Studies of Peripheral Thyroxine Distribution in Thyrotoxicosis and Hypothyroidism

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ABSTRACT Compartmental analysis of the peripheral distribution of labeled thyroxine was applied to various groups of subjects with thyrotoxicosis and hypothyroidism. It was observed that the hepatic incorporation of thyroxine was augmented in subjects with Graves' disease when compared to non-Graves' disease control groups at all levels of thyroid function. Decreased values of hepatic incorporation occurred in primary hypothyroid subjects. These lowered values were not acutely corrected by elevation of the serum thyroxine level, but were observed to be rectified after several months' therapy with exogenous thyroid hormone. These alterations of the hepatic thyroxine-^{131}I incorporation were independently verified by direct quantitative liver scintiscan determinations.

Employing a dual thyroxine tracer system, we were able to demonstrate that during the early phases of equilibration of a tracer dose of thyroxine, alterations in the rate of deiodination were observed to be present in the various thyroid disease states. Increased deiodination rates were found in subjects with Graves' disease and the reverse was noted in patients with primary hypothyroidism. Kinetic analysis of thyroxine compartmental distribution during this early phase of equilibration of a labeled thyroxine tracer indicated that the primary tissue uptake occurred in the liver. These findings supported the contention that the amount of labeled thyroxine incorporated in the liver may be directly related to the deiodination rate of thyroxine by that organ. The pathogenetic basis of these alterations is presently unknown.

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INTRODUCTION

Evidence that disturbances in the peripheral metabolism of thyroxine may occur in some forms of thyroid disease in man became apparent from the investigations of Lennon, Engbring, and Engstrom (1), and Ingbar and Freinkel (2). Lennon and coworkers studied the pattern of the acute disappearance of an injected tracer dose of thyroxine-^{131}I in various thyroid disease states. They found that during the 20–50 min postinjection period there was a relative acceleration in the thyroxine-^{131}I removal rates in thyrotoxic, eumetabolic, and hypothyroid Graves' disease patients. Interestingly, a reduced disappearance rate was noted in patients with primary hypothyroidism, and a normal or reduced rate was present in subjects with toxic nodular goiter and iatrogenic thyrotoxicosis. The investigations of Ingbar and Freinkel also supported the concept that patients with Graves' disease have alterations in thyroxine kinetics (2). Their studies focused on alterations in the fractional turnover rates of an equilibrated tracer dose of thyroxine-^{131}I both in subjects with thyrotoxic and eumetabolic Graves' disease. They observed an acceleration of the thyroxine turnover rates in both eumetabolic and toxic Graves' disease subjects and the reverse in primary hypothyroid patients. Ingbar, Freinkel,

Dowling, and Kumagai extended these observations to relatives of Graves' disease subjects in whom they also found an increased turnover rate present in some cases. These latter findings suggest that the alterations in thyroxine turnover might be an expression of an underlying inherited trait that antedates the onset of clinically detectable Graves' disease (3, 4). Collectively, these studies (1–4) appear to indicate that alterations in the manner of thyroxine distribution and disposal may play an integral part in the pathophysiology of Graves' disease as well as other forms of thyroid disease.

We have recently described several techniques for the measurement of the compartmental distribution and deiodination of extrathyroidal thyroxine in normal human subjects (5). These techniques allow the quantitative estimation of the thyroxine distribution in the plasma, extracellular fluid, hepatic, and extrahepatic tissue compartments, as well as a semiquantitative estimation of the relative deiodinating activities of the hepatic vs. the extrahepatic compartments. The purpose of this investigation was to apply these new techniques to the evaluation of peripheral thyroxine metabolism in subjects with Graves' disease and other thyroid disease states. Additionally, there will be described a newly devised method for the direct quantitative approximation of the hepatic incorporation of labeled thyroxine.

METHODS

The subjects employed for this study were obtained from the inpatient and outpatient services of the King County Hospital System in Seattle, Wash. and the Wadsworth Veterans Administration Hospital in Los Angeles, Calif. The patients' diagnoses were established by a combination of appropriate clinical and laboratory observations by one or both of the authors. Subjects with a history or physical findings of liver disease or obesity were excluded except where specifically stated. The protein-bound iodine (PBI) determinations were performed by the method described by Barker, Humphrey, and Soley (6). The triiodothyronine resin uptake tests (RU) were determined by a standard commercial method (Trisorb, Abbott Laboratories, North Chicago, Ill.). Thyroxine-^{131}I and thyroxine-^{125}I equally labeled in the 3′, 5′ positions were obtained from a commercial source (Abbott Laboratories) and stored in a sterile 1% serum albumin-saline solution at 4°C. Before initiating the study, the contaminating iodide was removed from the labeled thyroxine solutions by dialysis against a strong anion exchange resin (5, 7). An approximation method for the compartmental distribution of labeled thyroxine in plasma, extracellular fluid, hepatic, and extrahepatic tissue spaces was applied to all study subjects (5). This method employed the measurement of the plasma disappearance rate of thyroxine-^{131}I concomitantly with its uptake as observed in the liver and forearm. Plasma thyroxine distribution space was determined by extrapolation of the early plasma curve to the time of injection. The expansion of the labeled thyroxine distribution space during the first 3 hr postinjection was partitioned between thyroxine distribution into the extracellular fluid and hepatic thyroxine spaces. The proportion of the labeled thyroxine distribution entering the extracellular fluid space was assumed to be equal to the observed transcapillary passage of the labeled thyroxine into the forearm by 3 hr. The remainder was then considered to represent hepatic space. The further expansion of the thyroxine distribution space after the 3 hr period represented the entrance of labeled thyroxine into the extrahepatic tissue spaces. Justification for these assumptions was described in detail elsewhere (5). Additionally, a four compartmental mathematical model system devised by Berman (5, 8) was also used in some study subjects for comparison with the approximation method.

Hepatic thyroxine-^{131}I uptake employing a quantitative scintiscan method. It was apparent from our previous studies in normal subjects (5) and those of other investigators in man (9–16) and laboratory animals (17–19) that the hepatic uptake of thyroxine may be an important factor in determining the form of the initial portion of the thyroxine-^{131}I plasma disappearance curve and that the liver constituted a major site for tissue thyroxine sequestration. A need seemed apparent for the development of a more direct quantitative technique for measurement of thyroxine-^{131}I hepatic incorporation. The observation by Van Middlesworth, Turner, and Lipscomb (9), that the liver could be scintiscanned after the injection of a thyroxine-^{131}I tracer, seemed to offer an excellent opportunity for achieving this goal. The method devised for this study was to perform a liver scintiscan 3 hr after the injection of 20–60 μc of thyroxine-^{131}I tracer. This time period was selected because the peak liver uptake of labeled thyroxine was observed to plateau on or before the 3 hr period in all subjects we have thus far investigated, regardless of hepatic or thyroid status (5). The instrument employed for the scanning was a Picker Magnascanner III. A 0.340–0.390 Mev window and a zero background subtraction setting was used. The probe employed had a 19 hexagonal port collimator with a 4 inch focal length. This is the standard collimator employed for qualitative liver scintiscans. The liver outline was determined by inspection of the dot scan picture. The total liver counts were calculated by summing all of the dots incorporated within the area of the liver outline and multiplying them by the dot factor employed. The dot factor represents the number of counts accumulated in producing one dot on the scan picture. The amount of thyroxine-^{131}I administered varied depending on the anticipated fraction of the injected labeled thyroxine tracer which, judging from previous experience, would be incorporated into the liver. In all

cases, the accumulated gross counts over the liver were greater than 2500. The vascular background correction was approximated by counting a portion of the dot scan picture corresponding to the left lower portion of the abdomen which was equal in area to the hepatic scintiscan image. Standardization of the in vivo scintiscan was performed by scintiscanning a molded plastic liver phantom containing the appropriate fraction (15–40%) of the administered thyroxine dose. The liver phantom form was produced by casting in plaster of Paris a normal 1400-g liver at autopsy. The plaster casting was then used to construct a hollow acrylic plastic liver phantom. The walls of the phantom measured approximately ½ inch in thickness and the volume of the phantom contained 1400 ml. The phantom was filled with a 1% human serum albumin–normal saline solution which contained an appropriate percentage of the injected tracer dose. The phantom was positioned in a manner to conform as closely as possible to the in vivo geometry of the liver. A typical example of a scintiscan of the liver in vivo and the liver phantom are portrayed for comparison in Fig. 1. Scanning was performed at a 3 inch distance from the anterior surface of the phantom, while in the study subjects, scanning distance was 1 inch from the anterior chest wall.

