Journal of Clinical Investigation
Vol. 45, No. 6, 1966

The Kinetics of Distribution between Plasma and Liver of ^{131}I-labeled L-Thyroxine in Man: Observations of Subjects with Normal and Decreased Serum Thyroxine-binding Globulin *

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All but a few studies concerned with the peripheral metabolism of labeled thyroxine [1] in man have ignored the early phase of equilibration of labeled with unlabeled hormone in the tissues (see review in reference 1). Lennon, Engbring, and Engstrom (2) measured the rate of disappearance of radioactivity from the blood during the initial 50 minutes after intravenous injection of T_4-^{131}I in humans. They found that the rate of decline in the level of circulating tracer was abnormally slow in patients with hepatic disease and in untreated cases of hypothyroidism and abnormally rapid in thyrotoxic individuals. Haddad (3) analyzed the plasma curve in normal subjects and fit the data to a two compartment kinetics model representing plasma and tissue pools of thyroxine. More recently, Blomstedt and Plantin (4), using only the time curve of plasma disappearance and the rate of excretion of tracer, proposed a four compartment system to describe the distribution and metabolism of labeled T_4 in normal humans. Hepatic localization of radioactivity within hours after the intravenous administration of labeled T_4 was observed in several studies in man (5–9).

The purpose of the present investigation was to study in a quantitative manner the rate of exchange of labeled T_4 between plasma and liver in humans with neither thyroid nor hepatic disease. The method involves body sector counting with externally placed detectors and simultaneous measurement of the plasma concentration of protein-bound ^{131}I. Analysis of the data is based upon a two compartment kinetics model in which no restrictions are placed on the behavior of the plasma compartment.

In the course of this study we encountered two unrelated adult males in whose plasma T_4 binding by TBG was lacking, apparently on an idiopathic basis. These individuals offered an unusual opportunity to study the effects of alterations in plasma T_4 binding on the distribution of labeled T_4 *in vivo*. The results of the kinetics study and *in vitro* measurements of serum free T_4 are presented in these two patients and in nine control subjects with normal levels of TBG. Portions of this work have already appeared in preliminary form (10).

Methods

The specific activity of sodium L-thyroxine,[2] labeled with ^{131}I in the 3′ and 5′ positions, varied from 22 to 53 mc per mg thyroxine. Contamination with iodide-^{131}I never exceeded 6% of total ^{131}I, as determined by precipitation with TCA in the presence of normal human serum. Each lot of labeled T_4 was used within 2 weeks after it was received.

In vitro studies

T_4-binding capacity of serum TBG and TBPA was estimated by a modification (11) of the method of Robbins (12). Reverse-flow paper electrophoresis in 0.1-M ammonium carbonate buffer, pH 8.4, was performed of mixtures of serum and T_4-^{131}I at the following levels of added, unlabeled T_4: 1.00 and 4.50 μg per ml serum. TBG capacity was determined at the lower T_4 concentration and TBPA capacity at the higher level of added T_4. In each case, duplicate results varied by less than 7%.

Serum T_4 iodine concentration was measured[3] by a method of column chromatography and the ceric-arsenite reaction (13).

* Submitted for publication November 30, 1965; accepted March 2, 1966.

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[1] Abbreviations used in the text: T_4, thyroxine; TBG, thyroxine-binding globulin; TBPA, thyroxine-binding prealbumin; TCA, trichloroacetic acid.

[2] Abbott Laboratories, North Chicago, Ill.

[3] By Bio-Science Laboratories, Los Angeles, Calif.

The proportion of free T_4 in serum was estimated by the method of Lee, Henry, and Golub (14). Five-tenths μc L-T_4-^{131}I (0.015 μg) was added to 1 ml serum. The mixture was allowed to equilibrate at room temperature for 1 hour. The protein-bound and free fractions of T_4-^{131}I were separated by passing the mixture through a column of Sephadex (G-25, fine). Protein-bound radioiodine was eluted with 0.15-M sodium chloride after which the free fraction was eluted with 0.1-M sodium hydroxide. The proportion of free T_4 in serum, in per cent of total T_4, was computed from the ^{131}I content of free and bound fractions. The mean variation between duplicate determinations was 9%. Replicate determinations of per cent free T_4 done several weeks apart on a pool of normal serum yielded the following values: 0.044, 0.043, and 0.045. The concentration of free T_4, in millimicrograms T_4 iodine per 100 ml serum, was calculated from the per cent of free T_4 and the level of T_4 iodine.

In vivo studies

Before injection of tracer, 20 ml of venous blood was collected for determination of serum T_4 iodine, thyroxine-binding capacity, and free T_4. An additional 20 ml was transferred to a sterile vial containing heparin. To this blood was added 90 to 100 μc T_4-^{131}I (less than 3 μg). Exactly 17 ml of the blood containing the tracer was injected during a period of 1 minute into an antecubital vein. The remaining 3 ml was used as a counting standard. An indwelling polyethylene catheter was inserted into an antecubital vein in the opposite arm. Ten-ml samples of blood were withdrawn from the catheter at intervals of 5 or 10 minutes, from 10 to 90 minutes after injection of the tracer. Subsequent samples of blood were collected by venipuncture at 2, 4, and 6 hours, and thereafter at 24-hour intervals for 10 to 14 days. The samples were immediately transferred to tubes containing heparin. Plasma was separated by centrifugation. An aliquot was treated with 4 vol of 10% TCA, and the precipitate was washed once with 8 ml of TCA. The final precipitate was resuspended and assayed for radioactivity.

