abnormalities of plasma proteins. An additional group was composed of four treated hypothyroid patients who were taking 180 mg of desiccated thyroid daily after radioactive iodine treatment for hyperthyroidism. In the untreated myxedematous group there were six patients; one suffered severe pituitary myxedema and five had idiopathic myxedema. The hyperthyroid group comprised patients with Graves' disease or toxic nodular goiter demonstrated by clinical and biochemical findings, as well as by thyroid scanning.

In the liver disease group there was one patient with serum hepatitis during the icteric phase. The rest were patients with advanced alcoholic cirrhosis and hepatic failure.

In the estrogen-treated group, all but five patients were on estrogen therapy for carcinoma of the prostate; the five noncarcinomatous subjects were also euthyroid. The daily dosage of estrogen and duration of therapy are summarized in Table III.

All patients on DPH therapy were epileptic; the dosage of the drug was 300 to 400 mg daily by mouth. An additional dose of 500 mg of DPH was injected intravenously during the 50 minutes in which the study was performed. No side effects were observed by the use of this intravenous dose of DPH.

In addition, a single patient with idiopathic lack of TBG was studied who had no clinical evidence of thyroid dysfunction; he had a low serum protein-bound iodine (PBI) and normal radioactive iodine uptake (see Table V).

In several patients acute disappearance studies with both T_3 and T_4 were carried out.

Techniques of counting. The acute half-time of disappearance of the injected radioactivity was determined by serial counting; for convenience the Radicoil apparatus was employed (24) to equalize the volumes counted. An amount equal to 0.3 μc per kg of body weight of ^{131}I-labeled T_3 or ^{131}I-labeled T_4 solution was injected intravenously; this dose contained approximately 1 μg of carrier T_3 or T_4. Samples containing 4 ml of blood were withdrawn in oxalated tubes 10 minutes after injection and thereafter every 5 minutes from 20 to 60 minutes. The Radicoil was filled with each sample and counted for 10 minutes in a well-type scintillation detector.

The standard was prepared in the following manner: A dose of tracer equal to that injected into the patient was added to 10 ml of plasma, allowed to stand for 30 minutes, and made up to 1,000 ml with water. It was then stirred with a magnet for 5 minutes. The same Radicoil used for the patient was filled with this mixture and counted for 10 minutes.

In vitro methods. The thyroxine binding capacity of TBG was determined by the method of Ingbar (26). To each sample of serum was added a tracer dose of radioactive T_4 (2 μc per ml, 4 μg per 100 ml). These samples were enriched with stable L-T_4 in varied concentrations for determinations of thyroxine binding capacity. The percentage distribution of labeled L-T_3 among the binding proteins was similarly determined in samples of serum from the same patients; these sera were enriched with labeled T_3 (2 μc per ml, 4 μg per 100 ml). The electrophoresis was performed in a Durrum cell with Whatman 3 filter paper in Tris-maleate buffer, pH 8.6, and allowed to run about 18 hours at 120 v. After drying, the strips were counted in a radioactive scanner (Nuclear-Chicago) and the areas of radioactivity calculated by planimetry. This was followed by radioautography and staining of the strips.

Free thyroxine was measured by the method of Ingbar, Braverman, Dawber, and Lee (17); the same technique was utilized to measure free T_3.

The T_3 resin uptake test was performed with the Triosorb technique.[1]

Computation of results. Acute half-time of disappearance of labeled T_3 (T_3 acute half-time) and labeled T_4 (T_4 acute half-time) was determined by plotting the net counts per minute on semilogarithmic paper. The portion of the curve between 20 and 50 minutes was extrapolated back to zero time, and the half-time of disappearance of radioactivity was derived therefrom. The reasons for using this part of the regression curve have been discussed elsewhere (24, 25). Briefly, this period follows the initial phase of mixing, may be analyzed as a straight line, and precedes the phase of significant cellular metabolism. The disappearance of the radioactivity from the circulation has also been expressed as a percentage of the injected dose (standard) remaining in the blood at 20 and 50 minutes after the intravenous injection of the tracer. In addition, the decline of the blood radioactivity during this 30-minute interval has been expressed as a percentage of the 20-minute value (100%) remaining in the blood at 50 minutes. The percentage of the standard was used for the 20- and 50-minute values rather than the percentage of the injected dose per liter of blood because the exact volume of the Radicoil within

[1] Abbott Laboratories, North Chicago, Ill.

