Peripheral Serum Thyroxine, Triiodothyronine and Reverse Triiodothyronine Kinetics in the Low Thyroxine State of Acute Nonthyroidal Illnesses

A NONCOMPARTMENTAL ANALYSIS

ELAINE M. KAPTEIN, WILLIAM J. ROBINSON, DEBORAH A. GRIEB, and JOHN T. NICOLOFF, *Division of Endocrinology, Department of Medicine, University of Southern California School of Medicine, Los Angeles, California 90033*

ABSTRACT The low thyroxine (T_4) state of acute critical nonthyroidal illnesses is characterized by marked decreases in serum total T_4 and triiodothyronine (T_3) with elevated reverse T_3 (rT_3) values. To better define the mechanisms responsible for these alterations, serum kinetic disappearance studies of labeled T_4, T_3, or rT_3 were determined in 16 patients with the low T_4 state and compared with 27 euthyroid controls and a single subject with near absence of thyroxine-binding globulin. Marked increases in the serum free fractions of T_4 (0.070±0.007%, normal [nl] 0.0315±0.0014, $P < 0.001$), T_3 (0.696±0.065%, nl 0.310±0.034, $P < 0.001$), and rT_3 (0.404±0.051%, nl 0.133±0.007, $P < 0.001$) by equilibrium dialysis were observed indicating impaired serum binding. Noncompartmental analysis of the kinetic data revealed an increased metabolic clearance rate (MCR) of T_4 (1.69±0.22 liter/d per m^2, nl 0.73±0.05, $P < 0.001$) and fractional catabolic rate (FCR) (32.8±2.6%, nl 12.0±0.8, $P < 0.001$), analogous to the euthyroid subject with low thyroxine-binding globulin. However, the reduced rate of T_4 exit from the serum (Kii) (15.2±4.6 d^{-1}, nl 28.4±3.9, $P < 0.001$) indicated an impairment of extravascular T_4 binding that exceeded the serum binding defect. This defect did not apparently reduce the availability of T_4 to sites of disposal as reflected by the increased fractional disposal rate of T_4 (0.101±0.018 d^{-1}, nl 0.021±0.003, $P < 0.001$). The decreased serum T_3 binding was associated with the expected increases in MCR (18.80±2.22 liter/d per m^2, nl 13.74±1.30, $P < 0.05$) and total volume of distribution (26.55±4.80 liter/m^2, nl 13.10±2.54, $P < 0.01$). However, the unaltered Kii suggested an extravascular binding impairment comparable to that found in serum. The decreased T_3 production rate (6.34±0.53 μg/d per m^2, nl 23.47±2.12, $P < 0.005$) appeared to result from reduced peripheral T_4 to T_3 conversion because of decreased 5′-deiodination rather than from a decreased T_4 availability. This view was supported by the normality of the rT_3 production rate. The normal Kii values for rT_3 indicated a comparable defect in serum and extravascular rT_3 binding. The reduced MCR (25.05±6.03 liter/d per m^2, nl 59.96±8.56, $P < 0.005$) and FCR (191.0±41.19%, nl 628.0±199.0, $P < 0.02$) for rT_3 are compatible with an impairment of the rT_3 deiodination rate.

These alterations in thyroid hormones indices and kinetic parameters for T_4, T_3, and rT_3 in the low T_4 state of acute nonthyroidal illnesses can be accounted for by: (*a*) decreased binding of T_4, T_3, and rT_3 to vascular and extravascular sites with a proportionately greater impairment of extravascular T_4 binding, and (*b*) impaired 5′-deiodination activity affecting both T_4 and rT_3 metabolism.

This work was presented in part at the 6th International Congress of Endocrinology, Melbourne, Australia, February, 1980 (Abstract No. 364), and at the 61st Annual Endocrine Society Meeting, Wash., D. C., June, 1980 (Abstract No. 459).

Address reprint requests to Dr. Elaine M. Kaptein.

Received for publication 11 June 1981 and in revised form 3 November 1981.

INTRODUCTION

Patients with severe nonthyroidal illnesses frequently display decreased serum concentrations of total thy-

roxine (TT_4)[1] in association with normal serum thyrotropin (TSH) levels (1–5). In addition, total serum triiodothyronine (TT_3) levels are decreased, and total reverse T_3 (TrT_3) values are usually increased (1, 3–5), as in other nonthyroidal illnesses (6–9).

Despite the reduced circulating levels of TT_4 in these patients, free T_4 concentrations by equilibrium dialysis (FT_4D) (1, 5) and T_4 production rates (5) are usually normal. The low TT_4 values have been attributed to an acquired defect of serum T_4 binding (1, 5), possibly secondary to a nondialyzable serum inhibitor (10). Such a defect is compatible with the shortened residence time ($\bar{t}$) and accelerated metabolic clearance rates (MCR) for labeled T_4 observed in these patients (5). Other kinetic parameters of T_4, as well as those for T_3, and rT_3 have not been described in patients with the low T_4 state of nonthyroidal illnesses.

The present study was undertaken to examine, by noncompartmental analysis, the pattern of alterations occurring in binding, distribution, production, and disposal of T_4, T_3, and rT_3 during the low T_4 state of acute nonthyroidal illnesses.

METHODS

The study population consisted of 16 critically ill patients admitted to the Los Angeles County/University of Southern California Medical Intensive Care Unit for the treatment of acute nonthyroidal illnesses (Table I). There were seven females and nine males. Their ages ranged between 23 and 80 (mean 54±4 [SE]) yr. The severity of their illnesses was evidenced by the high mortality (94%) during their hospitalization. The median time from completion of study to death was 10 d, with a range of 5–52 d (Table I). The selection criteria included a serum TT_4 concentration of 3 μg/dl or less throughout the study period and a normal serum TSH level. Patients receiving pharmacological agents known to alter peripheral thyroid hormone metabolism, such as aspirin (11, 12), dilantin (13), or heparin (14, 15) were excluded as were patients with known or suspected hypothalamic or pituitary disease, head trauma (16), thyroid disease, or recent thyroid (17) or glucocorticoid hormone therapies (18, 19). Data are presented only from patients' with stable concentrations of TT_4, TT_3, and TrT_3 during the entire period of the kinetic study. The control data were derived from studies in 27 healthy euthyroid male individuals. Their ages ranged between 21 and 51 (36±2) yr. None received medications other than multivitamins. One healthy euthyroid male (age 72 yr) with near absence of serum thyroxine-binding globulin (TBG) levels (0.016 mg/dl) was included for purposes of comparison to previously reported data in similar subjects. The protocols were approved by the Institutional Review Board of the Medical Center and written informed consents were obtained from the patient or responsible relative.