Evaluation of the accuracy of the scintiscan method. The influence of scintiscan probe distance on the efficiency of the detection was determined by repetitive scintiscan measurements performed at varying distances from the liver phantom source. Normalizing the total counts produced by the phantom at 3 inches to be 100, we observed the relative alteration in counts at 2 inches to be 107, at 4 inches 94, and at 5 inches 99. The estimated in vivo counting distance from the anterior surface of the liver was easily encompassed by this 2–5 inch span. Thus, a maximum error of 11% of the net liver uptake counts could be produced by variation in probe distance. The attenuation of counts produced by the shielding effect of the anterior chest wall was appraised by the placement of an autopsy specimen of an anterior chest wall over the liver phantom. The chest wall employed was composed of the rib cage, associated muscle, and subcutaneous tissue structures. The average over-all thickness of the specimen was ⅝ inch. Its placement over the liver phantom produced a net 6% reduction in liver phantom counts. The ½ inch acrylic plastic walls of the liver phantom itself approximated the same shielding effect. Therefore, no correction was used for the chest wall shielding effect. It therefore appeared that neither the chest wall nor the scan distance variations should result in major errors in the hepatic scintiscan measurements.

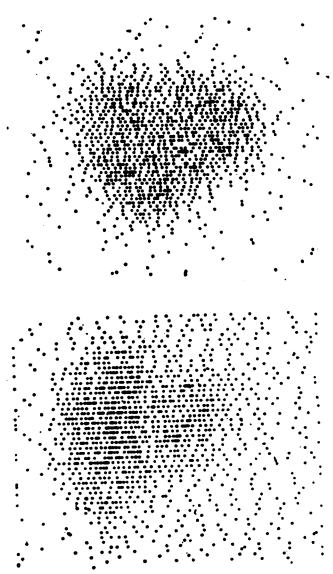

FIGURE 1 Comparison of dot scintiscan obtained from a liver phantom (upper scan) and an in vivo scan of a subject with thyrotoxic Graves' disease (lower scan) performed 3 hr after injection of a thyroxine-^{131}I tracer.

No attempts were made to correct for the errors produced from variations in the in vivo liver size and form, but in general, the in vivo scintiscan image corresponded fairly closely to that produced by the phantom itself, as illustrated in Fig. 1. The justification for the hepatic vascular background correction employed was based on the observation that a rapid scintiscan performed during the initial 10 min postinjection period resulted in a net hepatic uptake of 2% in a subject who eventually proved to have an uptake of 30% at 3 hr. Thus, the near zero time hepatic uptake value corresponded closely to the activity observed in the left lower quadrant of the abdomen. Additionally, a hepatic scintiscan performed using albumin-^{131}I instead of thyroxine-^{131}I revealed almost the same activity in the hepatic area as was observed in a similar area of the left lower quadrant.

Incorporated in this study was a previously described technique for the assessment of the relative deiodination rates of a labeled thyroxine tracer during the equilibration phase (5). This method involved the measurement of radioactive iodide activity in timed urine samples after the acute injection of a "pure" thyroxine-^{125}I tracer to subjects who had previously been equilibrated with a thyroxine-^{131}I tracer dose. The observed change in the ratio of ^{125}I to ^{131}I activities in the hourly urine samples allowed a qualitative analysis of the relative deiodination rates which occurred during the equilibration period. This method was applied to various forms of thyroid disease states in an attempt to ascertain if the altered manner of thyroxine incorporation into the tissue compartments might be reflected by changes in the rate of deiodination of the labeled thyroxine tracer by these compartments.

RESULTS

Compartmental distribution of thyroxine in hypothyroidism. 13 subjects with untreated hypothyroidism and 5 subjects with treated hypothyroidism were evaluated for their peripheral compartmental distribution of thyroxine (5). The

method employed allows an approximation of the plasma thyroxine distribution space, extracellular fluid distribution space, as well as hepatic and extrahepatic thyroxine compartments at equilibrium. The results are listed in detail in Table I. In the untreated hypothyroid subjects, the hepatic compartment contained only 9 ± 10 μg I/m^2 $\pm$ SD vs. a normal value of 51 ± 7 μg I/m^2, while the extrahepatic compartment contained 52 ± 26 μg I/m^2 vs. a nomal value of 122 ± 34 μg I/m^2. This reduction in the hepatic compartment was most prominent in those subjects with severe long-standing hypothyroidism. In some cases, there was no detectable hepatic incorporation of labeled thyroxine. Additionally, when an external scintillation probe was concomitantly placed over the liver, no detectable hepatic uptake was present in these severely hypothyroid subjects. When a measurable hepatic uptake was noted in subjects with milder forms of hypothyroidism, no apparent delay in the rate at which the liver achieved its peak was noted either by external liver probe analysis or indirectly from the early rapid drop in the forearm count rate. As noted in the treated hypothyroid group, subjects B.W. and L.N. had a repetition of their compartmental studies performed very shortly after the initiation of exogenous thyroid hormone therapy. In both cases a single large dose of stable thyroxine was administered intravenously (500 μg for B.W. and 1500 μg for L.N.). It is evident from the results of these repetitive studies, as shown in Table I, that the altered distribution of thyroxine was only minimally rectified by the acute elevation of the circulating thyroxine levels. In contrast, A.B. achieved a near normal compartmental distribution pattern after 6 months of therapy for hypothyroidism. It is of further interest that, in general, those patients with the most severe reduction in hepatic incorporation also displayed the lowest rate of extrathyroidal thyroxine turnover. This was most evident in subject A.B. when her turnover rate went from 5.87% before therapy to 10.5% after thyroid therapy.

Compartmental distribution of thyroxine in Graves' disease. This portion of the study pertains to the evaluation of 25 subjects with the established diagnosis of Graves' disease. These cases were divided into subgroups of 11 toxic, 10 eumetabolic, and 4 hypothyroid subjects on the basis of clinical and laboratory evaluation. The clinical status of each patient was relatively stable for 3 months or longer before the performance of the compartmental studies. Four of the eumetabolic subjects (J.L., V.R., H.B., and A.L.) had received no antithyroid therapy. The remainder of the eumetabolic and hypothyroid group had been treated with radioactive iodine except for subject M.F. who was treated by subtotal thyroidectomy. All thyrotoxic subjects were studied before the administration of any therapy. In the eumetabolic subjects, eumetabolism had been achieved for a period of 1 yr or longer, the longest period being 7 yr for subject M.S. and 8 yr for subject V.R.

