Journal of Clinical Investigation
Vol. 46, No. 5, 1967

Estimation of Rapidly Exchangeable Cellular Thyroxine from the Plasma Disappearance Curves of Simultaneously Administered Thyroxine-^{131}I and Albumin-^{125}I *

JACK H. OPPENHEIMER,† GERALD BERNSTEIN,‡ AND JULIAN HASEN §

(From the Endocrine Research Laboratory, Division of Medicine, Montefiore Hospital and Medical Center, and the Department of Medicine, Albert Einstein College of Medicine, Bronx, N. Y.)

Summary. A mathematical analysis of the plasma disappearance curves of simultaneously injected thyroxine-^{131}I and albumin-^{125}I allows the development of simple formulas for estimating the pool size and transfer kinetics of rapidly exchangeable intracellular thyroxine in man. Evidence is presented that the early distribution kinetics of albumin-^{125}I can be used to represent the expansion of the thyroxine-^{131}I–plasma protein complex into the extracellular compartment. Calculations indicate that approximately 37% of total body extrathyroidal thyroxine is within such exchangeable tissue stores. The average cellular clearance of thyroxine is 42.7 ml per minute, a value far in excess of the metabolic clearance of this hormone. Results of external measurements over the hepatic area and studies involving hepatic biopsies indicate that the liver is an important but probably not the exclusive component of the intracellular compartment. The partition of thyroxine between cellular and extracellular compartments is determined by the balance of tissue and plasma protein binding factors. The fractional transfer constants are inversely related to the strength of binding of each compartment and directly proportional to the permeability characteristic of the hypothetical membrane separating compartments. Appropriate numerical values for these factors are assigned. An increased fractional entrance of thyroxine-^{131}I into the cellular compartment was noted in a patient with congenital decrease in the maximal binding capacity of thyroxine-binding globulin and in three patients after the infusion of 5,5-diphenylhydantoin. Decreased intracellular space and impaired permeability characteristics were observed in five patients with hepatic disease. Studies of the rate of entrance of thyroxine-^{131}I and albumin-^{125}I into the pleural effusion of a patient with congestive heart failure suggested that transcapillary passage of thyroxine independent of its binding protein is not a predominant factor in the total distribution kinetics of thyroxine-^{131}I. The thesis is advanced that the distribution of thyroxine,

* Submitted for publication July 18, 1966; accepted January 12, 1967.

Supported by U. S. Public Health Service grant NB-03000 and contract U-1238 from the Health Research Council of New York City.

Presented in part at the Fifty-eighth Annual Meeting of the American Society for Clinical Investigation, May 2, 1966, Atlantic City, N. J., and abstracted in J. clin. Invest. 1966, **45**, 1054.

† Career Scientist of the Health Research Council of New York City.

Address requests for reprints to Dr. Jack H. Oppenheimer, Endocrine Research Laboratory, Division of Medicine, Montefiore Hospital and Medical Center, 111 E. 210th St., Bronx, N. Y. 10467.

‡ Postdoctoral fellow of the American Cancer Society.

§ U. S. Public Health Service postdoctoral research fellow.

both within the extracellular compartment and between the extracellular and intracellular compartments, is accomplished largely by the carrier protein and the direct transfer of thyroxine from one binding site to another. The concept of free thyroxine is reassessed in terms of this formulation.

Introduction

Albert and Keating (1) first demonstrated that a variety of tissues in the rat rapidly accumulate radioactivity after the intravenous injection of thyroxine-^{131}I. By external radioactive measurements in man, Myant and Pochin (2) showed that a significant fraction of administered thyroxine-^{131}I is concentrated in the hepatic area. Hazelrig (3), utilizing rat liver perfusion studies performed by Flock and co-workers, indicated that hepatic thyroxine is in rapid equilibrium with hormone in blood. The reversible transfer of thyroxine between liver and blood in the isolated rat liver preparation has recently been considered in detail by Gorman, Flock, Owen, and Paris (4). Pochin (5) and subsequently Cavalieri and Searle (6, 7) postulated a similar relationship in man on the basis of an analysis of the kinetics of thyroxine-^{131}I accumulation by the liver and the simultaneous disappearance of radioactivity from plasma.

In the present communication, the distribution kinetics of thyroxine-^{131}I are analyzed by comparing over a 4-hour period the plasma disappearance curves of simultaneously injected thyroxine-^{131}I and albumin-^{125}I. A model system is proposed that allows kinetic description of the exchange between thyroxine in the instantaneous volume of distribution of albumin-^{125}I and thyroxine outside this volume. The compartment comprising thyroxine outside the instantaneous volume of albumin-^{125}I distribution can be identified, as a first approximation, with the intracellular compartment of exchangeable hormone. The role of cellular and extracellular factors in determining the partition of thyroxine between these compartments is analyzed under a variety of physiological and pathological settings.

Methods

For the acute distribution studies 5 to 10 μc of thyroxine-^{131}I (SA 30 to 40 mc per mg) was mixed with an approximately equal amount of albumin-^{125}I (SA 0.25 to 1 μc per mg) in a solution of 1% human serum albumin.[1] Both thyroxine-^{131}I and albumin-^{125}I were obtained commercially[2] and utilized without further purification. The solution containing the two tracer materials was injected into an antecubital vein through the rubber segment of an intravenous infusion set. During the following 4 hours, serial blood samples were obtained from the contralateral arm through an indwelling catheter. Approximately 6 ml of blood was obtained at each time and anticoagulated with heparin. Plasma samples were assayed for ^{131}I and ^{125}I in a Packard autogamma spectrometer as previously described (8). Since we found that over 95% of ^{131}I and ^{125}I was precipitable with trichloroacetic acid (TCA), whole plasma was assayed for radioactivity rather than TCA precipitate unless otherwise stated. The simplifying assumption was made in our calculations that all radioactivity represented either thyroxine-^{131}I or albumin-^{125}I. Appropriate dilutions of the administered dose were assayed simultaneously, and the plasma concentration of radioactivity was expressed as a per cent of the administered dose per liter plasma. Calculations and the underlying theoretical considerations are considered in detail in the following section.

In a single study, the rate of disappearance of thyroxine-^{131}I and albumin-^{125}I from blood was compared to the rate of entrance of these materials into the pleural effusion of a 65-year-old man with congestive heart failure. Ten-ml samples of pleural fluid were withdrawn through an indwelling catheter at the same time that plasma samples were obtained from the antecubital vein. Since radioactive iodide existing as a contaminant in the radioactive preparation would tend to diffuse rapidly and thus contribute disproportionately to the radioactivity in the effusion in the early phase of the study, all plasma and pleural effusion samples were precipitated with equal volumes of 20% TCA and washed twice with 1% TCA before radioactive assay.

The calculated rate of intracellular accumulation of thyroxine was compared to the rate at which the liver concentrated radioactivity. Fifty to 100 μc thyroxine-^{131}I was injected intravenously into four patients with normal liver function. Plasma samples were obtained at frequent intervals. A collimated scintillation probe (2-inch sodium iodide crystal) was placed in direct skin contact at the center of hepatic dullness as determined by manual percussion. The external rate was determined over 1-minute intervals for the first 30 minutes after the injection, and for progressively longer intervals thereafter. The radioactivity detected by the probe can be considered to rise from two sources, hepatic parenchyma and blood

[1] Albumisol, Merck Sharp & Dohme, West Point, Pa.

[2] Abbott Laboratories, North Chicago, Ill.

(intercostal vessels, thoracic structures, and hepatic vessels). Thus,

$$H_t = C_t - B_t, \quad [1]$$

where H_t = counts per minute emanating from the hepatic parenchyma, C_t = counts per minute detected by the probe from all sources, and B_t = counts per minute originating from blood. We assumed that $B_t = B_0 (T_t/T_0)$, where B_0 is the contribution from blood at time $t = 0$, and T_t and T_0 are the concentrations of thyroxine-^{131}I in plasma at times t and 0, respectively. Since B_0 can be approximated from the initial counting rate over the hepatic area and since T_t and T_0 can be determined by measurement of plasma radioactivity, the term B_t can be evaluated and Equation 1 solved for H_t.

The effect of 5,5-diphenylhydantoin [3] on the acute distribution kinetics of thyroxine-^{131}I was evaluated in three patients with central nervous system disease. A combined dose of thyroxine-^{131}I and albumin-^{125}I was injected in the standard fashion described above. Three to four hours after injection of the isotopes, 150 mg of sodium diphenylhydantoin dissolved in 5 ml of the diluent provided by the manufacturer (40% propylene glycol and 10% ethanol adjusted to pH 12) was injected over a 3-minute interval into the rubber segment of a running intravenous infusion set. Samples were obtained from an indwelling catheter in an antecubital vein of the contralateral arm. During the infusion, blood pressure, pulse, and electrocardiogram were monitored. No adverse reactions were encountered.

The distribution kinetics of thyroxine-binding prealbumin (TBPA) and albumin were compared after the simultaneous injection of 10 μc TBPA-^{131}I and 10 μc albumin-^{125}I. TBPA was isolated from whole serum as previously described (9). After iodination with ^{131}I by the method of Greenwood, Hunter, and Glover (10), the preparation was further purified by starch gel electrophoresis (11).