Serum T_4 clearance studies were performed for 11 patients and 19 normal subjects, T_3 clearance studies for 5 patients and 12 normal subjects, and rT_3 clearance studies for 7 patients and 8 normal subjects. Tracer T_4 labeled with ^{125}I, T_3 labeled with ^{131}I (Amersham Corp., Arlington Heights, Ill., 50 and 1,200 μCi/μg sp act, respectively), or rT_3 labeled with ^{131}I in our laboratory (300 μCi/μg sp act) (20) was diluted in sterile 1% albumin-saline solution and dialyzed against anion exchange resin (Amberlite RIA 400, Rohm and Haas Co., Philadelphia, Pa.) to remove free iodide. The isotope solution was then sterilized by passing it through a 0.22-μm millipore filter (Millipore Corp., Bedford, Mass.) before injection. The thyroid gland uptake of radioiodine was minimized by the administration of either a saturated solution of potassium iodide (5 drops twice daily orally) or sodium iodide (0.5 g/d i.v.), with the first dose given at least 1 h before tracer injection. All subjects received a single intravenous bolus containing 50–100 μCi of labeled T_4, T_3, or rT_3. Blood samples were obtained at 0, 0.08, 0.25, 0.5, 1, 2, 3, 8, 24, 48, 72, and 96 h after tracer injection for the determination of labeled T_4 or T_3. For the rT_3 studies, samples were drawn at 0, 0.08, 0.16, 0.25, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h after tracer injection. An aliquot of labeled tracer diluted in pooled human serum served as the reference standard.

The ^{125}I- or ^{131}I-labeled hormone activity in the serum samples and standards was obtained by acidification (pH 4.0) and extraction of 2-ml aliquots of serum with 4 vol of ethyl acetate/butanol (9:1). 2 ml of the solvent phase was then placed in counting tubes and ^{125}I and ^{131}I activity determined by a dual-channeled automated gamma counter (Nuclear Chicago, Chicago, Ill.). Pooled serum containing ^{125}I-labeled albumin, $Na^{125}I$, and ^{125}I-labeled T_4, T_3, and rT_3 served as controls for each extraction procedure. This method extracted 57.0±1.3% of the labeled T_4, 54.7±1.2% of the T_3, 52.3±1.0% of the rT_3, 43.1±1.7% of the 3,3′ diiodothyronine (T_2), and 47.3±1.6% of the 3′,5′ T_2 into the solvent phase. The aqueous phase contained 99.7±0.1% of the iodoproteins and 98.1±0.3% of the free iodides. The mean serum data expressed as the percent injected dose per liter for T_4, T_3, and rT_3 in the normal subjects and sick patients are presented in Table II.

The kinetic parameters were calculated using noncompartmental analysis (21, 22). This assumes that the initial rapid distribution of labeled compound occurs in a central compartment that includes, but may also be identical to the plasma pool. The tracer then reversibly exchanges with an undetermined number of compartments that together represent the extravascular pool. Our analysis assumes that linearity and steady-state conditions prevail throughout the study.

All serum isotope concentrations, expressed as the percent injected dose per liter, were fitted to the sums of 1, 2, 3, and 4 exponentials. The tracer concentration (C^*) is described by the sum of exponentials and expressed by $C^* = \Sigma Ae^{-\alpha t}$ where A is the coefficient, α is the exponent and t is time. The largest number of exponentials that significantly lowers the residual sum of squares (RSS) compared with the next simplest model defines the best fit. This is determined by

[1] *Abbreviations used in this paper*: C^*, tracer concentration; FCR, fractional catabolic rate: FDR, fractional disposal rate; FF, free fraction (of T_3, rT_3, and T_4); FT_4D, free T_4 concentration by equilibrium dialysis; IVD, initial volume of distribution; Kii, rate of hormone exit from serum; MCR, metabolic clearance rate; PR, production rate; $\bar{t}$, residence time T_2, diiodothyronine; T_3, triiodothyronine; rT_3, reverse T_3; T_4, thyroxine; TBG, thyroxine-binding globulin; TT_3, total serum T_3; TSH, thyrotropin; TrT_3, total serum rT_3; TT_4, total serum thyroxine; TVD, total volume of distribution.

TABLE I
Patient Information

Case no.	Sex	Age	Total T_4	Disease state	Time to demise or recovery after study
		yr	*μg/dl*		*d*
1	M	50	2.7	Sepsis, respiratory failure	7
2	F	60	3.0	Sepsis, respiratory failure, diabetic ketoacidosis	5
3	F	59	1.2	Sepsis, respiratory failure	10
4	M	23	1.4	Sepsis, respiratory failure, acute renal insufficiency,‡ sickle cell crisis	Recovered
5	M	58	1.3	Sepsis, hepatic failure, acute renal insufficiency‡	5
6	M	43	3.0	Sepsis, respiratory failure	Recovered
7	F	80	1.9	Sepsis, dehydration	14
8	F	35	2.0	Sepsis, hepatic failure, acute renal insufficiency‡	17
9*	M	45	0.4	Trauma, sepsis, hypotension, acute renal insufficiency‡	7
10*	F	62	1.5	Respiratory failure, congestive heart failure	52
11*	M	41	1.6	Sepsis, hepatic failure, acute renal insufficiency,‡ hypotension, cardiopulmonary arrest	12
12*	M	54	1.5	Sepsis, hepatic failure, acute renal insufficiency,‡ hypotension	10
13	F	56	2.8	Sepsis, respiratory failure, hepatic failure, acute renal insufficiency‡	25
14*	M	65	1.4	Respiratory failure, diabetic ketoacidosis, hypotension	5
15	F	58	2.3	Sepsis, carcinoma	Recovered
16*	M	75	1.4	Sepsis, respiratory failure, hypotension	14

* Patients receiving dopamine therapy throughout the kinetic study.
‡ All patients with acute renal insufficiency were nonoliguric and did not require dialysis.

using an F test (23) where

$$F = \frac{Rss_k - Rss_j}{Rss_j} \cdot \frac{d.f._k}{d.f._k - d.f._j}.$$

The k and j identify the number of exponentials of the two models being compared where Rss_k is by definition larger than Rss_j. The d.f. is the degrees of freedom and equals the number of observation points minus two, less the number of parameters to be identified. The minimum number of exponentials required to obtain a significance level of <0.05 is chosen to define the serum disappearance curve. The kinetic parameters are related to the total curve of the isotope concentration from the time of injection of the labeled compound to the time when the isotope levels are negligible.

