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## The Kinetics of Distribution between Plasma and Liver of <sup>131</sup>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 <sup>1</sup> 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<sub>4</sub>-<sup>131</sup>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<sub>4</sub> in normal humans. Hepatic localization of radioactivity within hours after the intravenous administration of labeled T<sub>4</sub> 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<sub>4</sub> between plasma and liver in humans with neither thyroid nor hepatic disease. The method involves body sector counting with externally placed detectors and simultaneous meas-

urement of the plasma concentration of protein-bound <sup>131</sup>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<sub>4</sub> 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<sub>4</sub> binding on the distribution of labeled T<sub>4</sub> *in vivo*. The results of the kinetics study and *in vitro* measurements of serum free T<sub>4</sub> 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,<sup>2</sup> labeled with <sup>131</sup>I in the 3' and 5' positions, varied from 22 to 53 mc per mg thyroxine. Contamination with iodide-<sup>131</sup>I never exceeded 6% of total <sup>131</sup>I, as determined by precipitation with TCA in the presence of normal human serum. Each lot of labeled T<sub>4</sub> was used within 2 weeks after it was received.

#### *In vitro studies*

T<sub>4</sub>-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<sub>4</sub>-<sup>131</sup>I at the following levels of added, unlabeled T<sub>4</sub>: 1.00 and 4.50 µg per ml serum. TBG capacity was determined at the lower T<sub>4</sub> concentration and TBPA capacity at the higher level of added T<sub>4</sub>. In each case, duplicate results varied by less than 7%.

Serum T<sub>4</sub> iodine concentration was measured<sup>3</sup> by a method of column chromatography and the ceric-arsenite reaction (13).

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<sup>1</sup> Abbreviations used in the text: T<sub>4</sub>, thyroxine; TBG, thyroxine-binding globulin; TBPA, thyroxine-binding prealbumin; TCA, trichloroacetic acid.

The proportion of free  $T_4$  in serum was estimated by the method of Lee, Henry, and Golub (14). Five-tenths  $\mu\text{C}$   $L\text{-}T_4\text{-}^{131}\text{I}$  ( $0.015\text{ }\mu\text{g}$ ) 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  $T_4\text{-}^{131}\text{I}$  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  $^{131}\text{I}$  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  $\mu\text{C}$   $T_4\text{-}^{131}\text{I}$  (less than 3  $\mu\text{g}$ ). 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  $T_4\text{-}^{131}\text{I}$  injected was based on the TCA-insoluble  $^{131}\text{I}$  content of the blood and the volume of blood injected.<sup>4</sup>

Complete collections of urine were made during the initial 6 hours and from 6 to 24 hours after injection. Total  $^{131}\text{I}$  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  $^{131}\text{I}$ .

<sup>4</sup> Experiments were done to determine the proportion of  $T_4\text{-}^{131}\text{I}$  bound to erythrocytes *in vivo*. At 1, 4, and 24 hours after injection of the tracer, less than 2% of total protein-bound  $^{131}\text{I}$  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- $\times$  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  $^{131}\text{I}$  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<sup>5</sup> 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  $^{131}\text{I}$ , 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- $^{131}\text{I}$  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

<sup>5</sup> 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 <sup>131</sup>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-<sup>131</sup>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 <sup>131</sup>I thyroid uptake at 2 hours was 5% and at 24 hours, 9%. The basal metabolic rate was +18. The triiodothyronine-<sup>131</sup>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<sub>4</sub>-<sup>131</sup>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.

