The Distribution Kinetics of Triiodothyronine: Studies of Euthyroid Subjects with Decreased Plasma Thyroxine-Binding Globulin and Patients with Graves' Disease

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ABSTRACT The kinetics of distribution of 3,3',5-triiodo-L-thyronine (T_3) have been studied employing both a single-injection and a continuous infusion of T_3-^{131}I. External monitoring of radioactivity in the liver during the infusion permitted estimation of the hepatic distribution volume (V_H) and the one-way hepatic clearance (C_H) of the hormone. Among 10 euthyroid control subjects, V_H averaged 2.07 liters ±0.50 (SD), and the mean value for C_H, 231 ml of plasma per min (±64). In three euthyroid men whose plasma showed decreased binding capacity by thyroxine-binding globulin (TBG) abnormally high V_H and C_H values were found, the increase in C_H being proportional to the decrease in binding activity by plasma proteins. Among all 13 subjects, there was a high correlation (+ 0.86) between C_H and the proportion of free hormone in plasma, measured in vitro.

In four patients with hyperthyroid Graves' disease V_H ranged from 3.2 to 4.2 liters and C_H was elevated in every case, averaging 989 ml/min. The increase in C_H in this group was out of proportion to the elevation of free hormone fraction in plasma. Seven patients who were either euthyroid or hypothyroid after treatment of Graves' disease showed a slight but significant increase in C_H compared with the euthyroid controls without Graves' disease. The percentage of free hormone in the plasma of the treated group was normal or low and therefore could not explain the persistent elevation in unidirectional hepatic clearance observed.

The rate of accumulation of labeled T_3 in the tissues of the thigh during the interval from 10 to 60 min of the sustaining infusion of tracer was slow compared to the rate of equilibration in the liver and did not differ significantly among the various groups studied. These latter findings suggest that in slowly equilibrating tissues such as the thigh the kinetics of T_3 distribution are relatively insensitive to alterations in hormone-binding activity by plasma proteins.

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INTRODUCTION

There is accumulating evidence that 3,3',5-triiodo-L-thyronine (T_3) plays a more important role in a quantitative sense than is relatively low concentration in blood would suggest (1, 2). Although published estimates of total distribution volume vary widely (3–7), the reported values suggest that more than nine-tenths of the extrathyroidal pool of T_3 lies outside the vascular compartment. However, quantitative data in the human are lacking in regard to the rates of transfer of T_3 between plasma and tissue and the distribution of the hormone in extravascular sites. The rapidity with which labeled T_3 leaves the blood after a single injection of the tracer makes analysis of kinetics data difficult. In order to overcome this problem we have employed a method involving a continuous infusion of labeled T_3. During the period when the plasma concentration of tracer is maintained at a constant level, monitoring of radioactivity in various sites, such as the liver, provides a means of estimating the rate of penetration of hormone into tissues. This report describes the method and presents the results obtained in euthyroid subjects with either normal or decreased plasma TBG binding, patients with hyperthyroid Graves' disease, and individuals who have been treated for Graves' disease.

METHODS

The specific activity of L-triiodothyronine-^{131}I (T_3-^{131}I) labeled in the 3′ position, ranged from 40 to 60 mCi/mg. Before use, T_3-^{131}I was diluted one to three by volume in human serum albumin, 0.25 g/ml. This mixture was dialyzed for 24 hr in the cold against 50 volumes of sodium phosphate buffer, ionic strength 0.15, pH 7.4, in order to remove iodide-^{131}I. Before injection into the subjects, the dialyzed T_3-^{131}I) albumin solution was sterilized by Millipore filtration. A sample of the administered dose of T_3-^{131}I was diluted in normal plasma and the proteins were precipitated with tricholoroacetic acid (TCA). Calculation of the total T_3-^{131}I injected was based on the concentration of TCA-precipitable ^{131}I and the volume of the injected dose. In all but two studies, albumin space measurements were performed by injection of human serum albumin-^{125}I simultaneously with the injection of T_3-^{131}I.

Single injection studies. In each subject, both a single injection and an infusion study were done. In the single-injection study, approximately 25 μCi of T_3-^{131}I and 10 μCi of serum albumin-^{125}I were injected rapidly into an antecubital vein. Samples of blood were collected from the opposite arm at 3, 6, 10, 20, 30, and 40 min, and at 20-min intervals up to 2 hr after injection. Plasma was treated with 20% TCA, and the precipitated proteins were washed twice with 10% TCA. The washed precipitate was assayed for ^{131}I and ^{125}I. Extrapolation to time zero of the plasma-time curve for ^{125}I, in per cent of dose per liter, yielded an estimate of plasma volume (V_P).

Calculation of infusion rates. The plasma TCA-insoluble ^{131}I vs. time curve, obtained in the single-injection study, was approximated by three or four straight lines when plotted on semilogarithmic paper (Fig. 1). From the slope of each linear component an infusion rate, R, was calculated for the interval of time using the following formulae:

$$R_1 = D_0k_1$$
$$R_2 = (D_0 + R_1t_1)k_2$$
$$R_3 = [D_0 + R_1t_1 + R_2(t_2 - t_1)]k_3$$
etc.