TABLE I

*Acute disappearance of ^{131}I-labeled triiodothyronine (T_3)**

Patient	Sex and age	Serum PBI	24-hour thyroidal ^{131}I uptake	T_3 acute half-time	Blood radioactivity after injection of T_3		
					20 min	50 min	Difference
		μg/100 ml	%	*minutes*	*% of standard*†		%‡
(a) Euthyroid control							
V.M.	F 24	4.8		63	7.2	5.1	70.8
D.M.	F 26	3.9		72	6.8	5.1	75.0
A.G.	M 20	5.1		74	6.9	5.2	75.3
M.S.	F 23	6.2		72	6.1	4.6	75.4
A.M.	M 25	5.2		88	7.2	5.7	79.0
E.N.	F 27	4.6		69	6.9	5.1	73.9
M.C.	F 24	4.8		78	6.4	4.9	76.5
L.P.	F 40	5.8		55	9.1	6.2	68.1
G.S.	F 24	3.8		76	7.5	5.7	76.0
L.B.	F 45	5.9		84	7.3	5.7	78.0
C.D.	M 40	4 1		60	7.9	5.6	70.8
D.C.	M 46	5.9		81	6.2	4.8	77.4
Mean		5.0		72.7	7.1	5.3	74.7
SEM		±0.24		±2.8	±0.24	±0.14	±0.94
(b) Treated myxedema							
H.B.	M 59	3.6		84	6.4	5.0	78.1
C.C.	M 45	3.8		73	7.4	5.5	74.3
C.B.	F 54	4.1		75	7.8	5.8	74.3
H.I.	F 35	3.6		74	6.9	5.2	75.5
Mean		3.8		76.5	7.1	5.4	75.5
SEM		±0.12		±2.5	±0.31	±0.18	±0.90
p value				NS	NS	NS	NS
(c) Untreated myxedema							
E.K.	M 26	1.6	10	62	6.5	4.6	70.7
A.K.	F 58	2.2	5	73	8.7	6.5	74.7
E.C.	F 60	1.8	2	72	8.8	6.6	75.0
M.P.	F 73	1.6	3	82	7.9	6.1	77.2
R.J.	M 48	1.2	1	56	4.2	2.9	69.0
E.H.	M 58	2.3	3	73	8.3	6.2	74.8
Mean		1.8		69.3	7.4	5.5	73.6
SEM		±0.17		±3.7	±0.72	±0.60	±1.25
p value				NS	NS	NS	NS
(d) Hyperthyroid							
G.L.	F 42	9.6	63	77	8.4	6.5	77.3
M.B.	F 70	8.5	56	81	7.2	5.7	79.0
M.P.	F 54	13.6	48	74	7.3	5.5	75.3
C.F.	F 15	9.5	54	95	9.1	7.4	81.3
R.McD.	M 52	8.8	61	114	7.9	6.5	82.2
L.R.	F 35	11.6	54	77	9.4	7.2	76.5
T.F.	F 56	12.4	54	69	7.5	5.5	73.3
E.J.	M 55	14.4	60	87	7.8	6.1	78.2
S.L.	F 49	10.1	48	73	8.3	6.3	75.9
D.A.	F 49	20.1	93	71	8.1	6.0	74.0
Mean		11.9		81.8	8.1	6.3	77.3
SEM		±1.12		±4.4	±0.23	±0.21	±0.93
p value				NS	NS	.05	NS

* PBI = protein-bound iodine; p value = probability that there is no real difference between the groups tested and the control group; NS = not significant ($p > .05$).

† Although these values are expressed as per cent of the standard (see text), they do approximate the per cent of the injected radioactivity per liter of blood in all tables.

‡ This difference refers in all tables to the disappearance of radioactivity from the circulation between the 20- and 50-minute intervals utilized to derive a slope, expressed as the percentage of the 20-minute value (100%) remaining in the blood at 50 minutes after the injection of the labeled hormone.

TABLE I—(*Continued*)

Patient	Sex and age	Serum PBI	24-hour thyroidal ^{131}I uptake	T_3 acute half-time	Blood radioactivity after injection of T_3		
					20 min	50 min	Difference
		μg/100 ml	*%*	*minutes*	*% of standard†*		*%‡*
(e) Liver disease							
A.M.	F 39	3.8		75	7.8	5.9	76.1
R.W.	M 69	4.0		62	6.6	4.7	71.6
D.E.	F 52	3.6		73	7.6	5.6	74.5
P.W.	F 39	4.5		68	8.1	6.1	75.3
G.G.	M 79	3.9		57	7.0	4.8	68.7
R.C.	M 50	4.6		71	6.9	5.2	75.4
G.D.	M 59	5.1		60	6.9	4.8	69.5
R.M.	M 54	4.1		69	7.1	5.1	71.8
Mean		4.2		66.9	7.2	5.3	72.9
SEM		±0.18		±2.3	±0.18	±0.19	±1.01
p value				NS	NS	NS	NS

the counter was not known. However, the Radicoil had the advantage of counting all samples in the same volume.

