JCI The Journal of Clinical Investigation

Amendment history:
- Correction (August 1970)

Differences in primary cellular factors influencing the metabolism and distribution of 3,5,3′-L-triiodothyronine and L-thyroxine

Jack H. Oppenheimer, … , Gerald Bernstein, Martin I. Surks

J Clin Invest. 1970;49(5):1016-1024. https://doi.org/10.1172/JCI106301.

Research Article

Administration of phenobarbital, which acts exclusively on cellular sites, results in an augmentation of the liver/plasma concentration ratio of L-thyroxine (T4) in rats but no change in the liver/plasma concentration ratio of L-triiodothyronine (T3). Whereas phenobarbital stimulates the fecal clearance rate both of T3 and T4, it increases the deiodinative clearance rate of T4 only. These findings suggest basic differences in the cellular metabolism of T3 and T4. Further evidence pointing to cellular differences was obtained from a comparison of the distribution and metabolism of these hormones with appropriate corrections for the effect of differential plasma binding. The percentage of total exchangeable cellular T4 within the liver (28.5) is significantly greater than the corresponding percentage of exchangeable cellular T3 within this organ (12.3). Extrahepatic tissues bind T3 twice as firmly as T4. The cellular metabolic clearance rate (= free hormone clearance rate) of T3 exceeds that of T4 by a factor 1.8 in the rat. The corresponding ratio in man, 2.4, was determined by noncompartmental analysis of turnover studies in four individuals after the simultaneous injection of T4-^{125}I and T3-^{131}I. The greater cellular metabolic clearance rate of T3 both in […]

Differences in Primary Cellular Factors Influencing the Metabolism and Distribution of 3,5,3′-L-Triiodothyronine and L-Thyroxine

JACK H. OPPENHEIMER, HAROLD L. SCHWARTZ, HARVEY C. SHAPIRO, GERALD BERNSTEIN, and MARTIN I. SURKS

From the Endocrine Research Laboratory, Division of Medicine, Montefiore Hospital and Medical Center and Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10467

ABSTRACT Administration of phenobarbital, which acts exclusively on cellular sites, results in an augmentation of the liver/plasma concentration ratio of L-thyroxine (T4) in rats but no change in the liver/plasma concentration ratio of L-triiodothyronine (T3). Whereas phenobarbital stimulates the fecal clearance rate both of T3 and T4, it increases the deiodinative clearance rate of T4 only. These findings suggest basic differences in the cellular metabolism of T3 and T4. Further evidence pointing to cellular differences was obtained from a comparison of the distribution and metabolism of these hormones with appropriate corrections for the effect of differential plasma binding. The percentage of total exchangeable cellular T4 within the liver (28.5) is significantly greater than the corresponding percentage of exchangeable cellular T3 within this organ (12.3). Extrahepatic tissues bind T3 twice as firmly as T4. The cellular metabolic clearance rate (= free hormone clearance rate) of T3 exceeds that of T4 by a factor 1.8 in the rat. The corresponding ratio in man, 2.4, was determined by noncompartmental analysis of turnover studies in four individuals after the simultaneous injection of T4-^{125}I and T3-^{131}I. The greater cellular metabolic clearance rate of T3 both in rat and man may be related to the higher specific hormonal potency of this iodothyronine.

INTRODUCTION

It is widely recognized that 3,5,3′-L-triiodothyronine (T3) has a larger space of distribution and a more rapid fractional turnover rate than L-thyroxine (T4) both in man (1, 2) and the rat (3). Although these differences can be attributed, at least in part, to the fact that T4 is more firmly bound to plasma proteins than T3, no specific studies have been undertaken to determine to what extent intrinsic cellular factors may be involved. We became interested in this general question in connection with our studies of the effect of phenobarbital administration on thyroid hormone metabolism. Differences in the tissue response of T3 and T4 pointed to distinctive patterns of cellular handling of these hormones and prompted us to undertake a comparison of the cellular and plasma protein factors determining the distribution and metabolism of the thyroid hormones. The current report describes the results of these studies. Our findings indicate significant differences in the primary cellular determinants of T3 and T4 metabolism, factors which may be related to differences in the metabolic potency of these hormones.

METHODS

Male Sprague-Dawley rats (150–250 g) were supplied by Carworth Farms (New City, N. Y.) and maintained on a Purina Laboratory Chow diet.

Radioactive iodothyronines labeled in the phenolic ring with ^{125}I and ^{131}I (specific activity [SA] range for both T3 and T4: 30–50 mCi/mg) and ^{131}I-labeled human serum albumin (SA approximately 1 μCi/mg) were obtained from Abbott Laboratories, North Chicago, Ill. Radioactive T4 was purified by dialysis for 24 hr according to the method described by Schussler and Plager (4). This technique serves to remove ligands less tightly bound to plasma proteins than T4. Preparations of radioactive T3 were dialyzed for only 2 hr in order to remove contaminating radioactive iodide. After dialysis, preparations contained less than 5% contaminating radioactive material by paper chromatography in conventional solvent systems. All radioactive samples were counted to a statistical accuracy ±2% in a two-channel

Dr. Oppenheimer is a Career Scientist, Health Research Council of New York City (I-222). Dr. Shapiro is a Special Public Health Service Research Fellow.

Received for publication 13 August 1969 and in revised form 9 January 1970.

Autogamma Spectrometer (Packard). In order to insure geometric uniformity, whole tissue samples were digested in 2 N NaOH before counting. When both ^{131}I and ^{125}I were measured simultaneously, appropriate corrections were made for the "spill-over" of ^{131}I into 36 kev range at which ^{125}I was measured. All samples were compared with simultaneously counted aliquots of the original dose so as to obviate the necessity of additional corrections for physical decay. Radioactive doses were diluted in 1% human albumin and injected intravenously through the tail vein under light ether anesthesia. Blood samples were obtained from the cut tail and from the abdominal aorta at the end of the study.