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

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,

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

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,

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

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:

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

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<sub>4</sub> enter the system by way of the plasma (compartment 1).  $\rho_{1a}$  represents the steady state rate of endogenous hormone secretion into the plasma. T<sub>4</sub> in compartment 1 exchanges with that in the extravascular space of the liver (compartment 2). One way transfer rate constants are given as  $\lambda_{21}$  and  $\lambda_{12}$ . T<sub>4</sub> leaves the system from compartment 2 by a number of metabolic and secretory processes, all represented by  $\lambda_{02}$ . In addition to exchange with T<sub>4</sub> 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:

$$V_2 dC_2(t)/dt = \lambda_{21}C_1(t)V_1 - \lambda_{22}C_2(t)V_2, \quad [4]$$

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.  $\lambda_{21}$  is the

fraction of compartment 1 entering compartment 2 per unit time, and  $\lambda_{22}$  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,

$$V_2 C_2(t) = \lambda_{21} V_1 \int_0^t C_1 dt - \lambda_{22} V_2 \int_0^t C_2 dt.$$

If both sides are divided by  $V_1 \int_0^t C_1 dt$ ,

$$[V_2 C_2(t)] / (V_1 \int_0^t C_1 dt) = \lambda_{21} - \lambda_{22} (V_2 \int_0^t C_2 dt) / (V_1 \int_0^t C_1 dt). \quad [5]$$

If  $Q_P(t) = V_1 C_1(t)$  and  $q_L(t) = k \cdot V_2 C_2(t)$ , where  $k$  is a proportionality constant, Equation 5 may be written in terms of  $Q_P(t)$  and  $q_L(t)$ :

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

According to Equation 5a, a linear plot of  $q_L(t) / \int Q_P dt$  on the  $y$  axis against  $\int q_L dt / \int Q_P dt$  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  $q_L(t) / \int Q_P dt$  and  $\int q_L dt / \int Q_P dt$  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  $\lambda_{22}$  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  $H$ .<sup>6</sup>

<sup>6</sup> 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  $T_4$ -<sup>131</sup>I show uniform distribution of radioactivity. *c*) Labeled iodide released *in vivo* from  $T_4$ -<sup>131</sup>I. From the level of TCA-soluble <sup>131</sup>I in plasma and the diffusion space for iodide in liver, the error

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  $C_P(0)$ . 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,  $\rho_{21}$ , 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,  $\rho_{OT}$ , was calculated from  $S_T \times 0.693/\text{final } t_{1/2}$  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  $\mu\text{g}$  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  $\mu\text{g}$  per 100 ml. In T.R. the level of  $T_4$  iodine was 1.5  $\mu\text{g}$  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  $\mu\text{g}$   $T_4$  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  $\mu\text{g}$  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.<sup>7</sup>

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.

<sup>7</sup> 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<sub>4</sub> iodine, T<sub>4</sub>-binding capacity, and free T<sub>4</sub>\*

Subject	Age	Diagnosis	Serum T <sub>4</sub> iodine	T <sub>4</sub> -binding capacity		Serum free T <sub>4</sub>	
				TBG	TBPA	%	mμg T <sub>4</sub> I/100 ml
	years		μg/100 ml	μg T <sub>4</sub> I/100 ml			
<b>Controls</b>							
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	48		4.1	17	144	0.043	1.72
±SD			±0.7	±4	±54	±0.025	±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<sub>4</sub> = thyroxine; TBG = thyroxine-binding globulin; TBPA = thyroxine-binding prealbumin; ASHD = arteriosclerotic heart disease.

The concentration of free T<sub>4</sub> in the controls varied from 0.87 to 3.28 mμg T<sub>4</sub> 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<sub>4</sub> (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