A sample of the administered dose of blood containing labeled T_4 was diluted in pooled normal plasma, and the proteins were precipitated with TCA. Calculation of the total T_4-^{131}I injected was based on the TCA-insoluble ^{131}I content of the blood and the volume of blood injected.[4]

Complete collections of urine were made during the initial 6 hours and from 6 to 24 hours after injection. Total ^{131}I in the urine was measured and expressed as a per cent of the dose. All subjects were given 200 mg iodine per day in the form of Lugol's solution throughout the study in order to minimize thyroidal uptake of ^{131}I.

[4] Experiments were done to determine the proportion of T_4-^{131}I bound to erythrocytes *in vivo*. At 1, 4, and 24 hours after injection of the tracer, less than 2% of total protein-bound ^{131}I in whole blood is associated with cells (unwashed) after correction of the hematocrit for trapped plasma. The amount of tracer bound to red cells was, therefore, neglected.

Method of external counting over the liver

Monitoring of radioactivity in the region of the liver was done with a 2- × 2-inch scintillation detector fitted with a single-hole lead collimator. Signals from the detector were fed into a rate meter and a linear chart recorder running at a speed of 1.5 cm per minute. The face of the collimator was placed in contact with the subject at a point in the anterior axillary line at the level of the xiphoid process. The axis of the detector was at 45° above the horizontal and 110° from the long axis of the patient in a caudal direction. Five minutes after injection of the tracer final adjustments were made in the position of the detector in order to obtain a maximal counting rate. The skin was marked to permit accurate placement for subsequent counts.

Calibration of the detector

The field of the collimated detector was determined with a point source of ^{131}I in a water-filled phantom, approximately the size of a human trunk. The pattern of isoresponse lines so obtained was superimposed upon a scale drawing of a cross section through the human body at the level of the xiphoid process. It was apparent that the field of the detector includes a large portion of the liver and little extrahepatic tissue. (The counting rate at a depth of 16 cm along the axis of the detector was only 3% of the counting rate obtained with the source at the face of the collimator.)

Calibration of the detector was accomplished with a plastic model[5] consisting of a hollow vessel the size and shape of a human liver suspended within a larger cylindrical vessel with the contours of a human abdomen. The "liver" was filled with water containing a known quantity of ^{131}I, and the outer compartment was filled with water containing no radioactivity. At a standard "skin-to-liver" distance of 2.5 cm, the counting rate was determined and a calibration factor, F, in counts per minute per microcurie in the liver, was calculated. Variation in the depth of the "liver" beneath the "skin" resulted in a 6.8% change in counting rate per cm displacement.

Subjects

As control subjects, nine patients were selected from the Medical Service. All were adult males with chronic diseases not involving the liver or thyroid. None of the subjects were acutely ill at the time of the study. All were euthyroid by clinical assessment and according to the results of thyroid function tests, including serum protein-bound iodine and/or serum T_4 iodine level, triiodothyronine-^{131}I erythrocyte uptake, and in some cases thyroid radioiodine uptake determinations. None of the control subjects received drugs known to affect thyroxine binding.

Case reports

Patient J.G., a 43-year-old Caucasian male, was referred for evaluation of thyroid function because of symptoms of

[5] Organ-scanning Phantom, Alderson Research Laboratories, Long Island City, N. Y.

depression and fatigue and a low serum protein-bound iodine (PBI). He had been under psychiatric care for episodes of depression occurring over a period of years. There was chronic fatigue, but there were no other symptoms of hypothyroidism. He had taken various sedatives, mostly barbiturates, and Parnate (tranylcypromine), but he denied taking hormones or drugs known to affect serum thyroxine binding. Blood pressure was 130/80, pulse rate, 68. Skin and hair were normal. Thyroid gland was not enlarged. The remainder of the physical examination was negative. By clinical assessment he was euthyroid. The ^{131}I thyroid uptake at 2 hours was 6% (normal 5 to 12) and at 24 hours was 13% (normal 10 to 35). The basal metabolic rate was −11. The serum cholesterol was 170 mg per 100 ml. The triiodothyronine-^{131}I red cell uptake was 48% in 2 hours (normal 15 to 25). Serum PBI on two occasions was 1.5 and 2.2 μg per 100 ml. The following determinations were normal: hemoglobin, leukocyte count, and urine analysis. The total serum protein was 7.3 g per 100 ml, and the electrophoretic pattern was normal.

Patient T.R., a 76-year-old Serbian-born white male, was admitted to the hospital for elective repair of bilateral inguinal hernias. Except for a recent 10-pound weight loss and mild symptoms referable to the hernias, he had no complaints. He denied symptoms of hypo- and hyperthyroidism. The past medical history was negative. He had not taken drugs of any kind. Blood pressure was 170/90, pulse rate was 100 and regular, and weight was 67 kg. The thyroid gland was not enlarged. There was a soft systolic murmur at the apex. Inguinal hernias were easily reduced. The remainder of the physical examination was negative. Routine laboratory data, including hemoglobin, leukocyte count, fasting blood glucose, and urine analysis, were normal. An electrocardiogram revealed sinus tachycardia. Thyroid function tests were done because of the history of weight loss and mild tachycardia. The ^{131}I thyroid uptake at 2 hours was 5% and at 24 hours, 9%. The basal metabolic rate was +18. The triiodothyronine-^{131}I red cell uptake was 41%. The serum PBI ranged from 1.2 to 1.6 μg per 100 ml on five determinations done over a period of 6 months. The patient underwent a bilateral herniorrhaphy without complications. The T_4-^{131}I kinetics study was done 6 months after the surgery at a time when he was asymptomatic. He has been followed as an out-patient for the past 18 months and has been clinically euthyroid according to several examiners. In neither this patient nor in J.G. were living relatives available for study.