In our kinetic analysis we assumed that all radioactivity in plasma and tissues was in the form of iodide or the injected substance. This assumption was based on paper chromatographic analyses (hexane: tertitary amyl alcohol: 2 N ammonia) (5) of tissue and plasma extracts obtained 17 hr after the injection of 30 μCi T4-^{125}I and 30 μCi T3-^{125}I. 2 g equivalent of liver, kidney, and homogenate and 1 ml plasma were extracted with four volumes of 95% ethanol × 4. The pooled extracts were evaporated to dryness *in vacuo* and the residue taken up in methanol-ammonia (3:1). Over 90% of the radioactivity was in the form of the injected radioactivity and iodide. Since all experiments were conducted within 24 hr after injection of the tracers, the contribution of nonextractable iodine to the total tissue and plasma radioactivity was negligible (6, 7).

Because of variable deiodination (10–20%) during the chromatography of tissue and plasma samples, the contribution of iodide was determined by alternative techniques. In the case of plasma, concentration of radioactive iodide was assessed either by rapid paper electrophoresis or by trichloroacetic acid (TCA) precipitation. In the case of tissues, the iodide contribution was estimated either by TCA precipitation or from the product of the predetermined iodide space and the plasma iodide concentration. For liver and kidney, the iodide contribution could be effectively ignored since the estimated iodide concentration was consistently less than 10% of the total tissue radioactivity.

The effect of phenobarbital. The effect of phenobarbital stimulation on the liver/plasma concentration ratio of T3 and T4 was determined after 5 days of pretreatment with phenobarbital (100 mg/kg intraperitoneally) (8). Animals were sacrificed 35 min after intravenous injection of a combined dose of 2 μCi of T4-^{125}I and 1 μCi of T3-^{131}I. It was assumed on the basis of previous experiments that each gram of liver contained the equivalent of 0.1 ml of trapped plasma. Correction for trapped plasma reduced the apparent T4 concentration in the liver by 20% but had no substantial effect (less than 5%) on the liver concentration of T3 because of the high liver/plasma ratio for T3. Subcellular distribution of the iodothyronines was determined according to the method of Schneider and Hogeboom (9). It was assumed on the basis of our previous studies (10) that all trapped plasma proteins were included in the supernatant fraction.

The effect of phenobarbital on the metabolism of T3 was determined in a group of five control animals and five animals pretreated with phenobarbital for 5 days. The kinetic analysis was similar to that previously used to determine the metabolic clearance of T4 (11). 2 μCi of T3-^{125}I was injected intravenously and serial plasma samples were obtained 3, 17, and 24 hr after injection of the dose. Animals also received an intraperitoneal injection of 1 mg NaI in order to prevent thyroidal accumulation of radioactive iodide.

Plasma T3 concentrations were calculated as the difference between the plasma total ^{125}I concentration and the iodide-^{125}I concentration as determined by paper electrophoresis. A rectilinear plasma decay between 3 and 24 hr was observed on a semilogarithmic plot. The T3 distribution volume was obtained from the reciprocal of the zero time intercept and the metabolic clearance rate was calculated from the product of the total distribution volume and the fractional plasma disappearance rate. Urinary and fecal collections were also obtained during this period. Fecal pellets in the colon obtained after sacrificing the animals were pooled with excreted feces. The fecal clearance rate was estimated from the product of the metabolic clearance rate and the fraction of total excreted radioactivity in the feces. The deiodinative clearance rate was calculated from the product of the metabolic clearance rate and the fraction of total excreted radioactivity found in the urine. Electrophoresis of the urine indicated that over 95% of the radioactivity was in the form of iodide. The nature of the fecal radioactivity was not analyzed.

Comparison of cellular binding and metabolism of T3 and T4. The partition of T4 and T3 among various body compartments was assessed. Six animals were injected intravenously with a combined dose of 2 μCi albumin-^{131}I and 4 μCi T4-^{125}I and six animals were injected with 4 μCi T3-^{125}I only. Animals were placed in individual metabolic cages for separate collection of urine and feces. 17 hr after injection the animals were killed. Since the plasma disappearance curve of T4, T3, and albumin was in the exponential phase, distribution equilibrium could be assumed.[1] The livers and kidneys were separately removed and plasma samples were obtained. The stomach and the intestines were totally removed and the gut contents were expressed and pooled with the fecal collection. The gut and the remaining carcass were combined, passed through a manual meat grinder, and homogenized in a Waring Blendor. Weighed samples were solubilized with concentrated NaOH. Residual iodide in the carcass was estimated on the basis of the product of the plasma radioactive iodide concentration and an iodide space of 48.3 ml/100 g body weight estimated on the basis of plasma iodide disappearance kinetics in 10 separate animals. In the animals injected with a combined dose of albumin-^{131}I and T4-^{125}I appropriate corrections for tissue or carcass T4 bound to trapped plasma proteins were made on the basis of the albumin-^{131}I counting rates and the T4/albumin counting ratios in the plasma. In the animals injected with T3 alone corrections for T3 bound to trapped plasma proteins were made on the basis of the mean albumin distribution volume determined in the paired animals injected with the combined dose of albumin and T4. The assumption was made that the distribution volume of the T3-binding proteins is the same as that of serum albumin.