Replicate T_3 studies were carried out in six subjects among the various groups with no significant variations between the first and second studies. The method is thus reproducible.

Statistical analysis of the data was performed by the methods described by Snedecor (27) and Owen (28).

Results

Euthyroid (Tables Ia and II)

The mean T_3 acute half-time for the euthyroid group was 72.7 minutes, and the mean T_4 half-time was 73.4 minutes (Table II); the difference was not significant. Furthermore, the proportion of the 20-minute count remaining at 50 minutes was almost identical in the two groups, 74.7% in the T_3 group and 74.6% with T_4.

By contrast, however, at 20 minutes and throughout the entire interval of study the percentage of the standard remaining in the blood was markedly less in the T_3 group as compared to the T_4 results ($p < 0.01$). The mean percentage of the standard remaining at 20 minutes was 7.1% for T_3 and 14.7% for T_4.

Treated myxedema (Table Ib)

The mean T_3 acute half-time was 76.5 minutes; there is no difference from the euthyroid results. The percentage of the 20-minute count remaining at 50 minutes was 75.5, again consistent with the euthyroid values. The four patients in this group were given varying doses of stilbestrol for 3- to 4-week periods and restudied with T_3; the values

TABLE II

Acute disappearance rates of ^{131}I-labeled thyroxine (T_4): euthyroid control subjects

Subject	Sex and age	Serum PBI	T_4 acute half-time	Blood radioactivity after T_4 administration		
				20 min	50 min	Difference
		μg/100 ml	*minutes*	*% of standard*		*%*
D.K.	M 62	5.1	73	13.5	10.2	74.3
R.McN.	F 56	4.6	64	13.5	9.8	72.5
F.B.	M 31	5.0	62	14.1	9.9	70.3
J.W.	M 26	4.1	66	14.1	10.0	71.0
M.S.	M 73	3.8	80	18.9	14.6	77.6
C.McG.	M 75	5.2	74	14.6	10.7	73.5
S.H.	M 46	5.3	78	12.0	9.2	76.7
L.Mc.	F 20	4.5	80	18.0	13.9	77.3
M.L.	M 47	4.0	84	14.1	11.0	78.0
Mean		4.7	73.4	14.7	11.0	74.6
SEM		±0.19	±2.3	±0.74	±0.63	±0.98
p value*			NS	<0.01	<0.01	NS

* Probability that there is no real difference between the group tested and the T_3 normal control group.

TABLE III

*The effect of estrogen therapy on T_3 and T_4 acute disappearance rates**

Patient	Sex and age	Serum PBI	T_3 resin uptake test (normals 26–34%)	Estrogen therapy			Thyroxine binding capacity of TBG†		Proportion of T_3 bound to TBG *in vitro*		Acute half-time	Blood radioactivity after administration of labeled hormone		
				Drug	Daily dose	Duration of therapy	Control‡	Estrogen	Control‡	Estrogen		20 min	50 min	Difference
		μg/100 ml plasma	%		*mg*		*μg/100 ml plasma*		%		*minutes*	% *of standard*		%
(a) Patients studied with ^{131}I-labeled T_4														
S.H.	M 46	7.6	21	Stilbestrol	15	22 days	22.8	50.6	65.2	75.8	160	14.7	12.9	87.7
J.G.	M 64	5.7	23	Vallestril	9	1 year		51.1		72.6	124	12.7	10.7	84.2
J.C.	M 84	8.5	23	Vallestril	12	22 days	21.9	46.8	58.0	70.1	140	14.1	12.2	86.5
R.A.	M 70	5.8	20	Stilbestrol	3	75 days		45.4		73.4	138	13.9	12.0	86.3
M.M.	M 77	7.8	19	Stilbestrol	3	2 years		48.6		69.3	146	14.9	13.0	87.2
W.A.	M 75	7.5	23	Stilbestrol	3	1½ years		55.9		75.2	136	16.2	14.1	87.0
H.B.	M 59	8.7	20	Stilbestrol	30	21 days	25.3	50.4	65.3	78.2	166	17.3	15.3	88.4
G.S.	M 59	9.0	18	Stilbestrol	100	17 days§		55.8		73.6	186	13.7	12.3	89.7
F.T.	M 74	6.6	21	Vallestril	9	28 days	20.2	48.8	60.9	69.4	158	13.7	12.0	87.5
Mean		7.4	20.9				22.5	50.4	62.3	73.1	150.4	14.6	12.7	87.2
SEM		±0.40	±0.61				±1.06	±1.20	±1.78	±1.02	±6.3	±0.47	±0.44	±0.50
p value											<0.01	NS	<0.05	<0.01
(b) Patients studied with ^{131}I-labeled T_3														
L.A.	M 72	7.2	21	Vallestril	60	23 days	25.4	48.3	60.1	75.1	74	7.2	5.3	73.6
H.B.‖	M 59	9.5	20	Stilbestrol	30	28 days	22.6	51.6	63.0	78.4	72	6.9	5.2	75.3
C.C.‖	M 45	6.2	22	Stilbestrol	15	21 days	21.1	49.4	64.4	76.7	76	7.4	5.6	75.6
M.M.	M 77	6.9	27	Stilbestrol	3	2 years		46.3		71.8	84	6.4	5.0	78.1
A.F.	M 74	6.0	26	Stilbestrol	3	10 years		52.3		74.1	71	6.7	4.9	73.1
C.B.‖	F 54	6.2	16	Stilbestrol	10	21 days	19.8	49.6	58.3	69.8	76	7.6	5.8	76.3
H.I.‖	F 35	5.2	24	Stilbestrol	15	21 days	24.5	50.4	62.8	74.4	74	7.3	5.5	75.3
J.C.	M 84	8.5	24	Vallestril	9	50 days	21.9	48.6	58.0	68.2	84	7.5	5.8	77.3
G.S.	M 59	9.1	19	Stilbestrol	100	20 days§		55.8		75.9	76	7.4	5.6	75.6
J.A.	M 73	6.9	18	Stilbestrol	3	67 days		46.4		72.2	74	7.1	5.3	74.6
Mean		7.2	21.7				22.5	49.9	61.1	73.7	76.1	7.1	5.4	74.5
SEM		±0.45	±1.13				±0.86	±0.90	±1.09	±1.00	±1.4	±0.12	±0.10	±0.48
p value											NS	NS	NS	NS