The following parameters were calculated:

Initial volume of distribution (IVD) in liters per square meter. This represents the volume in which the tracer is distributed immediately and assumes that the tracer distributes uniformly in serum. It is expressed by the inverse of the

TABLE II

Serum Disappearance of T_4, T_3, and rT_3 in the Low T_4 State of Nonthyroidal Illness

Time (hours)		0.08	0.25	0.5	1	2	3	8	24	48	72	96
Serum T_4												
Sick	Mean	31.65	25.72	24.35	19.47	16.85	14.73	11.46	7.71	5.39	4.13	3.13
(n = 11)	±SE	1.85	1.43	1.49	1.07	0.91	0.81	0.93	0.78	0.47	0.42	0.37
Normal	Mean	30.77	25.95	21.79	16.59	13.13	11.42	9.02	7.42	6.31	5.70	5.06
(n = 19)	±SE	1.78	1.50	1.26	0.93	0.82	0.66	0.50	0.47	0.40	0.41	0.32
	P	NS	NS	NS	NS	<0.01	<0.005	<0.02	NS	NS	<0.02	<0.001
Serum T_3												
Sick	Mean	19.96	10.45	7.71	6.11	4.48	3.62	1.62	0.62	0.35	0.24	0.17
(n = 5)	±SE	2.81	2.23	1.95	1.42	0.91	0.77	0.14	0.072	0.077	0.054	0.059
Normal	Mean	17.12	13.24	11.20	9.08	6.09	5.27	2.57	1.12	0.48	0.20	0.11
(n = 12)	±SE	1.12	1.04	1.09	0.80	0.46	0.52	0.21	0.12	0.062	0.023	0.017
	P	NS	NS	NS	NS	NS	NS	<0.02	<0.005	NS	NS	NS

Time (hours)		0.08	0.16	0.25	0.33	0.5	1	2	3	4	6	8	12	16	24
Serum rT_3															
Sick	Mean	29.30	23.66	20.06	17.41	14.28	9.64	6.12	4.42	3.33	2.35	1.81	1.20	0.99	0.61
(n = 7)	±SE	1.29	1.58	1.97	1.99	2.31	2.44	1.84	1.35	1.12	0.90	0.71	0.47	0.43	0.21
Normal	Mean	24.79	19.03	15.12	11.49	7.30	3.45	1.45	1.10	0.83	0.66	0.53	0.35		
(n = 8)	±SE	0.92	0.82	0.68	0.60	0.61	0.39	0.18	0.13	0.11	0.11	0.093	0.079		
	P	<0.05	<0.05	=0.05	<0.05	<0.01	=0.01	<0.01	<0.01	<0.01	<0.05	NS	=0.05		

All data are expressed as the percent injected dose per liter. The T_4 and T_3 data for the normal and sick subjects were compared by unpaired t tests for equal or unequal variance. The rT_3 data were compared using the unpaired ranked sum test.

concentration at time zero. Therefore,

$$\text{IVD} = \frac{1}{\Sigma A} \cdot 100\%$$

MCR in liters per square meter per day. This is the volume of serum cleared of tracer per day and is calculated from the total area under the curve of the serum concentration of labeled hormones (21, 22) as

$$\text{MCR} = \frac{1}{\int_0^\infty \Sigma A e^{-\alpha t} \cdot dt} \cdot 100\% = \frac{1}{\Sigma \frac{A}{\alpha}} \cdot 100\%$$

$\bar{t}$, in days. This represents the time interval from entry of the labeled hormone into the vascular compartment to its irreversible loss from the circulation and is estimated by:

$$\bar{t} = \frac{\int_0^\infty t \cdot \Sigma A e^{-\alpha t} \cdot dt}{\int_0^\infty \Sigma A e^{-\alpha t} \cdot dt} = \frac{\Sigma \frac{A}{\alpha^2}}{\Sigma \frac{A}{\alpha}}$$

Total volume of distribution (TVD) in liters per square meter. This is the volume of the exchangeable hormone pool and is calculated from the MCR and $\bar{t}$. TVD = MCR · $\bar{t}$.

Pool size in micrograms per square meter. This quantity describes the total body content of exchangeable hormone and is calculated from the TVD and the serum concentration of hormone in micrograms per liter. The values of the serum concentration used in the calculation represent the mean TT_4 and TT_3 values from 0, 48, and 96 h and the mean TrT_3 values from times 0, 12, and 24 h of the kinetic study. Pool size = TVD · serum concentration.

Fractional catabolic rate (FCR) in percent per day. This is the fraction of the total hormone pool renewed (removed and replaced) each day. It is calculated from either the $\bar{t}$ or the MCR and TVD using one of the following equations: FCR = $1/\bar{t} \cdot 100\%$ = MCR/TVD · 100%.

Production rate (PR) in micrograms per square meter per day. The PR equals the disposal rate (DR) under steady-state conditions. The PR is estimated by calculating the DR: DR = PR = $1/\bar{t}$ · pool size.

Rate of hormone exit from serum (Kii) per day. This is the rate at which the labeled hormone moves from the vascular to the extravascular space. It is defined as Kii = $(dC^*/dt)_0 \cdot 1/C_0^*$. Since $C^* = \Sigma A e^{-\alpha t}$, then $C_0^* = \Sigma A$ and $(dC^*/dt)_0 = d(\Sigma A e^{-\alpha t})/dt = -\Sigma A \alpha$. Therefore, Kii = $-\Sigma A \alpha / \Sigma A$.

Fractional disposal rate (FDR) per day. This is the fraction of the total amount of labeled hormone leaving the circulation irreversibly and hence, no longer exchanging with the vascular compartment. It is calculated as follows: FDR = MCR/(Kii · IVD).

All serum samples were assayed in duplicate for TT_4, TT_3, and TrT_3 concentrations by standard double-antibody radioimmunoassay techniques (24, 25). The serum TSH concentrations were measured by a commercial method (Abbott Diagnostics, Diagnostic Products, North Chicago, Ill.). Free T_4 levels were determined by equilibrium dialysis (FT_4D) (26) courtesy of Nichols Institute, San Pedro, Calif. Free T_3 and rT_3 were also measured by equilibrium dialysis (26, 27) with purification of the tracer by polyacrylamide gel filtration before dialysis (28), and of the dialysate by resin after dialysis (29). All free hormone levels were determined on 0 time samples. Serum TBG levels were measured by radioimmunoassay (30) courtesy of Nichols Institute.

All results are expressed as a mean±1 SEM. The data were analyzed for significance using unpaired Student's *t* test for unpaired data with equal and unequal variances or unpaired ranked sum test (31). Correlation matrices were calculated using programs from the Biomedical Data Processing package (32).