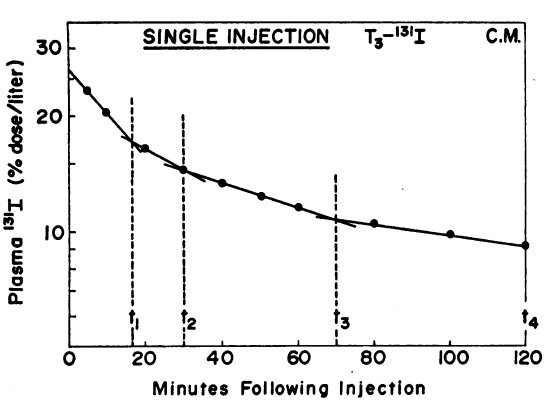

FIGURE 1 Analysis of the time-course of the plasma concentration of TCA-insoluble ^{131}I after a single injection of T_3-^{131}I into a control subject. The values for plasma concentration, solid circles, were approximated by four straight lines on a semilogarithmic plot. The slope of each straight segment was used to calculate the infusion rate for the corresponding time-interval. (See text for details.)

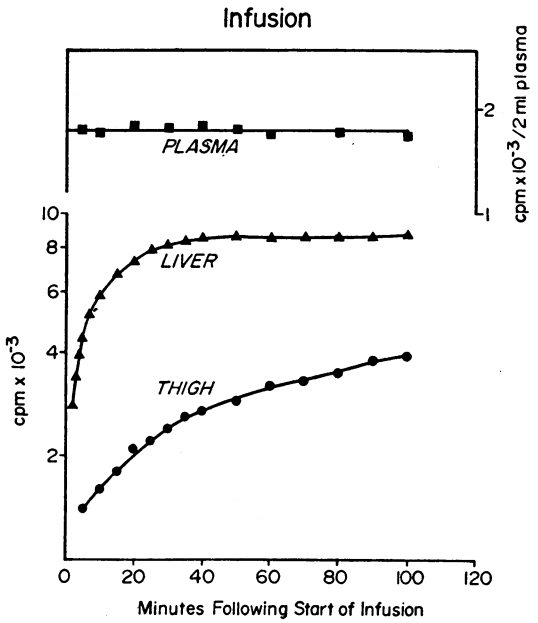

FIGURE 2 Data obtained in a control subject during the infusion of T_3-^{131}I. A priming dose of the tracer was injected rapidly at time zero, followed immediately by an infusion at decreasing rates. The concentration of TCA-insoluble ^{131}I in plasma is shown by squares, radioactivity over the liver by triangles, and radioactivity over the thigh by solid circles.

Where D_0 is the priming dose, in milliliters, and k_1 is the slope of the plasma-time curve (single-injection data) during any interval i. For example, k_2 is given by $(\log C_2 - \log C_1)/t_2 - t_1$, where C_1 and C_2 are the plasma concentration at time t_1 and t_2, respectively, after the single injection. This formulation is an extension of one developed previously to calculate the infusion rate of labeled glucose in an assumed single-compartment system (8). The objective is to achieve a constant level of tracer in the plasma extending from the initial priming dose throughout the period of infusion.

The continuous infusion experiments were begun on the 3rd or 4th day after the single injection. The patient was recumbent for at least 30 min before and during the period of the infusion. A sample of blood was collected for determination of the residual radioactivity in the plasma. A priming dose, exactly 3.0 ml of a solution containing approximately 15 μCi of T_3-^{131}I in dilute albumin, was rapidly injected intravenously and followed immediately by an infusion of additional solution containing the same concentration of tracer. The infusion rates were controlled within 5% of the calculated rates by means of a Harvard pump (Harvard Apparatus Co., Dover, Mass.). In most of the subjects studied, the infusion was continued for 120 min; in some cases, the infusion was terminated after 90 min. The volume infused during the entire period of the study ranged from 9 to 12 ml, including the priming dose. Samples were collected from a vein in the opposite arm at 3, 6, and 10 min after injection of the priming dose, and at 10- to 20-min intervals thereafter until the end of the infusion. Plasma was assayed for TCA-precipitable ^{131}I.

Body surface counting of radioactivity was performed continuously during the infusion by means of two fixed detectors, one over the liver and the other over the mid-thigh.

The exact position of the hepatic detector was determined during the single-injection study as the point giving the maximum counting rate at 10 min after injection. The instrumentation used and the method of calibrating the hepatic detector have been described (9). Scintiscans of the abdomen were made in several cases at the end of the infusion of T_3-^{131}I and in other cases within 30 min after the single injection of the label. In every scan, the highest concentration of ^{131}I was in the liver, which showed a uniform pattern of distribution. The kidneys were visualized in posterior views taken as early as 30 min after injection of T_3-^{131}I, but the apparent renal concentration of label was lower than that in the liver.

Hepatic distribution volume (V_H) (*See Fig. 2*). The following expression was used:

$$V_H = (L_{max} - L_0)/C_P^{(eq)} \times F$$

Where V_H is the hepatic distribution volume for T_3 at equilibrium, in liters, L_{max} is the maximum counting rate over the liver (at distribution-equilibrium), L_0 is the counting rate over the liver at time zero, determined by extrapolation of the intial portion of the heaptic uptake curve; $C_p^{(eq)}$ is the concentration of ^{131}I in plasma at equilibrium, in microcuries per liter; and F is a calibration factor relating counts per minute observed over the liver to microcuries contained within the liver. (See reference 9 for detailed description of the method of calibration.)