The relative strength of binding of T4 and T3 to plasma proteins was assessed by simultaneous equilibrium dialysis of tracer quantities of T4-^{131}I and T3-^{125}I added to the same serum samples (13). The ratio of the net strength of plasma binding of T4, b_4, to the net strength of plasma binding of T3, b_3, was calculated from the expression

$$\frac{b_4}{b_3} = \frac{1 - DF_4}{1 - DF_3} \times \frac{DF_3}{DF_4}$$

where DF_3 and DF_4 are the dialyzable fractions of T3 and T4 as previously defined (13). This relationship is apparent from the following considerations. The net strength of plasma protein is defined as the sum of the products of the

[1] In other studies we have shown that the tissue/plasma concentration ratio of T3 and T4 has achieved a constant value by 3–5 hr (12).

association constant (k_i) of each of n classes of binding sites and the concentration of unoccupied binding sites P_i. Thus either for T3 or T4

$$b = \sum_{1}^{n} k_i P_i$$

From the law of mass action as pointed out by Robbins and Rall (14).

$$\sum_{1}^{n} k_i P_i = \frac{\sum_{1}^{n} TP_i}{T}$$

where TP_i is the concentration of the hormone-binding class i complex and T, the concentration of free hormone. Since $T = (DF)(c)$ and $\sum_{1}^{n} TP_i = (1 - DF)c$, where DF is the dialyzable fraction as determined by equilibrium dialysis and c is the total hormone in the system, it follows that

$$\sum_{1}^{n} k_i P_i = \frac{1 - DF}{DF}$$

and

$$b_4 = \frac{1 - DF_4}{DF_4}$$

and

$$b_3 = \frac{1 - DF_3}{DF_3}$$

Studies in man. In order to ascertain possible differences in the intrinsic cellular handling of T3 and T4 in man, studies were performed in two euthyroid convalescing patients and two healthy normal volunteers. A combined dose of 40 μCi T3-^{131}I and 20 μCi T4-^{125}I was injected intravenously. Blood samples were obtained 10 min and 2, 5, 10 and 24 hr after injection. Thereafter, samples were obtained twice a day for 5 days and subsequently once daily until 2 wk after injection. All subjects received 5 drops of Lugol's solution three times a day during the study. Plasma samples were subjected to TCA precipitation to remove inorganic iodide and extracted four times with ethanol in order to measure nonextractable iodine as previously described (6). The difference between the TCA-precipitable iodine and nonextractable iodine was considered to be iodothyronine. Data were analyzed by the noncompartmental approach proposed by Tait (15). Thus, the metabolic clearance rate (MCR) expressed in liters per day was calculated from the expression

$$MCR = \frac{100}{\int_0^\infty c\,dt}$$

where c = per cent dose injected per liter plasma and t = time (days). The integral expression was evaluated as follows. The concentration of labeled hormone c was plotted on semilogarithmic paper and a convenient time t_m corresponding to concentrations c_m was chosen during the early phase of exponential removal from plasma. A constant fractional removal rate (λ) was generally attained within 1–2 days after injection. Thus, the integral expression could be subdivided into two components

$$\int_0^\infty c\,dt = \int_0^{t_m} c\,dt + \int_{t_m}^\infty c_m e^{-\lambda t} dt$$

$\int_0^{t_m} c\,dt$ was evaluated graphically from a plot on Cartesian coordinates. Since

$$\int_{t_m}^\infty c_m e^{-\lambda t} dt = \frac{c_m}{\lambda} e^{-\lambda t_m}$$

the second integral could be calculated.

RESULTS

Comparison of the effect of phenobarbital administration on the distribution and turnover of T3 and T4 in the rat. A difference in the effect of phenobarbital administration on the partition of T4 and T3 between liver and plasma is demonstrated by the data summarized in Table I. Phenobarbital treatment resulted in an 82% increase in the liver/plasma (L/P) concentration ratio of T4-^{125}I, in agreement with previous results (8), but no change in the L/P concentration ratio of simultaneously administered T3-^{131}I. Previous experiments had indicated that phenobarbital does not affect the plasma binding of T4 (8). Similarly in the current studies, equilibrium dialysis revealed no significant difference between the binding of T3 by plasma proteins in control and in phenobarbital-treated animals (control DF [n = 11]: 36.8 ±7.1 [SD] %; phenobarbital [n = 12]: 31.6 ±5.7 [SD] %). Thus, we concluded that the failure to demonstrate an increase in the L/P concentration ratio of T3 was due to failure of phenobarbital administration to induce an over-all increase in the binding of T3 by hepatocellular tissue.

Results of the subcellular fractionation studies summarized in Table I may provide at least a partial explanation of this difference. A much larger fraction of total cellular T3 is associated with the supernatant fraction (36.9%) than in the case of T4 (17.6%). Conversely, a larger proportion of cellular T4 is bound to microsomes (45.8%) than is T3 (33.0%). Previous studies have suggested that the increase in total hepatocellular binding of T4 is due largely to microsomal proliferation (10). Since T3 is associated to a larger extent than T4 with nonmicrosomal sites, the increase in microsomal binding indicated in Table I might not be reflected as clearly in the L/P ratio of T3 as in the case of T4. Redistribution of T3 among subcellular fraction during homogenization, however, cannot be rigorously excluded.

Other differences in the response of T3 and T4 to the administration of phenobarbital were found in turnover experiments. In previous studies (7) we observed that phenobarbital administration resulted in a 30% increase in the turnover rate of T3-^{125}I whereas a 61% increase had been noted with T4 (11). In an additional study (Table II), we again noted only a 38% increase in the metabolic clearance rate of T3-^{125}I after phenobarbital treatment. The relative increase in fecal clear-

TABLE I

Effect of Phenobarbital on L/P Ratio and Subcellular Distribution of Injected Iodothyronines*