RESULTS

The mean serum disappearance curves for labeled T_4, T_3 and rT_3 of the study patients and the normal subjects are shown in Fig. 1 and Table II and the noncompartmental parameters are summarized in Tables III–V. The pattern of serum disappearance of T_4 during the first 24 h in the patients was similar or slower than in the normal subjects, while the subsequent disappearance rate was greater in the patients. The disappearance patterns of T_3 were similar in both groups during the early and late phases. In contrast, the mean rT_3 disappearance curve in the patients was retarded throughout the study period relative to the control group.

It is evident from Fig. 1 that the serum disappearance curves for both the normal subjects and the patients did not fit 1 exp. and 4 exp. were not required to describe the curves for any of the individuals studied. Indeed, these curves were best described by the sum of 3 exp in 20 of 30 subjects for T_4, in 14 of 17 for T_3, and in 12 of 15 for rT_3. The disappearance curves in the remaining subjects best fit the sum of 2 exp. The numbers of curves fitting 2 or 3 exp in the two groups were not significantly different when compared by Fisher's exact test (33).

During the time of the T_4 tracer studies in the patients, the serum TT_4 concentrations remained stable with a mean of 1.7±0.2 μg/dl at time 0, 1.8±0.2 μg/dl at 48 h, and 1.8±0.3 μg/dl at 96 h of the study. Despite the markedly decreased serum TT_4 values, the FT_4D were within the normal range in 8 of 11 patients (Table III). This was secondary to an increased percent free fraction of T_4 (FFT_4). There were no significant correlations among these parameters. In comparison, a markedly decreased serum level of TT_4 with an increased FFT_4 was also noted in the one healthy euthyroid subject with near absence of TBG.

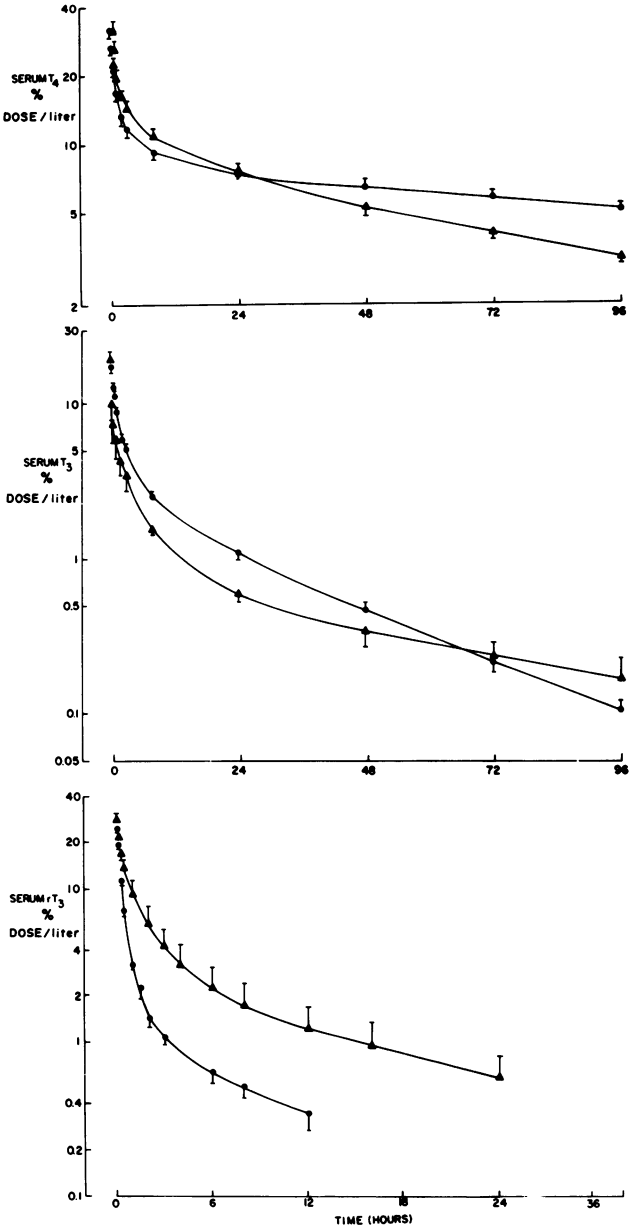

FIGURE 1 The serum disappearance curves of T_4, T_3, and rT_3 in patients with the low TT_4 state of nonthyroidal illnesses (▲) and in normal subjects (●). Each data point represents the mean±1 SEM. The serum rT_3 counts after 12 h in the normal subjects were too low to be accurately assessed.

The mean PR of T_4 in the patients (27.9±3.7 μg/d per m^2) was significantly lower than the normal mean (50.3±3.4 μg/d per m^2, $P < 0.001$) but within the normal 95% confidence limits in eight of the patients. It should be noted that three patients receiving dopamine therapy had lower values of T_4 PR (14.0±2.4 μg/d per m^2) than the other eight patients (33.1±3.4 μg/d per

TABLE III

T_4 Kinetics in the Low T_4 State of Nonthyroidal Illness

Case no.	TT_4	FFT_4	Free T_4	$\bar{t}$	IVD	TVD	MCR	FCR	PR	T_4 pool	Kii	FDR
	μg/dl	%	ng/dl	d	liter/m²		liter/d/m²	%/d	μg/d/m²	μg/m²	d^{-1}	d^{-1}
Sick patients												
1	2.7	0.076	2.05	3.85	2.04	4.54	1.18	26.0	32.4	122.5	6.6	0.088
2	3.0	0.041	1.23	2.56	1.88	4.22	1.65	39.1	51.1	130.6	11.4	0.077
3	1.2	0.040	0.48	2.25	1.59	7.58	3.37	44.4	39.0	88.0	36.2	0.059
4	1.4	0.074	1.04	3.70	2.39	6.09	1.65	27.0	23.7	87.8	4.7	0.148
5	1.3	0.058	0.75	3.77	2.30	6.70	1.78	26.5	22.2	83.8	5.6	0.139
6	3.0	0.045	1.35	5.22	1.56	5.84	1.12	19.2	34.6	180.9	38.9	0.019
7	1.9	0.070	1.33	2.14	2.00	4.12	1.92	46.8	36.6	78.2	9.2	0.104
8	2.0	0.094	1.88	2.66	2.44	3.35	1.26	37.7	25.3	67.0	2.5	0.209
9*	0.4	0.069	0.28	2.84	2.43	7.47	2.63	35.4	10.0	28.4	41.3	0.026
10*	1.5	0.100	1.50	3.69	1.72	3.35	0.91	27.1	13.7	50.3	7.2	0.074
11*	1.6	0.107	1.70	3.18	2.00	3.73	1.17	31.5	18.4	59.5	3.5	0.168
Mean	1.8	0.070	1.24	3.26	2.03	5.18	1.69	32.8	27.9	88.8	15.2	0.101
±SE	0.2	0.0070	0.17	0.27	0.10	0.48	0.22	2.6	3.7	12.8	4.6	0.018
P‡	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Normal subjects (n = 19)												
Mean	7.1	0.0315	2.21	8.98	1.55	6.31	0.73	12.0	50.3	433.3	28.4	0.021
±SE	0.4	0.0014	0.13	0.56	0.076	0.44	0.05	0.8	3.4	26.4	3.9	0.003
Low TBG subject	1.7	0.055	0.94	4.12	1.13	12.20	2.96	24.3	50.4	207.4	192.0	0.014

* Patients receiving dopamine.