The most notable alteration observed in the compartmental analysis in the subjects with Graves' disease was the relative augmentation in the quantity of thyroxine estimated to be incorporated into the liver compartment. This was 250 ± 81 μg I/m^2 $\pm$ 1 SD in the toxic subjects and 117 ± 38 μg I/m^2 in the eumetabolic subjects; these values were significantly greater than the 51 ± 7 μg I/m^2 observed in the control subjects. This relative increase of hepatic incorporation seemed to be extended even to the hypothyroid Graves' disease group. The 32 ± 4 μg I/m^2 observed to be in the hepatic compartment of subjects with hypothyroid Graves' disease was significantly greater than 9 ± 10 μg I/m^2 found in the hepatic compartment of subjects with primary hypothyroidism. Although the hepatic thyroxine compartment of the Graves' disease groups appeared to be grossly enlarged, and these groups statistically different from their non-Graves' disease counterpart ($P < 0.01$), there is great difficulty in properly matching these patients with non-Graves' disease subjects. Possibly the most satisfactory standard for comparison would be to relate the hepatic compartment size relative to the PBI level. This comparison is graphically represented in Fig. 2. It is evident that the hepatic compartment of subjects with Graves' disease generally exceeds that of the non-Graves' disease subjects at all PBI levels with the difference being less evident at the lower PBI ranges. In contrast, the extrahepatic pool size did not appear to be greatly enlarged in the toxic Graves' disease groups, as listed in Table I, with the exception of subject C.K. The toxic and eumetabolic Graves' disease groups displayed an increased fractional rate of thyroxine disposal of $17.34 \pm 4.56\%$ $\pm$ 1 SD for the toxic group and

TABLE I

Estimation of Thyroxine Turnover Kinetics and

Category	Subject	Sex	Age	m^2	PBI	K	TDS
					μg/100 ml	*%*	*liters*
Primary hypothyroidism	A. B.	F	72	1.24	1.0	5.05	12.3
	B. W.	F	85	1.31	0.4	5.87	12.3
	V. T.	F	80	1.31	0.7	4.95	14.7
	B. D.	M	45	1.94	0.6	5.77	19.6
	L. N.	M	30	2.20	0.8	6.87	14.0
	M. G.	F	50	1.93	1.8	7.85	12.7
	H. L.	M	78	1.67	1.7	4.40	16.1
	A. P.	M	73	1.83	1.3	5.77	12.7
	S. Y.	F	56	1.99	2.9	7.87	10.8
	J. C.	M	58	1.94	1.9	9.24	15.9
	Mean		63	1.74	1.3	6.36	14.1
	SD±		18	0.34	.8	1.55	2.6
Primary hypothyroidism, treated	B. W.	F	72	1.34	4.0	6.30	12.0
	L. N.	M	30	1.96	9.9	7.95	13.0
	A. B.	F	72	1.17	3.4	10.50	10.5
	G. D.	F	69	1.94	4.1	9.90	11.2
	L. W.	F	64	1.85	9.4	8.45	9.9
Graves' disease-toxic	C. K.	M	33	1.87	17.5	23.90	13.7
	A. P.	F	40	1.84	12.5	23.10	9.6
	L. R.	F	69	1.27	10.3	19.30	10.5
	F. G.	F	66	1.37	10.2	21.00	12.5
	C. F.	M	47	1.81	10.1	15.00	12.2
	J. K.	M	52	1.75	9.9	19.80	14.7
	K. J.	M	43	2.15	9.6	9.11	13.9
	R. S.	M	40	1.80	9.3	14.70	14.1
	E. V.	F	51	1.50	9.2	13.10	12.1
	S. R.	F	28	1.67	9.1	17.33	10.8
	A. C.	M	60	2.02	8.7	14.40	11.0
	Mean		48	1.70	10.6	17.34	12.3
	SD±		13	0.27	2.5	4.56	1.7
Graves' disease, eumetabolic	C. F.	M	61	1.87	7.8	15.75	10.0
	L. H.	M	51	1.83	7.5	11.72	13.9
	A. L.	M	32	1.60	7.4	15.40	11.6
	H. B.	M	65	1.72	7.2	10.98	12.7
	S. K.	F	27	1.71	6.6	13.86	9.4
	A. S.	F	50	1.94	5.8	10.20	12.7
	M. S.	F	24	1.73	5.1	10.40	9.8
	J. L.	F	28	1.74	5.0	13.80	13.9
	V. R.	M	52	1.97	4.1	9.00	14.5
	J. L.	F	40	1.68	3.4	12.16	12.2
	Mean		43	1.78	6.0	12.32	12.1
	SD±		15	0.12	1.5	2.29	1.8

Abbreviations used are as follows: PBI, protein-bound iodine; K, daily fractional rate of thyroxine turnover; TDS, thyroxine distribution space; PTDS, plasma thyroxine distribution space; ETDS, extracellular fluid thyroxine iodine distribution space; HC/m^2, hepatic thyroxine iodine content per square meter of body surface; EHC/m^2, extrahepatic thyroxine iodine content per square meter of body surface; D/m^2, daily turnover of thyroxine iodine per square meter of body surface.

* Triosorb, Abbott Laboratories, North Chicago, Ill.

PTDS	ETDS	HC/m²	EHC/m²	D/m²	Resin uptake*	Comment
liters	*liters*	*μg I*	*μg I*	*μg/day*		
2.20	2.56	0	61	5.0	—	Precoma
2.20	2.30	0	21	2.2	—	Precoma
2.58	3.07	1	47	3.9	19.5	Precoma
3.85	4.55	3	32	3.5	18.3	Severe
4.00	2.63	4	23	3.5	—	Severe
3.00	2.20	20	50	9.3	21.0	
2.73	2.08	5	110	7.2	15.4	
2.90	2.70	5	45	5.2	18.7	
2.64	1.83	22	70	12.4	22.5	
3.89	2.88	26	63	14.4	27.0	
3.00	2.68	8.6	52	6.7		
0.68	0.76	10	26	4.3		
2.20	2.20	15	212	22.6	—	2 days treated
4.10	3.16	56	234	52.2	—	7 days treated
2.20	1.90	32	154	32.0	—	4 months treated
3.40	3.00	32	70	23.4	23.5	1 yr treated
2.63	2.53	92	212	42.5	40.5	1.5 yr treated
3.80	3.31	395	222	306	50.3	Atrial fibrillation
2.26	2.48	186	137	151	52.1	Exophthalmos
2.90	2.60	279	127	164	44.7	
2.40	2.65	372	183	195	50.3	
3.02	3.00	270	75	102	43.3	Exophthalmos
3.28	3.28	260	200	165	46.1	Atrial fibrillation
3.82	3.40	181	117	56.5	33.0	
3.08	3.12	268	141	107	42.1	
2.88	2.48	231	170	97.2	44.7	Exophthalmos
3.00	2.30	168	128	102	43.5	
3.30	2.30	141	91	68	—	
3.07	2.81	250	145	137.6	45.0	
.49	.42	81	45	148	6.8	
2.80	2.27	100	105	65.7	37.7	Exophthalmos
3.08	3.02	160	160	66.8	36.0	Exophthalmos
2.78	2.72	160	123	82.6	39.2	
2.75	3.00	170	126	58.4	32.5	Exophthalmos
2.60	3.32	78	58	50.3	33.0	Exophthalmos
3.13	3.62	91	91	38.7	—	Exophthalmos
2.81	1.49	83	83	30.0	26.5	
3.40	3.00	141	63	55.1	41.5	
3.05	2.05	118	88	27.2	28.1	Exophthalmos
2.78	2.93	66	65	30.0	29.9	
2.92	2.74	117	96	50.5	33.8	
0.24	0.63	38	33	18.7	4.8	