			Per cent Iodothyronine in			
		L/P*	Nuclei	Mitochondria	Microsomes	Supernatant
T4	(A) Control	0.513 ±0.024	23.4 ±0.9	13.2 ±1.4	45.8 ±2.0	17.6 ±1.2
	(B) Phenobarbital	0.938 ±0.058	14.6 ±0.7	11.0 ±1.0	59.7 ±1.7	14.6 ±1.4
T3	(C) Control	8.99 ±0.44	21.6 ±1.0	9.39 ±0.84	33.0 ±1.6	36.0 ±1.1
	(D) Phenobarbital	8.75 ±0.38	20.5 ±0.9	8.63 ±0.69	40.4 ±1.2	30.5 ±1.4
P	A vs. B	<0.001	<0.001	NS	<0.001	NS
	C vs. D	NS	NS	NS	<0.005	<0.01
	A vs. C	<0.001	NS	<0.05	<0.001	<0.001
	B vs. D	<0.001	<0.001	NS	<0.001	<0.001

Determinations made 35 min after intravenous injection of a combined dose of T4-^{125}I and T3-^{131}I.
11 control animals and 11 phenobarbital-treated animals were used. Results are expressed as mean ±SE.
NS, not significant (paired *t* test).
* L/P = liver/plasma concentration ratio.

ance rate of T3-^{125}I was similar to that previously noted for T4 (83%) but no increase in deiodinative clearance rate could be detected for T3. Thus, the increase in the metabolic clearance rate of T3 after phenobarbital treatment was due largely to an increase in fecal clearance rate, presumably secondary to an enhanced biliary disposition of T3.

Comparison of the distribution and metabolism of T3 and T4 in the rat: estimation of the relative cellular binding and metabolism of T3 and T4. The finding that phenobarbital administration results in differences in the cellular handling of T3 and T4 prompted a comparison of the peripheral distribution and metabolism of these hormones with specific reference to the role of plasma binding proteins and cellular factors. Table III summarizes the results of studies in six rats given a combined intravenous dose of albumin-^{131}I and T4-^{125}I and in six rats injected with T3-^{125}I only. Animals were

TABLE II

Effect of Phenobarbital on Iodothyronine Turnover

		k_t	V_t	Metabolic clearance rate	Deiodinative clearance rate	Fecal clearance rate
		hr^{-1}	*(ml/100 g)*	*(ml/100 g per hr)*	*(ml/100 g per hr)*	*(ml/100 g per hr)*
T3	Control	0.079 ±0.001	265 ±26	20.9 ±1.3	10.9 ±0.9	10.0 ±0.1
	Phenobarbital	0.103 ±0.012	284 ±47	28.8 ±0.9	10.4 ±0.7	18.3 ±0.6
	P	<0.005	NS	<0.005	NS	<0.001
	Phenobarbital / Control	1.30	1.07	1.38	0.95	1.83
T4*	Phenobarbital / Control	1.34	1.19	1.61	1.40	1.85

k_t = fractional turnover rate of total body pool of T3; V_t = total distribution volume. Clearance rates and distribution volume expressed per 100 g body weight.
* Data from Oppenheimer, Bernstein, and Surks (11).

TABLE III

Distribution and Metabolism of T3 and T4 in the Rat

	T4*	T3*	P
Body weight, *g*	212 ±13	214 ±14	NS
Residual body iodothyronine, *%D*	42.5 ±1.9	20.5 ±1.0	<0.001
Protein-bound iodothyronine, *%D*	20.1 ±0.9	0.98 ±0.04	<0.001
Cellular iodothyronine, *%D*	22.4 ±1.4	19.5 ±1.0	NS
% in Carcass	67.8 ±1.7	83.7 ±0.7	<0.001
% in Kidney	3.65 ±0.15	3.96 ±0.11	NS
% in Liver	28.5 ±1.7	12.3 ±0.7	<0.001
$t_{\frac{1}{2}}$, *hr*	14.0 ±0.7	7.43 ±0.23	<0.001
Distribution space, *ml/100 g body weight*			
Total	16.4 ±0.7	165 ±10	<0.001
Cellular	8.71 ±0.6	157 ±10	<0.001
Total metabolic clearance rate, *ml/100 g body weight*	0.829 ±0.05	15.3 ±0.6	<0.001
Fecal	0.449 ±0.034	7.20 ±0.32	<0.001
Deiodinative	0.380 ±0.038	8.11 ±0.57	<0.001
L/P	0.562 ±0.028	5.33 ±0.33	<0.001
K/P	0.388 ±0.031	8.34 ±0.43	<0.001
R/P	0.071 ±0.006	1.52 ±0.10	<0.001

Abbreviations: L/P, K/P, and R/P, liver, kidney, and carcass/plasma concentration ratio.
All determinations made 17 hr after injection of isotopes. Six animals in T4 and six animals in T3 group.
* Mean ±SEM indicated.

killed 17 hr after injection. At this time isotopic distribution had been achieved and only negligible quantities of tissue and plasma iodoprotein were present.

The data confirm previous observations that T3 is primarily an intracellular hormone (3). Only about 5% of residual body isotopic T3 was associated with the plasma proteins whereas 47% of total body T4 tracer was bound to plasma proteins. Although the partition of iodothyronines between plasma and tissues is clearly related to the strength of plasma protein binding of the thyroid hormones, the distribution of the iodothyronines within components of the cellular compartment is independent of plasma protein binding. It was of interest that whereas 28% of the total cellular pool of T4-^{125}I was situated in the liver, only 12% of total cellular T3 was intrahepatic. The proportion of total cellular hormones within the kidneys was approximately the same for T3 (4.0%) and T4 (3.7%). Thus, a substantially higher percentage of total cellular T3 (84%) was associated with remaining carcass as compared to T4 (68%).