Analysis of data

Correction of the time curve of hepatic radioactivity for tracer in plasma. The counting rate over the liver at any time after injection of tracer is a function of the quantity of label in the extravascular space of the liver and the amount in the blood contained in that portion of the liver being monitored, neglecting any contribution made by radioactivity in nonhepatic tissues within the collimated field of the detector.

$$L(t) = [C_L(t)v_L + C_P(t)v_P]DE, \qquad [1]$$

where L(t) is the observed counting rate, in counts per minute, recorded by the hepatic detector at time t, $C_L(t)$ is the concentration of tracer in the extravascular space of the liver, in fraction of the injected dose per gram of liver, at time t, v_L is the virtual mass of liver, in grams, within the collimated field of the detector, $C_P(t)$ is the concentration of label in the plasma, in fraction of the dose per liter of plasma, at time t, v_P is the virtual volume of plasma, in liters, within the collimated field, D is the injected dose, in microcuries, and E is the over-all detection efficiency in counts per minute per microcurie, which is assumed to be identical for label in liver and in the plasma within the collimated field.

If one assumes instantaneous mixing of tracer within the vascular compartment at the time of injection,

$$L(o) = C_P(o)v_PDE, \qquad [2]$$

where L(o) and $C_P(o)$ are estimated from the respective time curves for L(t) and $C_P(t)$ by extrapolation of the initial (10 to 60 minutes) portion to t = 0 (see Figure 1).

If one substitutes for v_PDE from Equation 2 in Equation 1 and rearranges,

$$C_L(t)v_LDE = L(t) - [C_P(t)/C_P(o)]L(o). \qquad [3]$$

Let $C_L(t)v_LDE = q_L(t)$, the hepatic counting rate at time t, corrected for radioactivity in the plasma. Let $C_P(t)/C_P(o) = Q_P(t)$, the fraction of the injected dose in the entire plasma, since it is assumed that at time zero the entire dose is in the vascular (plasma) compartment. Then, Equation 3 becomes:

$$q_L(t) = L(t) - Q_P(t)L(o). \qquad [3a]$$

Note that $q_L(t)$ is directly proportional to $C_L(t)$, since v_L, D, and E are constant with time in any given subject. Values of q_L from t = 0 to 6 hours were computed from L(t), L(o), $C_P(t)$, and $C_P(o)$ (see Figure 1).

Kinetic analysis. Figure 2 shows the kinetics model upon which the analysis is based. Both labeled and unlabeled (endogenous) T_4 enter the system by way of the plasma (compartment 1). ρ_{in} represents the steady state rate of endogenous hormone secretion into the plasma. T_4 in compartment 1 exchanges with that in the extravascular space of the liver (compartment 2). One way transfer rate constants are given as λ_{21} and λ_{12}. T_4 leaves the system from compartment 2 by a number of metabolic and secretory processes, all represented by λ_{02}. In addition to exchange with T_4 in the liver, compartment 1 exchanges with one or more extrahepatic tissue sites, all indicated in the model by compartment 3. The kinetics of the extrahepatic tissue compartments are not considered in the present analysis.

If one assumes instantaneous mixing of tracer in compartment 1 at the time of injection, the differential equation describing the time course of tracer in compartment 2 is:

$$V_2dC_2(t)/dt = \lambda_{21}C_1(t)V_1 - \lambda_{22}C_2(t)V_2, \qquad [4]$$

where $C_1(t)$ and $C_2(t)$ are the concentrations at time t of tracer in compartments 1 and 2, respectively, and V_1 and V_2 are the volumes of the compartments. λ_{21} is the

fraction of compartment 1 entering compartment 2 per unit time, and λ_{22} is the fraction of compartment 2 leaving that compartment per unit time by all routes ($\lambda_{22} = \lambda_{12} + \lambda_{02}$).

If both sides of Equation 4 are integrated,

$$V_2C_2(t) = \lambda_{21}V_1 \int_0^t C_1dt - \lambda_{22} V_2 \int_0^t C_2dt.$$

If both sides are divided by $V_1 \int_0^t C_1dt$,

$$[V_2C_2(t)]/(V_1 \int C_1dt) = \lambda_{21} - \lambda_{22}(V_2 \int C_2dt)/(V_1 \int C_1dt). \quad [5]$$

If $Q_P(t) = V_1C_1(t)$ and $q_L(t) = k \cdot V_2C_2(t)$, where k is a proportionality constant, Equation 5 may be written in terms of $Q_P(t)$ and $q_L(t)$:

$$q_L(t)/\int Q_Pdt = \lambda_{21} \cdot k - \lambda_{22}(\int q_Ldt/\int Q_Pdt). \quad [5a]$$

According to Equation 5a, a linear plot of $q_L(t)/\int Q_Pdt$ on the y axis against $\int q_Ldt/\int Q_Pdt$ on the x axis will yield a straight line with a y intercept of $\lambda_{21} \cdot k$ and a slope of $-\lambda_{22}$. It is emphasized that this method of analysis involves no assumptions regarding the number and kinetic behavior of extrahepatic compartments, that is, no restrictions have been placed on the time course of tracer in the plasma compartment.

The experimentally determined time curves of $q_L(t)$ and $Q_P(t)$ were integrated by planimetry at 10-minute intervals from 10 to 120 minutes in each subject. The values of $q_L(t)/\int Q_Pdt$ and $\int q_Ldt/\int Q_Pdt$ were computed at each 10-minute interval and plotted as a linear function (see Figure 3). In every case the points approximated a straight line. The value of λ_{22} was obtained from the slope of the line. (See Appendix for an alternative method of analysis.)