* TBG = thyroxine-binding globulin; Vallestril = commercial name of methallenestril; p value = probability that there is no real difference between the groups tested and the normal control group (a, Table II; b, Table I).

† Mean of at least two determinations.

‡ The control values were obtained on sera of the patients before commencement of estrogen treatment.

§ Patient G.S. had been previously taking 4 mg stilbestrol daily for 45 days.

‖ T_3 acute disappearance studies performed on these four patients before estrogen therapy are tabulated in Table I (b).

obtained after estrogen therapy are described below with the estrogen-treated group.

Untreated myxedema (Table Ic)

The mean T_3 acute half-time for this group was 69.3 minutes, and the percentage of the 20-minute count remaining at 50 minutes was 73.6. Both figures are not significantly different from the result of euthyroid subjects. The results of the patient with pituitary myxedema were consistent with the results of the remaining patients with primary myxedema. However, the normal acute disappearance of T_3 in the myxedematous patients was markedly different from the delayed acute appearance of T_4 observed in similar patients (24, 25).

Hyperthyroid (Table Id)

The mean T_3 acute half-time for this group was 81.8 minutes; this was slightly but not significantly greater than control values. The percentage of the 20-minute count remaining at 50 minutes was 77.3, also not significantly different from normal values. Patients with Graves' disease and toxic nodular goiter were included in the same group, since there was no appreciable difference in the results obtained in these two types of hyperthyroidism.

Liver disease (Table Ie)

The mean T_3 acute half-time was 66.9 minutes, showing no significant difference from euthyroid control values; the percentage of the 20-minute count at 50 minutes was 72.9. One patient (P.W.) had acute serum hepatitis; the results obtained did not differ from the remainder of the group. The other patients were all suffering from advanced alcoholic cirrhosis and liver failure.

Estrogen-treated (Table III)

In vitro. There was a consistent increase in the T_4 binding capacity of TBG in all patients studied, with a mean of 50.4 μg per 100 ml of plasma [findings similar to those previously reported in pregnancy (5, 29)]; variations in the compounds and the doses employed did not produce appreciable differences in the results. As expected, there was a good correlation between the binding capacity of TBG, the T_3 resin uptake test, and the PBI. The mean percentage distribution of T_3 among the binding proteins showed 61.7% bound to TBG, 31.5% to albumin, and the rest to globulins cathodal to TBG. In our studies, performed at pH 8.6, no binding to TBPA was observed; this was in agreement with all previous observations (9–16).