The $t = 0$ correction (L_0), although variable from one subject to another, averaged 40% of L_{max}. This correction represents circulating radioactivity both within the liver and in extrahepatic tissues within view of the detector. (See Appendix for a discussion of the validity of this correction.)

Unidirectional hepatic clearance (C_H). Points on the time-curve of hepatic radioactivity, L_t, were subtracted from the maximum value, L_{max}, achieved during the infusion. The resulting data, $L_{max} - L_t$, when plotted against time on semi-logarithmic coordinates, fit a single exponential function for at least two half-times. The slope, λ, of the derived exponential function was used to calculate one-way hepatic clearance, C_H, from the formula: $C_H = V_H \cdot \lambda \cdot 1000$, were C_H is in milliliters per minute, V_H is in liters, and λ is in minutes^{-1}.

Extrahepatic distribution (V_{EH}). The apparent volume of distribution of the equilibrating tracer increases with time after the single injection of T_3-^{131}I. The distribution volume outside the vascular (plasma) compartment, V_{EV}, at any time, t, was calculated from the expression: $V_{EV}(t) = 100/C_P(t) - V_P$, where $C_P(t)$ is the concentration of ^{131}I (TCA-insoluble) in plasma at time t, expressed in per cent of administered dose per liter, and V_P is the plasma volume, in liters. It is assumed that equilibration in the liver is completed within 20 min after the single dose of T_3-^{131}I. This permits calculation of the apparent volume of distribution in extrahepatic tissues (V_{EH}) at any time t beyond 20 min after injection:

$$V_{EH}(t) = V_{EV}(t) - V_H$$

Subjects. As control subjects, 10 male adults were studied. All were patients who had chronic, nondebilitating disease, were euthyroid, gave no history of thyroid disease, and were receiving no drugs known to affect plasma binding of thyroid hormones. Serum total T_4 and free T_4 concentration were normal in every case. All had normal hepatic function by clinical and routine laboratory evaluation.

Four patients with moderately severe hyperthyroidism (diffuse toxic goiter) were studied. All had mild exophthalmos, and one also had pretibial myxedema (subject P.B.). Subject W.P. had been begun on antithyroid medication, methimazole, 10 mg every 8 hr, 10 days previously, but he was still clinically thyrotoxic at the time of the study.

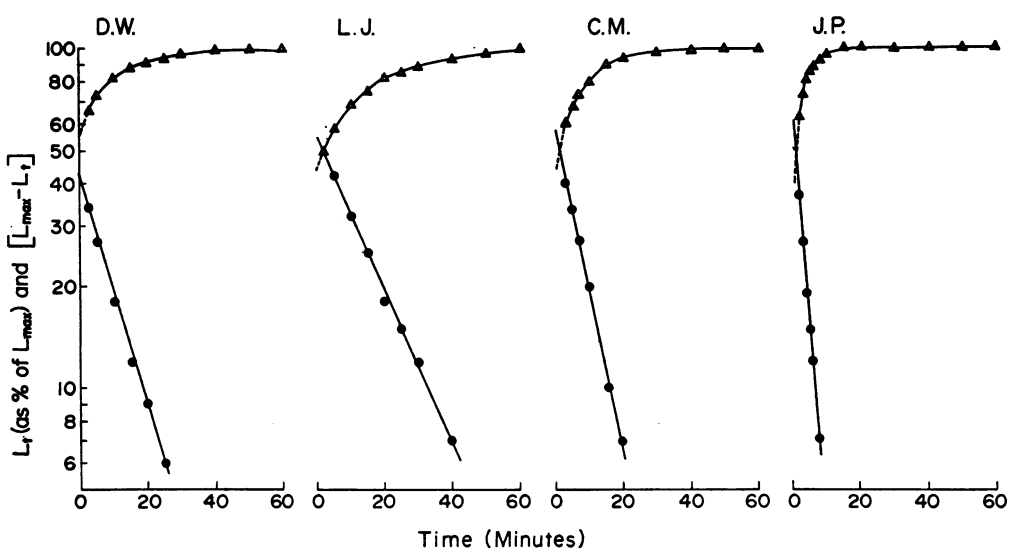

FIGURE 3 The time-course of hepatic radioactivity during the infusion of T_3-^{131}I in three control subjects and in one hyperthyroid patient (J. P.). The points depicting hepatic radioactivity, L_t (triangles), have been normalized to the maximum value, L_{max}, reached during the infusion. The derived values, $L_{max} - L_t$ (solid circles), fit a single exponential function with slope, λ. Extrapolation of this slope to time zero permitted estimation of the background radioactivity which was used in calculation of V_H.