Estimation of hepatic T_4 space, H, and pool size, S_L. The hepatic T_4 space is defined as the volume of distribution of T_4 in the extravascular liver, referred to the concentration of T_4 in plasma. The space is estimated from the formula: $H = [q_L(eq)]/[C_P(eq)DF]$, where H is in liters, D is the injected dose in microcuries, F is the calibration factor, in counts per minute per microcurie in the liver (see Methods), and $q_L(eq)$ and $C_P(eq)$ are values of $q_L(t)$ and $C_P(t)$ at a time eq after distribution equilibrium of tracer. In each subject the ratio $q_L(t)/C_P(t)$ was computed at 4, 6, and 24 hours. In six controls and in J.G. and T. R. the ratios both at 4 and at 6 hours were within 6% of the ratio at 24 hours. In these subjects, therefore, distribution equilibrium between plasma and liver was nearly reached by 4 hours. In the remaining three controls, values obtained at 6 hours, when the ratio was within 5% of the final ratio, were used in the calculation of H.[6]

The hepatic pool size, S_L, in micrograms T_4 iodine, was calculated as the product of the hepatic space, in liters, and the serum concentration of T_4 iodine.

The total plasma volume, V_P, was obtained from the reciprocal of the value of $C_P(o)$. The plasma pool size, S_P, was derived from $V_P \times$ serum level of T_4 iodine.

The hepatic T_4 clearance (one way), C_H, was calculated from the product, $H \times \lambda_{22}$. The plasma to liver T_4 flux, ρ_{21}, was obtained from $S_L \times \lambda_{22}$.

The total extrathyroidal T_4 distribution volume, V_T, was estimated from the intercept at time zero of the final slope of the plasma time curve (from days 2 to 14). S_T, the total extrathyroidal T_4 iodine pool, was obtained from $V_T \times$ serum concentration of T_4 iodine. The daily T_4 disposal rate, ρ_{OT}, was calculated from $S_T \times 0.693/$final $t_{\frac{1}{2}}$ of plasma curve.

Results

In vitro studies. Table I presents the pertinent data for each of the control subjects and for J.G. and T.R. Among the controls the concentration of serum T_4 iodine varied from 3.1 to 5.3 μg per 100 ml. The normal range by this method is 3.2 to 6.4 (13). In J.G. serum T_4 iodine was determined on days 1 and 14 of the kinetics study, and identical results were obtained, 2.1 μg per 100 ml. In T.R. the level of T_4 iodine was 1.5 μg per 100 ml on the first day of the study.

Thyroxine-binding capacity of serum TBG was too low to measure in J.G. and T.R. even at trace levels of added T_4. In the controls TBG capacity ranged from 11 to 23 μg T_4 iodine per 100 ml (mean 17). This range is similar to that obtained by this method in a series of normal individuals (11). TBPA capacity varied from 57 to 237 μg per 100 ml in the controls and was within these limits in J.G. and T.R. The variation in TBPA capacity is wider than that previously reported for normal subjects (11).

Lee, Henry, and Golub (14), using the method of gel filtration, obtained values for per cent free T_4 in healthy subjects ranging from 0.026 to 0.074, which is slightly narrower than that found in the present study, 0.018 to 0.086.[7]

[6] The estimation of H involves several potential sources of error: *a*) Variations in position and size of the liver from one individual to another. *b*) Uneven distribution of tracer within the liver. Scintillation scans of the liver 4 hours after administration of T_4-^{131}I show uniform distribution of radioactivity. *c*) Labeled iodide released *in vivo* from T_4-^{131}I. From the level of TCA-soluble ^{131}I in plasma and the diffusion space for iodide in liver, the error in H from this source is less than 1%. *d*) Radioactivity in extrahepatic tissues in the field of the detector, especially the chest wall. During the initial 4 hours the counting rate over the thigh was found to decrease at nearly the same rate as the level of tracer in plasma. If one assumes that the tissues of the chest wall and the thigh exhibit similar kinetics of T_4 distribution, then the error from this source may be neglected.

[7] The wide variation in the level of TBPA and in per cent free T_4 is probably due to the presence of chronic

TABLE I

*Results of in vitro measurements of serum T_4 iodine, T_4-binding capacity, and free T_4**

Subject	Age	Diagnosis	Serum T_4 iodine	T_4-binding capacity		Serum free T_4	
				TBG	TBPA		
	years		*μg/100 ml*	*μg T_4 I/100 ml*		%	*mμg T_4 I/100 ml*
Controls							
R.C.	26	Rheumatoid arthritis	3.1	23	98	0.028	0.87
G.C.	69	ASHD	4.8	20	181	0.039	1.88
S.R.	51	ASHD	4.1	16	57	0.044	1.80
M.R.	56	Diabetes mellitus	5.3	18	105	0.018	0.95
E.L.	44	ASHD	3.8	14	237	0.086	3.27
T.J.	40	Slowly resolving pneumonia	3.1	11	169	0.031	0.96
H.P.	44	Peptic ulcer	4.3	16	174	0.034	1.46
D.F.	53	Hypertension	4.2	18	157	0.024	1.01
J.N.	50	Hypertension	3.9	20	116	0.084	3.28
Control mean ±SD	48		4.1 ±0.7	17 ±4	144 ±54	0.043 ±0.025	1.72 ±0.96
J.G.	43	Low TBG	2.1	<1	177	0.123	2.58
T.R.	76	Low TBG	1.5	<1	150	0.127	1.90

* T_4 = thyroxine; TBG = thyroxine-binding globulin; TBPA = thyroxine-binding prealbumin; ASHD = arteriosclerotic heart disease.

The concentration of free T_4 in the controls varied from 0.87 to 3.28 mμg T_4 iodine per 100 ml. The values obtained in J.G. and T.R. were within this range (Table I).

disease in our control subjects. Others have found low TBPA levels (15, 16) and elevated per cent free T_4 (16) in patients with acute and chronic illness.