TABLE I—(*Continued*)

Category	Subject	Sex	Age	m^2	PBI	K	TDS
					μg/100 ml	*%*	*liters*
Graves' disease-hypothyroid	M. F.	F	37	1.52	4.0	10.23	7.2
	J. S.	F	40	1.56	2.5	9.76	11.4
	J. N.	F	70	1.65	2.5	9.49	12.1
	G. G.	M	19	2.35	1.8	9.65	15.3
	Mean		42	1.77	2.7	9.78	11.5
	SD±		21	0.39	0.9	.32	3.3
Toxic nodular goiter	I. C.	F	80	1.56	10.9	8.45	11.0
	I. B.	F	50	1.65	8.0	10.50	9.6
	E. G.	F	77	1.57	8.0	13.60	8.0
	Mean		69	1.59	9.0	10.85	9.5
	SD±		17	0.04	1.7	2.60	1.5
Miscellaneous	P. M.	F	40	1.60	11.2	8.67	14.0
	A. D.	M	59	2.04	4.6	12.20	11.8
	G. H.	M	66	1.71	5.2	13.30	9.8
	R. L.	M	59	1.85	2.5	23.20	13.5
Control (n = 13)	Mean		47	1.69	5.5	10.66	10.4
	SD±		20	0.20	1.1	1.66	1.4

$12.32 \pm 2.29\%$ for the eumetabolic Graves' disease group as compared to the control group values of $10.66 \pm 1.66\%$. The hypothyroid Graves' disease group also displayed a surprisingly high fractional disposal rate of $9.78 \pm 0.32\%$. This is in marked contrast with the $6.36 \pm 1.55\%$ value observed in the non-Graves' disease hypothyroid group. These results again support the concept that there may be a direct relationship in these conditions between hepatic incorporation and the fractional disposal rate of thyroxine. Indeed, a correlation coefficient of 0.839 was obtained for these two parameters in the control, hypothyroid, and Graves' disease groups, but, an even higher correlation coefficient value of 0.925 was observed between the hepatic uptake and the net disposal rate as illustrated in Fig. 3.

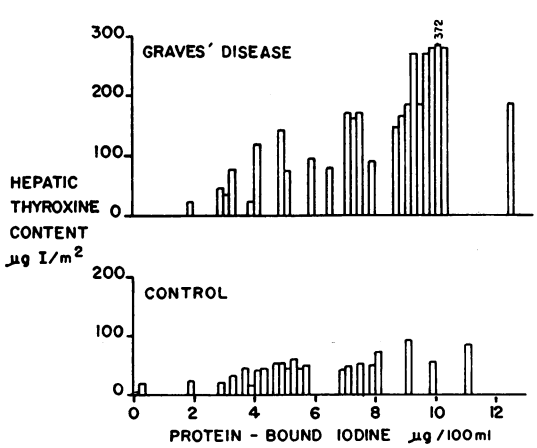

FIGURE 2 Comparison of the hepatic thyroxine content and protein-bound iodine levels for Graves' disease and non-Graves' disease subjects.

Thyroxine distribution in toxic nodular goiter. In contrast to the Graves' disease subjects, no apparent alteration in the relative distribution of hepatic vs. extrahepatic thyroxine pools was apparent in this group, as shown in Table I and Fig. 2 in the non-Graves' disease group. The fractional turnover rate of thyroxine in these subjects was also noted not to be markedly increased. Patient L.W., a subject with treated primary hypothyroidism, should probably be included in this portion of the result section since she appeared to be chronically over-treated with exogenous desiccated thyroid which resulted in an elevated PBI level of 9.4 μg/100 ml as seen in Table I. It is of interest that her compartmental distribution of thyroxine was quite similar to that found in a toxic nodular goiter group.

Thyroxine distribution in subjects with liver diseases and low thyroxine-binding globulin lev-

PTDS	ETDS	HC/m²	EHC/m²	D/m²	Resin uptake*	Comment
liters	*liters*	*μg I*	*μg I*	*μg/day*		
2.38	1.28	22	45	19.4	21.5	14 yr posttherapy
2.25	1.90	35	81	17.8	—	9 yr posttherapy
3.00	2.05	47	60	17.4	—	25 yr posttherapy
3.50	3.10	23	43	11.3	—	8 months posttherapy
2.78	2.08	32	57	16.5	—	
0.58	.76	12	18	3.6		
2.27	3.00	83	320	64.9	39.5	—
2.56	1.87	50	200	48.9	—	—
2.35	1.99	72	115	55.4	31.0	—
2.39	2.29	68	212	56.4		
.15	.62	17	103	8.0		
2.95	2.70	0	585	85.0	—	Acute hepatitis
3.28	2.72	18	103	32.5	—	Laennec's cirrhosis
2.50	1.93	40	123	39.6	—	Laennec's cirrhosis
3.20	3.20	73	23	42.3	56.6	Low-thyroxine-binding globulin
2.72	2.70	51	122	34.7	31.7	
0.47	.69	7	34	3.6	2.85	

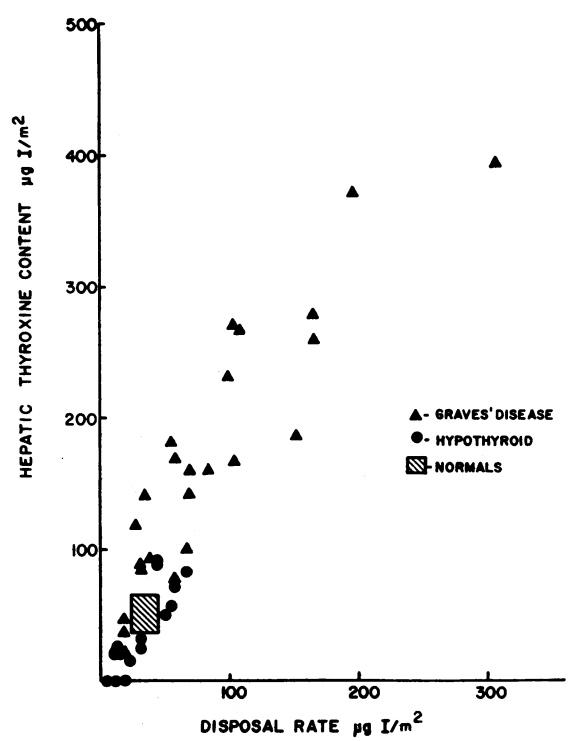

FIGURE 3 Comparison of thyroxine disposal rates and hepatic thyroxine content for normal, Graves' disease, and hypothyroid subjects. Shaded area encloses the two standard deviation limits for the control group.

els. Three subjects with liver disease were included in this present study for the purpose of demonstrating that liver disease will cause a marked decrease in the hepatic thyroxine uptake. In subject P.M., with acute infectious hepatitis, virtually no hepatic uptake of thyroxine could be demonstrated. As in the hypothyroid subject A.B., this lack of hepatic uptake was qualitatively verified by external counting over the area of the liver. It is of interest that the disposal rate of 85 μg I/m² was in the range that was observed in mildly thyrotoxic subjects. In mild alcoholic cirrhosis, subject G.H. was found to have a minimal alteration in compartmental distribution, but in subject A.D., with severely decompensated cirrhosis, there was a marked decrease in hepatic thyroxine uptake. In subject R.L., who has a near absence of thyroxine-binding globulin on an idiopathic basis, there was a calculated elevation of 73 μg I/m² in the hepatic incorporation of thyroxine and a reduction to 23 μg I/m² in his extrahepatic thyroxin compartment. The thyroxine-binding globulin saturation capacity for this subject was 7 μg T_4/100 ml which compares with the normal range in our laboratory of 15–25 μg T_4/100 ml.