Seven patients were studied who had a history of hyperthyroid Graves' disease. Patient N.M. had received an 18 month course of treatment with propylthiouracil and was euthyroid at the same time of the study, 1 yr after the drug had been discontinued. All other patients had received radioiodine therapy from 6 months (J.P.) to 15 yr (J.V.) previously. Patients H.B. and W.B. were clinically hypothyroid; the other cases in this group were euthyroid. Two of the

TABLE I

Results of In Vitro Determinations of Serum T_4 and In Vivo Studies of T_3 Distribution

		T_4I	FT_4F	V_P	V_H	C_H	$V_{EH}^{(t)}$ 20 min	$V_{EH}^{(t)}$ 60 min	$V_{EH}^{(t)}$ 120 min	$V_{EH}^{(60)}/V_{EH}^{(20)}$
		μg/100 ml	*% of control*	*liters*	*liters*	*ml/min*		*liters*		
Controls										
C. M.		4.4	100	3.20	2.26	224	1.02	3.04	5.27	3.0
L. J.		2.9	98	3.31	2.16	115	2.85	6.33	9.43	2.2
D. W.		5.2	77	3.15	1.75	110	2.29	4.50	7.40	2.0
S. F.		5.3	103	2.43	1.52	265	3.84	7.35	10.40	1.9
E. L.		3.2	129	3.02	2.36	298	4.72	10.52	14.5	2.2
J. H.		3.8	95	3.21	3.15	242	1.87	4.94	7.74	2.6
R. M.		3.9	125	3.05	1.65	261	4.91	8.70	12.5	1.8
W. M.		4.1	118	3.01	2.01	268	3.84	7.88	11.8	2.0
K. C.		5.7	115	3.13	2.30	301	4.45	9.57	15.6	2.1
M. G.		5.2	88	2.10	1.51	224	2.67	5.65	8.59	2.1
Mean		4.4	105	2.96	2.07	231	3.25	6.85	10.32	2.2
±SD		±1.0	±16	±0.36	±0.47	±64	±1.24	±2.26	±3.11	±0.3
Thyrotoxic Graves'										
P. B.		13.8	156	2.90	3.20	888	5.69	11.4	16.7	2.0
J. P.		11.4	172	2.33	3.54	1170	3.93	8.66	12.5	2.2
M. M.		8.6	150	2.30	4.24	840	2.25	5.16	7.86	2.3
W. P.		6.3	162	2.55	3.98	1058	3.85	9.32	14.6	2.4
Mean			160*	2.52	3.74*	989*	3.93	8.64	12.9	2.2
±SD			±8	±0.24	±0.40	±132	±1.22	±2.24	±3.27	±0.1
Treated Graves'										
J. P.		5.6	100	2.10	2.06	508	1.76	4.59	9.96	2.6
N. M.		7.3	81	2.36	2.29	396	2.07	4.77	7.40	2.3
J. V.		4.3	110	2.53	3.53	351	2.19	5.24	8.35	2.4
J. R.		5.4	93	3.15	2.66	392	0.48	3.19	5.36	6.6
M. H.		5.8	102	2.48	1.85	427	3.64	6.57	11.1	1.8
H. B.		3.5	79	1.88	2.02	324	2.14	4.95	7.95	2.3
W. B.		1.5	74	3.15	1.95	387	1.14	4.42	7.72	3.9
Mean			91	2.52	2.34	398*	1.92‡	4.82‡	8.26‡	3.1
±SD			±13	±0.45	±0.55	±54	±0.91	±0.94	±1.71	±1.5
Low TBG	TBG Cap.§									
M. G.	0.046	2.4	140	2.31	2.74	475	3.22	6.64	10.4	2.1
E. L.	0.064	2.2	151	2.10	2.96	494	3.84	7.14	11.0	1.9
J. A.	0.034	2.3	189	2.88	3.88	486	4.90	10.4	15.2	2.1
Mean			160*	2.43	3.19*	485*	3.99	8.06	12.2	2.0
±SD			±21	±0.33	±0.49	±8	±0.69	±1.67	±2.14	±0.1

T_4I = total serum thyroxine iodine; FT_4F = serum free T_4 fraction, in per cent of value obtained in normal serum; V_P = plasma volume; V_H = hepatic T_3 distribution volume at equilibrium; C_H = one-way hepatic clearance; $V_{EH}^{(t)}$ = extrahepatic T_3 distribution volume at time t. (All volumes have been corrected to 1.73 m^2 body-surface area.)

* Significantly different from control mean ($P < 0.01$).

‡ Significantly different from control, thyrotoxic, and low TBG mean values ($P < 0.05$).

§ Binding capacity of serum TBG, in μg T_4I per 100 ml.

latter group, J.V. and M.H., were taking replacement therapy (L-thyroxine). J.P. was studied both before ^{131}I therapy and after he was rendered euthyroid.

The study included three euthyroid male subjects who exhibited a decrease in plasma TBG-binding capacity. One of these cases (M.G.) was a control subject who was restudied after a period of 4 wk on methyltestosterone, 100 mg a day. In the other two individuals, the decrease in TBG was on an idiopathic basis.

Total serum thyroxine iodine was measured chemically (10). The T_4-binding capacity in serum TBG was estimated by paper electrophoresis in ammonium carbonate buffer as described previously (9).

The proportion of free T_4 in serum (free T_4 fraction) was determined indirectly using a method of adsorption by Sephadex[1] described elsewhere (11). This technique provides an estimate of the change in free T_4 fraction relative to a control reference serum; the results are expressed as a per cent of the control value.[2]

RESULTS

The kinetics data obtained in a typical infusion experiment are shown in Fig. 2. A constant ^{131}I level in plasma was reached in all cases as early as 3 min after the administration of the priming dose (time zero) and remained unchanged throughout the period of the infusion. Radioactivity over the liver rose rapidly during the initial 20–40 min reaching a constant level which was maintained during the remainder of the study. The counting rate over the thigh rose more slowly than that over the liver and continued to increase during the period of the infusion.