‡ All P values are for unpaired t tests.

m^2, $P < 0.01$) and had the only PR values below the 95% confidence limits of normal. In addition, the T_4 pool size was reduced in the dopamine-treated compared with the untreated patients (46.1±9.2 vs. 104.9±13.3 μg/m^2, $P < 0.05$) and in both groups compared to normal. The serum disappearance curves and all other parameters were similar in the dopamine-treated and untreated patients.

The mean $\bar{t}$ of T_4 in the patients was shortened to 36% of normal and the MCR (232%), FCR (273%), and FDR (481%) were significantly accelerated. Except for a normal FDR, the same pattern was noted in the one subject with near absent TBG. Despite these findings of accelerated peripheral metabolism, the Kii was significantly retarded in the patients to 54% of normal. This is in contrast to the markedly increased Kii observed in the subject with near absent TBG. It should be noted that the IVD was increased (131%) and the TVD was reduced (82%) in the patients.

The serum concentrations of TT_3 in the patients were markedly decreased and remained stable throughout the T_3 study with a mean value of 34±4 ng/dl at time 0, 34±3 ng/dl at 24 h, and 37±4 ng/dl at 48 h. Although the serum FFT_3 values were increased, the free T_3 values were decreased in all patients because of the marked reduction in TT_3 levels, as shown in Table IV. The mean T_3 PR was markedly decreased (27%) as was the T_3 pool size (47%). Although the magnitude of the decrements in mean T_3 PR (27%) and mean serum TT_3 (22%) were comparable, there was no significant correlation between these two parameters. The mean T_3 MCR (137%) and TVD (203%) were increased while the mean Kii value was not significantly different from normal. The one dopamine-treated patient had values similar to those of the other patients.

During the serum rT_3 kinetic studies, the serum concentrations of TrT_3 were significantly elevated and remained stable in the patients with a mean value of 89±22 ng/dl at time 0, 89±24 ng/dl at 12 h, and 95±26 ng/dl at 24 h of the study. The mean $FFrT_3$ value was increased (304%) as was the mean free rT_3 level (777%). Despite the increased $FFrT_3$, the mean $\bar{t}$ was prolonged (362%) while the MCR (42%) and FCR (30%) values were significantly reduced. Furthermore, the Kii values were normal or decreased, and the mean rT_3 PR and FDR values were not significantly different from normal. There were no significant differences

TABLE IV

T_3 Kinetics in the Low T_4 State of Nonthyroidal Illness

Case no.	Total T_3	FFT_3	Free T_3	$\bar{t}$	IVD	TVD	MCR	FCR	PR	T_3 pool	Kii	FDR
	ng/dl	*%*	*pg/dl*	*d*	*liter/m²*		*liter/d/m²*	*%/d*	*μg/d/m²*	*μg/m²*	*d^{-1}*	*d^{-1}*
Sick patients												
3	30	0.906	272	0.64	2.10	13.21	20.73	157.0	6.18	3.9	63.6	0.155
5	42	0.588	247	3.15	0.85	43.02	13.65	31.7	5.67	17.9	277.2	0.058
6	25	0.605	151	1.07	2.17	23.24	21.65	93.2	5.41	5.8	126.6	0.079
7	34	0.783	266	1.09	4.19	26.67	24.47	91.7	8.39	9.1	37.4	0.156
12*	45	0.626	282	1.97	2.28	26.62	13.50	50.7	6.07	12.0	87.4	0.068
Mean	35	0.696	244	1.59	2.32	26.55	18.80	84.9	6.34	9.7	118.4	0.203
±SE	4	0.065	24	0.45	0.54	4.80	2.22	21.6	0.53	2.5	42.3	0.022
P	<0.001	<0.001	<0.001	NS	NS	<0.01	<0.05‡	NS	<0.005	<0.02	NS	NS
Normal subjects (n = 12)												
Mean	162	0.310	503	0.96	2.56	13.10	13.74	116.8	23.47	20.7	124.1	0.135
±SE	5	0.034	46	0.08	0.25	2.54	1.30	15.6	2.12	2.5	48.5	0.035

* Patient receiving dopamine.

‡ Unpaired ranked sum test; all other *P* values are for unpaired *t* tests.

noted between the dopamine-treated and untreated patients (Table V).

DISCUSSION

The results of the present study demonstrate that multiple alterations occur in the peripheral hormone metabolism of T_4, T_3, and rT_3 during the low T_4 state of acute critical nonthyroidal illnesses. These alterations are presumably responsible for the decreased serum levels of TT_4 and TT_3 and for the elevated levels of TrT_3 observed in these patients. It should be emphasized that the magnitude of the changes in the serum levels and the kinetic parameters were similar within

TABLE V

rT_3 Kinetics in the Low T_4 State of Nonthyroidal Illness

Case no.	Total rT_3	$FFrT_3$	FrT_3	$\bar{t}$	IVD	TVD	MCR	FCR	PR	rT_3 pool	Kii	FDR
	ng/dl	*%*	*pg/dl*	*d*	*liter/m²*		*liter/d/m²*	*%/d*	*μg/d/m²*	*μg/m²*	*d^{-1}*	*d^{-1}*
Sick patients												
1	60	0.410	246	1.74	1.24	24.24	13.93	57.5	8.4	14.6	96.0	0.117
8	166	0.500	870	0.92	1.83	6.33	6.86	108.4	11.4	10.5	8.9	0.424
10*	61	0.329	201	0.31	1.44	9.29	30.27	325.7	18.5	5.7	64.3	0.327
11*	175	0.359	628	1.09	1.55	9.91	9.10	92.2	15.9	17.3	13.8	0.427
14*	107	0.639	684	0.40	2.30	10.21	25.36	248.4	27.0	10.9	26.2	0.422
15	24	0.213	51	0.54	1.96	23.75	43.66	183.8	10.4	5.7	77.0	0.289
16*	51	0.374	191	0.31	1.30	14.26	46.18	324.1	23.4	7.2	87.4	0.406
Mean	92	0.404	404	0.76	1.66	14.00	25.05	191.0	16.4	10.3	53.4	0.345
±SE	22	0.051	114	0.02	0.15	2.65	6.03	41.9	2.64	1.7	13.7	0.043
P	<0.05‡	<0.001	<0.025	<0.05	NS	NS	<0.005	<0.02	NS	<0.02	NS	NS
Normal subjects (n = 8)												
Mean	40	0.133	52	0.21	1.59	10.56	59.96	628.0	24.8	4.2	84.0	0.460
±SE	1.6	0.007	3.0	0.04	0.095	1.37	8.56	199.0	3.5	0.6	8.0	0.053

* Patients receiving dopamine therapy.