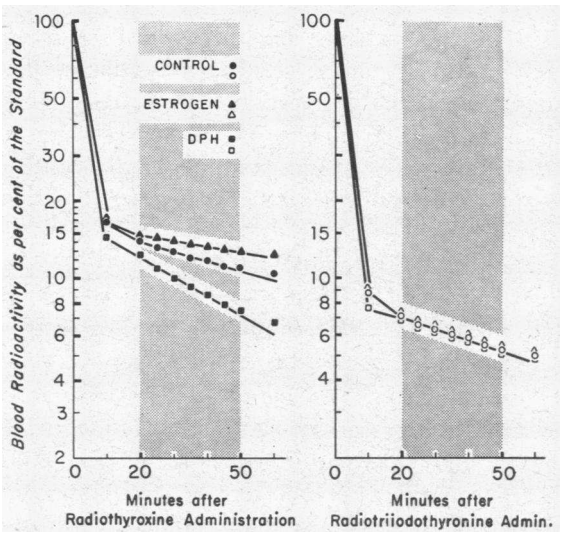

FIG. 1. EFFECTS OF ESTROGEN AND DIPHENYLHYDANTOIN (DPH) ON THE ACUTE RATES OF DISAPPEARANCE OF ^{131}I-LABELED THYROXINE AND ^{131}I-LABELED TRIIODOTHYRONINE. The points are expressed as per cent of the standard (see text).

In vivo. There was no appreciable difference between the 20-minute counts in the estrogen-treated patients and the control group in the T_4 studies. However, the estrogen-treated patients studied with T_4 had a mean T_4 acute half-time of 150 minutes, which was significantly higher than control values ($p < 0.01$). The retention of T_4 within the blood was also shown by the percentage radioactivity of the 20-minute count remaining in the circulation at 50 minutes (mean 87.2%). This is 12% more than the control values ($p < 0.01$) and 25% more than the results in the patients given DPH. The T_3 acute half-time, on the other hand, had a mean of 76.1 minutes, which was not significantly different from the T_3 control group or the T_3 results in the patients taking DPH. The slopes derived by plotting on semilogarithmic paper the points between the 10 and 60 minutes are shown in Figure 1, which depicts the results obtained in the estrogen- and

DPH-treated patients and the control group for both T_3 and T_4 studies. It is apparent that marked alterations of the disappearance of T_4 result from changes in TBG binding induced by estrogen or DPH. No such changes were observed in the T_3 disappearance rates in estrogen- and DPH-treated patients.

Four estrogen-treated patients (H.B., M.M., J.C., and G.S.) had studies with both labeled T_3 and labeled T_4. The results obtained were consistent with the rest of the respective group.

One patient (G.S.) was given very large doses of stilbestrol for carcinoma of the prostate; when the T_3 study was performed, he had been taking 100 mg of stilbestrol daily for 17 days before the study and had previously been taking 4 mg of the same drug daily for 45 days. This was reflected in a high T_4 binding capacity of TBG. The disappearance rates of T_3 and T_4 in this patient did not differ significantly from the others in this group.

Four patients (C.C., H.B., C.B., and H.I.) were studied with T_3 before and after the administration of estrogen; in these, as in all patients given estrogen therapy, there was no significant change in the T_3 acute half-time from control values.

Diphenylhydantoin (DPH)-treated (Table IV)

The mean T_3 acute half-time in these patients was 71.3 minutes, similar to control values. The T_4 half-time, on the other hand, was 44.6 minutes, which was markedly reduced compared to the control group ($p < 0.01$). The proportion of the 20-minute count remaining in the blood at 50 minutes also clearly showed the accelerated disappearance of T_4 from the blood in this group; the mean value was 62.3% as opposed to 74.6% in the control group ($p < 0.01$). Furthermore, the 20-minute T_4 values were significantly reduced by DPH administration. All patients in the DPH-treated group studied with T_4 showed similar disappearance rates of this hormone, whereas in those studied with T_3, the results varied among the individual patients. Only two patients were studied with both hormones. In both such studies the results agreed with the appropriate group. The mean

TABLE IV

The effect of diphenylhydantoin on acute disappearance rates of T_3 and T_4

Patient	Sex and age	Serum PBI	T_3 resin uptake test	Acute half-time	Blood radioactivity after administration of labeled hormone		
					20 min	50 min	Difference
		μg/100 ml	%	*minutes*	*% of standard*		%
(a) Patients studied with labeled T_4							
D.R.	M 49	4.1	37	50	13.4	8.7	64.8
D.E.	M 33	3.7	31	45	12.3	7.8	63.4
M.C.	F 19	5.3	26	42	11.1	6.7	60.3
G.P.	M 33	3.8	30	45	12.9	8.0	62.0
T.C.	F 20	3.9	29	43	11.3	6.9	61.0
K.G.	M 43	4.1	33	43	11.6	7.3	62.9
T.R.	M 36	3.8	37	44	11.8	7.3	61.8
Mean		4.1	31.9	44.6	12.1	7.5	62.3
SEM		±0.21	±1.55	±1.0	±0.32	±0.26	±0.57
p value				<0.01	<0.01	<0.01	<0.01
(b) Patients studied with labeled T_3							
A.S.	M 65	3.6		72	7.0	5.2	74.3
U.K.	F 23	3.2	45	60	8.3	5.8	69.8
D.R.	M 49	3.9	38	84	6.9	5.4	78.2
T.C.	F 20	3.7	34	58	7.7	5.4	70.1
D.B.	F 26	4.4	37	72	6.7	5.0	74.6
H.H.	M 30	3.1	36	92	5.9	4.7	79.6
R.C.	M 35	4.8	36	58	7.3	4.6	63.2
J.D.	M 72	3.4	34	74	7.2	5.4	75.0
Mean		3.8	36.4	71.3	7.1	5.2	73.1
SEM		±0.21	±1.45	±4.4	±0.25	±0.14	±1.86
p value				NS	NS	NS	NS