To determine whether thyroxine-binding proteins are present in the soluble fraction of liver homogenates, we performed the following experiment on two occasions. Portions of diagnostic liver biopsies obtained at the time of laparotomy were weighed, homogenized in 10 vol of 0.1 M physiologic saline (assuming a tissue density of 1), and centrifuged at 100,000 $\times$ g in a Spinco preparative ultracentrifuge for 60 minutes. Serum samples were also obtained from the same patients at the time of surgery. Serum was diluted in saline to the same extent as the liver biopsies. Radioactive thyroxine (0.03 μg per ml solution) was added to the diluted serum, to the supernatant of the liver homogenate, to a mixture of equal parts of diluted serum and supernatant liver homogenate, and to buffer alone. All preparations were then subjected to conventional paper electrophoresis (glycine acetate, pH 8.6), and the distribution of radioactivity was assessed in a paper strip scanner (12).

To obtain a direct estimate of hepatic thyroxine uptake, we gave four patients scheduled to undergo abdominal laparotomy a combined dose of thyroxine-^{131}I and albumin-^{125}I 3 to 4 hours before the anticipated time of abdominal exposure. Serial plasma samples were obtained and analyzed in the standard manner. Portions of diagnostic biopsies obtained at the time of laparotomy were lightly blotted and weighed. The contribution of thyroxine-^{131}I in residual blood in the tissue specimen to the observed ^{131}I counting rate could be assessed from the counting rate of albumin-^{125}I in the tissue and the ratio of ^{131}I to ^{125}I counting rates in a peripheral plasma sample obtained at the time of the biopsy. Thus, the net tissue uptake of thyroxine-^{131}I could be calculated from the difference in total counting rate of ^{131}I of the sample and the contribution from residual blood within the specimen.

Theoretical analysis of the acute distribution kinetics of thyroxine-^{131}I. As a first approximation, the plasma disappearance curves of thyroxine-^{131}I and albumin-^{125}I can be analyzed without reference to the effects of metabolic transformation of either substance for the first 4 hours after intravenous injection. If we assume single compartmental kinetics and a normal half-life for thyroxine and albumin, less than 2% of the administered thyroxine and less than 1% of the administered albumin will be catabolized in 4 hours.[4] Thus, the volumes of distribution of thyroxine-^{131}I and of albumin-^{125}I at any time t during the first 4 hours after the injection of the tracers can be obtained from the reciprocal of their plasma concentrations.

In the Results section experimental data will be presented to substantiate the following assumptions: *1)* The distribution kinetics of the thyroxine-^{131}I–plasma protein complex can be represented by those of albumin-^{125}I. *2)* Mixing of thyroxine in the extracellular compartment is largely governed by the mixing of the carrier protein. *3)* The rapid escape of thyroxine-^{131}I from the albumin distribution space after intravenous injection is due predominantly to the intracellular accumulation of tracer.

N_t, the per cent of administered thyroxine-^{131}I outside the albumin distribution space at any time t during the first 4 hours after injection, will be the product of the instantaneous concentration of thyroxine-^{131}I (T_t) expressed as per cent of the dose per liter and the differences in the volumes of distribution of thyroxine-^{131}I and albumin-^{125}I expressed in liters ($V_T - V_A$). Since $V_T = (100/T_t)$ and $V_A = (100/a_t)$, where a_t is the instantaneous concentration of albumin-^{125}I, it follows that

$$N_t = 100T_t[(1/T_t) - (1/a_t)] = 100\ [1 - (T_t/a_t)]. \quad [2]$$

Analysis of the acute distribution kinetics of thyroxine-^{131}I and albumin-^{125}I is complicated by the finding that the initial distribution volume of thyroxine is frequently larger than that of albumin as determined by extra-

[3] Dilantin, Parke, Davis, Detroit, Mich.

[4] The urinary excretion of ^{131}I was measured in four patients after the intravenous injection of thyroxine-^{131}I. Thyroidal accumulation of ^{131}I was blocked by the oral administration of Lugol's solution. The average 4-hour urinary excretion was 3% of the dose. Although this value is higher than the calculated metabolic transformation, the discrepancy can be attributed to the preferential early excretion of iodide-^{131}I contaminating the administered dose.

polation to $t = 0$. It is unclear whether this discrepancy is the artifactual result of inadequacy in the extrapolation technique and the difficulties in frequent early plasma sampling or whether there is a true increase in the initial thyroxine distribution volume.[5] For the purposes of simplicity we have assumed, somewhat arbitrarily, that the initial differences in distribution volumes between albumin and thyroxine are real but do not represent the effects of intracellular accumulation. Thus, we have defined I_t, the per cent of administered thyroxine-^{131}I accumulated in the cellular compartment, as $I_t = N_t - N_0$, where N_0 represents the initial value of N_t as determined by extrapolation. If we substitute into Equation 2,

$$I_t = 100[(T_0/a_0) - (T_t/a_t)]. \quad [3]$$

The per cent of thyroxine remaining in the extracellular compartment at time t, E_t, will then be $100 - I_t$. Alternative and probably equally justified interpretations of the initial discrepancy would lead to results substantially similar to those presented here since the volume difference under consideration is relatively small (less than 5%).

Curves illustrating I_t as a function of time are presented in the Results. It can be seen that I_t achieves a plateau value between 3 and 4 hours after the administration of the isotopes. The shape of the intracellular accumulation curve suggests that it can be described by an exponential growth curve of the general form

$$I_t = I_{max}(1 - e^{-\lambda t}). \quad [4]$$

The adequacy of this function in describing the function I_t can be graphically tested. If we rearrange the terms of Equation 4,

$$I_{max} - I_t = I_{max}e^{-\lambda t}. \quad [5]$$

The linearity of $(I_{max} - I_t)$ as a function of t on a semilogarithmic plot with an intercept of I_{max} on the ordinate is also illustrated in the following section. In all subjects and patients that we have studied to date Equation 4 appears to describe the observed intracellular accumulation curve within the limits of experimental error.

The function described by Equation 4 is known to represent the accumulation of isotope in one of the compartments of a closed two compartmental system as is illustrated in Figure 1. The properties of a closed two compartmental model have previously been discussed by Solomon (14). Isotope is injected into compartment E at $t = 0$. The defining equations of the system are

$$(dE_t/dt) = -(k_E)E_t + (k_I)I_t, \text{ and} \quad [6]$$

$$(dI_t/dt) = -(k_I)I_t + (k_E)E_t, \quad [7]$$

where E_t and I_t represent the per cent of the injected dose within compartments E and I at any time t, k_E is the fractional transfer constant from compartment E to compartment I, and k_I is the fractional transfer constant in the opposite direction.

Solution of Equations 6 and 7 will indicate that at any time t

$$I_t = [100k_E/(k_I + k_E)][1 - e^{-(k_I+k_E)t}], \quad [8]$$

and

$$E_t = \frac{100k_I}{k_I + k_E} + \frac{100k_E}{k_I + k_E}[e^{-(k_I+k_E)t}]. \quad [9]$$

Thus, at $t = \infty$, I_t will reach a maximal value of

$$I_{max} = 100k_E/(k_E + k_I), \quad [10]$$

and E_t will reach a minimal value at equilibrium of

$$E_{min} = 100k_I/(k_E + k_I). \quad [11]$$

Moreover,

$$\lambda = k_I + k_E. \quad [12]$$

The volume of compartment E can be defined as $V_E = E_{min}/T_{eq}$, where E_{min} and T_{eq} represent the per cent of the dose in the extracellular compartment and the simultaneous plasma concentration of thyroxine-^{131}I in the plasma when the two compartments come to equilibrium relative to each other. Analogously, the volume of compartment I can be defined as $V_I = I_{max}/T_{eq}$. Although the volume of the intracellular compartment is a useful concept, no immediate anatomical significance should be assigned to it.[6]

[5] One possible source of the discrepancy between the extrapolated initial distribution volume of thyroxine-^{131}I and albumin-^{125}I is the presence of contaminating radioactive iodide and of radioactive thyronines (3,5,3′-triiodothyronine and 3,5′,3′-triiodothyronine) in the thyroxine preparations used (13). Such contaminants, being less tightly bound to plasma proteins than thyroxine, may also be responsible for a slight overestimation of the fractional rate of exit of thyroxine from the plasma compartment. The significance of these contaminants is further discussed in connection with the pleural effusion study.

[6] Actually, the volumes of compartments E and I (not to be confused with the volumes of distribution of thyroxine-^{131}I and albumin-^{125}I) are expanding during the period of observation. Initially, the volume of compartment E is that of the plasma volume, about 3.2 L. At the equilibrium time, the volume of E is 4.2 L. A similar relative increase occurs in compartment I. Nevertheless, the interchange of thyroxine between these compartments can be treated as though we were dealing with two static compartments, the volumes of which were determined at equilibrium time. The reason for this lies in the fact that fractional exchange of thyroxine between the two compartments is relatively independent of the compartment size during the period of observation.