Analysis of the hepatic curves, in four representative studies, is illustrated in Fig. 3. The linearity of the derived curve, log ($L_{max} - L_t$), extended over at least two, and usually three half-times, justifying the assumption that hepatic T_3 can be considered to be a single compartment in a steady state (See Appendix.)

The values for hepatic distribution volume at equilibrium (V_H) and for one-way hepatic T_3 clearance (C_H) are given in Table I. The hyperthyroid patients showed an elevation in V_H compared to the nonGraves' disease controls and a marked increase in C_H to about four times the mean control value.

V_H was lower, on the average, among the treated Graves' disease patients than in the hyperthyroid patients and was not significantly different from the mean control value for V_H. On the other hand, C_H was, in every case of treated Graves' disease, increased above the normal (control) range, but was not as high as in the hyperthyroid patients.

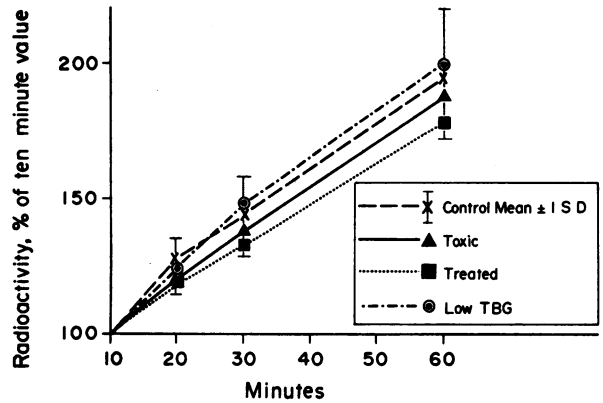

FIGURE 4 The time-course of radioactivity monitored over the thigh during the sustaining infusion of T_3-^{131}I. The counting rate at 20, 30, and 60 min after the start of the infusion was expressed as a per cent of the 10 min value. Only the mean ratios are shown for each group of subjects. In none of the groups is the mean ratio different from that in the controls.

Both V_H and C_H were significantly elevated above the control values in the three individuals with low plasma TBG.

The apparent distribution volumes for T_3 in extrahepatic tissues (V_{EH}) at 20, 60, and 120 min after the single injection of T_3-^{131}I are listed in Table I. Within each group of subjects there was wide variation among individual values for V_{EH} at each time interval. Only in the case of treated Graves' disease were the mean values for V_{EH} significantly different from those of any other group. Among all of the subjects in the study, there is a moderate correlation ($+0.66$, $P < 0.001$) between V_{EH} at 20 min and serum free T_4 fraction. No correlation exists, however, between the free T_4 fraction and the ratio of V_{EH} at 60 min to that at 20 min. The increment in V_{EH} during intervals beyond 20 min was, on the average, not significantly different comparing one group with another (Table I).

Continuous monitoring of radioactivity over the thigh during the sustaining infusion of T_3-^{131}I revealed a rapid rise in counting rate during the initial 3–6 min after injection of the priming dose. This phase presumably represents, for the most part, distribution of the tracer throughout the vascular spaces of the thigh. Subsequently in all subjects, radioactivity in the thigh rose more slowly and at nearly a constant rate for approximately 20–30 min, increasing somewhat more gradually during the remainder of the period of infusion. The relative change in thigh "uptake" was expressed as a per cent of the 10-min counting rate. Since the plasma concentration of tracer remained constant

[1] Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

[2] For the purpose of the present study, the serum free T_4 fraction is taken as an estimate of the relative proportion of total T_3 which is not bound to plasma protein. The validity of this approach is supported by data indicating a high correlation ($+0.93$) between the serum free T_4 fraction, determined by the present Sephadex method, and the per cent free T_3, by equilibrium dialysis, in samples of serum from patients over a wide range of thyroid function (unpublished observations).

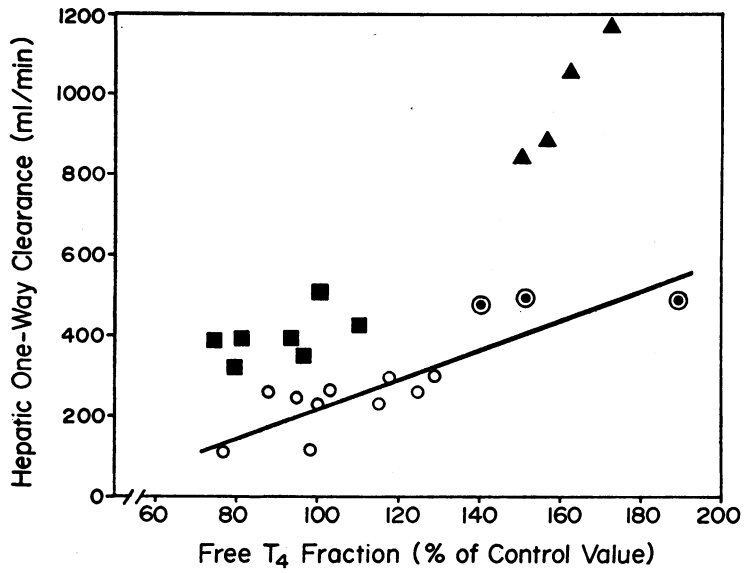

FIGURE 5 A comparison between the unidirectional hepatic clearance of T_3 (C_H) and the free T_4 fraction in serum in 10 control subjects (open circles), 3 euthyroid individuals with decreased plasma TBG (filled circles), 4 hyperthyroid Graves' disease patients (solid triangles), and 7 patients with treated Graves' disease (solid squares). The serum free T_4 fraction, determined by the Sephadex uptake of labeled T_4, is expressed as a per cent of the result in a normal reference serum. The regression line was derived by the method of least squares for values obtained only in the 13 subjects without Graves' disease.