‡ Unpaired ranked sum test; all other *P* values are for unpaired *t* test.

this patient group, despite a marked variation in the underlying diagnoses. This included the dopamine-treated patients in whom the only difference was a lower T_4 PR and pool size. This uniformity suggests that the factors responsible for these alterations may be similar. The disturbances in thyroid hormone kinetic parameters could, theoretically, result from abnormalities of intravascular and extravascular binding and distribution, as well as of hormone production and disposal.

The marked reduction of T_4, T_3, and rT_3 binding to serum carrier proteins in the low T_4 state was one of the most consistent findings of the present study. This alteration was principally manifested by the two- to fourfold increase in the percent free fraction (FF) values for T_4, T_3, and rT_3 as determined by equilibrium dialysis. This finding has been previously reported in a variety of acute and chronic nonthyroidal illnesses (27). The reduced serum binding could be due to decreased serum concentration and/or affinity of TBG. Serum TBG levels by radioimmunoassay were available in patients 4, 5, and 6 of the present study and the values were 2.7, 1.3 and 2.9 mg/dl (normal range: 1.7–5.1 mg/dl). Similar TBG levels were observed in two other comparable groups of critically ill patients with nonthyroidal illnesses who had serum TT_4 levels below 3 μg/dl (5, 34). Serum TBG values determined by radioimmunoassay (5) were within the normal range in 9 of 12 patients (mean 2.5±0.3 mg/dl, normal: 3.4±0.1), and those measured by antibody binding (34) (Corning Immunophase, Corning Medical, Medfield, Mass.) were normal in 8 of 10 patients (mean 10.9±1.8 μg/ml, normal: 16.2±1.1). These findings indicate that serum TBG levels are minimally reduced in patients with the low T_4 state of nonthyroidal illness and would only partially account for the observed increase in the FF of T_4, T_3, and rT_3. This suggests the presence of an additional defect in the binding affinity of TBG for these thyroid hormones (1, 5, 10). Theoretically, a decrease in TBG affinity or concentration should result in similar alterations in serum thyroid hormone kinetics. This reduction of hormone binding to serum carrier proteins could contribute to the observed alterations in distribution and metabolism of these hormones.

The kinetic consequences of reduced serum T_4 binding to carrier proteins should mimic the alterations observed in healthy euthyroid individuals with reduced serum levels of TBG. Indeed, the findings in our patients of accelerated MCR and FCR values and reduced $\bar{t}$ and T_4 pool size are similar to the abnormalities reported in healthy euthyroid individuals with reduced TBG concentrations (35), as well as the low TBG patient whose kinetic parameters were evaluated as part of this study (Table III). In addition, the decreased serum binding of T_4 should permit an accelerated Kii as seen in our one patient with a low TBG concentration, and as previously reported (36, 37). However, our data show that Kii was significantly retarded in patients with the low T_4 state suggesting impaired egress of T_4 from serum.

The decrease in the rate of T_4 egress from serum could result from (*a*) reduced activity of a specific transport system for T_4, (*b*) impaired extravascular T_4 binding or (*c*), an increased serum concentration of a compound(s) that competes with T_4 for either transport or tissue binding sites. Impaired transport of T_4 out of serum should result in a reduced MCR, FCR, and FDR. The finding of an increased MCR and FDR for T_4 in our patients probably negates this possibility. There is considerable evidence that extravascular T_4 uptake is influenced by the quantity and affinity of T_4 binding proteins on each side of the plasma membrane (36–39). Therefore, a decrease in extravascular binding of T_4, of a greater magnitude than the impairment in serum binding could account for a reduced Kii in these patients. Furthermore, this type of defect would be compatible with the increased MCR, FCR, and FDR values and the decreased $\bar{t}$ and TVD observed during the low T_4 state. The nature of the factor(s) responsible for the reduced binding of T_4 are not elucidated by our studies; however, the extravascular and serum protein binding defect may be the result of a common factor. This concept is supported by the observation that heparin administration is capable of reducing T_4 binding both in serum and extravascular sites (15).

The metabolic consequences of decreased extravascular T_4 binding are not defined. However, Jennings et al. (40) have suggested that the impaired hepatic uptake of T_4 into the perfused rat liver after fasting is caused by decreased tissue binding and may, in turn, be responsible for the decreased T_3 production. In addition, Felicetta et al. (41) have shown that ipodate, a potent inhibitor of T_4 to T_3 conversion (42, 43) displaces T_4 from liver. If the decreased tissue binding of T_4 were responsible for reduced T_3 production in our patients, one would expect a comparably impaired conversion of T_4 to rT_3. However, the normal rT_3 PR in our patients provides indirect evidence to suggest that no impairment of free T_4 availability to sites of deiodination and disposal exists. Furthermore, there is evidence to suggest that factors other than reduced uptake of T_4 into tissues could be responsible for the decreased T_3 PR. Reduced 5′-deiodination of T_4 to T_3 in nonthyroidal disorders has been suggested by in vitro studies (44) to be responsible for the decreased T_3 production. A plausible assumption would be that reduced extravascular binding of T_4 does not result in decreased tissue availability of free T_4. Hence, de-

creased tissue binding of thyroid hormones may be analogous to reduced serum binding, which results in reduced total levels, but normal free hormone availability (35, 37).

The increased FFT_3 observed in our patients and in other nonthyroidal illnesses (27) suggests a decreased binding of T_3 to serum proteins. This abnormality could account for the accelerated MCR and increased TVD, which are also seen in healthy euthyroid individuals with low concentrations of TBG (35, 45). Because one would expect Kii to be enhanced in the presence of decreased serum T_3 binding (45), the normal Kii values in our patients suggest a comparable defect in serum and extravascular binding.

The T_3 PR was markedly reduced in our patients, which is a feature common to other nonthyroidal illnesses (6–8). T_3 production could be reduced as a result of decreased free T_4 availability or to impaired enzymatic deiodination of T_4. Because both, the T_4 PR and free T_4 levels were in the low normal range in these patients, reduced T_4 availability would not appear to be a major factor. Furthermore, the reduction in the T_3 PR to 27±2% of the normal mean was significantly ($P < 0.01$) greater than the decrease in T_4 PR of 56±7% suggesting that impaired deiodination of T_4 to T_3 was involved. In vitro data indicate that the 5′-deiodinase activity may be reduced in nonthyroidal disorders (44); however, the extent to which these factors contribute to the decreased T_3 PR in vivo is not defined.