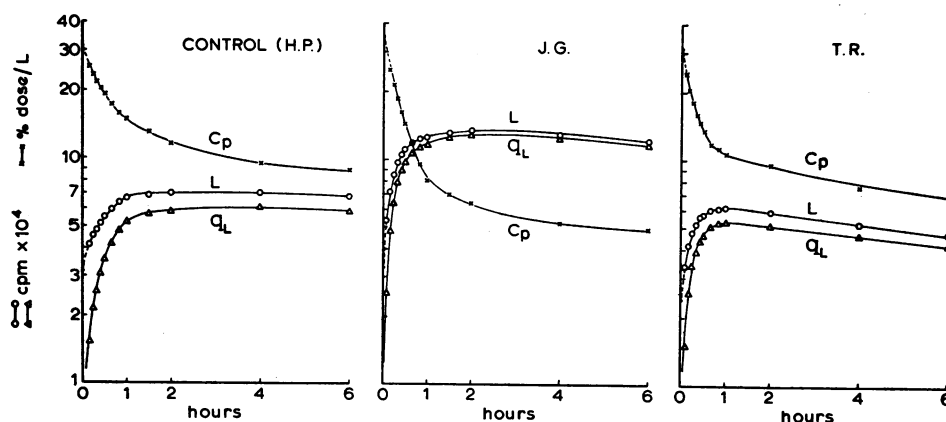


FIG. 1. THE TIME COURSE OF HEPATIC RADIOACTIVITY AND THE CONCENTRATION OF PROTEIN-BOUND-<sup>125</sup>I (PB<sup>125</sup>I) IN THE PLASMA. The observed counting rate recorded over the liver, L (open circles), and the level of PB<sup>125</sup>I in the plasma, C<sub>P</sub> (crosses), are shown in a typical 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<sub>P</sub>(o) and v<sub>P</sub>, 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<sub>L</sub> (triangles) represents the time course of hepatic radioactivity corrected for label in the plasma. (For derivation of q<sub>L</sub> 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 $\lambda_{22}$	Hepatic $T_4$ clearance $C_H$	Plasma to liver flux $\rho_{11}$
	$L$	$\mu g T_4 I$	$L$	$\mu g T_4 I$	$min^{-1}$	$ml/min$	$\mu g T_4 I/min$
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
$\pm SD$	$\pm .81$	$\pm 39$	$\pm .50$	$\pm 30$	$\pm 0.0021$	$\pm 5$	$\pm 0.35$
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<sup>2</sup> 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  $\mu g T_4$  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  $\mu g T_4$  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 $t_{1/2}$	Total daily $T_4$ disposal rate* $\rho_{OT}$
	$L$	$\mu g T_4 I$	days	$\mu g T_4 I/day$
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
$\pm SD$	$\pm 2.2$	$\pm 124$	$\pm 1.1$	$\pm 13$
Low TBG				
J.G.	21.5	452	4.4	71
T.R.	16.7	250	2.4	72

\* Corrected to 1.73 m<sup>2</sup> body surface area.

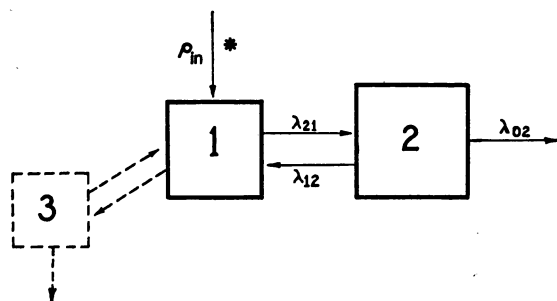


FIG. 2. KINETICS MODEL DESCRIBING THE DISTRIBUTION OF <sup>125</sup>I-LABELED THYROXINE (T<sub>4</sub>). Compartment 1 represents plasma; compartment 2, the extravascular space of the liver; and compartment 3, all nonhepatic tissues. Tracer(\*) enters the system via compartment 1. Unlabeled T<sub>4</sub> (endogenous) enters at a rate,  $\rho_{in}$ . 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<sub>4</sub> 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<sub>4</sub> disposal rate,  $\rho_{OT}$ , ranged from 36 to 70  $\mu$ g iodine per day in the controls. The values in J.G. and T.R. were 71 and 72  $\mu$ g per day. In a larger group of normal men, aged 48 to 59, Gregerman and associates (17) found a mean disposal rate of  $63.1 \pm 14.4$  (SD). The values for  $\rho_{OT}$  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 <sup>125</sup>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<sub>4</sub> 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.<sup>8</sup> 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<sub>4</sub> disposal rate is normal. The finding of a normal level of free T<sub>4</sub> in the serum of our patients is consistent with the presently accepted view regarding the importance of free T<sub>4</sub> in determining the metabolic status of the individual. The hypothesis that the unbound fraction of plasma T<sub>4</sub> 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<sub>4</sub> have direct bearing on the postulated role of free T<sub>4</sub> 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<sub>4</sub> (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<sub>4</sub> 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 non-hepatic tissues.