during the study, no correction was made for circulating label. Fig. 4 shows, for each of the groups of subjects, the average thigh ratios obtained. In the controls the mean values were 1.26 ±0.10 SD, 1.44 ±0.14, and 1.97 ±0.24 at 20, 30, and 60 min, respectively. In none of the other groups did the mean ratio differ significantly from the control value. Extension of the thigh "uptake" beyond 60 min revealed no significant differences among the various groups (not shown in Fig. 4).

In an effort to determine whether the fractional rate of transfer of T_3 from plasma to liver is limited by the availability of free hormone in plasma, we compared the individual values for C_H with those for free T_4 fraction. As shown in Fig. 5, a high degree of correlation was found among the subjects without Graves' disease ($r = +0.86$; $P < 0.01$). In contrast, the patients with Graves' disease, both treated and untreated, showed values for C_H which were higher than predicted from the free hormone fraction.

DISCUSSION

The results of the present study permit a comparison of T_3 and T_4 in regard to distribution in man. The hepatic distribution volume (V_H) for T_3 is about one-half that for T_4, determined in our previous study in a different but comparable group of control subjects (9). Given the nearly 10-fold greater binding activity by plasma proteins of T_4 compared with T_3 (12), the observed difference in V_H between the two hormones implies that the liver binds T_4 nearly 20 times more strongly than T_3. The difference between T_4 and T_3 can be stated also in terms of the proportion of total-body hormone which is in the liver after equilibration of tracer is complete. Using average values from the literature (13), the total T_3 space in a 70 kg man is about 40 liters, nearly 3.5 times the T_4 space. The liver contains at equilibrium approximately 30% of the total T_4 outside of the thyroid gland (9). In contrast, according to the results of the present study, only about 5% (2 liters/40 liters) of the total T_3 pool is within the liver. Thus, the hepatic content of T_3 is much less than that of T_4 both in absolute terms and as a fraction of the total-body hormone.

These findings in man agree, in general, with the observations in several species of animals. Thus in the rat (14), the dog (15), and the rabbit (16) at early time intervals after injection of either labeled hormone, the liver takes up approximately 25% of the dose. In the case of T_4, the liver retains a large fraction of the dose; most of the remainder is in extracellular fluid (15). Hepatic content of labeled T_3, on the other hand, declines more rapidly with time as the tracer accumulates

in cells of extrahepatic tissues, for the most part, skeletal muscle (16).

In our control subjects the one-way clearance (C_H) of T_3, a measure of the fractional rate of transfer from plasma to liver, averaged 231 ml of plasma per min which is nearly 5 times greater than the corresponding value for T_4 (9). This finding of a difference between the two hormones in respect to hepatic clearance is consistent with the known difference in binding activity of plasma proteins and supports the thesis that the entry of the hormone into the liver is limited to the free hormone. Further evidence for this view is provided by the observations made in the present study of euthyroid individuals with decreased binding by plasma TBG. Among these subjects, the increase in C_H above control levels was directly proportional to the decrease in overall binding of thyroid hormone by plasma proteins (Fig. 5).

In spite of the relatively low hepatic space for T_3, the one-way clearance by liver is slightly higher than the total unidirectional clearance by all other tissues combined. Assuming a linear rate of change in V_{EH} during the initial 20 min after the single injection of labeled T_3, the one-way clearance by extrahepatic tissues is approximately 160 ml/min (3.25 liters/20 min) in our control subjects. The liver, therefore, receives about one out of every two molecules of T_3 that leave the circulation. It must be emphasized that this process is *not* net (metabolic) clearance. In man the total-body metabolic clearance rate of T_3 is approximately 15 ml/min (13), which is less than one-tenth the one-way hepatic clearance obtained in the control subjects of the present study. Therefore, more than nine-tenths of the T_3 which enters the liver per unit time must return to the plasma in unaltered form.

Analysis of the distribution kinetics data for extrahepatic tissues presents a more complex problem than in the case of the liver. The increase in the apparent distribution volume in extrahepatic tissues, V_{EH}, as a function of time after the single-injection of T_3-^{131}I reflects several processes which probably occur simultaneously. These include diffusion of tracer out of the plasma, equilibration in the interstitial compartment, and penetration into cells. Interpretation of the kinetics data is complicated further by the likely possibility that extrahepatic tissues are heterogeneous with regard to the rate of equilibration. Studies in experimental animals have shown that organs differ markedly in the rate of uptake of labeled T_3 after intravenous injection of the tracer (14–16). In general, liver and kidney take up the label rapidly; most other tissues, including skeletal muscle, equilibrate slowly. The results of the present study demonstrate in man a similar difference between liver, and perhaps kidneys, on the one hand, and the thigh on the other, with respect to the rate of equilibration of labeled T_3.