Since residual body hormone at 17 hr was known, the fractional disappearance rate and the corresponding $t_{\frac{1}{2}}$ of T3 and T4 could be calculated if one assumes that the fractional disappearance is constant throughout the initial distribution period. This assumption is made in all single-compartment approximations of thyroid hormone turnover. The average metabolic half-time of T4 was calculated to be 14.0 hr, in reasonable agreement with the $t_{\frac{1}{2}}$ obtained from plasma measurements alone, 15.1 hr (11). The $t_{\frac{1}{2}}$ of T3 in the total body experiments was 7.4 hr compared to 8.8 hr from the plasma disappearance curve (Table II). The total distribution volume of T4 in these experiments was 16.4 ml/100 g body weight, compared to 15.6 ml/100 g from plasma measurements. The total distribution volume for T3, 165 ml/100 g, however, was less than the value of 265 ml/100 g in Table II. The total metabolic clearance rate per 100 g body weight for T4 was 0.83 ml/hr (plasma kinetics, 0.70 ml/hr) and for T3, 15.3 ml/hr (plasma kinetics, 20.9 ml/hr).

In these experiments, the total amount of tracer hormone deiodinated was determined from the sum of the residual body iodide and the urinary iodide excretion. The fecal excretion was estimated from the sum of radioactivity in the gut and in the feces. On the basis of these values the fecal and deiodinative clearance rates were calculated (Table III). The fecal clearance rate of T4 constituted about 54% of the total metabolic clearance rate, in confirmation of previous studies (11). Similarly, the fecal clearance rate of T3 was found to be 47% of the metabolic clearance rate.

As is apparent from the Appendix, the total metabolic clearance rate, fractional turnover rate, and distribution spaces are dependent on both primary cellular factors and on the strength of plasma protein binding of the thyroid hormones. In order to assess the cellular contributions to the observed differences in these parameters and in order to calculate the ratio of the cellular binding of T3 and T4 in individual tissues, the comparative strength of plasma protein binding of T4 and T3 was

TABLE IV

Comparison of Intrinsic Cellular Handling of T3 and T4 in the Rat

Parameter	Symbol	Ratio of T3/T4
Fractional removal rate from pooled cellular compartment	k_c	1.02
Binding, pooled cellular	B_c	1.73
Cellular metabolic clearance rate	$B_c k_c$	1.77
Liver binding	b_L	0.91
Kidney binding	b_K	2.12
Carcass	b_R	2.06

Data based on studies summarized in Table III. For calculations see details in Appendix (equations 3, 4, 7, and 9).

TABLE V

Differential Cellular Metabolism of T3 and T4 in Man

Subject	Age	Sex	Wt	MCR		$\frac{(MCR)_3}{(MCR)_4}$	$\frac{(b_p)_4}{(b_p)_3}$	$\frac{(B_c k_c)_3}{(B_c k_c)_4}$
				T3	T4			
			kg	*liters/day*				
L. G.	57	M	73.2	28.3	1.30	21.8	8.40	2.59
J. M.	70	F	35.0	16.8	0.68	24.7	10.2	2.42
J. O.	41	M	88.0	28.1	1.25	22.5	9.68	2.42
M. S.	35	M	79.5	25.5	1.02	25.0	11.50	2.17
Mean			69.0	24.7	1.06	23.5	9.94	2.40

L. G., patient recovering from cerebrovascular accident; J. M., convalescent patient following lobar pneumonia; J. O. and M. S., laboratory controls. MCR, total metabolic clearance rate; b_p, strength of plasma protein binding; $B_c k_c$, cellular metabolic clearance rate. See Appendix and text for further explanation of terms. Subscript 3 and 4 refer to T3 and T4 respectively.

determined by simultaneous equilibrium dialysis of isotopic tracers of T3 and T4. Measurements in seven individual rat sera and multiple determinations of a single pool of six rat sera indicated T4 was bound to rat plasma 10.4 times as tightly as T3.

The cellular handling of iodothyronines can be expressed in terms of two variables, the fractional removal of hormone from the pooled cellular compartment (k_c) and the binding of hormone by the pooled cellular compartment (B_c). The product of these two terms can be defined as the cellular metabolic clearance rate ($k_c B_c$) and can be shown to be numerically equivalent to the free hormone clearance rate (Appendix, equation 10). The cellular metabolic clearance rate is a measure of the total metabolic clearance rate corrected for effects of plasma binding. It is also possible to calculate the cellular binding of individual tissues. The ratios of these functions (T3/T4), derived as indicated in the Appendix (equations 3, 4, 7, and 9), are listed in Table IV. It is apparent that the net cellular binding and the cellular metabolic clearance rate of T3 exceed those of T4. Similarly, the cellular binding of T3 by kidney and carcass exceeds that of T4. In the case of the liver, however, the strength of binding of both hormones is approximately the same. In essence, these results show that the increased distribution volume of T3 and its greater metabolic clearance rate cannot be attributed exclusively to diminished plasma binding of T3. These results also indicate that the increased binding of T3 by extrahepatic tissues is primarily responsible for the smaller fraction of total cellular T3 within the liver as compared to T4.

Comparison of the cellular metabolism of T3 and T4 in man. The results of studies in the rat prompted us to determine whether differences also exist between the cellular metabolism of T3 and T4 in man. The opportunity to obtain multiple plasma samples in man facilitated adoption of a noncompartmental kinetic analysis. Results of studies in four subjects without thyroidal disease are summarized in Table V. In each subject the plasma binding ratio of T4 and T3 was determined by simultaneous equilibrium dialysis of T4-^{131}I and T3-^{125}I. The ratio of the metabolic clearance rates of T3/T4 averaged 23.5; the ratio of plasma protein binding T4/T3 averaged 9.94; and the ratio of the cellular metabolic clearance rates averaged 2.40. Thus, in man as well as the rat, differences in the metabolic clearance between T3 and T4 cannot be attributed exclusively to differential plasma protein binding. The cellular compartment metabolizes T3 more rapidly than T4.