TABLE V

Results from patient with lack of TBG (patient G.F.) compared with control values*

	Acute half-time	Blood radioactivity after administration of labeled hormone			Serum free (unbound) hormone	Thyroxine binding capacity		T_3 distribution	
		20 min	50 min	Difference		TBG	TBPA†	Albumin	TBG
	minutes	*% of standard*		%	*% total circulating hormone*	*μg/100 ml plasma*		%	
T_4									
Euthyroid control mean values	73.4	14.7	11.0	74.6	0.054‡	22.9‡	123‡		
Range					0.050–0.059	18.2–26.8	108–141		
Patient G.F.§	39.0	9.2	5.3	57.7	0.098	Nil	173.3		
T_3									
Euthyroid control mean values	72.7	7.1	5.3	74.7	0.61‡			31.5‡	61.7‡
Range					0.56–0.67			27–36	55–67
Patient G.F.§	69.0	6.0	4.4	73.3	0.69			90	See below‖

* Routine thyroid function tests on patient G.F.: T_3 resin uptake, 60% (normals 26–34%); PBI, 2.1 μg per 100 ml; 24-hour thyroidal ^{131}I uptake, 30%.

† TBPA = thyroxine-binding prealbumin.

‡ Normal values for this laboratory.

§ Mean of four determinations.

‖ 10% of the radioactivity was present in the area cathodal to albumin in the α- and β-globulin areas.

PBI in the DPH group was 3.9 μg per 100 ml, whereas the mean T_3 resin uptake test was 34.0%.

Patient with idiopathic lack of TBG (Table V)

In vitro. In this patient (G.F.) the binding capacity of TBG was so low that it could not be determined even with very low concentrations of added stable T_4; his blood was thus devoid of appreciable TBG. In the electrophoresis there was a greater percentage than normal of T_4 in the TBPA zone. The *in vitro* addition of stable L-T_4 in varying concentrations showed the hormone to be bound primarily to TBPA and secondarily to albumin. The binding capacity of TBPA averaged 173.3 μg per 100 ml in several determinations. Radioautographs of the electrophoretic distribution of T_3 and T_4 among the thyroxine-binding proteins in serum from this patient showed a total lack of radioactivity in the TBG area after distribution of T_4. T_4 was bound mainly by TBPA and T_3 by albumin; there was a small amount of T_3 radioactivity in the area cathodal to albumin. The T_4 studies showed no trailing of radioactivity.

The serum free T_4 found in this patient was 0.098% of the total concentration of circulating thyroxine, which, as expected, was significantly higher than the normal control values in our laboratory (0.054%). The serum free T_3 was 0.69% of the bound hormone, normal control value 0.61%.

In vivo. The acute disappearance of T_4 was very rapid (half-time, 39 minutes), slightly faster than the values in the DPH-treated group. The 20-minute value for T_4 was markedly reduced (to 9.2% of the standard) and approximated the T_3 20-minute value. However, the T_3 acute half-time was 69 minutes, not significantly different from the normal control group.

Discussion

After intravenous injection, tracer amounts of L-T_4 and L-T_3 initially disappear rapidly from the blood to reach equilibrium in the body pool in 12 to 48 hours; subsequently the disappearance of the labeled hormone proceeds slowly in an exponential manner, and the slope so derived is considered to represent solely turnover of the injected hormone in a steady state (30, 31). The binding of thyroxine to the plasma proteins has been demonstrated to be a factor in the regulation of the disappearance rate of T_4 when followed for several days after injection. Thus, an increase in the thyroxine binding capacity of TBG is followed by a delay in the disappearance of thyroxine from the circulation (21), and the opposite effect is seen after depression of the thyroxine binding capacity of TBG (6). Similar effects on the turnover of T_4 have also been observed in patients with familial overproduction of TBG (32, 33) and in those with lack of TBG (26, 34, 35). It has also been shown

that TBPA plays an important role in the turnover of T_4 under certain circumstances (23). Observations in hyperthyroid and hypothyroid patients (30, 36, 37) suggested that the metabolic rate also appears to be a factor in the disappearance of thyroxine in this later slow phase.