An alternative kinetic scheme would involve an unrestricted two compartmental model analogous to that originally employed by Berson and Yalow (15) and more recently by Cavalieri and Searle (7). In such a system an interchange between the plasma compartment and compartment I would occur, but no restrictions would be placed on the movement of thyroxine out of the plasma compartment into the interstitial compartment. Solution of such a model would yield essentially similar results. The fractional transfer constants from the plasma compartment to the intracellular compartment k_p would

The nature of the equilibrium time deserves comment. This is the theoretical point at which the flux of thyroxine-^{131}I from the extracellular compartment to the intracellular compartment is equal to the flux in the opposite direction. On the time scale used in these studies, the point is determined as the midpoint of the I_{max} plateau. At this point the specific activity of thyroxine in both compartments can be assumed to be equal. Since the concentration of unlabeled thyroxine can be determined from the chemical protein-bound iodine (PBI), the exchangeable pools of thyroxine in both compartments can be calculated from the data at hand. Thus,

$$Z_I = V_I(\tau), \qquad [13]$$

and

$$Z_E = V_E(\tau), \qquad [14]$$

where Z_I and Z_E are the pool sizes of thyroxine in compartments I and E, respectively, and τ is the concentration of circulating thyroxine in plasma. It is apparent that if thyroxine-^{131}I accumulation in the intracellular compartment were followed for a longer period and plotted on a time scale of days rather than hours, the apparent plateau level of I_t would be reduced to a maximal point with a subsequent downward deflection of the curve representing the net effects of thyroxine metabolism and the further distribution of thyroxine into the extracellular compartment.

Data will be presented to indicate that the transfer of thyroxine from the extracellular compartment to the intracellular compartment is dependent in part on the strength of thyroxine binding by the plasma proteins. The model system proposed can be used to quantitate this effect of protein binding. Thus, the fractional transfer constant k_E can be expressed as

$$k_E = h/b_E, \qquad [15]$$

where h is a permeability factor (liters per minute) dependent on the properties of the hypothetical composite membrane separating compartments including its surface area and effective porosity[7] and b_E is a term (liters) representing over-all binding of thyroxine by plasma proteins in the extracellular compartment. The strength of thyroxine binding by plasma proteins can be represented by the expression $P = \sum_{1}^{n} k_i(M_i - TP_i)$, where k_i is the apparent association constant of the ith species of a total of n binding sites, M_i the molar concentration of the ith binding sites, and TP_i, the molar concentration of the ith thyroxine binding site complex. This expression is, in essence, a measure of the effective concentration of unoccupied thyroxine binding sites in plasma. The value P can be evaluated by equilibrium dialysis. In an equilibrium dialysis system in which serum is diluted by a factor of 150, $P = 150/DF$, where DF represents the dialyzable fraction (8). The over-all binding by the extracellular compartment can be obtained by multiplying P and V_E, the volume of the extracellular compartment at the equilibrium time. Thus, if we substitute into Equation 15 and rearrange terms,

$$h = (150/DF)(V_E)(k_E). \qquad [16]$$

The value for k_E can be determined as follows. The function λ can be determined graphically from a plot of log $(I_{max} - I_t)$ against t (Equation 5). From Equations 10 and 11, it follows that

$$I_{max}/(100 - I_{max}) = (k_E/k_I), \qquad [17]$$

and from Equation 12 and 17

$$k_E = \lambda(I_{max}/100), \qquad [18]$$

and

$$k_I = \lambda[1 - (I_{max}/100)]. \qquad [19]$$

If we substitute into Equation 16,

$$h = \lambda(150/DF)(V_E)(I_{max}/100). \qquad [20]$$

Lastly, since V_E will vary with body size, it is convenient for the purpose of comparisons to express the permeability factor per liter extracellular volume at equilibrium. Thus,

$$(h/V_E) = (150\lambda/DF)(I_{max}/100). \qquad [21]$$

Analogously, the fractional transfer constant from the intracellular to the extracellular compartment, k_I, can be expressed as

$$k_I = h/b_I, \qquad [22]$$

where h is the same permeability factor used in Equation 15 and numerically defined in Equation 20, and b_I represents net intracellular binding. It should be emphasized that the intracellular binding may differ markedly in terms of its physicochemical characteristics from plasma protein binding. It may be useful to regard intracellular binding simply as the effective retarding mechanism serving to maintain thyroxine within the cellular compartment. Again, b_I is the product of a concentration and a volume factor. Since the volume of the intracellular compartment is expressed in terms of plasma equivalents at the equilibrium time, the binding factor per unit volume is the same as that for plasma ($P = 150/DF$).

$$b_I = (150/DF)(V_I). \qquad [23]$$

The final picture that emerges from this formulation is that the partition of thyroxine between tissues and the extracellular compartments will be the net resultant of the balance of measurable tissue and protein binding factors. An increased I_{max} could result either from increased tissue binding or decreased extracellular binding or both. Conversely, a diminished I_{max} could represent either increased plasma protein binding or diminished cellular binding or both. If we assume normal permeability characteristics of the hypothetical membrane, increased binding by extracellular proteins should result

---

simply be related to k_E by the following relationship:

$k_E/k_p = V_a/V_E$, where V_a is the plasma volume.

[7] This is not to suggest that the exchange between the extracellular and intracellular compartments is necessarily a passive phenomenon. The concept of effective porosity is used purely in an operational sense and can be represented by a number of physical models any of which could depend on active cellular metabolism.

in a diminished k_E but not affect k_I. Similarly, diminished binding by plasma proteins should result in a high k_E but leave k_I unchanged.

Results

Evidence supporting the use of albumin-^{125}I in tracing the distribution of thyroxine-^{131}I into the extracellular space

Distribution of the thyroxine–binding proteins. The plasma disappearance curves of simultaneously injected TBPA-^{131}I and albumin-^{125}I for the first 4 hours are illustrated in Figure 2. Similar results were observed in a second study. Plasma levels of the two iodinated proteins do not differ appreciably during the period of observation. The data indicate that the acute distribution kinetics of TBPA and albumin are similar and consonant with previous demonstrations that the space of TBPA distribution is the same as that of serum albumin (16, 17).

Unfortunately, purified TBG is not available for similar kinetic studies. Indirect techniques

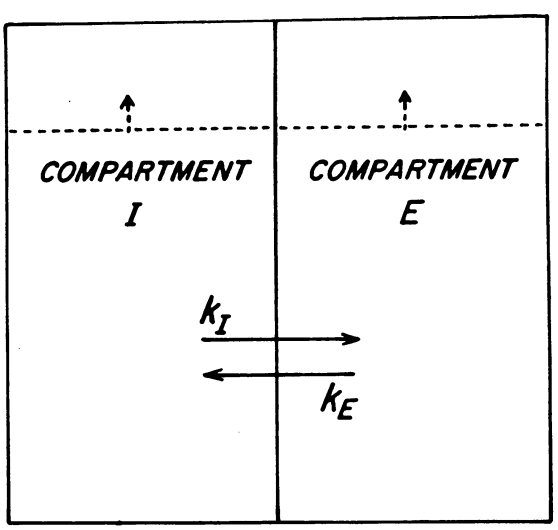

FIG. 1. SCHEMATIC REPRESENTATION OF MODEL SYSTEM EMPLOYED TO REPRESENT THE INTERCHANGE OF THYROXINE BETWEEN INTRACELLULAR (I) AND EXTRACELLULAR (E) COMPARTMENTS. During the 4 hours of observation, the size of each compartment increases. The initial state is indicated by the broken line, and the direction of expansion by the broken arrows. The fractional interchange (k_E, k_I) remains unaltered during the period of expansion. Mathematically, this model can be treated as a quasistatic system with the dimensions of the final compartments represented by the solid lines.

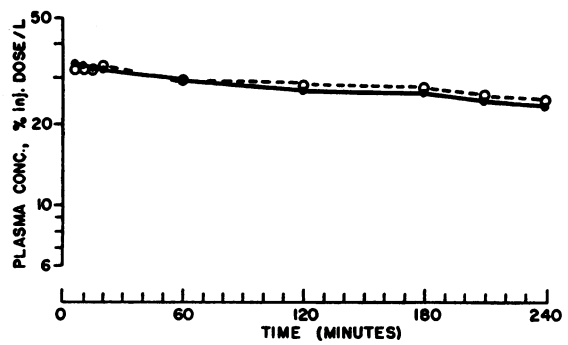

FIG. 2. PLASMA DISAPPEARANCE CURVES OF SIMULTANEOUSLY INJECTED THYROXINE-^{131}I–BINDING PREALBUMIN (————) AND ALBUMIN-^{125}I (- - - -).

were, therefore, applied to obtain information about the distribution of this protein. As will be shown subsequently, a large fraction of administered thyroxine-^{131}I leaving the plasma compartment enters the liver. If intrahepatic thyroxine exists as a thyroxine–TBG complex, one should be able to detect TBG in the soluble fraction of liver homogenates. Results of two separate experiments failed to disclose evidence of any strong soluble intracellular binding protein capable of competing effectively with plasma proteins for thyroxine. Thyroxine-^{131}I added to the supernatant fraction migrated electrophoretically as a single peak with a mobility identical to that of thyroxine-^{131}I alone. Moreover, when the supernatant fraction was mixed with an equivalent volume of appropriately diluted serum, the mobility and distribution of thyroxine-^{131}I were identical to those of an equivalent dilution of serum in saline. These results suggest that TBG is not present in the soluble fraction of liver homogenates.