DISCUSSION

The results of these studies point to important differences in the cellular handling of T3 and T4. This conclusion is apparent from an analysis of the effects of phenobarbital on T3 and T4 metabolism, a comparison of the subcellular distribution of T3 and T4, and calculation of intrinsic cellular determinants of hormonal distribution and metabolism. In particular, our studies suggest that a smaller fraction of the total cellular T3 is situated within the liver than is the case with T4. The failure to demonstrate an increase in deiodinative clearance rate of T3 after phenobarbital administration also raises the possibility that the liver makes a substantially smaller contribution to the total deiodination of T3 in comparison to its role in the deiodination of T4. This concept is supported by recent studies from our labortory in which we have demonstrated that the ratio of the concentration of nonextractable iodine in liver to other tissues after injection of radioactive T3 is less than the corresponding ratio after the injection of T4-^{125}I (7). In these studies we have also supplied evidence that there is a direct correlation between deiodination and the formation of iodinated tissue proteins.

On the other hand, the finding that the proportion of total hormonal flux cleared via the fecal route is the

same for T3 and T4 suggests that a larger fraction of hepatocellular T3 is excreted into the bile per unit time. This conclusion is based on two assumptions: (*a*) that fecal radioactivity results exclusively from the difference between biliary excretion and enteric reabsorption, and (*b*) that the fractional rate of enteric reabsorption of T3 is the same as that of T4. The increased metabolic clearance rate of T3 after phenobarbital administration would appear to result largely from an increase in fecal and, presumably, an enhanced biliary clearance rate. No increase in deiodinative clearance rate was detected in contrast to our previous observations with T4. The anatomic basis for the differential hepatocellular handling of T3 and T4 remains obscure. The finding that a large proportion of hepatocellular T3 is associated with the cytosol highlights the possible importance of soluble intracellular binding proteins such as those described by Hamada, Torizuka, and Miyake (16).

The conclusion that the proportion of total cellular iodothyronine within the liver is larger for T4 than for T3 confirms the earlier findings of Van Arsdel, Hogness, Williams, and Elgee (3). Our results are also consonant with the findings of Heninger, Larson, and Albright who found that T3/T4 ratio in liver was lower than that in most other tissues after equilibration with dietary $^{125}I^-$ (17). These observations mitigate against the possibility that the differential isotopic distribution which we and Van Arsdel et al. observed do not reflect nonradioactive hormone distribution. Lastly, Cavalieri, Steinberg, and Searle, by use of constant infusion techniques, have estimated that the liver distribution space of T3 in normal males is 2.75 liters (18). Assuming a total cellular distribution of space of T3 of 33 liters (= total T3 distribution space of 40 liters [5] — albumin space of 7 liters [19]) the hepatic space of T3 would constitute only 8.3% of the total cellular T3 space. The same authors have estimated that the hepatic distribution space of T4 is 3.8 liters (20). The cellular distribution volume of T4 estimated from the difference of the total distribution volume given by these authors, 12.4 liters, and the protein distribution space of 7 liters is 5.4 liters. Thus, hepatic T4 would appear to constitute 68% of the total cellular space. Although, these calculations are subject to considerable experimental error, the striking difference between T3 and T4 in hepatic contribution to total exchangeable iodothyronine strongly supports the findings of this report.

The difference in the partition of T3 between liver and extrahepatic tissue theoretically could be due either to increased binding by extrahepatic tissues or to decreased hepatocellular binding or to both. Our analysis would suggest that the primary factor responsible for the observed data in the rat is increased binding of T3 by extrahepatic tissues. This conclusion was derived from an analysis of the tissue/plasma concentration ratio after establishment of isotopic distribution and measurement of the relative strength of plasma protein binding of T3 and T4 by equilibrium dialysis. We determined that T4 was bound to plasma proteins about 10 times as avidily as T3 both in man and the rat. This finding agrees with previous estimates (21, 22).

The observation that binding of T3 by extrahepatic tissues exceeds that of T4 raises the possibility that the increased binding in these tissues may be linked to degradative biochemical processes and thus to the over-all increase in the cellular metabolism of T3. An important distinction must be drawn between metabolically linked and nonmetabolically linked binding.[2] Nonmetabolically linked binding is exemplified by plasma protein binding. According to current concepts, perturbation in plasma protein binding will not result in any changes in hormonal flux under steady-state conditions (14, 23). This would suggest that there is no specificity in the interaction between the plasma binding proteins and critical intracellular sites since a specific interaction would lead to intracellular redistribution and to an alteration in hormonal flux. In the case of intracellular binding, however, it is theoretically possible to have both metabolically linked and nonmetabolically linked binding sites. Thus, phenobarbital administration results in an increase in hepatocellular binding of T4 which is apparently linked with increased cellular metabolism (11). As indicated in the present experiments, an increase in intracellular binding of T3 after phenobarbital may not be of sufficient magnitude to be detected in the gross measurements of the liver/plasma concentration ratio. Thus one could postulate that the proportion of metabolically linked intracellular sites to the total intracellular sites in rat liver is greater for T4 than for T3.

Our studies in man clearly demonstrate an increase in cellular metabolic clearance rate of T3 in comparison to T4. These observations gain additional importance since a noncompartmental model was employed which would obviate any possible error inherent in single-compartment analysis. Nevertheless, certain fundamental differences in the kinetics of distribution and metabolism between these species exist. As pointed out above, a crude estimate of the cellular space of T3 distribution is about 33 liters. Thus, the ratio of the cellular distribution space to the plasma distribution volume (7 liters) is 4.7. Similar calculations in the rat lead to a ratio of $157/8 = 19.6$. Since the ratio of plasma binding T4 and T3 is about 10 in both species, it is apparent that there is actually a decrease in the over-all cellular T3 binding in man in comparison to T4. Despite this, in man there

[2] "Metabolism" in this sense refers simply to irreversible transformation of hormone and not to metabolic events induced by the hormone.

is a larger cellular clearance rate of T3, a finding which is related to the fractional removal rates from the cellular pools of T3 and T4 (k_c) which can be calculated to be about 0.7 and 0.2 (day^{-1}) respectively. The difference in the cellular metabolic clearance rates in man is therefore determined by the fractional removal of the intracellular hormones whereas in the rat, the difference in cellular metabolic clearance rates is determined by cellular binding of T3 and T4. The calculated fractional removal rates of intracellular T3 and T4 in the rat are approximately the same.