The binding of rT_3 to serum carrier proteins also appeared to be impaired since the $FFrT_3$ in our patients was increased as has been reported in other nonthyroidal illnesses (27). The finding that Kii was normal in the face of decreased serum binding is compatible with a comparable decrease in serum and tissue rT_3 binding. An alternate explanation for these changes might be an impaired transport of rT_3 into tissues. This should result in reduced MCR, FCR, and FDR values for rT_3. Indeed, MCR and FCR are reduced but FDR values are essentially normal, making this possibility unlikely. Similar kinetic alterations for rT_3 have been reported in nonthyroidal illnesses by Chopra et al. (9) and in fasting (46) and have been attributed to impaired 5′-deiodination of rT_3. An abnormality in this enzyme step could account for the reduced production of T_3 from T_4 as well as the impaired MCR and FCR of rT_3 in our patients.

The results of the present study indicate that two major abnormalities could account for the disturbances in T_4, T_3, and rT_3 metabolism present in patients with the low T_4 state of nonthyroidal illnesses. These include decreased binding of T_4, T_3, and rT_3 to both vascular and extravascular sites, and impaired 5′-deiodination of T_4 to T_3.

ACKNOWLEDGMENTS

The authors would like to thank the staff of the Medical Intensive Care Unit particularly Dr. W. S. Wheeler and Dr. M. Davenport for their clinical assistance, Mr. G. Adachi for his technical assistance, Mrs. Anne Santo for graphical presentations, Mrs. Valery Bourbeau for typing the manuscript and the nurses on the General Clinical Research Center.

This work was supported in part by National Institutes of Health grant AM-11727 and General Clinical Research grant RR-43. Dr. Kaptein is a recipient of a National Institutes of Health General Clinical Research Center Clinical Associate Physician grant (RR-43).

REFERENCES

1. Chopra, I. J., D. H. Solomon, G. W. Hepner, and A. A. Morgenstein. 1979. Misleadingly low free thyroxine index and usefulness of reverse triiodothyronine measurement in nonthyroidal illnesses. *Ann. Intern. Med.* **90:** 905–912.
2. Wood, D. G., J. Cyrus, and E. Samols. 1980. Low T4 and low FT4I in seriously ill patients: concise communication. *J. Nucl. Med.* **21:** 432–435.
3. Eisenberg, D., H. Silberman, J. Ryan, R. Shafer, J. Weiss, E. M. Kaptein, J. T. Nicoloff, and C. A. Spencer. 1980. Prognostic significance of thyroid function tests in critically ill patients. *Surg. Forum.* **31:** 211–213.
4. Slag, M. E., J. E. Morley, M. K. Elson, T. W. Crowson, F. Q. Nuttall, and R. B. Shafer. 1981. Hypothyroxinemia in critically ill patients as a predictor of high mortality. *JAMA (J. Am. Med. Assoc.).* **245:** 43–45.
5. Kaptein, E. M., D. A. Grieb, C. A. Spencer, W. S. Wheeler, and J. T. Nicoloff. 1981. Thyroxine metabolism in the low T4 state of critical nonthyroidal illnesses. *J. Clin. Endocrinol. Metab.* **53:** 764–771.
6. Nomura, S., C. S. Pittman, J. B. Chambers, M. W. Buck, and T. Shimizu. 1975. Reduced peripheral conversion of thyroxine to triiodothyronine in patients with hepatic cirrhosis. *J. Clin. Invest.* **56:** 643–652.
7. Carter, J. N., C. J. Eastman, J. M. Corcoran, and L. Lazarus. 1976. Inhibition of conversion of thyroxine to triiodothyronine in patients with severe chronic illness. *Clin. Endocrinol.* **5:** 587–594.
8. Lim, V. S., V. S. Fang, A. I. Katz, and S. Refetoff. 1977. Thyroid dysfunction in chronic renal failure. *J. Clin. Invest.* **60:** 522–534.
9. Chopra, I. J. 1976. An assessment of daily production and significance of thyroidal secretion of 3,3′,5′-triiodothyronine (reverse T3) in man. *J. Clin. Invest.* **58:** 32–40.
10. Chopra, I. J., G. N. Chua Teco, A. H. Nguyen, and D. H. Solomon. 1979. In search of an inhibitor of thyroid hormone binding to serum proteins in nonthyroidal illnesses. *J. Clin. Endocrinol. Metab.* **49:** 63–69.
11. Musa, B. U., R. S. Kumar, and J. T. Dowling. 1968. Effects of salicylates on the distribution and early plasma disappearance of thyroxine in man. *J. Clin. Endocrinol. Metab.* **28:** 1461–1464.
12. Larsen, P. R. 1972. Salicylate-induced increases in free triiodothyronine in human serum: evidence of inhibition of triiodothyronine binding to thyroxine-binding globulin and thyroxine-binding prealbumin. *J. Clin. Invest.* **51:** 1125–1134.
13. Larsen, P. R., A. J. Atkinson, H. N. Wellman, and R. E. Goldsmith. 1972. The effect of diphenylhydantion on