Evaluation of the possible influence of differential transcapillary migration of thyroxine and its binding proteins. Current estimates based on equilibrium dialysis of serum against aqueous buffer have suggested that from 0.030 to 0.050% of total circulating thyroxine is unbound (8, 18–20). If this free fraction diffused very rapidly across the capillary endothelium, it would be theoretically possible for thyroxine to distribute itself in the extracellular space more rapidly than its binding proteins. The kinetic analysis proposed in this paper assumes that such independent diffusion is quantitatively unimportant. The ex-

perimental findings underlying this assumption will be presented.

Since interstitial fluid in man is generally unavailable for sampling, the movement of simultaneously injected thyroxine-^{131}I and albumin-^{125}I from the plasma into the pleural effusion of a 65–year–old man was studied. The pleural fluid had an albumin concentration of 2.1 g and a globulin concentration of 1.7 g per 100 ml. The concomitant plasma albumin and globulin concentrations were 3.4 and 3.6 g per 100 ml, respectively. The PBI concentration of the effusion was 3.4 μg per 100 ml, and that of the plasma was 6.1 μg per 100 ml. It should be noted parenthetically that the ratio of PBI concentrations in pleural fluid and plasma, 0.56, approximates within experimental error the ratio of albumin concentrations, 0.62. Also, the distribution of tracer thyroxine-^{131}I among binding proteins after paper electrophoresis was identical in both fluids. These findings suggest that the movement of thyroxine–binding proteins across this capillary bed is similar to that of serum albumin.[8]

The concentration of thyroxine-^{131}I and albumin-^{125}I in plasma and pleural fluid is illustrated in Figure 3. At the end of 4 hours, the specific activities of both tracers in the pleural fluid were less than one-tenth that of plasma. Because of the slow movement of both tracers the radioactivity in pleural fluid up to 40 minutes was found to be predominantly derived from contaminating iodide present in the original shipments of the tracers. Accordingly, all samples, both of pleural fluid and plasma were precipitated with TCA to remove the bulk of iodide. Nevertheless, the problem of coprecipitation of iodide with thyroxine (12) prevented accurate measurements until the 60–minute pleural sample.

An estimate of the relative fractional rate of entrance of thyroxine-^{131}I and albumin-^{125}I into the pleural fluid can be obtained from the follow-

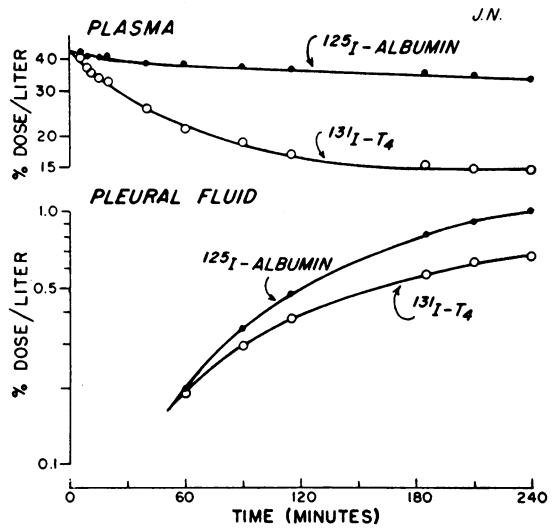

FIG. 3. CONCENTRATIONS OF THYROXINE-^{131}I (T_4) AND ALBUMIN-^{125}I IN PLASMA AND PLEURAL FLUID AFTER SIMULTANEOUS INJECTION OF TRACERS. All samples were precipitated with trichloroacetic acid.

ing considerations. Assume that the movement of both tracers from the plasma compartment to the pleural compartment obeys first–order kinetics. Since the movement of both tracers is slow and the plasma–pleural system far from equilibrium, it can be assumed that the net movement of tracers out of the pleural fluid is negligible during the interval under consideration. Thus,

$$(c_T)_t(V_e) = (k_{T,i})V_a \int_0^t T_t dt, \qquad [24]$$

where $(c_T)_t$ is the concentration of thyroxine-^{131}I in the pleural fluid at time t (per cent dose per liter); V_e, the volume of the pleural effusion (liters); $k_{T,i}$, the fractional transfer constant from the plasma compartment into the pleural compartment (minutes^{-1}); V_a, the plasma volume (liters); and T_t, the concentration of thyroxine-^{131}I in plasma at time t (per cent dose per liter). Since values from 0 minutes to 60 minutes are unreliable, the function described by Equation 24 is more appropriately evaluated between 60 and 240 minutes and can be rewritten

$$[(c_T)_{240} - (c_T)_{60}]V_e = (k_{T,i})V_a \int_{60}^{240} T_t dt. \qquad [25]$$

[8] This suggestion, of course, is predicated on the assumption that the fractional removal rates of the various components in the pleural space (PBI, TBPA, TBG, and albumin) are similar. Moreover, the pleural fluid should not be considered necessarily representative of the extravascular fluid in general. In this connection, however, it is of interest to note that the ratio of the concentrations of PBI to protein in thoracic duct lymph is the same as that in serum (7).

Similarly,

$$[(c_a)_{240} - (c_a)_{60}]V_e = (k_{a,i})V_a \int_{60}^{240} a_t dt, \quad [26]$$

where $(c_a)_t$ is the concentration of albumin-^{125}I in the pleural fluid at any time t; $k_{a,i}$, the fractional transfer constant of albumin-^{125}I from the plasma compartment to the pleural compartment; and a_t the concentration of albumin-^{125}I in the plasma at any time t.

From Equations 20 and 21, it follows that

$$\frac{k_{T,i}}{k_{a,i}} = \left[\frac{(c_T)_{240} - (c_T)_{60}}{(c_a)_{240} - (c_a)_{60}}\right] \frac{\int_{60}^{240} a_t dt}{\int_{60}^{240} T_t dt}. \quad [27]$$

Values for c_T and c_a are directly available, and the integral expressions can be evaluated graphically. The above ratio of the fractional transfer constants was determined to be 1.25. Thus, the possibility exists that there is a 25% faster ingress of thyroxine into the pleural fluid and that this difference could be due to independent diffusion of hormone. It appears likely, however, that the calculated ratio of 1.25 is an overestimation. Recent observations (13) have indicated that Abbott thyroxine-^{131}I is contaminated with several per cent radioiodinated 3,5,3′-L-triiodothyronine and 3,5′,3′-L-triiodothyronine. These substances are much less strongly bound to plasma proteins than thyroxine. Thus, material appearing in the pleural fluid in the early phase of the equilibrium period could contain a disproportionate contribution from these iodinated contaminants.

Even if we assume that the ratio of the fractional transfer constants is correct, calculations based on this figure would suggest that the effects of independent transcapillary diffusion of thyroxine are negligible in terms of the over–all distribution kinetics of thyroxine. In this patient initial clearance of albumin-^{125}I from plasma, estimated from the graphically determined initial fractional exit rate and the plasma volume, is 6.24 ml per minute. Similarly, the total clearance of thyroxine from the plasma in this patient is 37.6 ml per minute. If independent thyroxine diffusion is 25% of the albumin clearance, the clearance of thyroxine from plasma by means of independent diffusion is 1.56 ml per minute. Thus, independent diffusion would constitute only 4.1% [= (1.56/37.6) × 100] of the total thyroxine clearance. Since the 25% figure is probably an overestimation, as pointed out above, the effects of independent diffusion are minimal.

This analysis is based on the assumption that permeability characteristics of the pleural capillary bed in the patient studied are typical of other capillary beds that allow only slow transcapillary passage of protein. The fact that tracer thyroxine is cleared initially at a sixfold greater rate than tracer albumin from the plasma compartment can readily be attributed to the more rapid rate of equilibration of protein across selected capillary beds such as the hepatic sinusoids (21). A small pool of rapidly exchangeable protein in such beds would not greatly influence the apparent initial disappearance rate of albumin-^{131}I from the plasma but would serve to facilitate a rapid and direct exchange of thyroxine from plasma protein to cellular receptor sites.

The transport of thyroxine from the interstitial space into the cells would thus not be limited by the diffusion of thyroxine in an aqueous medium or by the transcellular movement of plasma binding proteins.