Previous work has indicated that in man the liver plays an important role in the distribution of T<sub>4</sub>. In early studies using <sup>125</sup>I-labeled *dl*-T<sub>4</sub>, Albert and Keating (5) noted high levels of radioactivity in the hepatic region during the first hours

<sup>8</sup> The alternative possibilities, that accelerated degradation of plasma TBG occurs, or that TBG is synthesized in an altered form incapable of binding T<sub>4</sub>, 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  $5.2 \text{ L} \pm 0.7$  (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  $\mu\text{g } T_4$  per g) to that in plasma (in  $\mu\text{g } T_4$  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  $5.8 \mu\text{g}$  per 100 g. The level in plasma was  $1.5 \mu\text{g}$  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

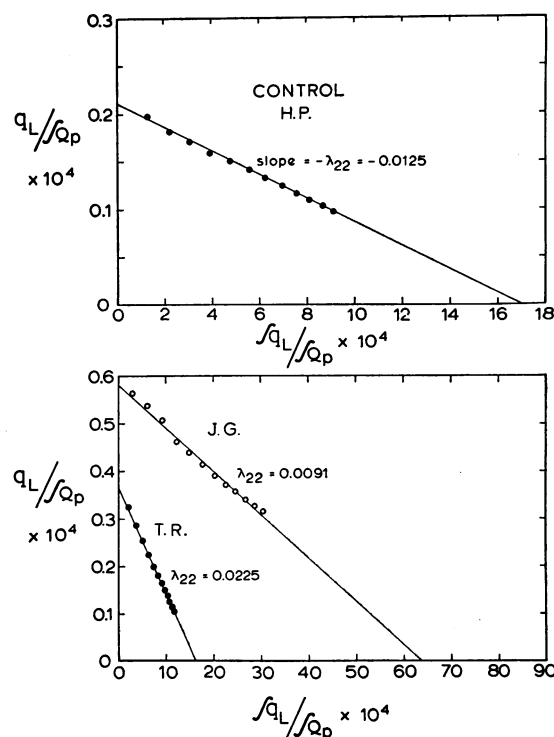


FIG. 3. ESTIMATION OF  $\lambda_{22}$ . The time curves of the hepatic counting rate ( $q_L$ ) and the fraction of the injected dose in the entire plasma ( $Q_P$ ) (see text) were integrated at intervals of 10 minutes, from 10 to 120 minutes after injections. At each interval the ratio  $q_L/Q_P$  was plotted on the ordinate against  $\int q_L/Q_P$  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  $\lambda_{22}$  are  $\text{minutes}^{-1}$ .

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  $\mu$ g  $T_4$  iodine per hour). The rate of deiodination in the liver of man is not known but probably does not exceed 1  $\mu$ g  $T_4$  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  $\mu$ g  $T_4$  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  $\mu$ g  $T_4$  iodine per hour. This is less than 5% of the flux from plasma to liver (118  $\mu$ g 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 pre-labeled with  $T_4$ -<sup>131</sup>I were perfused with blood containing  $T_4$ -<sup>125</sup>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 ( $T_4$ )- $^{131}\text{I}$  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  $T_4$ - $^{131}\text{I}$  between plasma and liver was approached by 4 hours after injection of tracer. The hepatic  $T_4$  distribution volume (space) averaged  $3.80 \text{ L} \pm 0.50 \text{ (SD)}$ , which was 30% of the total  $T_4$  space, after complete equilibration. The mean hepatic clearance (one way) was  $48 \text{ ml plasma per minute} \pm 5 \text{ (SD)}$ . 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  $C_1(t)V_1$ ,

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

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  $\lambda_{22}$  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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