In vivo studies. The disappearance of tracer from the plasma and the rise in hepatic radioactivity were more rapid during the initial 60 minutes in subjects J.G. and T.R. than in the control (Figure 1). In all subjects the maximal counting rate over the liver was reached by 2 hours after injection. Thereafter the hepatic and plasma

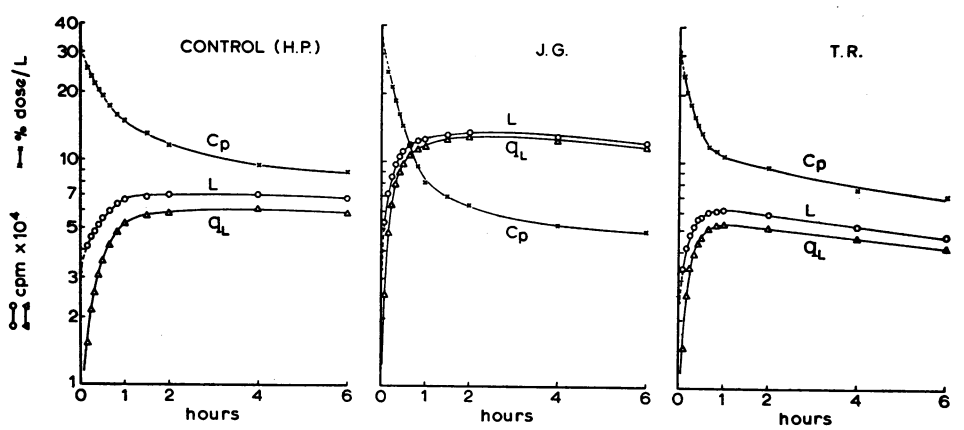

FIG. 1. THE TIME COURSE OF HEPATIC RADIOACTIVITY AND THE CONCENTRATION OF PROTEIN-BOUND-^{131}I ($PB^{131}I$) IN THE PLASMA. The observed counting rate recorded over the liver, L (open circles), and the level of $PB^{131}I$ in the plasma, C_P (crosses), are shown in a typical control subject, H.P., and in patients J.G. and T.R., whose plasma thyroxine-binding globulin (TBG) was low. The initial portion of the plasma curve was extrapolated to time zero in order to obtain $C_P(o)$ and v_P, the virtual volume of plasma within the collimated field (see text). Similarly, the hepatic curve, L, was extrapolated to yield L(o). The curve q_L (triangles) represents the time course of hepatic radioactivity corrected for label in the plasma. (For derivation of q_L see Analysis of Data).

TABLE II

*Plasma and liver T_4 distribution volume, pool size, hepatic clearance, and plasma to liver flux**

Subject	Plasma volume V_P	Plasma T_4 pool size S_P	Hepatic T_4 distribution volume H	Hepatic T_4 pool size S_L	Rate constant λ_{22}	Hepatic T_4 clearance C_H	Plasma to liver flux ρ_{21}
	L	*μg T_4 I*	*L*	*μg T_4 I*	*min^{-1}*	*ml/min*	*μg T_4 I/min*
Controls							
R.C.	3.18	98	3.26	101	0.0160	52	1.62
G.C.	2.67	128	3.96	190	0.0123	49	2.34
S.R.	4.75	195	4.32	177	0.0115	50	2.04
M.R.	2.76	146	2.78	147	0.0165	46	2.43
E.L.	2.64	100	3.90	148	0.0100	39	1.48
T.J.	2.14	66	3.95	122	0.0135	53	1.65
H.P.	2.76	119	4.38	188	0.0125	55	2.35
D.F.	4.05	170	3.94	166	0.0117	46	1.94
J.N.	2.90	113	3.75	146	0.0122	46	1.78
Mean	3.09	126	3.80	154	0.0129	48	1.96
±SD	±.81	±39	±.50	±30	±0.0021	±5	±0.35
Low TBG							
J.G.	2.98	63	14.1	296	0.0091	128	2.70
T.R.	3.34	50	5.56	83	0.0225	125	1.87

* All values corrected to 1.73 m^2 body surface area.

time curves approached a similar gradual rate of decline.

Table II presents the individual values for plasma volume (V_P) and plasma T_4 pool (S_P). In J.G. and T.R. S_P was approximately one-half the control mean due to the low serum concentration of T_4 in these two patients. In the controls the calculated hepatic space (H) averaged 3.80 L (range 2.78 to 4.38), and the hepatic pool size (S_L) averaged 154 μg T_4 iodine (range 101 to 190). In J.G. H was nearly four times and S_L about twice the control mean. In contrast, T.R. showed a slightly elevated H and a relatively low value for S_L.

The hepatic one way clearances of plasma T_4 (C_H) in subjects J.G. and T.R. were similar and about 2.6 times the control mean (48 ml per minute).

Because the determination of serum T_4 iodine at low levels involves considerable error, the values for pool sizes, flux, and disposal rates are probably less reliable than the estimates of distribution volume and clearance in subjects J.G. and T.R.

Table III gives the values for total T_4 distribution volume, V_T. The mean in the control group (12.4 L) agrees closely with the value of 12.0 L obtained by Gregerman, Gaffney, and Shock (17) in a group of 18 normal men, aged 48 to 59, and by Oddie, Fisher, and Epperson (18), who found an average of 12.1 L in 20 normal subjects. Earlier studies (reviewed in reference 1) yielded lower values of V_T, the over-all mean equalling 9.4 L. There is no apparent explanation for the systematic difference between these two groups of results.

Total extrathyroidal T_4 (S_T) in the controls varied from 301 to 697 μg T_4 iodine (Table III).