Comparison of the rates of intracellular and hepatic accumulation of thyroxine-^{131}I. Results

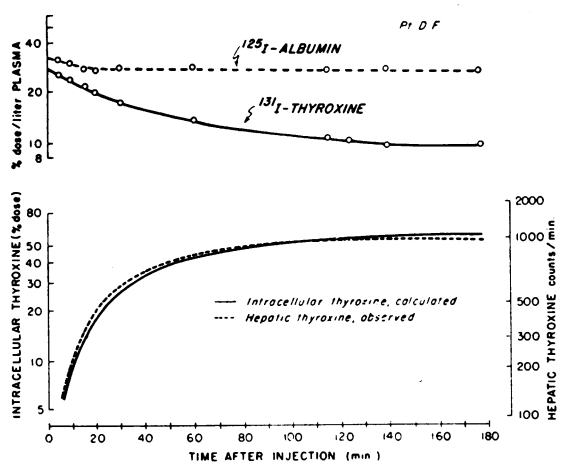

FIG. 4. COMPARISON OF RATES OF HEPATIC UPTAKE AND INTRACELLULAR ACCUMULATION OF THYROXINE-^{131}I. Hepatic uptake was calculated from the observed external counting rate over the liver with an appropriate correction for the falling levels of blood thyroxine detected by the probe (see text). Intracellular accumulation was calculated from the plasma disappearance curves of thyroxine-^{131}I and albumin-^{125}I.

illustrated in Figure 4 are representative of one of four studies in which the intracellular and hepatic accumulations of thyroxine-^{131}I were simultaneously determined. Since the absolute fraction of hormone taken up by the liver cannot be readily determined from external measurements alone, only the rates of these processes can be meaningfully compared. The close correspondence of these rates suggests either that the liver is the predominant component of the intracellular compartment or that the kinetic characteristics of nonhepatic components resemble those of the liver. Under any circumstances these findings lend credence to the basic assumptions underlying our analysis.

Results of the analysis (Table I)

The results of a representative study in a normal subject are illustrated in Figure 5. Here, initial distribution volume of thyroxine as determined by simple extrapolation is approximately 4.5% greater than the initial distribution volume of albumin. Thyroxine rapidly leaves the vascular compartment, but by 200 minutes after injection, the fractional rates of egress of thyroxine-^{131}I and albumin-^{125}I are similar. In the 4-hour period, the fall of albumin-^{125}I is 15.5% of the initial concentration, whereas the decline in thyroxine-^{131}I during the same interval is 62%. The calculated per cent of the dose in the intracellular compartment rises to a plateau value of 51.1%. The linearity of a semilogarithmic plot of $(I_{max} - I_t)$ as a function of time, which forms the basis of the two compartmental analysis, is illustrated in Figure 6. Kinetic data on six normal subjects are summarized in Table I.

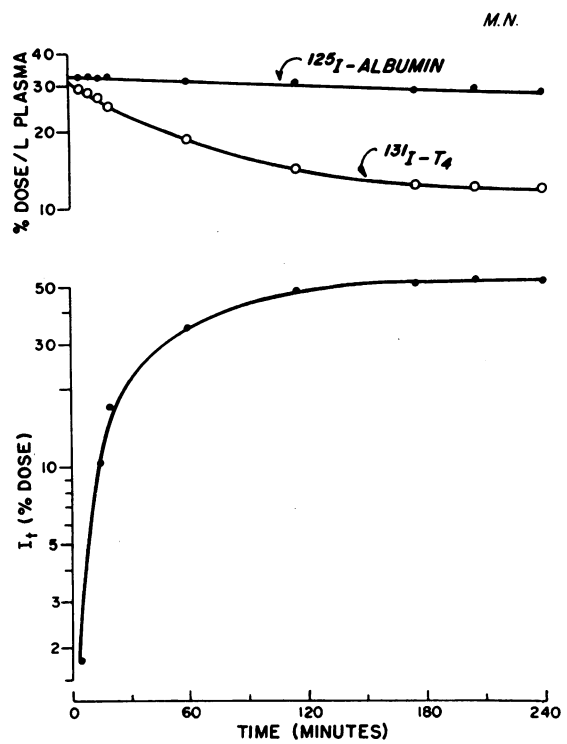

FIG. 5. REPRESENTATIVE STUDY IN A NORMAL SUBJECT (M.N.). I_t = intracellular accumulation, calculated from the plasma disappearance curves of thyroxine-^{131}I and albumin-^{125}I.

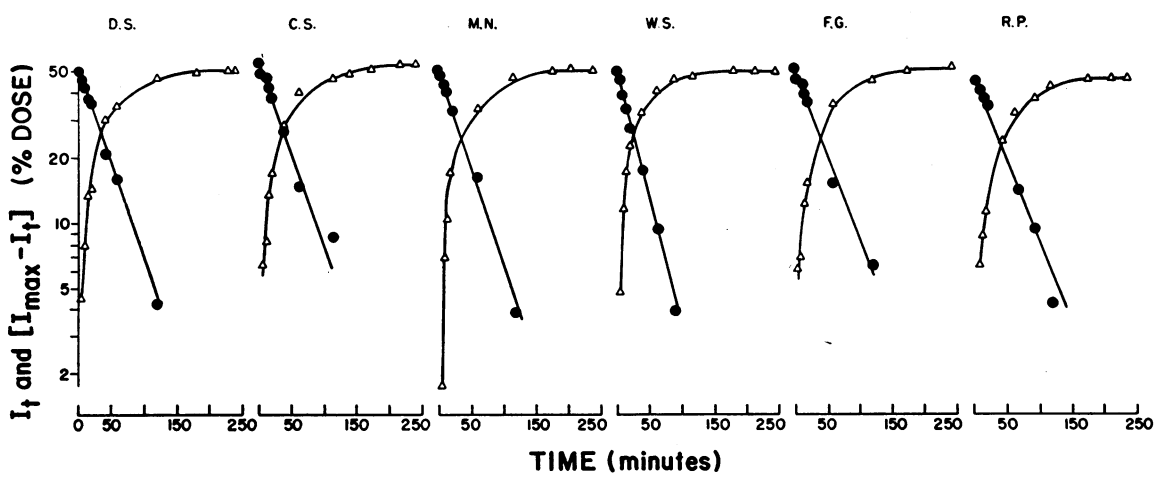

FIG. 6. LINEAR RELATIONSHIP OF LOG $(I_{max} - I_t)$ AS A FUNCTION OF TIME IN THE GROUP OF SIX NORMAL SUBJECTS. The intracellular thyroxine-^{131}I accumulation (I) is plotted on the same scale. $\triangle = I_t$; $\bullet = I_{max} - I_t$.

TABLE I

*Results of kinetic and serum protein binding studies**

	Age	Sex	Weight	Serum thyroxine	Maximal binding capacity		DF	V_A	I_{max}	λ	k_I	k_E	V_E	V_I	b_E	b_I	Z_E	Z_I	h/V_E
					TBPA	TBG													
	years		*kg*	*μg/100 ml*	*μg thyroxine/100 ml*		$\times 10^2$	*L*	%	$min^{-1} \times 10^2$	$min^{-1} \times 10^2$	$min^{-1} \times 10^2$	*L*	*L*	$L \times 10^{-4}$	$L \times 10^{-4}$	*μg*	*μg*	min^{-1}
A. Normal subjects																			
FG	50	M	77.3	7.19	309	18.3	4.25	3.12	52.4	17.7	8.43	9.27	3.97	4.36	1.40	1.54	285	313	32.7
MN	31	M	79.5	7.65	244	19.0	3.78	3.08	51.1	18.2	8.90	9.30	3.66	3.82	1.45	1.52	280	292	36.9
DS	57	M	88.6	7.34	355	18.4	3.46	4.08	50.8	19.2	9.45	9.75	5.72	5.90	2.48	2.56	420	433	42.3
RP	26	M	90.9	7.49	311	18.1	3.43	3.03	47.8	16.1	8.40	7.70	4.00	3.66	1.75	1.60	300	274	33.7
CS	55	F	56.4	6.73	199	16.0	4.16	3.01	55.0	16.5	7.42	9.08	3.57	4.37	1.28	1.58	240	294	32.7
WS	48	M	86.4	7.80	357	22.2	4.28	3.08	51.1	30.1	14.72	15.38	4.42	4.62	1.55	1.62	345	360	53.9
		Average	79.9	7.37	296	18.7	3.89	3.23	51.4	19.6	9.55	10.08	4.22	4.46	1.65	1.74	312	328	38.7
B. Liver disease†																			
AG	40	M	78.6	9.18	43	31.4	4.67	3.12	25.6	18.2	13.54	4.66	3.95	1.36	1.27	.43	363	125	15.0
AL	24	M	63.4	9.63	150	22.0	4.83	3.03	33.1	17.7	11.84	5.86	4.03	1.99	1.25	.62	388	192	18.2
DH	36	F	43.6	3.36	46	30.0	12.15	3.33	16.5	5.6	4.88	.92	4.29	0.85	0.53	.10	144	29	1.1
WL	45	M	105.0	6.73	83	17.1	8.17	5.40	16.9	17.3	14.38	2.92	6.57	1.33	1.20	.24	442	90	5.4
JL	38	M	85.9	7.49	134	32.4	4.47	3.55	40.9	15.7	9.28	6.42	4.86	3.36	1.63	1.13	364	252	21.5
		Average	75.3	7.28	91.2	26.6	6.86	3.69	26.6	14.9	10.74	4.16	4.74	1.78	1.18	0.50	340	138	12.2
C. Congenital decrease in TBG																			
TH	17	F	63.6	4.3	156	5.1	13.87	2.30	73.6	31.5	8.30	23.20	2.94	8.21	0.74	1.71	126	352	35.2

* Abbreviations and symbols: TBPA, thyroxine-binding prealbumin; TBG, thyroxine-binding globulin; DF, dialyzable fraction (8, 12); V_A, initial distribution volume of albumin-^{125}I; I_{max}, maximal per cent of administered ^{131}I within cellular compartment, see Equations 4 and 10; λ, see Equations 4, 5, and 12; k_I, fractional transfer constant from intracellular to extracellular compartments; k_E, fractional transfer constant from extracellular compartment; V_E, volume of extracellular compartment at equilibrium time; V_I, volume of intracellular compartment at equilibrium time; b_E, net binding of extravascular compartment [= (150/DF) × V_E]; b_I, net binding of intracellular compartment [= (150/DF) × V_I]; Z_E, thyroxine pool in extracellular compartment at equilibrium time (Equation 14); Z_I, thyroxine pool in intracellular pool at equilibrium time (Equation 13); h/V_E, corrected permeability factor (Equation 21).