Quantitative liver thyroxine uptake employing a scintiscan method. The results of the compart-

TABLE II

Estimation of the 3 Hr Hepatic Uptake of Labeled Thyroxine by External Liver Scintiscan Method

Category	Subject	Age	Sex	PBI	Resin uptake*	% Hepatic uptake	Comment
Control	C. H.	42	M	5.5	30.4	26.7	Peptic ulcer
	D. A.	45	M	5.0	29.4	15.8	Peptic ulcer
	J. G.	42	M	5.3	31.6	15.5	Chronic pyleonephritis
	H. H	44	M	5.8	27.6	13.8	Arterioscl. heart disease
	A. C.	46	M	6.0	35.3	19.5	Psychoneurosis
	M. F.	30	M	4.5	29.9	16.1	Peptic ulcer
	R. M.	44	M	7.1	30.2	22.2	Chronic pyelonephritis
	R. T.	33	M	6.0	32.7	20.0	Normal
	D. F.	44	M	5.0	34.7	18.8	Functional bowel disease
	D. G.	53	M	4.6	26.7	20.8	Mild diabetes
	R. B.	43	F	6.1	29.2	18.6	Normal
	T. T.	46	M	6.2	31.2	23.4	Normal
	P. S.	18	F	4.9	32.0	25.0	Normal
	Mean			5.54	30.84	19.71	
	SD±			0.747	2.48	3.88	
Primary hypothyroidism	H. W.	55	M	2.5	18.8	8.6	
	M. S.	70	F	2.0	21.4	15.4	1 gr of thyroid/day
	B. B.	80	M	2.0	18.7	10.4	
	A. D.	70	M	1.5	13.2	13.2	
	R. L.	46	M	1.5	20.8	14.8	
	Mean			1.9	18.6	12.48	
	SD±			0.418	3.24	2.91	
Toxic Graves' disease	W. B.	48	M	16.1	50.0	23.6	
	T. F.	47	M	16.0	52.4	28.8	
	H. M.	32	M	14.2	48.0	38.3	
	L. G.	35	M	14.2	49.8	37.6	
	A. T.	38	M	14.1	42.5	38.8	
	J. H.	19	M	13.1	48.2	27.8	
	P. S.	48	M	13.0	42.9	31.3	
	J. C.	59	M	12.4	37.2	34.0	
	C. K.	33	M	12.2	40.3	25.0	
	N. O.	36	M	12.1	48.8	22.0	
	R. E.	42	M	10.0	42.0	27.4	
	Mean			13.4	45.65	30.42	
	SD±			1.78	4.84	6.02	
Eumetabolic Graves' disease	T. L.	24	M	7.8	38.9	33.3	Stable 6 months, no treatment
	L. H.	51	M	6.0	30.1	39.0	Stable 1 yr post ^{131}I
	K. J.	49	M	4.0	29.3	25.1	Stable 1 yr, post ^{131}I
	L. L.	49	F	4.5	29.1	21.8	Stable 1 yr, post ^{131}I
	L. H.	65	M	5.0	30.1	31.0	Stable 1 yr, post ^{131}I
	C. P.	58	M	6.6	28.0	23.9	Stable 3 yr, post ^{131}I
	K. O.	39	M	6.6	32.4	24.8	Stable 5 yr, post ^{131}I
	R. L.	45	M	5.6	27.3	24.6	Stable 6 yr, post ^{131}I
	L. S.	49	M	7.2	31.1	25.3	Stable 6 yr, post ^{131}I
	L. B.	33	F	5.9	27.2	25.5	Stable 6 yr, post ^{131}I
	F. V.	44	M	4.2	31.0	25.7	Stable 9 yr, post ^{131}I
	L. A.	68	M	4.1	26.2	25.9	Stable 10 yr, post ^{131}I
	T. N.	69	M	7.0	34.8	38.3	Stable 15 yr, post ^{131}I
	Mean			5.73	30.42	28.02	
	SD±			1.28	3.45	5.57	

PBI, protein-bound iodine.

* Trisorb, Abbott Laboratories, North Chicago, Ill.

TABLE II—(*Continued*)

Category	Subject	Age	Sex	PBI	Resin uptake*	% Hepatic uptake	Comment
Hypothyroid Graves' disease	E. H.	42	M	1.6	17.6	28.4	9 yr post ^{131}I
	J. G.	47	M	2.0	26.9	21.1	2 yr post ^{131}I
	R. B.	38	M	2.5	21.3	18.6	6 months post ^{131}I
	H. H.	48	M	2.0	18.9	14.3	6 yr post ^{131}I
	Mean			2.025	21.175	20.58	
	SD±			0.369	4.11	5.91	
Toxic nodular goiter	R. D.	65	M	12.0	37.2	24.6	
	J. P.	70	M	10.0	41.8	22.8	
	Mean			11	39.5	23.7	
	SD			1.41	3.25	1.27	
Miscellaneous	R. L.	59	M	2.5	56.6	42.0	Idiopathic low TBG
	Q. C.	52	M	2.0	41.7	30.8	Idiopathic low TBG
	R. B.	38	M	5.9	32.1	9.4	Severe Laennec's cirrhosis
	J. M.	41	M	17.6	41.0	16.3	Thyrotoxicosis factitia
	R. B.	42	M	15.1	38.2	18.3	Thyrotoxicosis factitia

mental analysis of thyroxine distribution revealed that the hepatic uptake of thyroxine could be substantially altered in a variety of thyroid disease states. It appeared desirable to establish a direct technique for the quantitative measurement of the hepatic incorporation of labeled thyroxine which was independent of the indirect methods previously employed. To accomplish this purpose, a second group of subjects were evaluated for their hepatic thyroxine-^{131}I uptakes using a quantitative external scintiscan technique. While patients were, with few exceptions, different individuals from those employed for the previous compartmental analysis study, the clinical classification of these subjects was the same as previously employed. The compartmental analysis results revealed that the hepatic uptake values at 3 hr should be on the average 22.5% for control and 16.0% for primary hypothyroid subjects, while in Graves' disease subjects they would be 28.6% for toxic, 25.6% for eumetabolic, and 19.7% for hypothyroid subjects. The corresponding values employing the 3 hr hepatic scintiscan method were found to be 19.7% for control and 12.5% for primary hypothyroid subjects, while in Graves' disease subjects they were 30.4% for toxic, 28.0% for eumetabolic, and 20.6% for hypothyroid subjects as listed in Table II. In those subjects, the concomitant measurement of hepatic thyroxine uptake values and compartmental analysis by the approximation method were performed. Close correlation between these methods regarding the 3 hr hepatic uptake values were observed as recorded in Table III. No alteration in the 3 hr hepatic thyroxine uptake percentages were observed in subjects with toxic nodular goiter and factitial thyrotoxicosis, while an increased hepatic uptake was noted in two subjects with an idiopathic decrease in serum thyroxine-binding globulin. A marked decrease was found in one patient with severe Laennec's cirrhosis.