TABLE III

Total T_4 distribution volume, extrathyroidal pool size, and degradation rate

Subject	Total distribution volume* V_T	Total extrathyroidal T_4 pool* S_T	Final plasma $t_{\frac{1}{2}}$	Total daily T_4 disposal rate* ρ_{OT}
	L	*μg T_4 I*	*days*	*μg T_4 I/day*
Controls				
R.C.	11.8	365	6.4	40
G.C.	10.5	504	8.3	42
S.R.	17.0	697	7.3	66
M.R.	11.8	627	6.2	70
E.L.	13.2	502	5.8	60
T.J.	9.7	301	5.8	36
H.P.	11.0	475	5.0	66
D.F.	14.3	600	8.3	50
J.N.	12.5	488	7.0	48
Mean	12.4	507	6.7	53
±SD	±2.2	±124	±1.1	±13
Low TBG				
J.G.	21.5	452	4.4	71
T.R.	16.7	250	2.4	72

* Corrected to 1.73 m^2 body surface area.

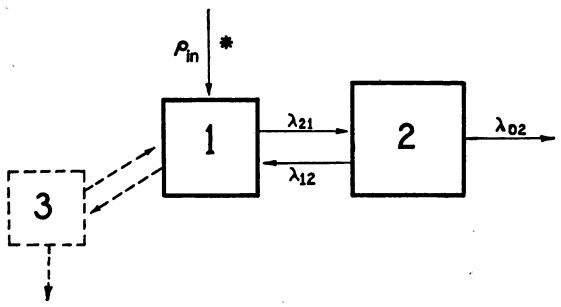

FIG. 2. KINETICS MODEL DESCRIBING THE DISTRIBUTION OF ^{131}I-LABELED THYROXINE (T_4). Compartment 1 represents plasma; compartment 2, the extravascular space of the liver; and compartment 3, all nonhepatic tissues. Tracer(*) enters the system via compartment 1. Unlabeled T_4 (endogenous) enters at a rate, ρ_{in}. Fractional rate constants for distribution and metabolism are indicated adjacent to the appropriate arrows. The analysis in the present study is concerned with the distribution between compartments 1 and 2; the kinetic behavior of compartment 3, which may consist of several compartments, is not considered.

In J.G. the relatively high V_T was balanced by the low serum T_4 to yield a value of S_T within the control range. In T.R., V_T was slightly elevated, and S_T was approximately one-half the control mean.

The daily T_4 disposal rate, ρ_{OT}, ranged from 36 to 70 μg iodine per day in the controls. The values in J.G. and T.R. were 71 and 72 μg per day. In a larger group of normal men, aged 48 to 59, Gregerman and associates (17) found a mean disposal rate of 63.1 ± 14.4 (SD). The values for ρ_{OT} obtained in patients J.G. and T.R. are, therefore, probably not significantly different from normal and are consistent with the eumetabolic state in these individuals.

Urinary excretion of tracer during distribution equilibration. Control subjects excreted in the urine from 3.0 to 6.5% of the administered dose during the initial 6 hours of the study. In J.G. and T.R. urinary ^{131}I was 4.2 and 2.6% of the dose, respectively, in the same interval. The cumulative urinary excretion during the period of 0 to 24 hours averaged 10.3% in the controls (range = 6.0 to 14.0), 8.0% in J.G., and 7.3% in T.R. Thus the initial rapid clearance of tracer from the plasma and the abnormally high values of V_T in patients J.G. and T.R. cannot be attributed to excessive loss via the urine.

Discussion

An idiopathic decrease in T_4 binding by TBG has been reported as an isolated finding in euthyroid individuals (19–21) and in six members of a family (22). The underlying abnormality is presumably a genetically determined defect in the synthesis of TBG.[8] The two patients described in the present study resemble previously reported patients in that they are euthyroid, the serum PBI is abnormally low, and the daily T_4 disposal rate is normal. The finding of a normal level of free T_4 in the serum of our patients is consistent with the presently accepted view regarding the importance of free T_4 in determining the metabolic status of the individual. The hypothesis that the unbound fraction of plasma T_4 governs both the rate of degradation of the hormone and its metabolic effects has received support from several lines of evidence that have already been reviewed elsewhere (1, 15, 23). The observations made in the present study on the kinetics of distribution of labeled T_4 have direct bearing on the postulated role of free T_4 in determining the rate of entry of hormone into tissues. This question will be considered again later in the discussion.

In a number of animal species the liver rapidly accumulates label after intravenous injection of tracer doses of T_4 (24). In the rat nearly one-third of the dose is taken up by the liver within 1 minute (25). Roche and co-workers (26) found that in the dog 12% of the dose was localized in the liver by 60 minutes after injection. Brown-Grant and Tata (27), studying the distribution of labeled T_4 in the rabbit, found approximately 15% of the dose in the extravascular space of the liver 20 minutes after injection. In both the dog and the rabbit distribution equilibration between plasma and liver occurred several times more rapidly than that between plasma and nonhepatic tissues.

Previous work has indicated that in man the liver plays an important role in the distribution of T_4. In early studies using ^{131}I-labeled *dl*-T_4, Albert and Keating (5) noted high levels of radioactivity in the hepatic region during the first hours

[8] The alternative possibilities, that accelerated degradation of plasma TBG occurs, or that TBG is synthesized in an altered form incapable of binding T_4, cannot be excluded.

after injection of tracer. Friis (8) estimated that 25 to 30% of the dose of labeled L-T_4 is taken up by the liver within 1 hour in normal humans. Pochin (9), using a method of profile body scanning, has estimated that nearly one-half of the injected dose of L-T_4- ^{131}I is localized in the liver within 2 hours after administration. The calculated hepatic distribution volume in his group of seven normal subjects averaged 5.2 L ± 0.7 (SE). The reason for the difference between the mean value for hepatic T_4 space in the controls of the present study and the value obtained by Pochin is not clear but may involve methodological factors, differences in the subjects themselves, or both.