thyroxine metabolism in man. *J. Clin. Invest.* **49:** 1266–1279.
14. Schatz, D. L., R. H. Sheppard, G. Steiner, C. S. Chandarlapaty, and G. S. DeVeber. 1969. Influence of heparin on serum free thyroxine. *J. Clin. Endocrinol. Metab.* **29:** 1015–1022.
15. Schwartz, H. L., A. R. Schadlow, D. Faierman, M. I. Surks, and J. H. Oppenheimer. 1973. Heparin administration appears to decrease cellular binding of thyroxine. *J. Clin. Endocrinol. Metab.* **36:** 598–600.
16. Rudman, D., A. S. Fleisher, M. H. Kutner, and J. F. Raggio. 1977. Suprahypophyseal hypothyroidism and hypogonadism during prolonged coma after head trauma. *J. Clin. Endocrinol. Metab.* **45:** 747–754.
17. Krugman, L. G., J. M. Hershman, I. J. Chopra, G. A. Levine, A. E. Pekary, D. L. Geffner, and G. N. Chua Teco. 1975. Patterns of recovery of the hypothalamic-pituitary thyroid axis in patients taken off chronic thyroid therapy. *J. Clin. Endocrinol. Metab.* **41:** 70–80.
18. Nicoloff, J. T., D. Fisher, and M. Appleman. 1970. The role of glucocorticoids in the regulation of thyroid function in man. *J. Clin. Invest.* **49:** 1922–1929.
19. Gamstedt, A., G. Janerot, B. Kagedal, and B. Soderholm. 1979. Corticosteroids and thyroid function. *Acta Med. Scand.* **205:** 379–383.
20. Kochupillai, N., and R. S. Yalow. 1978. Preparation, purification, and stability of high specific activity of ^{125}I-labeled thyronines. *Endocrinology.* **102:** 128–135.
21. Guripde, E., and J. Mann. 1970. Interpretation of isotopic data obtained from blood-borne compounds. *J. Clin. Endocrinol. Metab.* **30:** 707–718.
22. Bianchi, R., G. C. Zucchelli, D. Gianessi, A. Pilo, G. Mariani, A. Carpi, and M. G. Toni. 1977. Evaluation of triiodothyronine (T_3) kinetics in normal subjects, in hypothyroid, and hyperthyroid patients using specific antiserum for the determination of labeled T_3 in plasma. *J. Clin. Endocrinol. Metab.* **46:** 203–214.
23. Boxenbaum, H. G., S. Riegelman, R. M. Elashoff. 1974. Statistical Estimations in Pharmacokinetics. *J. Pharmacokinet. Biopharm.* **2:** 123–148.
24. Chopra, I. J. 1974. A radioimmunoassay for measurement of 3,3′,5′-triiodothyronine (reverse T_3). *J. Clin. Invest.* **54:** 583–592.
25. Challand, G. S., W. A. Ratcliffe, and J. G. Ratcliffe. 1975. Semi-automated radioimmunoassays for total serum thyroxine and triiodothyronine. *Clin. Chim. Acta.* **60:** 25–32.
26. Sterling, K., and M. A. Brenner. 1966. Free thyroxine in human serum: simplified measurement with aid of magnesium precipitation. *J. Clin. Invest.* **45:** 153–163.
27. Chopra, I. J., U. Chopra, S. R. Smith, M. Reza, and D. H. Solomon. 1975. Reciprocal changes in serum concentrations of 3,3′,5′-triiodothyronine (reverse T_3) and 3,3′-5-triiodothyronine (T_3) in systemic illnesses. *J. Clin. Endocrinol. Metab.* **41:** 1043–1049.
28. McDonald, L. J., N. Robin, and L. Siegel. 1978. Free thyroxine in serum as estimated by polyacrylamide gel filtration. *Clin. Chem.* **24:** 652–656.
29. Jiang, N. A., and K. A. Tur. 1977. Determination of free thyroxine in serum by radioimmunoassay. *Clin. Chem.* **23:** 1679–1683.
30. Levy, R. P., J. S. Marshal, and N. L. Velayo. 1971. Radioimmunoassay of human thyroxine-binding globulin (TBG). *J. Clin. Endocrinol. Metab.* **32:** 372–381.
31. Dixon, W. J., and F. L. Massey Jr. 1969. Introduction to Statistical Analysis. McGraw-Hill Book Co., Inc., New York. 3rd edition. 114, 119, 344.
32. Dixon, W. J., and M. B. Brown. 1977. Biomedical Computer Programs, P-series. University of California Press, Berkeley, Calif. 170–184.
33. Maxwell, A. W. 1971. Analyzing Qualitative Data. Methuen and Co., Ltd., London. 23–26.
34. Kaptein, E. M., S. S. MacIntyre, J. M. Weiner, C. A. Spencer, and J. T. Nicoloff. 1981. Free thyroxine estimates in nonthyroidal illness: Comparison of eight methods. *J. Clin. Endocrinol. Metab.* **52:** 1073–1077.
35. Nicoloff, J. T., J. C. Low, J. H. Dussault, and D. A. Fisher. 1972. Simultaneous measurement of thyroxine and triiodothyronine peripheral turnover kinetics in man. *J. Clin. Invest.* **51:** 473–483.
36. Oppenheimer, J. H., G. Berstein, and J. Hansen. 1967. Estimation of rapidly exchangeable cellular thyroxine from the plasma disappearance curves of simultaneously administered [^{131}I]thyroxine and ^{125}I-albumin. *J. Clin. Invest.* **46:** 762–777.
37. Cavalieri, R. R., and G. L. Searle. 1966. The kinetics of the distribution between plasma and liver of ^{131}I-labeled L-thyroxine in man: observation of subjects with normal and decreased serum thyroxine-binding globulin. *J. Clin. Invest.* **45:** 939–949.
38. Gorman, C. A., E. V. Flock, C. A. Owen, and J. Paris. 1966. Factors affecting exchange of thyroid hormone between liver and blood. *Endocrinology.* **179:** 391–405.
39. Hillier, A. P. 1971. The mechanism of thyroxine transport from plasma to tissue binding sites. *J. Physiol. (Lond.).* **217:** 635–639.
40. Jennings, A. S., D. C. Ferguson, and R. D. Utiger. 1979. Regulation of the conversion of thyroxine to triiodothyronine in the perfused rat liver. *J. Clin. Invest.* **64:** 1614–1623.
41. Felicetta, J. V., W. L. Green, and W. B. Nelp. 1980. Inhibition of hepatic binding of thyroxine by cholecystographic agents. *J. Clin. Invest.* **65:** 1032–1040.
42. Burgi, H., C. Wimpfheimer, A. Burger, W. Zaunbauer, H. Rosler, and T. Lemarchand-Beraud. 1976. Changes of circulating thyroxine, triiodothyronine, and reverse triiodothyronine after radiographic contrast agents. *J. Clin. Endocrinol. Metab.* **43:** 1203–1210.
43. Wu, S-Y., I. J. Chopra, D. H. Solomon, and L. R. Bennett. 1978. Changes in circulating iodothyronines in euthyroid and hyperthyroid subjects given ipodate (Oragrafin), an agent for oral cholecystography. *J. Clin. Endocrinol. Metab.* **46:** 691–697.
44. Balsam, A., and S. H. Ingbar. 1978. The influence of fasting, diabetes, and several pharmacological agents on the pathways of thyroxine metabolism in rat liver. *J. Clin. Invest.* **62:** 415–424.
45. Cavalieri, R. R., M. Steinberg, and G. L. Searle. 1970. The distribution kinetics of triiodothyronine: studies of euthyroid subjects with decreased plasma thyroxine-binding globulin and patients with Grave's disease. *J. Clin. Invest.* **49:** 1041–1050.
46. Suda, A. K., C. S. Pittman, T. Shimizu, and J. B. Chambers. 1978. The production and metabolism of 3,5,3′-triiodothyronine and 3,3′,5′-triiodothyronine in normal and fasting subjects. *J. Clin. Endocrinol. Metab.* **47:** 1311–1319.