Mathematical model analysis of thyroxine distribution. In a previous study employing normal subjects, a hypothetical system was developed for the compartmental distribution of thyroxine (5). The model system employed was that described by Berman and Schoenfeld (20), and was based on a set of differential equations which portrayed the size and kinetic interplay of a theoretical four compartmental model. The restrictions placed on the model system were the same as previously employed in normal subjects with the exception that the estimation of the 3 hr hepatic uptake value was

TABLE III

Comparison of the 3 Hr Hepatic Uptake by the Compartmentaland Liver Scan Methods

Subjects	Diagnosis	Per cent 3-hr hepatic uptake: Compartmental analysis	Per cent 3-hr hepatic uptake: Liver scan
R. L.	Low TBG	45.8	42.0
C. K.	Toxic Graves'	32.0	25.0
L. H.	Eumetabolic Graves'	39.0	39.0

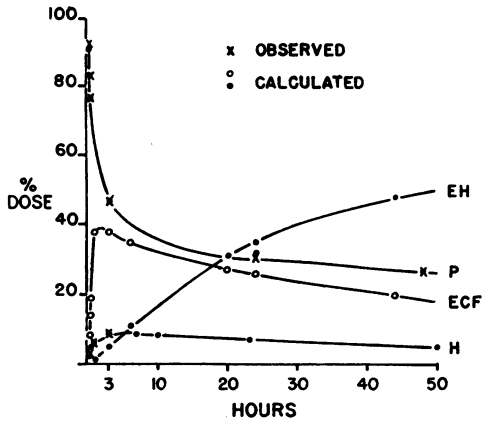

FIGURE 4 Computer analysis of the temporal pattern of labeled thyroxine distribution in the major body fluid and tissue compartments in a subject with primary hypothyroidsm. *EH, P, ECF,* and *H* represent the extrahepatic, plasma, extracellular fluid, and hepatic compartments, respectively.

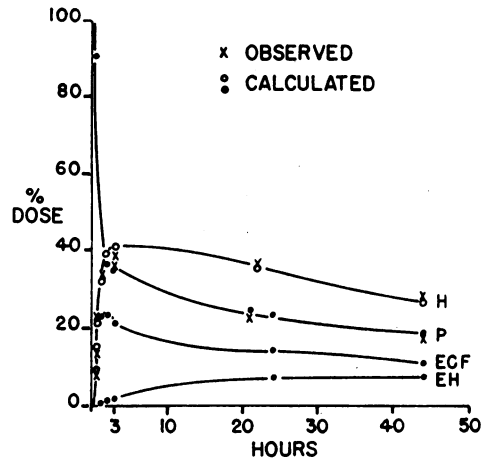

FIGURE 5 Computer analysis of the temporal pattern of labeled thyroxine distribution in the major body fluid and tissue compartments in a subject with thyrotoxic Graves' disease. *H, P, ECF,* and *EH* represent hepatic, plasma, extracellular fluid, and extrahepatic compartments, respectively.

obtained by the approximation method rather than from hepatic biopsy data as previously employed (5). Close agreement was observed between the approximation method estimates and the mathematical model system in all thyroid disease states studied. Typical examples of this close correlation are graphically demonstrated in Figs. 4 and 5, in which the results from a hypothyroid and a toxic Graves' disease subject are portrayed.

The change in rate of deiodination of an equilibrating tracer dose of thyroxine in thyroid disease states. The results from both compartmental analysis and hepatic thyroxine scintiscan studies revealed an increase in hepatic incorporation of thyroxine in Graves' disease and a decrease in primary hypothyroidism. The question then arose, if the hepatic thyroxine uptake is variable, would the contribution of the liver to the total thyroxine disposal rate also be variable. In other words, if the hepatic uptake were elevated, would the hepatic thyroxine disposal rate be elevated and vice versa. Because the hepatic uptake of a tracer dose of thyroxine is accomplished long before that of the extrahepatic tissues (5), any major alteration in the disposal rate attributable to the liver would be most apparent during the first few hours after the injection of the thyroxine tracer. We have previously described a method for measurement of the relative deiodination rate for labeled thyroxine throughout the time course of its equilibration (5). The method employed dual tracers of thyroxine, one of which was in an equilibrated state and the other in the process of achieving equilibration. The relative rates of deiodination of these two tracers were assessed by measuring the ratio of the two labeled iodide isotopes as they were excreted in timed urine samples after the administration of the second (unequilibrated) tracer. The

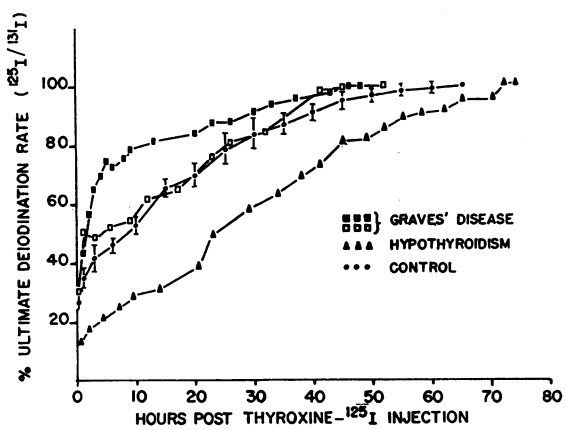

FIGURE 6 The change in the rate of deiodination of labeled thyroxine during the equilibration period as measured in serial timed urine samples for normals and subjects with primary hypothyroidism and Graves' disease. The vertical bar represents the value for one standard error of the mean for the control group. The open and the solid squares represent the values for two patients with thyrotoxic Graves' disease. After equilibration of the two tracers has been achieved, the established ratio was normalized as 100%.

TABLE IV

3-Hr Thyroxine Deiodination Values in Thyroid Disease

Category	Subject	Age	Sex	PBI	3-hr deiodination rate	HC/m²
				μg/100 ml	%	
Control	L. N.	24	F	5.7	39.8	48
	A. S.	50	M	7.6	40.0	54
	G. H.	66	M	5.2	46.0	45
	H. G.	40	M	5.0	48.2	—
	M. B.	19	F	3.4	36.9	50
	E. A.	39	M	5.2	47.5	60
	J. A.	36	M	6.0	49.0	45
	J. S.	46	F	5.4	47.5	39
	J. G.	46	M	5.5	50.6	52
	J. N.	45	F	4.6	48.0	45
	Mean	41.1		5.4	45.4	48.67
	SD±	13.2		1.1	4.7	6.16
Primary hypothyroidism	B. D.	45	M	0.6	11.9	3
	L. N.	30	M	0.8	13.9	4
	A. P.	72	M	1.3	28.5	5
	H. L.	78	M	1.7	22.3	2
	S. Y.	56	F	2.9	37.8	22
	Mean	56.2		1.5	20.9	7.8
	SD±	19.6		0.9	14.9	7.98
Eumetabolic Graves' disease	J. L.	40	F	3.5	76.5	66
	M. F.	45	F	4.0	59.7	22
	V. R.	52	M	4.1	38.7	118
	K. J.	43	M	7.8	65.5	117
	Mean	45		4.9	60.1	80.8
	SD±	5.1		2.0	15.9	46.1
Thyrotoxic disease	A. P.	40	F	12.5	63.0	186
	E. V.	42	F	9.2	56.3	231
	L. H.	51	M	8.2	79.0	200
	N. O.	35	M	8.0	66.0	—
	A. T.	40	M	11.9	80.0	—
	C. K.	33	M	17.5	58.5	—
	Mean	40.2		11.2	67.1	206
	SD±	6.3		3.6	10.2	23