The kinetic difference between rat and man may be a simple reflection of the percentage of total cellular binding sites which are metabolically linked. Since it is difficult experimentally to determine the proportion of cellular binding sites which are metabolically linked it is more convenient for most purposes simply to determine the cellular metabolic clearance rate, $B_c k_c$, which is numerically equal to the free hormone clearance rate, a concept previously used by Ingbar, Braverman, Dawber, Lee (21). Determination of total cellular binding, however, can be useful under certain circumstances in obtaining indications about the precise tissue or organ in which metabolic changes occur. Thus, demonstration of increased hepatocellular binding of T4 after phenobarbital administration was helpful in inferring that the increase in total turnover was due to the liver.

It is interesting to speculate about the relationship between the increased cellular metabolic clearance of T3 and its greater specific metabolic potency compared to T4 (24). It has been suggested that the biological action of thyroid hormones is related to their metabolism (25). The finding that the ratio of the cellular metabolic clearance rate of T3 to T4 is about the same in man and the rat despite differences in the cellular binding relationship supports this concept. If the increased specific hormonal potency is related to the increased cellular metabolic clearance rate, one would also have to infer that such differences are due to extrahepatic sites since our data would suggest that the liver probably deiodinates a smaller proportion of total body T3 than T4. The proportion of thyroid hormone which is cleared via the enteric tract probably is not related to the metabolic action of the hormone (26). Although these considerations are speculative they are capable of critical testing in further experiments.

APPENDIX

Under equilibrium conditions the total body pool of an exchangeable strongly protein-bound hormone may be considered to be distributed between plasma protein and cellular compartments. For T3 and T4, the total amount of hormone in the "free" form can be neglected as a first approximation. If we assume validity of single-compartment kinetics for the sake of simplicity, the following relationships can be derived. Let

B_c = binding of the lumped cellular compartment (liters)
b_p = over-all strength of plasma binding which can be experimentally defined by equilibrium dialysis. Thus, $b_p = \frac{1}{DF} - 1$, where DF is the dialyzable fraction
V_a = the plasma protein distribution volume (liters)
V_t = the total hormonal distribution volume (liters)
V_c = cellular distribution volume (liters) where $V_c = V_t - V_a$

The partition of hormone between plasma protein and cells can be regarded as a function of the total cellular and plasma protein binding. Thus,

$$\frac{B_c}{V_a b_p} = \frac{V_c}{V_a}$$

and therefore

$$B_c = b_p V_c \tag{1}$$

Similarly, for an individual tissue or organ we can define cellular binding b_i so that

$$\frac{b_i}{b_p} = \frac{c_i^*}{c_p^*}$$

where c_i^* and c_p^* are the radioactive concentrations of hormone in tissue i and plasma, respectively, so that

$$b_i = \left(\frac{c_i^*}{c_p^*}\right) b_p \tag{2}$$

In order to compare the relative strength of cellular binding for T3 and T4 as in Table IV, the following expression can be used

$$\frac{(B_c)_3}{(B_c)_4} = \frac{(b_p)_3}{(b_p)_4} \cdot \frac{(V_c)_3}{(V_c)_4} \tag{3}$$

and

$$\frac{(b_i)_3}{(b_i)_4} = \frac{(b_p)_3}{(b_p)_4} \cdot \frac{\left(\frac{c_i^*}{c_p^*}\right)_3}{\left(\frac{c_i^*}{c_p^*}\right)_4} \tag{4}$$

where the subscripts 3 and 4 refer to T3 and T4, respectively. The turnovers of hormone, expressed in micrograms per day, can be defined

$$S = k_t V_t c_p \tag{5}$$

where k_t = the total fractional removal rate from the body (day^{-1}) and c_p = the concentration of nonradioactive hormone in plasma (μg/liter).

By definition, $k_t V_t$ = MCR, the total metabolic clearance rate (liters/day) and under steady-state conditions

$$S = k_c V_c c_p \tag{6}$$

where k_c = fractional removal rate of hormone from the lumped cellular compartment. Thus from equations 5 and 6, $k_t V_t = k_c V_c$ and

$$k_c = \frac{k_t V_t}{V_c} \tag{7}$$

The cellular metabolic clearance rate is defined as $B_c k_c$. From equations 1 and 7 it follows that

$$B_c k_c = k_t V_t b_p = (\text{MCR}) b_p \tag{8}$$

and

$$\frac{(B_c k_c)_3}{(B_c k_c)_4} = \frac{(MCR)_3}{(MCR)_4} \cdot \frac{(b_p)_4}{(b_p)_3} \quad (9)$$

The free hormone clearance rate is equal to $\frac{S}{T}$ where T = free hormone concentration in plasma. Since $T = \frac{c_p}{b_p}$

$$\frac{S}{T} = \frac{(MCR)c_p}{\left(\frac{c_p}{b_p}\right)} = (MCR)\,(b_p) \quad (10)$$

Thus, the cellular metabolic clearance rate is the same as the free hormone clearance rate.

By simple rearrangements and substitution, the conventionally used kinetic terms V_t and k_t can be expressed in terms of primary cellular and plasma protein determinants and the plasma protein distribution volume.

Thus,

$$V_t = V_a + \frac{B_c}{b_p} \quad (11)$$

and

$$k_t = \frac{k_c B_c}{V_a b_p} + B_c. \quad (12)$$

ACKNOWLEDGMENTS

We wish to thank Mr. Modesto Martinez, Mr. Francisco Martinez, and Mr. Jose Guerra for their expert technical assistance and Mrs. Marian Zullo for her secretarial assistance.