By studying the first 50 minutes after injection of the labeled hormone ("acute disappearance") we eliminated the metabolic factor, since degradation is insignificant during the 30-minute period utilized to derive a slope. Furthermore, previous studies in our laboratory have shown that this phase is unaffected by changes in the metabolic rate or of blood flow: Thus the increased metabolic rate of malignant disease, or that induced by warming the body in a bath (which also increases blood flow), had no effect on acute disappearance rates (38). We felt that this early and rapid phase of T_3 or T_4 disappearance might be dependent at least in large part on binding properties in the circulation and could be studied in the absence of other factors attendant on the later phase of dynamic equilibrium.

By 20 minutes after injection, the disappearance of T_3 from the circulation was much greater than that of T_4, although the subsequent 20- to 50-minute slopes of T_3 and T_4 disappearance were similar in the control group. The explanation of this rapid 20-minute loss of T_3 is not clear, although it is related to an increased distribution space for T_3, which may be readily calculated from the 20-minute counts and the standard counts.

Presumably the T_3 diffuses very rapidly into this initial volume of distribution within the first few minutes. After that diffusion, which is virtually completed by 10 minutes (see Figure 1), any short part of the curve (capable of analysis) may be arbitrarily chosen for study, at least until the time that cellular metabolism becomes a significant factor. Results do not differ qualitatively when any part of the curve between 10 and 90 minutes is analyzed (39), although there are then more variables and even more problems in analysis of the data. Furthermore, the 20- to 50-minute interval was found to give the best statistical separation (for the T_4 studies) among the various groups studied. Hence it seems justifiable to arbitrarily pick the 20- to 50-minute interval for analysis of acute disappearance for both hormones. It must nevertheless be conceded that the 20- to 50-minute period is possibly more sensitive to measurements of the rate of egress of T_4 from the vascular compartment than to measurements of the rate of egress of T_3.

The larger initial volume of distribution for T_3 is very likely related to binding properties. The explanation for the lower 20-minute values for T_3 (as compared to T_4), while the 20- to 50-minute slopes are so similar, is not known with certainty. One might speculate that the role of the specific protein TBG may be important in this regard, since the initial volume of distribution for T_4 can be enlarged by decreasing TBG. It may be that TBG acts very rapidly to bind T_4, thus reducing its initial volume of distribution; the binding of T_3 to presumably nonspecific binding sites may be somewhat slower, thus permitting a wider initial volume of distribution. However, by the end of the first 10 minutes, this phase would appear to be virtually complete in each instance, thus allowing a comparison of later parts of the curve. This hypothesis is given credence by the observation that in the absence of TBG (Table V), the 20-minute count for T_4 approaches that for T_3, and the volumes of distribution for T_4 and T_3 are thus similar under these circumstances. Furthermore, these data are similar to observations made by Brown-Grant and Tata (40) in rabbits. These authors found a much larger volume of distribution for T_3 (compared to T_4) within 5 minutes after injection and correlated these observations with the binding affinities of both hormones for TBG (which in rabbits are similar to man).

It is evident that the volume of distribution at 10 or 20 minutes is comprised of the blood volume plus a variable amount of extravascular space. Although a reduction in TBG appeared to cause a larger volume of distribution for T_4 (as represented by lower 20-minute counts), an increase in TBG (e.g., with estrogen treatment) caused no significant decrease in the initial volume of T_4 distribution. A possible reason for this apparent inconsistency may be that the blood volume represents the irreducible minimum for the initial volume of distribution. The mean volume of distribution at 20 minutes for the T_4 studies was 6.87 L for the normal control group. This value is not much greater than the blood volume and presumably could not be appreciably contracted by increasing TBG.

In the T_4 studies, alterations induced in TBG binding of T_4 caused predictable changes in T_4 acute disappearance in the 20- to 50-minute interval. Thus the presence of increased unsaturated TBG binding capacity (estrogen therapy, myxedema) was associated with a marked decrease in the T_4 acute disappearance rates [as previously demonstrated (25)], whereas reduced unsaturated TBG binding capacity (DPH treatment, idiopathic lack of TBG, hyperthyroidism) was accompanied by acceleration of the T_4 acute disappearance rates.

By contrast, T_3 acute disappearance rates were not significantly altered from control values by either an increased or decreased TBG binding capacity. This observation differed from the *in vitro* findings, in which T_3 binds to TBG (albeit less strongly than T_4); furthermore in these *in vitro* studies, increased available TBG binding capacity resulted in increased TBG binding of T_3, and vice versa.