† All patients had Laennec's cirrhosis except A.L., who had infectious hepatitis.

TABLE II

Thyroxine-^{131}I content of liver biopsies

Patient	Age	Sex	Weight	Estimated* liver wt	% Dose/g liver	T/P†	Estimated hepatic uptake	I_{max}‡	Estimated % I_{max} in liver
	years		*kg*	*kg*			%	%	
SB	60	F	59.1	1.18	.0439	3.29	51.9	63.4	81.8
ZF§	35	F	51.8	1.04	.0115	0.46	11.9	27.4	43.5
AM	26	F	60.4	1.21	.0275	1.91	33.2	47.9	69.4
JR	77	M	78.2	1.56	.0276	3.01	43.2	52.3	82.6

* Liver weight estimated to be 2% of body weight.
† T/P = tissue/plasma ratio = per cent dose per gram liver/per cent dose per milliliter plasma at time of biopsy.
‡ I_{max} = maximal fraction of administered dose thyroxine-^{131}I calculated to be within intracellular compartment.
§ Fatty infiltration, indicated by liver biopsy, may account for relatively low I_{max} and hepatic uptake.

Results of hepatic biopsies obtained approximately 4 hours after the injection are indicated in Table II. If we assume that the weight of the liver is 2% of the body weight, the thyroxine-^{131}I content of liver is on the average approximately 70% of the calculated I_{max}. The important contribution of the liver to intracellular accumulation of thyroxine-^{131}I is also indicated by results of studies in five patients with liver disease due to Laennec's cirrhosis or acute hepatitis (Table I). Figure 7 illustrates the characteristic changes in the plasma disappearance curve of thyroxine-^{131}I. The calculated intracellular accumulation of thyroxine (I_{max}) was markedly

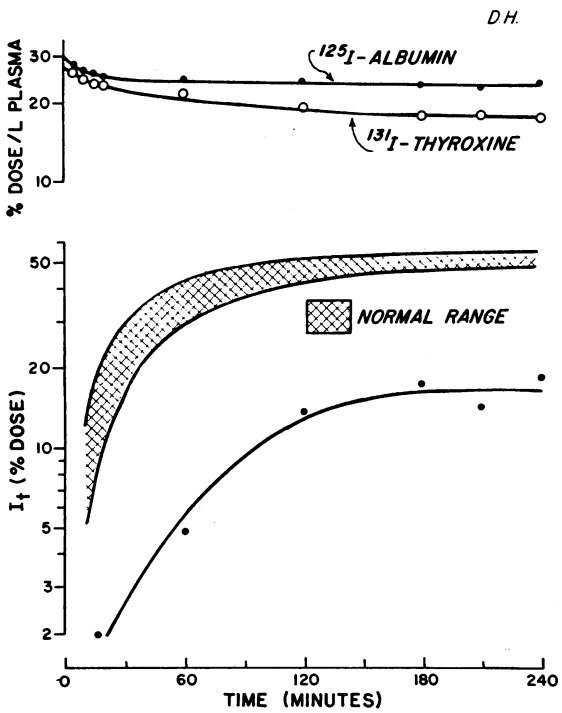

FIG. 7. PLASMA DISAPPEARANCE AND CALCULATED INTRACELLULAR ACCUMULATION OF THYROXINE-^{131}I IN A PATIENT (D.H.) WITH SEVERE LIVER FAILURE DUE TO LAENNEC'S CIRRHOSIS. Range of intracellular accumulation in normal subjects is indicated by crosshatched area. Note the relatively small splay between thyroxine-^{131}I and albumin-^{125}I curves in comparison to similar curves in the normal subject illustrated in Figure 5.

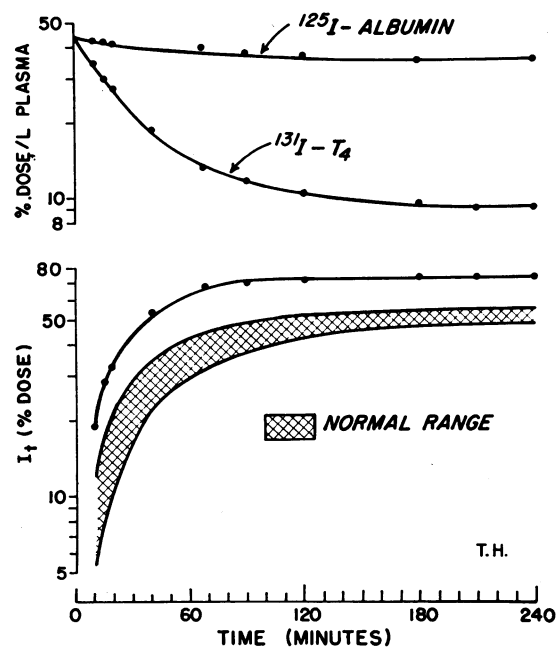

FIG. 8. PLASMA DISAPPEARANCE AND CALCULATED INTRACELLULAR ACCUMULATION OF THYROXINE-^{131}I IN A PATIENT (T.H.) WITH A CONGENITAL DEPRESSION IN THE MAXIMAL BINDING CAPACITY OF THYROXINE-BINDING GLOBULIN. Note the wide splay between thyroxine-^{131}I and albumin-^{125}I curves in comparison to that in the normal subject illustrated in Figure 5. Normal range of intracellular uptake is illustrated by crosshatched area.

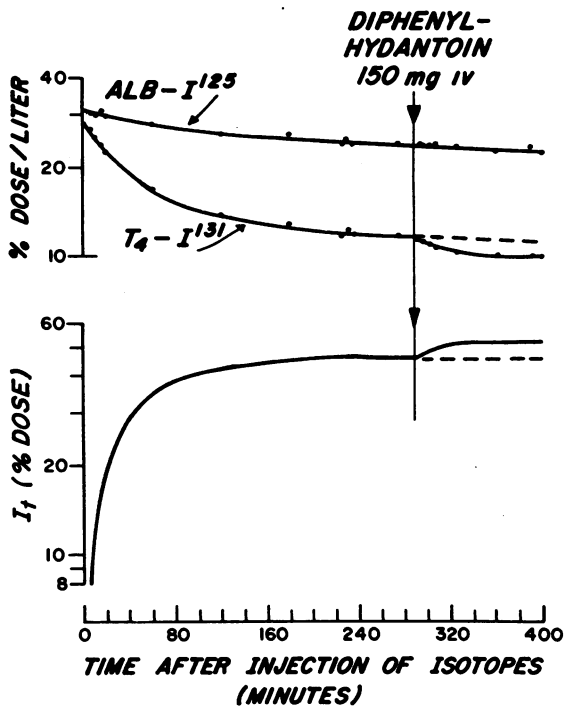

FIG. 9. EFFECT OF ACUTE INFUSION OF 150 MG DIPHENYLHYDANTOIN ON THE PLASMA DISAPPEARANCE OF THYROXINE-^{131}I AND THE CALCULATED INTRACELLULAR ACCUMULATION CURVE.

reduced in this group of patients. The fractional transfer constant from extracellular to cellular compartments, k_E, was low, although the fractional transfer constant in the opposite direction was normal. Since the net binding by serum proteins as measured by equilibrium dialysis was either normal or diminished, the reduced fractional entrance into the cellular compartment must be attributed to a change in the permeability function (h/V_E). Such changes in permeability can be interpreted either in terms of alterations in the effective porosity of the plasma membrane or in terms of disruption of a metabolically dependent transport system for thyroxine. Consonant with these observations, the calculated intracellular pool of thyroxine was also reduced.

The effect of alterations in the plasma thyroxine–binding proteins is illustrated by a study in a 17–year–old female with a genetically determined reduction in the maximal binding capacity of TBG (Table I, Figure 8).[9] The concentration of thyroxine-^{131}I falls precipitously after injection, leading to the calculated increase in k_E and I_{max}. The values for k_I and the permeability factor h/V_E are within the normal range. Although the calculated extracellular pool of thyroxine at the equilibrium time is reduced, the calculated intracellular pool is normal. Thus, the observed changes in the partition of tracer can be attributed exclusively to an alteration in the maximal binding capacity of TBG.

The role of binding proteins in regulating the partition of thyroxine-^{131}I between extracellular and cellular compartments is also apparent from the effects of the intravenous injection of diphenylhydantoin, a drug known to displace thyroxine from TBG and lower the serum PBI (22–24). Studies were carried out in three patients. Diphenylhydantoin (150 mg) was infused when the intracellular accumulation curve had reached a plateau value. Results in a representative study are indicated in Figure 9. Within minutes after the injection, there was a downward deflection of the plasma thyroxine-^{131}I curve. No change in the albumin-^{125}I curve was noted. The calculated intracellular accumulation rose from 46 to 52% of the administered dose.