results of such an approach are illustrated in Fig. 6 for normal, hypothyroid, and Graves' disease subjects. When the two isotopes achieve full equilibration, the ratio of the iodide tracers in the urine maintains a fixed ratio. The value of this ratio was normalized as 100%. Values obtained before achieving the ultimate ratio were then expressed as a percentage. In subjects with Graves' disease, there was an augmentation of the early deiodination rate of thyroxine, and in subjects with primary hypothyroidism a marked reduction was noted. These findings correlate with the augmented proportion of labeled thyroxine found in the liver of Graves' disease patients and the diminished values found in patients with primary hypothyroidism. The maximum differences in the deiodination rate appeared to occur during the 3–4 hr urine collection period. The differences between the two groups became less apparent as equilibration was approached. For this reason, a comparison of Graves' disease, normal, and hypothyroid groups was made employing the 3 hr urinary col-

lection values. The results of the comparison of the 3 hr deiodination rates are listed in Table IV. The observed 3-hr value was expressed as a percentage of the ultimate ratio value. This value was then multiplied by the ratio of the 3 hr thyroxine distribution volume/ultimate thyroxine distribution volume in order to compensate for the relative differences in specific activities of the unequilibrated and equilibrated thyroxine tracers in these various clinical disease states. In other words, after 3 hr of equilibration in subjects with Graves' disease, the thyroxine tracer may have achieved 75% of its ultimate distribution volume, while in a subject with primary hypothyroidism, this may only be 50%. These differences are illustrated in Fig. 7. Using this method, the 3-hr deiodination rate in normal subjects was found to be 45.5 ± 4.7% ± 1 SD which was in contrast to the primary hypothyroid group which demonstrated a 20.9 ± 14.9% value. Elevated values were observed for the eumetabolic (60.1 ± 15.9%) and the thyrotoxic Graves' disease subjects (67.1 ± 10.2%). A gross correlation between hepatic thyroxine incorporation and the 3 hr deiodination rate in these groups was observed ($r = 0.73$). Although the relative 3-hr deiodination rates of these three groups were significantly different ($P < 0.01$), there was a wide variability noted within each group, especially in the Graves' disease subjects.

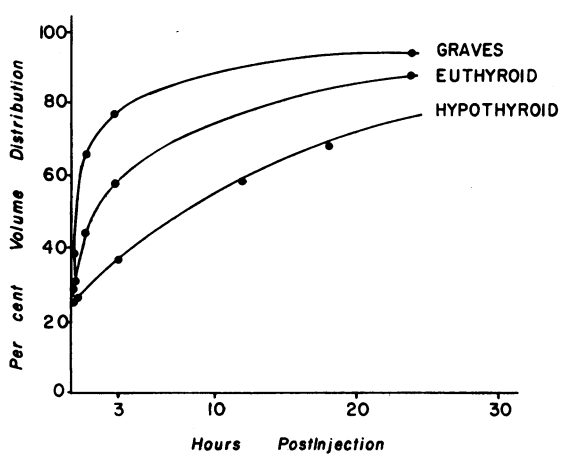

FIGURE 7 The relative percentage of the ultimate distribution volume for a thyroxine-^{131}I tracer was measured during the period of isotonic equilibration in a normal subject and in patients with primary hypothyroidism and Graves' disease.

DISCUSSION

The results of these compartmental studies in the various thyroid disease states are consistent as to the direction of alterations in hepatic incorporation of thyroxine. In subjects with Graves' disease, it appeared that an increased proportion of labeled thyroxine was taken up and deiodinated by the peripheral tissues, and that this occurred primarily in the liver. In subjects with primary hypothyroidism the reverse was observed. The increase in hepatic thyroxine uptake found in subjects with thyrotoxic Graves' disease was also observed in the eumetabolic and hypothyroid patients with Graves' disease when compared to their non-Graves' disease counterparts. Results of urinary deiodination studies employing dual thyroxine tracers indicated that the labeled thyroxine incorporated into the liver promptly became available for deiodination. It might, therefore, be expected that the amount of thyroxine incorporated into the liver should then be directly proportional to the net hormonal disposal rate. The close correlation observed between the hepatic incorporation values and the net hormonal disposal rate in primary hypothyroid and Graves' disease subjects would seem to verify this relationship. This is graphically illustrated in Fig. 3. This relationship was not so apparent in subjects with toxic nodular goiter and factitial thyrotoxicosis, when the increased tissue thyroxine incorporation was approximately equally partitioned between the hepatic and extrahepatic tissue compartments.

The question of why subjects with Graves' disease persist in maintaining a relatively large hepatic thyroxine pool in the eumetabolic or even hypothyroid state is one of the most puzzling problems which has evolved from this study. This finding would appear to be consistent with the observations of Ingbar and Freinkel (2), which indicated a persistence of elevated thyroxine turnover rates in such subjects. In addition, these results would also support the observations of Lennon and coworkers (1) as to the presence of rapid early thyroxine disappearance rates in patients with Graves' disease. This would be the expected result since the liver is the major tissue site for the early uptake of thyroxine. It is doubtful that alterations in total serum thyroxine-binding protein properties could explain these changes in distribution and disposal since the T_3

resin uptake values obtained in the eumetabolic and hypothyroid Graves' disease groups revealed no major alteration to be present. Additionally, restoration of normal thyroxine-binding capacities for thyroxine-binding globulin and thyroxine-binding prealbumin in treated Graves' disease subjects has recently been observed by Braverman, Foster, and Ingbar (21). At present, the most likely explanation would appear to be that an increase in the number of intracellular hepatic thyroxine-binding sites occurs in subjects with Graves' disease which persists after therapeutic measures to lower the serum thyroxine levels to normal or to below normal ranges. The reverse would also seem to be true in the early treatment phase of subjects with primary hypothyroidism. The studies on hypothyroid subjects B.W. and L.N., revealed that a rapid elevation of thyroxine levels to normal concentrations did not result in restoration of a normal hepatic thyroxine pool size. These findings support the contention that hepatic thyroxine binding sites were decreased in patients with primary hypothyroidism and that a prolonged period of eumetabolism was necessary to restore the hepatic thyroxine pool size to normal as was observed in subjects A.B., G.D., and L.W. The factors which regulate these intracellular thyroxine-binding sites are presently unknown.

The investigations of Lennon and coworkers (1), Ingbar and Freinkel (2), and this present study, are relatively consistent in that they describe distinct qualitative alterations in the manner in which Graves' disease subjects handle labeled thyroxine. Not all investigations have been able to confirm these qualitative differences. Webster, Britton, Volpe, and Ezrin (22), employing essentially the same methodology as Lennon and coworkers (1), found that the acute disappearance of labeled thyroxine was directly related to the circulating levels of thyroxine and indirectly to the concentration of thyroxine-binding globulin, no matter which thyroid disease state was considered. They concluded from their findings that there was no unique qualitative difference in the manner in which Graves' disease or primary hypothyroid subjects handled thyroxine. There is no apparent explanation for these disparate results. These apparently conflicting results indicate the complexity of the factors which affect peripheral thyroxine metabolism and only serve to point up the need for the development of more direct investigative approaches.

The hepatic thyroxine content and hepatic deiodination rates were not as closely correlated as the hepatic thyroxine content and thyroxine disposal rates. One reason for this finding may be that the technique employed for the estimation of hepatic thyroxine deiodination rates was one which required that the equilibrating thyroxine tracer be of the highest purity. Contamination of labeled thyroxine from commercial sources with a variety of other labeled thyronines has previously been described (23) and was noted to be present in some of our shipments. Even in cases where the apparent purity of the delivered isotope allowed this technique to be employed, it is quite possible that minor degrees of contamination were still present and could account for some of the variations observed in the groups studied. Despite all of these shortcomings, there was no difficulty in clearly distinguishing differences between the major thyroid disease groups.