In patients with liver disease, preliminary observations (39) confirm the marked delay in T_4 acute disappearance rates similar to those found by Lennon, Engbring, and Engstrom (24). However, contrary to the estrogen results, there was no significant increase in TBG binding capacity in the liver disease group (39). Early data from our laboratory suggest that a delay in the handling of T_4 by the liver in this group may be a major factor in the slowing of the T_4 acute disappearance rates as previously observed (41). In any event, the T_3 acute disappearance values were again unchanged from control values.

It therefore seems evident that changes in TBG binding capacity exert a profound effect on T_4 acute disappearance, but no appreciable effect on T_3 acute disappearance rates. Thus T_3 is apparently not bound to TBG *in vivo*. An alternative explanation might be that there is TBG binding of T_3 *in vivo,* but of such a minor magnitude that significant changes in T_3 acute disappearance cannot be detected after alterations in TBG capacity. The fact that in the T_3 studies the lowest 20-minute value was found in the patient with lack of TBG suggests that TBG may possibly play a minor role in at least the initial phase of T_3 transport. It is evident that there is T_3 binding to sites within the circulation, since the T_3 acute disappearance curves so closely approximate the T_4 curves for the control group; however, our data suggest that TBG is at least not a major site.

Furthermore, when there was no appreciable TBG present in the circulation (i.e., in the patient lacking TBG) or when TBG binding of T_4 was reduced (e.g., by DPH) the T_4 acute disappearance rates were actually faster than T_3 slopes; this suggests that in the absence of TBG, T_3 binds more readily to sites within the circulation than does T_4.

T_3 acute disappearance rates were not influenced by metabolic factors as judged by the normal results in hyperthyroidism and hypothyroidism. Indeed, in hyperthyroidism, there appeared to be a slight delay in the T_3 acute disappearance rates, but these results were not significantly different from control values. [This lack of statistical significance may have been the result of the comparatively small number of hyperthyroid patients, since Hales and Dobyns (42) have shown that T_3 is retained significantly in hyperthyroid patients when compared to normal subjects. This aspect should be explored further.]

Although it appears from our data that T_3 does not bind significantly to TBG *in vivo,* it is clearly evident that it does bind well to TBG *in vitro,* although with much less affinity than T_4. The reasons for this disparity are not clear, since the unequal binding competition between T_3 and T_4 could not entirely explain the apparent total lack of *in vivo* TBG binding of T_3. The *in vitro* measurements are performed under unphysiological conditions. Whereas previous observations in this laboratory have shown reduced TBG binding of T_3 at a physiological pH in starch gel electrophoresis (borate buffer) (16), Braverman and Ingbar have demonstrated TBG binding of T_3 at pH 7.4 in agar gel (phosphate buffer) (15). If there is binding of T_3 to TBG within the circulation, it is masked, perhaps as a result of conditions of pH, blood flow, T_3–T_4 competition, and other unknown factors obtaining *in vivo*. Under these circumstances T_3 apparently binds mainly to nonspecific sites such as various proteins and erythrocytes, which may be competitive with TBG under physiological conditions; this may then result in the apparent lack of TBG binding of T_3 as demonstrated by our acute disappearance studies.

Summary

The acute half-time of disappearance of ^{131}I-labeled 3,5,3′-L-triiodothyronine (T_3) from the circulation between 20 and 50 minutes after intravenous injection was determined in normal persons, in patients with thyroid disease, in patients with hepatic cirrhosis, in subjects given either diphenylhydantoin sodium or estrogens, and in one person with idiopathic lack of TBG. In some of these patients the same study was done with L-thyroxine (T_4). The thyroxine binding capacity of thyroxine-binding globulin (TBG) was determined by filter paper electrophoresis in Tris-maleate buffer (pH 8.6). It was found that labeled T_3 half-time is not significantly shorter than labeled T_4 half-time and, unlike the latter, shows no significant variation among euthyroid, hyperthyroid, myxedematous, and cirrhotic patients and those lacking TBG. The labeled T_4 half-time markedly decreased after intravenous administration of diphenylhydantoin; by contrast, no significant effect was observed in labeled T_3 half-time under the same circumstances. Findings similar to the diphenylhydantoin results were obtained in the patient with idiopathic lack of TBG. In the estrogen-treated group, labeled T_4 half-time increased to values twice the control. In these patients also, no significant changes were seen in the labeled T_3 half-time; however, the *in vitro* binding of radioactive T_3 by TBG in sera of patients in this group showed a significant increase, as revealed by electrophoresis and by the labeled T_3 resin uptake test. It therefore appears that there is virtually no *in vivo* binding of T_3 by TBG; alternatively, if there is T_3-TBG binding *in vivo*, it must be of such minor magnitude that it cannot be demonstrated by acute disappearance studies.

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