Discussion

A number of assumptions underlying this analysis deserve special consideration. In interpreting the results of the plasma disappearance curves and in estimating thyroxine accumulated in the hepatic samples, we have tacitly assumed that serum albumin is predominantly extracellular. Although intracellular albumin (or material immunologically related to albumin) has been demonstrated by immunofluorescence (25, 26), this is not considered to be quantitatively significant in relation to the mass of extracellular protein (27). Moreover, radioautographic studies carried out in the rat during the initial distribution period of albumin and thyroxine revealed no intracellular localization of albumin-^{125}I but diffuse cytoplasmic distribution of thyroxine-^{125}I (28). This observation justifies, in part, the use of the term "intracellular," since selective concentration of thyroxine-^{125}I in the interstitium or on the surface of cell membranes could not be demonstrated.

[9] Kindly referred to our attention by Dr. B. Segal.

We have also assumed that the distribution of TBG does not differ significantly from that of albumin and TBPA. Direct proof of this assumption must await the purification of TBG so that the kinetics of labeled TBG can be measured. Nevertheless, our failure to find TBG in the soluble fraction of liver homogenate serves largely to exclude TBG as the transport vehicle across the hepatic cell membrane. If TBG were indeed responsible for the intracellular accumulation of thyroxine, one would expect to find at least two to three times the concentration of TBG in liver as in plasma, based on the relative concentrations of thyroxine in liver and plasma. Our data do not exclude the possibility that TBG may be strongly associated with particulate cellular elements, but in such an event it would be difficult to assign to intracellular TBG the transport function that would be required by a rapid two way exchange across the cell membrane. Moreover, other studies have failed to demonstrate greater thyroxine binding by particulate elements than by the cell sap of hepatic cells (29, 30). Lastly, the argument previously advanced by Cavalieri and Searle (6) strongly militates against any possible role of TBG in transcellular transport. In patients with congenitally low TBG one would not expect increased hepatic uptake of administered tracer thyroxine if TBG were indeed responsible for transcellular thyroxine transport.

The importance of the liver in the extravascular distribution of thyroxine is illustrated by the diminished intracellular thyroxine in patients with liver disease, by the close correspondence of the rates of intracellular and hepatic uptake of thyroxine-^{131}I, and by the liver biopsy data. Estimates based on the normal histological specimens listed in Table II (i.e., excluding ZF) suggest that approximately 78% of the calculated intracellular thyroxine is intrahepatic. If we assume a normal intracellular volume of thyroxine distribution of 4.5 L, our estimate of intracellular hepatic thyroxine will be 3.5 L. This figure is in excellent agreement with the recent calculations of Cavalieri and Searle (7) for extravascular hepatic thyroxine space, 3.8 L $\pm$ 0.5 (SD). This correspondence serves to strengthen the underlying assumptions made in our analysis and to confirm our radioautographic data suggesting only negligible concentration of thyroxine in interstitial hepatic spaces.[10]

Since 78% of the calculated intracellular thyroxine space can be attributed to cellular hepatic uptake, the question arises as to the partition of the remaining 22%. Preliminary data obtained from experimental animals (rabbit, dog, rat) suggest that the kidney also makes a significant contribution to the net intracellular thyroxine compartment. In a patient with hepatic cirrhosis and a diminished thyroxine-^{131}I uptake by the liver, the renal outlines were clearly demonstrated by external radioactive scanning 4 hours after the administration of thyroxine-^{131}I. In normal subjects, the kidneys are not seen under these circumstances, probably because of the interference from hepatic concentration of radioactivity. Insufficient data are available to make a quantitative estimate of renal thyroxine uptake in man or to assess the contribution of other tissues. The technical limitations in our study previously cited also do not allow us to rigorously exclude the possibility that a small proportion of the remaining 22% of radioactivity is in fact associated with plasma proteins outside the instantaneous albumin-^{125}I distribution space.

The rapid exit of thyroxine from the albumin distribution volume clearly cannot be attributed to the effects of metabolic transformation of thyroxine. The average normal cellular clearance of thyroxine ($V_E k_E$) is 42.7 ml per minute. If we assume an average thyroxine distribution volume in these patients of 12.7 L based on a mean weight of 79.8 kg (31) and a thyroxine half-life of 6.0 days, the metabolic clearance can be estimated to be approximately 1.0 ml per minute. It follows that thyroxine must return from the intracellular compartment without having undergone effective metabolic transformation. This conclusion is in essential agreement with the findings of others (4, 5, 7).

[10] Rapid equilibrium of albumin-^{131}I between the vascular and interstitial spaces of the liver has been established (21). Calculations based on the three normal hepatic biopsy specimens listed in Table II indicate that the average total thyroxine distribution volume per gram of wet tissue is 2.88 ml plasma. The average albumin distribution volume in the same specimens was 0.14 ml plasma. Thus 20.5 (= 2.88/0.14) times as much thyroxine is associated with the cellular components as with plasma proteins in the vascular and extravascular spaces.

The fraction of administered thyroxine-^{131}I within the intracellular compartment (I_{max}) was evaluated at a time between 3 and 4 hours after injection of the isotopes, when the intracellular and extracellular compartments were in equilibrium relative to each other. At this time, however, albumin-^{125}I and presumably the thyroxine-^{131}I–plasma protein complex are not yet completely distributed in the total extravascular protein space. Several days are required for complete distribution equilibrium of isotopic albumin. The proportion of total body thyroxine in the rapidly exchangeable tissue pools may thus be less than I_{max}. The partition of unlabeled thyroxine between cellular and extracellular thyroxine can be estimated from the following considerations. If we assume that the ratio of extravascular to intravascular albumin as determined by multicompartmental analysis is 1.33 (32), the final average albumin-^{125}I distribution volume in our series of normal subjects can be estimated to be 7.53 L. Thus, the average distribution volume of thyroxine in these subjects, estimated from the sum of the final albumin-^{125}I distribution space and the intracellular distribution space of thyroxine, is 12.0 L. This is in excellent agreement with the predicted value of 12.7 L for the thyroxine distribution space calculated for an average weight of 79.8 kg from the empirical formula proposed by Oddie, Meade, and Fisher (31). Our findings would, therefore, suggest that 37.3% (= 4.47/12.00) of total body thyroxine is situated within rapidly exchangeable tissue stores. Thus, in the normal group, the average estimated total body pool of thyroxine is 885 μg, of which 329 μg is intracellular. Further data supporting the validity of our estimate of intracellular thyroxine were derived from two additional studies in which the total distribution volume of thyroxine, estimated by extrapolation to $t = 0$ (33),[11] was compared to the value representing the sum of the total albumin distribution volume obtained by the equilibrium time method (35) and the intracellular thyroxine distribution volume. In one patient the extrapolated thyroxine volume was 11.6 L, whereas the sum of the albumin and intracellular spaces was 11.2 L. In the other patient, the corresponding values were 8.8 and 9.2 L.

The results of our studies strongly support one of our underlying assumptions, namely, that the distribution of labeled thyroxine into the extracellular space is largely, if not completely, determined by the distribution kinetics of the carrier proteins. Diffusion of thyroxine independent of its carrier proteins does not appear to make a significant contribution to the kinetics of extravascular distribution. Thyroxine appears to leave the vascular tree in association with its carrier proteins through openings in the endothelial membrane. The mechanism of such transcapillary movement of protein has been extensively discussed by Mayerson, Wolfram, Shirley, and Wasserman (21).

The transfer of thyroxine from the extracellular to the cellular compartment probably also occurs as the consequence of an interaction between plasma protein and membrane receptor sites. Thus, the concept of free thyroxine may not be necessary in describing the acute distribution kinetics of the hormone. Measurement of the dialysis fraction (or per cent free thyroxine) by equilibrium dialysis, however, provides an index inversely related to the over–all strength of plasma protein binding. The absolute concentration of free thyroxine, determined from the product of the dialyzable fraction and the total thyroxine concentration in plasma, would then be proportional to absolute rate of unidirectional transfer.

Robbins and Rall (36) have postulated that the turnover rate of thyroxine is proportional to the concentration of free thyroxine in plasma. Since the metabolism of thyroxine is related to cellular function, it would be of interest to determine in future studies whether the turnover of thyroxine is in fact more closely related to the exchangeable intracellular pool. We should anticipate that primary changes in plasma protein binding would result in similarly directed and proportional shifts in free thyroxine and the intracellular pool. On the other hand, primary changes in cellular binding would produce oppositely directed changes in free thyroxine and the intracellular pool.

The nature of intracellular binding and the character of the reversible thyroxine transport across the cell surface have not been established.

[11] The extrapolation method along with other single compartmental analyses currently used for approximating the thyroxine distribution volume may result in an overestimation of the space. As pointed out by Rall, Robbins, and Lewallen (34), however, this overestimation probably does not exceed 10% of the true value.