Our observations of an increase in the size of the hepatic thyroxine compartment in subjects with idiopathic low thyroxine-binding globulin, and a decreased hepatic thyroxine compartment in patients with hepatic disease, confirms the recent investigations of Cavalieri and Searle (10, 24). Although our calculated values differ somewhat from theirs, primarily because of the different mathematical models employed, the qualitative alterations are similar. The findings that the liver concentrates thyroxine quite well in subjects with idiopathic low thyroxine-binding globulin, infers that hepatic tissue-binding sites are not involved in this disorder.

The compartmental analysis method employed in this study revealed that the extracellular fluid distribution of thyroxine was on the average equal to that of the plasma compartment in virtually all conditions studied. The early extravascular uptake pattern of thyroxine in the forearm was employed in determining the size of the extracellular fluid thyroxine space (5). The assumption was made that the other extracellular fluid compartments of the body possessed approximately the same kinetic pattern as that observed in the forearm. Also, all extrahepatic tissues were grouped together for purposes of simplifying the analysis.

It is quite obvious that these various compartments of the body are not homogenous and that these estimates should be considered only as rough approximations. Since direct measurements of the extracellular fluid and extrahepatic compartments were not obtained in this study, the solution obtained from either the approximation or the mathematical model systems cannot be considered entirely unique. In spite of these limitations, these model systems do appear to be conceptually useful for the future design of experiments which may establish more exactly the kinetic characteristics of the extracellular fluid and extrahepatic thyroxine compartments. Although the precision of the measurement of the extracellular fluid and extrahepatic tissue thyroxine pools may be in question, this would not appear to invalidate the observations of the differences found for the liver pool size in these various disease states.

ADDENDUM

Since the submission of this investigation for publication, Musa, Kumar, Ogilvie, and Dowling (25) have published in abstract form, that the hepatic space in subjects with thyrotoxic Graves' disease, toxic nodular goiter, and hypothyroidism is increased, normal, and decreased, respectively. They have employed the method of Cavalieri and Searle (10) for estimation of the hepatic space. In addition, they observed no correlation of these alterations in hepatic thyroxine space with binding capacities of either thyroxine-binding globulin or thyroxine-binding prealbumin levels.

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REFERENCES

1. Lennon, E. J., N. H. Engbring, and W. W. Engstrom. 1961. Studies of the rate of disappearance of labeled thyroxine from the intravascular compartment. *J. Clin. Invest.* **40**: 996.
2. Ingbar, S. H., and N. Freinkel. 1958. Studies of thyroid function and the peripheral metabolism of I^{131}-labeled thyroxine in patients with treated Graves' disease. *J. Clin. Invest.* **37**: 1603.
3. Ingbar, S. H., N. Freinkel, J. T. Dowling, and L. F. Kumagai. 1956. Abnormalities of iodine metabolism in euthyroid relatives of patients with Graves' disease. *J. Clin. Invest.* **35**: 714. (Abstr.)
4. Ingbar, S. H. 1960. Clinical and physiologic implications of thyroxine turnover in man. *In* Clinical Endocrinology. I. E. B. Astwood, editor. Grune & Stratton, Inc., New York. 91.
5. Nicoloff, J. T., and J. T. Dowling. 1968. Estimation of thyroxine distribution in man. *J. Clin Invest.* **47**: 26.
6. Barker, S. B., M. J. Humphrey, and M. H. Soley. 1951. The clinical determination of protein-bound iodine. *J. Clin. Invest.* **30**: 55.
7. McCall, M. S., and M. F. Camp. 1961. A simple technique for prolonging the storage life of I^{131}-labeled proteins and polymers. *J. Lab. Clin. Med.* **58**: 772.
8. Berman, M. 1960. Application of differential equations to the study of the thyroid system. *In* Proceedings of the 4th Berkeley Symposium on Mathematical Statistics and Probability. Jerzy Neyman, editor. University of California, Berkeley. **4**: 87.
9. Van Middlesworth, L., J. A. Turner, and A. Lipscomb. 1963. Liver function related to thyroxine metabolism. *J. Nucl. Med.* **4**: 132.
10. 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.
11. Blomstedt, B., and L. O. Plantin. 1965. The extrathyroidal distribution of ^{131}I thyroxine. *Acta Endocrinol.* **48**: 536.
12. 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.)
13. Pochin, E. E. 1964. Liver concentration of thyroid metabolites. *In* Dynamic Clinical Studies with Radioisotopes. R. M. Kniseley and W. N. Tauxe, editors. Atomic Energy Commission–Technical Information Document No. 7678, Oak Ridge. 413.
14. Inada, M., and K. Sterling. 1967. Thyroxine turnover and transport in Laennec's cirrhosis of the liver. *J. Clin. Invest.* **46**: 1275.
15. Vannotti, A., and T. Béraud. 1959. Functional relationships between the liver, the thyroxine-binding protein of serum, and the thyroid. *J. Clin. Endocrinol. Metab.* **19**: 466.
16. Roche, J., and R. Michel. 1960. On the peripheral metabolism of thyroid hormone. *Ann. N. Y. Acad. Sci.* **86**: 454.
17. Ford, D. H., K. R. Corey, and J. Gross. 1957. The localization of thyroid hormones in the organs and tissues of the guinea pig: an autoradiographic and chromatographic study. *Endocrinology.* **61**: 426.

18. Gorman, C. A., E. V. Flock, C. A. Owen, Jr., and J. Paris. 1966. Factors affecting exchange of thyroid hormones between liver and blood. *Endocrinology.* **79**: 391.
19. Albert, A., and F. R. Keating, Jr. 1952. The role of the gastrointestinal tract, including the liver, in the metabolism of radiothyroxine. *Endocrinology.* **51**: 427.
20. Berman, M., and R. Schoenfeld. 1956. Invariants in experimental data on linear kinetics and the formulation of models. *J. Appl. Phys.* **27**: 1361.
21. Braverman, L. E., A. A. Foster, and S. H. Ingbar. 1967. Thyroid hormone transport in the serums of patients with treated thyrotoxicosis. *In* Annual Meeting of the American Thyroid Association, Inc. Ann Arbor. 35. (Abstr.)
22. Webster, B. R., A. Britton, R. Volpé, and C. Ezrin. 1967. Further studies on the rate of disappearance of labeled thyroxine from the intravascular compartment of man, with reference to the role of thyroxine binding proteins. *Acta Endocrinol.* **55**: 497.
23. Volpert, E. M., M. Martinez, and J. H. Oppenheimer. 1967. Radioiodinated impurities in commercial preparations of ^{131}I-thyroxine and their effect on the measurement of free thyroxine in human serum by equilibrium dialysis. *J. Clin. Endocrinol. Metab.* **27**: 421.
24. Cavalieri, R. R., and G. L. Searle. 1965. Role of the liver in the distribution of I^{131} thyroxine in man. *In* The Fifth International Thyroid Conference, Rome. C. Cassano and M. Andreoli, editors. Academic Press, Inc., New York.
25. Musa, B. U., R. S. Kumar, J. T. Ogilvie, and J. T. Dowling. 1968. Hepatic thyroxine kinetics in man in states of altered thyroid function. *Clin. Res.* **16**: 149. (Abstr.)