The moderate degree of correlation (+ 0.66) between the values of the serum hormone fraction and those of V_{EH} at 20 min after the single injection suggests that the level of free T_3 in the circulation is an important determinant of the rate of diffusion of T_3 into rapidly equilibrating extrahepatic tissues. The absence of a correlation between the free hormone fraction and increments in V_{EH} during later intervals could be explained by postulating that the rate of diffusion of T_3 into slowly equilibrating tissues is relatively insensitive to alterations in plasma binding activity. The data regarding the thigh accumulation during the infusion of labeled T_3 are consistent with this formulation. In view of the complexity of the kinetics in extrahepatic tissues, the above interpretation is offered only as a tentative hypothesis.

The dichotomy with regard to the distribution kinetics of T_3 between rapidly equilibrating sites (e.g., liver) and relatively slow equilibrating tissues (e.g., thigh) provides a possible explanation for the observations of Zaninovich, Farach, Ezrin, and Volpé (17). They reported that the rate of disappearance of labeled T_3 from the plasma during the interval from 20 to 50 min after rapid injection of the tracer into humans was not affected by alterations in the plasma TBG, even though such alterations are known to result in large changes in the proportion of free T_3 in vitro. On the basis of their studies these workers suggested that T_3 is not bound to any appreciable extent to TBG in vivo. The results of the present study, on the other hand, by demonstrating that the rate of entry of T_3 into liver is a function of the binding activity of plasma TBG, point to a significant in vivo role for TBG. Since equilibration of tracer between plasma and liver is virtually completed within 20 min after a single injection of labeled T_3, the decrement in plasma content of label between 20 and 50 min, the period studied by Zaninovich et al., probably reflects distribution of T_3 into extrahepatic tissues, a process which we postulate to be insensitive to alterations in plasma binding of the hormone. A similar explanation for the findings of Zaninovich et al. has recently been offered by Musa, Kumar, and Dowling (18).

Recently Woeber, Hecker, and Ingbar (19) have obtained more direct evidence for *in vivo* binding of T_3 by plasma proteins. After injection of a large loading dose of T_4 into normal subjects, they observed a significant increase in the volume of distribution of a tracer dose of labeled T_3. The increment in the T_3 space was evident within 20 min after administration of the tracer and persisted throughout the period of equilibration in the body. The authors concluded that the T_4

load, by displacing T_3 from extracellular binding sites, caused a redistribution of the latter hormone, probably into the liver. Their findings, together with the results of the present study, offer strong evidence for significant binding of T_3 by TBG in vivo.

In a number of studies devoted to the late phase of T_3 kinetics, after tracer equilibration is complete, a decrease in binding activity by TBG has been associated with a decreased fractional rate of T_3 turnover (20–22). These findings, being directly opposite to the alterations observed in the case of T_4, have been offered as further evidence that TBG is of little or no significance in the transport of T_3 in vivo (22). An alternative interpretation, however, can be given. When binding activity of TBG is diminished, T_4 is redistributed from extracellular to intracellular sites (23) particularly into the liver (9). This shift in T_4 occurs both in subjects with a primary decrease in TBG activity (9, 23) and in normal subjects given an acute loading dose of T_4 (24). An increase in hepatic T_4 content under these circumstances might compete effectively with hepatic T_3 for metabolic disposal even in the face of enhanced hepatic uptake of T_3. The result would be a decrease in the fractional turnover (disposal) rate of T_3. Thus, the apparently contradictory effects of diminished TBG binding activity on the fractional turnover rates of T_4 and T_3 can be explained on the basis of shifts in T_4 distribution with the attendant effects on T_3 metabolism. There is then no need to discard the idea that TBG binds both hormones in vivo.

The kinetics of T_3 distribution among the subjects with Graves' disease, treated and untreated, appear to be altered in a manner not explicable simply on the basis of known shifts in binding equilibrium. Thus, the increases in C_H observed in both the hyperthyroid and treated patients were out of proportion to the increase in the percentage of free hormone (Fig. 5). Hepatic blood flow has been measured by the Bromsulphalein method in patients with hyperthyroidism and found to be not significantly different from that in euthyroid individuals, 879 ml of blood per min per m^2 body-surface area in patients vs. 812 in controls (25). This average blood flow corresponds to an hepatic plasma flow of about 900 ml/min per 1.73 m^2. Thus, the average unidirectional hepatic clearance of T_3, in our patients with hyperthyroid Graves' disease, nearly equals hepatic plasma flow. This suggests that the rate of entry of T_3 into the liver from plasma may be limited, in these cases, by hepatic blood flow rather than by the level of free hormone in the circulation. This somewhat surprising conclusion requires confirmation by further study, such as simultaneous measurements in the same subjects of T_3 distribution kinetics and hepatic blood flow.