It is known that subcellular fractions can bind thyroxine (29, 30). To what extent such binding contributes to the influx of thyroxine *in vivo* and to what extent active metabolic processes are required for the bidirectional movement of the hormone must be determined in future studies.

Lennon, Engbring, and Engstrom (37) first reported the slowed disappearance of intravenously injected thyroxine-^{131}I in patients with liver disease. An increased fractional disappearance of thyroxine-^{131}I in patients with hyperthyroidism was also noted by these authors. Similar observations have been made in our laboratory and can be attributed, in our opinion, to the diminished binding of thyroxine by plasma proteins in this disease.

The diminished hepatic thyroxine space and decreased hepatic clearance in patients with liver disease described in the earlier communication by Cavalieri and Searle (6) correspond well with the relative changes in intracellular distribution and clearance of thyroxine reported here. Similarly, the alterations in hepatic distribution kinetics in two subjects with congenitally low TBG values recently reported by the same authors (7) correspond with similar shifts noted in our patient. It is apparent that cellular function must be considered in conjunction with the concentration and the affinities of the thyroxine–binding plasma proteins in assessing the extrathyroidal factors that govern the concentrations of circulating thyroxine.

The methods described in this paper for estimating intracellular thyroxine and characterizing the distribution kinetics of this hormone are technically simple since they require only the measurement of serial plasma samples and the application of uncomplicated formulas. If the investigator wishes to confine his attention to the measurement of the function I_{max}, the procedure can be even further simplied since the number of plasma samples required can be significantly reduced.

Acknowledgments

We thank Dr. A. Aaron Yalow for helpful discussions and for his critical review of this manuscript. Mr. Modesto Martinez, Mr. Frank Martinez, and Mr. Ruben Almazan rendered expert technical assistance. The secretarial assistance of Miss Joan Tomes is gratefully acknowledged.

References

1. Albert, A., and F. R. Keating, Jr. The role of the gastrointestinal tract, including the liver, in the metabolism of radiothyroxine. Endocrinology 1952, **51**, 427.
2. Myant, N. B., and E. E. Pochin. The metabolism of radiothyroxine in man. Clin. Sci. 1950, **9**, 421.
3. Hazelrig, J. B. The impact of high speed automated computation on mathematical models. Proc. Mayo Clin. 1964, **39**, 841.
4. Gorman, C. A., E. V. Flock, C. A. Owen, Jr., and J. Paris. Factors affecting exchange of thyroid hormones between liver and blood. Endocrinology 1966, **79**, 391.
5. Pochin, E. E. U. S. Atomic Energy Commission report TID-7678. Oak Ridge Institute of Nuclear Studies, 1964, p. 413.
6. Cavalieri, R. R., and G. L. Searle. The role of the liver in the distribution of ^{131}I-thyroxine in man *in* Current Topics in Thyroid Research, Proceedings of the Fifth International Thyroid Conference, C. Cassano and M. Andreoli, Eds. New York, Academic Press, 1965, pp. 336–45.
7. Cavalieri, R. R., and G. L. Searle. 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. J. clin. Invest. 1966, **45**, 939.
8. Oppenheimer, J. H., and M. I. Surks. Determination of free thyroxine in human serum: a theoretical and experimental analysis. J. clin. Endocr. 1964, **24**, 785.
9. Oppenheimer, J. H., M. I. Surks, J. C. Smith, and R. Squef. Isolation and characterization of human thyroxine–binding prealbumin. J. biol. Chem. 1965, **240**, 173.
10. Greenwood, F. C., W. M. Hunter, and J. S. Glover. The preparation of ^{131}I–labelled human growth hormone of high specific radioactivity. Biochem. J. 1963, **89**, 114.
11. Smith, J. C., G. Bernstein, M. I. Surks, and J. H. Oppenheimer. A method of preparative starch-gel electrophoresis. Biochim. biophys. Acta (Amst.) 1966, **115**, 81.
12. Oppenheimer, J. H., R. Squef, M. I. Surks, and H. Hauer. Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. J. clin. Invest. 1963, **42**, 1769.
13. Volpert, E., M. Martinez, and J. H. Oppenheimer. Radioiodinated impurities in commercial preparations of ^{131}I-thyroxine and their effect on the measurement of free thyroxine by equilibrium dialysis. J. clin. Endocr. 1967, **27**, 421.
14. Solomon, A. K. Equations for tracer experiments. J. clin. Invest. 1955, **28**, 1297.
15. Berson, S. A., and R. S. Yalow. The iodide trapping and binding functions of the thyroid. J. clin. Invest. 1955, **34**, 186.

16. Oppenheimer, J. H., M. I. Surks, G. Bernstein, and J. C. Smith. Metabolism of iodine-131–labeled thyroxine–binding prealbumin in man. Science 1965, **149**, 748.
17. Socolow, E. L., K. A. Woeber, R. A. Purdy, M. T. Holloway, and S. H. Ingbar. Preparation of I^{131}–labeled human serum prealbumin and its metabolism in normal and sick patients. J. clin. Invest. 1965, **44**, 1600.
18. Schussler, G. C., and J. E. Plager. Free thyroxine in human serum (abstract). Clin. Res. 1965, **13**, 248.
19. Ingbar, S. H., L. E. Braverman, N. A. Dawber, and G. Y. Lee. A new method for measuring free thyroid hormone in human serum and an analysis of the factors that influence its concentration. J. clin. Invest. 1965, **44**, 1679.
20. Sterling, K., and M. A. Brenner. Free thyroxine in human serum: simplified measurement with the aid of magnesium precipitation. J. clin. Invest. 1966, **45**, 153.
21. Mayerson, H. S., C. G. Wolfram, H. H. Shirley, Jr., and K. Wasserman. Regional differences in capillary permeability. Amer. J. Physiol. 1960, **198**, 155.
22. Oppenheimer, J. H., L. V. Fisher, K. M. Nelson, and J. W. Jailer. Depression of the serum protein–bound iodine level by diphenylhydantoin. J. clin. Endocr. 1961, **21**, 252.
23. Wolff, J., M. E. Standaert, and J. E. Rall. Thyroxine–displacement from serum proteins and depression of serum protein–bound iodine by certain drugs. J. clin. Invest. 1961, **40**, 1373.
24. Oppenheimer, J. H., and R. R. Tavernetti. Studies on the thyroxine–diphenylhydantoin interaction: effect of 5,5′-diphenylhydantoin on the displacement of L-thyroxine from thyroxine–binding globulin (TBG). Endocrinology 1962, **71**, 496.
25. Coons, A. H. The localization of antigen in tissue cells by means of fluorescein–labeled antibody *in* The Nature and Significance of the Antibody Response, A. M. Pappenheimer, Ed. New York, Columbia University Press, 1953, p. 200.
26. Gitlin, D., B. H. Landing, and A. Whipple. The localization of homologous plasma proteins in the tissues of young human beings as demonstrated with fluorescent antibodies. J. exp. Med. 1953, **97**, 163.
27. Gitlin, D., and C. A. Janeway. Some isotopic studies on the distribution and metabolism of plasma proteins *in* Advances in Biological and Medical Physics, C. A. Tobias and J. H. Lawrence, Eds. New York, Academic Press, 1960, vol. **7**, p. 249.
28. Sutton, C., and J. H. Oppenheimer. Unpublished observations.
29. Tata, J. R., L. Ernster, and E. M. Suranyi. Interaction between thyroid hormones and cellular constituents. I. Binding to isolated subcellular particles and sub-particulate fractions. Biochim. biophys. Acta (Amst.) 1962, **60**, 461.
30. Heninger, R. W., F. C. Larson, and E. C. Albright. Intracellular distribution of iodine–containing compounds in rat liver, kidney and heart. Endocrinology 1966, **78**, 61.
31. Oddie, T. H., J. H. Meade, Jr., and D. A. Fisher. An analysis of published data on thyroxine turnover in human subjects. J. clin. Endocr. 1966, **26**, 425.
32. Beeken, W. L., W. Volwiler, P. D. Goldsworthy, L. E. Garby, W. R. Reynolds, R. Stogsdill, and R. S. Stemler. Studies of I^{131}-albumin catabolism and distribution in normal young male adults. J. clin. Invest. 1962, **41**, 1312.
33. Sterling, K., J. C. Lashof, and E. B. Man. Disappearance from serum of I^{131}–labeled L-thyroxine and L-triiodothyronine in euthyroid subjects. J. clin. Invest. 1954, **33**, 103.
34. Rall, J. E., J. Robbins, and C. G. Lewallen. The thyroid *in* The Hormones, G. Pincus, V. K. Thiman, and E. B. Astwood, Eds. New York, Academic Press, 1964, vol. 5, p. 320.
35. Pearson, J. D., N. Veall, and H. Vetter. A practical method for plasma albumin turnover studies. Strahlentherapie Sonderbaende 1958, **38**, 290.
36. Robbins, J., and J. E. Rall. The interaction of thyroid hormones and protein in biological fluids. Recent Progr. Hormone Res. 1957, **13**, 161.
37. Lennon, E. J., N. H. Engbring, and W. W. Engstrom. Studies of the rate of disappearance of labeled thyroxine from the intravascular compartment. J. clin. Invest. 1961, **40**, 996.