This work was support by U. S. Public Health Service Grant NB03000 and Army Contract DA-49-193-MD-2967.

REFERENCES

1. Rall, J. E., J. Robbins, D. Becker, and R. W. Rawson. 1953. The metabolism of labeled L-triiodothyronine, L-thyroxine, and D-thyroxine. *J. Clin. Invest.* **32**: 596. (Abstr.)
2. Sterling, K., J. C. Lashof, and E. B. Man. 1954. Disappearance from serum of I^{131}-labeled L-thyroxine and L-triiodothyronine in euthyroid subjects. *J. Clin. Invest.* **33**: 1031.
3. Van Arsdel, P., J. R. Hogness, R. H. Williams, and N. Elgee. 1954. Comparative distribution and fate of I^{131}-labeled thyroxine and triiodothyronine. *Endocrinology.* **55**: 332.
4. Schussler, G. C., and J. E. Plager. 1967. Effect of preliminary purification of ^{131}I-thyroxine on the determination of free thyroxine in serum. *J. Clin. Endocrinol. Metab.* **27**: 242.
5. Bellabarba, D., R. E. Peterson, and K. Sterling. 1968. An improved method for chromatography of iodothyronines. *J. Clin. Endocrinol. Metab.* **28**: 305.
6. Surks, M. I., and J. H. Oppenheimer. 1969. Formation of iodoprotein during the peripheral metabolism of 3,5,3'-triiodo-L-thyronine-^{125}I in euthyroid man and rat. *J. Clin. Invest.* **48**: 685.
7. Surks, M. I., H. L. Schwartz, and J. H. Oppenheimer. 1969. Tissue iodoprotein formation during the peripheral metabolism of the thyroid hormones. *J. Clin. Invest.* **48**: 2168.
8. Bernstein, G., S. A. Artz, J. Hasen, and J. H. Oppenheimer. 1968. Hepatic accumulation of ^{125}I-thyroxine in the rat: augmentation by phenobarbital and chlordane. *Endocrinology.* **82**: 406.
9. Schneider, W. C., and G. H. Hogeboom. 1950. Intracellular distribution of enzymes; distribution of succinoxidase and cytochrome oxidase activities in normal mouse liver and in mouse hepatoma. *J. Nat. Cancer Inst.* **10**: 969.
10. Schwartz, H. L., G. Bernstein, and J. H. Oppenheimer. 1969. Effect of phenobarbital administration on the subcellular distribution of ^{125}I-thyroxine in rat liver: importance of microsomal binding. *Endocrinology.* **84**: 270.
11. Oppenheimer, J. H., G. Bernstein, and M. I. Surks. 1968. Increased thyroxine turnover and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *J. Clin. Invest.* **47**: 1399.
12. Oppenheimer, J. H., M. I. Surks, and H. L. Schwartz. 1969. The metabolic significance of exchangeable cellular thyroxine. *Recent Progr. Hormone Res.* **25**: 381.
13. Oppenheimer, J. H., R. Squef, M. I. Surks, and H. Hauer. 1963. Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. *J. Clin. Invest.* **42**: 1769.
14. Robbins, J., and J. E. Rall. 1960. Proteins associated with the thyroid hormones. *Physiol. Rev.* **40**: 415.
15. Tait, J. F. 1963. The use of isotopic steroids for the measurement of production rates *in vivo*. *J. Clin. Endocrinol. Metab.* **23**: 1285.
16. Hamada, S., J. Torizuka, and T. Miyake. 1966. Cellular thyroxine-binding proteins and their physiological significance. Gumma Symposia on Endocrinology. **3**: 153.
17. Heninger, R. W., F. C. Larson, and E. C. Albright. 1963. Iodine-containing compounds of extrathyroidal tissues. *J. Clin. Invest.* **42**: 1761.
18. Cavalieri, R. R., M. Steinberg, and G. L. Searle. 1969. Distribution kinetics of I^{131}-triiodothyronine in man. 51st Meeting of the Endocrine Society. Abstract No. 77.
19. Beeken, W. L., W. Volwiler, P. D. Goldsworthy, L. E. Garby, W. R. Reynolds, R. Stogsdill, and R. S. Stemler. 1962. Studies of I^{131}-albumin catabolism and distribution in normal young male adults. *J. Clin. Invest.* **41**: 1312.
20. Cavalieri, R. R., and G. L. Searle. 1966. The kinetics of distribution between plasma and liver of ^{131}I-labeled L-thyroxine in man: observations of subjects with normal and decreased serum thyroxine-binding globulin. *J. Clin. Invest.* **45**: 939.
21. Ingbar, S. H., L. E. Braverman, N. A. Dawber, and G. Y. Lee. 1965. A new method for measuring free thyroid hormone in human serum and an analysis of the factors that influence its concentration. *J. Clin. Invest.* **44**: 1679.
22. Nauman, J. A., A. Nauman, and S. C. Werner. 1967. Total and free triiodothyronine in human serum. *J. Clin. Invest.* **46**: 1346.
23. Oppenheimer, J. H. 1968. Role of plasma proteins in the binding, distribution, and metabolism of the thyroid hormones. *N. Engl. J. Med.* **278**: 1153.
24. Barker, S. B. 1964. Physiological activity of thyroid hormones and analogues. *In* The Thyroid, Vol. I. R. Pitt-Rivers and W. R. Trotter, editors. Butterworths, London. 199–236.
25. Galton, V. A., and S. H. Ingbar. 1962. Observations on the effects of the metabolism of thyroid hormones in Necturus Maculosus. *Endocrinology.* **71**: 369.
26. Irvine, C. H. G. 1969. Qualitative and quantitative aspects of thyroxine metabolism in sheep. *Endocrinology.* **85**: 662.