If a normal hepatic mass of 1,400 g is assumed, the concentration ratio of unlabeled T_4 in liver (in μg T_4 per g) to that in plasma (in μg T_4 per ml) is, on the average, nearly 3 to 1. Although there are no chemical data on the tissue concentration of T_4 in humans, some information is available in animals. Carr and Riggs (28) determined the PBI content of various extrathyroidal tissues of the dog. In three normal animals the concentration of PBI in liver exceeded that in other tissues and averaged 5.8 μg per 100 g. The level in plasma was 1.5 μg per 100 ml. The average liver to plasma PBI ratio was 4 to 1, somewhat higher than the ratio predicted for the human in the present study. Of course, specific chemical determination of T_4 in human tissues would be desirable.

A liver to plasma concentration ratio greater than 1 implies hepatic binding of T_4. One can speculate about the possible mechanism of such binding. The plasma proteins that bind T_4 (TBG, TBPA, and albumin) are probably all synthesized in the liver. An intrahepatic pool of these proteins could account for at least a portion of the T_4 concentrated in liver. Studies of the distribution of labeled serum albumin in humans, however, indicate that the liver contains only a small pool of exchangeable albumin (29). The results of similar investigations have recently been reported on ^{131}I-labeled prealbumin in which there was no evidence for selective hepatic accumulation of TBPA (30, 31). It would appear, therefore, that neither albumin nor TBPA could account for the high hepatic content of T_4 in man. Although there are no data on the distribution of TBG, the results of the present studies on subjects lacking TBG are relevant to this question. If hepatic

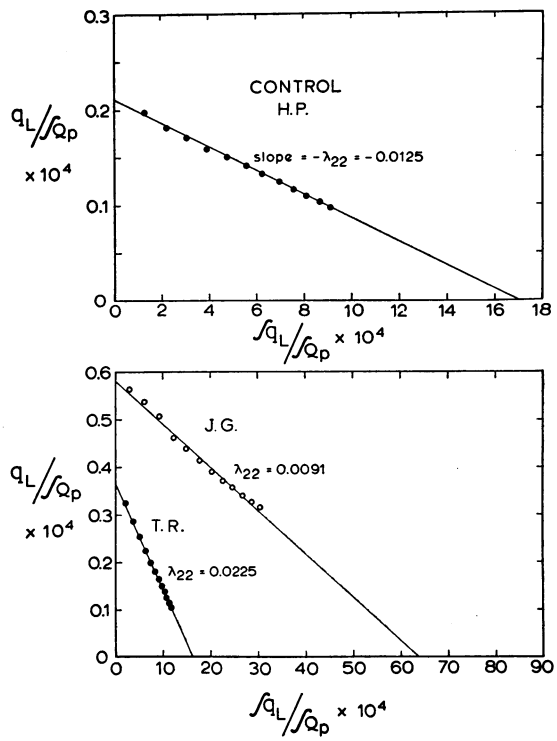

FIG. 3. ESTIMATION OF λ_{22}. The time curves of the hepatic counting rate (q_L) and the fraction of the injected dose in the entire plasma (Q_P) (see text) were integrated at intervals of 10 minutes, from 10 to 120 minutes after injections. At each interval the ratio $q_L/\int Q_P$ was plotted on the ordinate against $\int q_L/\int Q_P$ on the abscissa. The straight line drawn through the points has a slope = $-\lambda_{22}$. Above are shown the results in control subject H.P.; below, the data for subjects with low plasma TBG, J.G. (open circles) and T.R. (closed circles). The units of λ_{22} are minutes^{-1}.

binding of T_4 in normal subjects were due to a higher concentration of TBG in liver than in plasma, then one would expect an individual lacking TBG to exhibit a normal or low hepatic T_4 space and a low hepatic pool, assuming that in such a case TBG would be lacking in the liver as well as in plasma. In both subjects of the present study the hepatic T_4 space was higher than normal. The hepatic pool size was high in one patient and low in the other (Table II). These results would indicate that TBG does not play an important role in the binding of T_4 within the liver.

It is likely, therefore, that most of the intrahepatic T_4 is reversibly bound to sites on or within the cells of the liver. Consistent with this possibility is the finding by Lennon and associates (2) that in patients with hepatocellular disease the

initial rate of clearance of labeled T_4 from the blood is abnormally slow. We have made estimates of hepatic T_4 space and pool size in patients with cirrhosis and hepatitis. Even with normal plasma TBG capacity and free T_4 levels, the hepatic T_4 pool is usually diminished in such cases (10). Factors other than the state of hepatic function may influence the ability of the liver to bind T_4. Gregerman and co-workers (17) have shown that there is a progressive decline in total T_4 distribution volume and in extrathyroidal pool of organic iodine with increasing age, especially over age 60. This may explain the large difference in calculated hepatic T_4 pool size between our two subjects, J.G. and T.R. (there was no evidence that T.R. had significant hepatic disease). There may, of course, be other unknown factors that influence the hepatic T_4 pool.