Other explanations might be considered for the disproportionately high unidirectional hepatic clearance of T_3 observed in patients with Graves' disease. One possibility is that the rate of entry of T_3 into the liver in normal individuals might be limited by the rate of dissociation of the hormone from its binding sites on plasma proteins. One might expect the rate of debinding to be abnormally rapid in patients with hyperthyroidism since, in the latter case, a greater than normal proportion of circulating T_3 is associated with secondary binding proteins, i.e., albumin. This would not explain, however, the observed difference in C_H between the hyperthyroid patients and the individuals with decreased plasma TBG, nor would it explain the small but significant increase above control values of C_H in treated cases of Graves' disease. Furthermore, attempts at measurement of debinding rates for T_4 have indicated that this parameter is probably not of physiologic importance at normal rates of tissue perfusion (26).

Both in treated and untreated patients with Graves' disease the unidirectional hepatic clearance of T_3 was elevated out of proportion to the circulating free hormone fraction. This suggests that an abnormality may exist in the liver itself in this disorder. One possibility is that the hepatic cell membrane is altered in such a way as to permit an increased rate of exchange of the hormone between the plasma and intracellular binding sites. Although there is no evidence for its existence, an active transport process governing the entry of thyroid hormones into the liver might explain these results. Such a process could be abnormally active in Graves' disease. Whatever explanation is invoked, the persistence of the abnormality for many years after treatment of hyperthyroidism must be taken into account.

Recent measurements of total T_3 concentration in plasma indicate that abnormally high levels of this hormone may be found in some patients with this disease after their hyperthyroidism has been controlled (2). Whether this phenomenon is related to the abnormal distribution kinetics of T_3 observed in the present study remains to be determined.

Nearly a decade ago, Hales and Dobyns (27) reported studies of T_3 metabolism in Graves' disease. The disappearance of label from the plasma during the initial hours after a single injection of T_3-^{131}I was delayed and the radioactivity over the liver declined more abruptly in patients with Graves' disease, both in hyperthyroid and in treated cases, compared to normal controls. These authors suggested that the liver might play a special role in the peripheral distribution and metabolism of T_3 in Graves' disease. Since they presented no data obtained during the initial 60 min after injection of the labeled T_3 and since their subjects received propylthiouracil during the study, it would be difficult to compare the findings of Hales and Dobyns with the results of the present study. Nicoloff and Dowling (28) have demonstrated an increase in hepatic

space (and content) of T_4 both in untreated Graves' disease and in some patients who have been rendered euthyroid. The latter findings are in accord with the earlier observations of Ingbar and Freinkel (29), who noted an increase in the fractional turnover rate of T_4 in some patients long after successful treatment of their hyperthyroidism associated with diffuse toxic goiter. The weight of evidence, including the present observations with regard to the kinetics of T_3 distribution, supports the view that an abnormality in the peripheral distribution and metabolism of thyroid hormones is a feature of Graves' disease.

APPENDIX

The method employed for estimating the hepatic distribution volume, V_H, rests on two important assumptions. First, it is assumed that the phantom used for calibration of the hepatic detector closely simulates in vivo conditions. The scintiscans made at the end of the infusion of T_3-^{131}I showed a uniform distribution of ^{131}I within the liver; the hepatic image in every case corresponded reasonably well to the size and shape of the phantom.

The second major assumption concerns the correction for "background," defined as the radioactivity in vascular spaces and in extrahepatic tissue within "view" of the hepatic detector. In the calculation of V_H it is assumed that the background (L_0) remains constant during the period of hepatic equilibration, i.e., the initial 30–40 min of the infusion. This assumption is supported by the observed constancy in counting rate over the liver after hepatic equilibration is achieved (Figs. 2 and 3) and by the constant level of tracer in the plasma during the entire period of the infusion. In order to test the accuracy of the background correction, quantitative scintiscans were done in five of the subjects of this study. The scanning method was virtually identical to that employed by Nicoloff and Dowling for estimating hepatic T_4 content in man (28), except that the scans were made at the end of the infusion of T_3-^{131}I. The plastic phantom of the liver was used as a standard. Estimates of V_H by the quantitative scanning method ranged from −15 to +12% of the values obtained by the fixed detector method (average difference = −3%). Thus, the reasonably good agreement between the two methods of estimating V_H for T_3 tends to support the validity of the second assumption.

The following additional assumptions underlie the method of estimating V_H and C_H: metabolic transformation of labeled T_3 during the 2-hr period of infusion is negligible. A steady state exists during the same interval, i.e., the rate of entry of unlabeled T_3 into the liver equals the rate of loss from the liver by all routes. The concentration of labeled T_3 in the plasma of the antecubital veins (site of sampling) is the same as that in the plasma entering the liver, the level of tracer in both sites being constant during the sustaining infusion. Finally, single-compartment kinetics are assumed for hepatic T_3. This latter assumption appears justified in view of the single-exponential character of the hepatic accumulation curve in all subjects.

In a recent study of thyroid hormone distribution in man, Musa et al. (18), using a single injection of labeled T_3 and a method of external monitoring similar to ours, estimated hepatic T_3 distribution volume, uncorrected for background, in five normal subjects. They obtained a mean value of 3.6 liters ±0.97 SD. Applying our average correction for background (L_0/L_{max}) to their data yields a value for V_H of 2.2 liters. This result agrees well with the mean V_H of 2.07 liters for the control subjects.

Until more direct measurements of hepatic T_3 distribution volume and unidirectional clearance rate are made, the absolute values obtained in this study must be regarded as tentative. Nevertheless, the conclusions based on comparisons among the different groups of subjects would appear to be valid.

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