Once within the liver, T_4 is subject to several pathways of degradation and excretion: *a*) the hormone is secreted into the bile, mostly as the glucuronide- and sulfate-conjugated derivatives; *b*) deiodination of T_4 occurs within the liver, the released iodide returning to the plasma; *c*) T_4 may escape from the liver via the lymphatics, eventually returning to the circulation. Myant (32) has measured the biliary clearance of labeled T_4 in humans with biliary fistulas and has found an average clearance of about 20 ml plasma per hour (1 μg T_4 iodine per hour). The rate of deiodination in the liver of man is not known but probably does not exceed 1 μg T_4 iodine per hour. Loss of T_4 from the liver via lymphatics may be estimated by assuming that two-thirds of the lymph flowing through the thoracic duct arises in the liver. If the concentration of T_4 in hepatic lymph were equal to that in plasma and if the thoracic duct flow rate were 100 ml per hour (33), no more than 3 μg T_4 iodine would leave the liver per hour by this route. The total flux of T_4 out of the liver by the three pathways mentioned above could be no greater than 5 μg T_4 iodine per hour. This is less than 5% of the flux from plasma to liver (118 μg per hour) estimated from the kinetics data in the present study. Therefore, more than 95% of the T_4 entering the liver in a given interval of time must return to the plasma directly in unchanged form. The bidirectionality of exchange of labeled T_4 between plasma and liver has recently been documented by work of Flock and Owen (34) in which isolated rat livers prelabeled with T_4-^{131}I were perfused with blood containing T_4-^{125}I.

The question arises whether T_4 diffuses from plasma into the liver only as the free hormone. In the subjects lacking TBG the values for free T_4 in serum, as a per cent of total T_4, agreed closely and were approximately 2.9 times the mean value in the control group (Table I). The hepatic T_4 clearance (one way) in these individuals was 2.7 times the mean clearance in the controls (Table II). This direct correlation between the proportion of free T_4 in serum and the hepatic T_4 clearance argues for the proposition that T_4 enters the liver in unbound form, rather than as TBG-bound T_4. The present results do not exclude the possibility that TBPA may participate in the transcapillary diffusion of T_4. However, we have in other studies (35) examined the effect of salicylate on the kinetics of T_4 distribution in humans. This drug inhibits binding of T_4 to TBPA *in vitro* (36, 37) and presumably *in vivo,* also. After acute administration of salicylate to one normal subject there was a transient but significant increase in hepatic T_4 clearance and in hepatic T_4 space. This finding indicates that free T_4 is more important than TBPA-bound T_4 in the diffusion of the hormone into liver.

The possible physiological significance of hepatic concentration of T_4 in man remains to be considered. Nearly one-third of the total extrathyroidal pool of hormone is located in the liver. This pool exchanges with circulating T_4 rapidly in comparison with the rate of exchange in other tissues. The hepatic pool of T_4 may be regarded as a buffer that acts to modulate abrupt changes in the level of circulating hormone. There is some evidence for this concept in the studies of Ingbar and Freinkel (15). These workers administered a single large dose of unlabeled T_4 (4 mg) intravenously to a human subject several days after giving a tracer dose of labeled T_4. They found within minutes a fall in the plasma level of tracer coincident with a rise in radioactivity over the liver. The movement of tracer from plasma to liver presumably reflected movement of unlabeled T_4 in the same direction. After restoration of distribution equilibrium, approximately 24 hours later, the plasma disappearance time curve was displaced downward and declined at a rate not

very different from the rate preceding the injection of the loading dose. As the authors pointed out, interpretation of kinetics data in a nonsteady state is difficult. Suffice it to say that the liver appeared to participate in the early phase of redistribution of hormone after the acute increase in the level of circulating T_4 produced by the loading dose. Although such marked and rapid changes in plasma T_4 level would not occur in nature, measurable increases in the concentration of free T_4 in plasma have been noted in acutely and chronically ill patients (16) and after surgical stress (38). In such situations the liver might serve as a modulator by minimizing the effects on other tissues of increased levels of free T_4. Studies are in progress to investigate the role of the liver on the peripheral distribution and metabolism of T_4 in various physiological and pathological states.

Summary

A method is described for kinetic analysis of exchange of thyroxine (T_4)-^{131}I between plasma and extravascular space of the liver in humans.

Nine euthyroid subjects with normal levels of thyroxine-binding serum globulin (TBG) were studied as controls. Distribution equilibrium of T_4-^{131}I between plasma and liver was approached by 4 hours after injection of tracer. The hepatic T_4 distribution volume (space) averaged 3.80 L $\pm$ 0.50 (SD), which was 30% of the total T_4 space, after complete equilibration. The mean hepatic clearance (one way) was 48 ml plasma per minute $\pm$ 5 (SD). In two euthyroid individuals whose plasma was virtually devoid of TBG, estimates of the hepatic T_4 space were higher than in the controls but varied widely. The hepatic T_4 clearances were similar in these patients and nearly 2.7 times the mean control value. The calculated plasma to liver flux was approximately normal due to the low serum concentration of T_4 in these subjects.

The proportion of free T_4 in serum, measured *in vitro,* correlated with the hepatic T_4 clearance in patients with low TBG, indicating that the level of free rather than bound T_4 determines the entry of hormone into liver. The physiologic implications of the relatively large rapidly exchanging hepatic T_4 pool are discussed.

Appendix

An alternative method of analysis is possible, based upon the approach used by Berson and Yalow (39) in a study of thyroidal iodide kinetics. If Equation 4 is divided by $C_1(t)V_1$,

$$[V_2 dC_2(t)/dt]/[C_1(t)V_1] = \lambda_{21} - \lambda_{22}\,[C_2(t)V_2]/[C_1(t)V_1].$$

If one substitutes,

$$[dq_L(t)/dt]/[Q_P(t)] = \lambda_{21}\cdot k - \lambda_{22}[q_L(t)]/[Q_P(t)].$$

The latter differential equation is in the same form as Equation 5a but avoids the necessity of integration. Estimates were made of $dq_L(t)/dt$ at 10-minute intervals, from 10 to 120 minutes, by taking tangents to the curve, $q_L(t)$. A linear plot of $[q_L(t)/dt]/[Q_P(t)]$ against $q_L(t)/Q_P(t)$ yielded in every case a value for λ_{22} within $\pm$5% of that obtained by integration, but the scatter of points was greater. Therefore, the integral method was preferred.

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