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Research Article

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Role of Serum Carrier Proteins in the Peripheral Metabolism and Tissue Distribution of Thyroid Hormones in Familial Dysalbuminemic Hyperthyroxinemia and Congenital Elevation of Thyroxine-binding Globulin

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Abstract

To investigate the role of thyroxine-binding globulin (TBG) and albumin in the availability of thyroid hormones to peripheral tissues, comprehensive kinetic studies of thyroxine (T_4) and triiodothyronine (T_3) were carried out in eight subjects with familial dysalbuminemic hyperthyroxinemia (FDH), in four subjects with inherited TBG excess, and in 15 normals. In high-TBG subjects, the reduction of T_4 and T_3 plasma clearance rates (by 51% and 54%, respectively) was associated with normal daily productions; T_4 and T_3 distribution volumes were significantly reduced. In FDH subjects T_4 clearance was less reduced (by 31%) than in high TBG; consequently T_4 production rate was significantly increased (by 42%); T_4 and T_3 distribution volumes and T_3 clearance rate were unchanged. Increased T_3 peripheral production in FDH (by 24%) indicates that T_4 bound to abnormal albumin is more available to tissues than T_4 carried by TBG, thus suggesting an important role of albumin in T_4 availability to the periphery.

Introduction

Familial dysalbuminemic hyperthyroxinemia (FDH)[1] and familial elevation of thyroxine-binding globulin (TBG) are two hereditary abnormalities of the interaction between thyroid hormones and their binding proteins, both leading to euthyroid hyperthyroxinemia (1–7). In the former, the serum thyroid hormone excess is due to an albumin molecule with an abnormal binding site having a much greater affinity for thyroxine (T_4); in the latter, hyperthyroxinemia is due to an increased serum concentration of TBG. Absolute T_4 and triiodothyronine (T_3) serum free concentrations are, however, normal in both conditions. Although it is widely recognized that only the unbound hormone fraction is available to enter tissues, some experimental evidence suggests that also a portion of albumin-bound T_4 is readily available for transport (8, 9). In contrast, more recent studies in FDH (10) and in rats injected in the portal vein with serum from FDH subjects (11), have ruled out a specific role for albumin, different from TBG, in the transport of T_4 into cells. It should be noted that this conclusion has been based on T_4 kinetic studies, whereas the data on T_3 kinetics are few and incomplete (1).

Because most of the circulating T_3 normally arises from T_4 to T_3 conversion in peripheral tissues rather than from direct thyroidal secretion, we carried out simultaneous T_4 and T_3 turnover studies in subjects with altered thyroid hormone–binding protein interaction, aiming at a further definition of the role of TBG and albumin in the transport and distribution of thyroid hormones. Both noncompartmental and multicompartmental methods were used for data analysis. The latter approach appears to be particularly useful inasmuch as it allows estimating the partition of extrathyroidal hormones among the various tissues and measuring the unbiased T_3 production rate (PR) and the total body T_3 pool (Q_t) and a direct in vivo quantification of the T_3 thyroidal secretion rate (SR). The overall T_4 to T_3 conversion ratio (CR), and the relative contribution to peripheral T_3 neogenesis of the "fast or slow" exchanging tissue pools can also be assessed when using a multicompartmental description of the thyroid hormone system. The noncompartmental analysis, in contrast, was also used to compare the kinetic results of the present study with those previously reported.

Methods

Subjects, protocol, and analytical procedures

A total of 28 turnover studies were performed: eight subjects from five families with FDH, four men and four women, ranging in age 31–71 yr; four women from three families with congenital elevation of TBG, aged 48–67 yr. 15 normal subjects, 10 men and 5 women, served as the control group. All subjects were euthyroid by clinical and laboratory data (Table I). Measurements of total T_4, total T_3, thyrotropin (TSH), and reverse T_3 (rT_3) in the serum were performed by specific radioimmunoassays (RIA) as previously reported (12). Total T_4 and total T_3 serum concentrations were also measured after extraction with ethanol-butanol 1:1 to rule out the possibility that the abnormal serum albumin in FDH interferes in the RIA to yield artifactually high values. Free T_3 was measured by Liso-Phase RIA (Sclavo, Milan, Italy); TBG was measured by Immo-Phase (Corning Medical, Medfield, MA). The percentages of free thyroxine were measured by equilibrium dialysis using a modification of the method of Sterling and Brenner (13, 14). Serum concentrations of thyroxine-binding prealbumin and albumin were measured by radial

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1. *Abbreviations used in this paper:* CR, conversion ratio; FCR, fractional catabolic rate; FDH, familial dysalbuminemic hyperthyroxinemia; IDV, initial distribution volume; MC, multicompartmental; MCR, plasma (or metabolic) clearance rate; NC, noncompartmental; Q_t, total extrathyroidal body pool; rT_3, reverse triiodothyonine; SR, secretion rate; T_3, triiodothyronine; T_4, thyroxine; TBG, thyroxine-binding globulin; TDV, total distribution volume; TR, transfer rate.

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immunodiffusion (Behring, Berlin, Federal Republic of Germany). Thyrotropin-releasing hormone (TRH) stimulation tests were performed by intravenous bolus injection of 200 μg of TRH (Biodata, Milan). The distribution of tracer concentrations of ^{125}I-T_4 among serum carrier proteins of subjects with FDH was determined by polyacrylamide gel electrophoresis in Tris glycine buffer, pH 8.9 (15).

Informed consent was obtained from each subject before the tracer experiment. All subjects received 10 drops of saturated potassium iodide solution per day, starting 2 d before, throughout the study. ^{125}I-T_4 (calculated sp act 200 μCi/μg) and ^{131}I-T_3 (sp act 240 μCi/μg) were prepared in our laboratory by the chloramine-T technique using a labeling procedure described previously (16). A measured dose containing ^{125}I-T_4 (50 μCi) and ^{131}I-T_3 (30 μCi) was injected intravenously as a single bolus. Venous blood samples were taken at frequent intervals for the first 24 h (0.08, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 18, 24 h), then every 12, 24 h until 144–192 h after injection, and the plasma disappearance curves of ^{125}I-T_4 and ^{131}I-T_3 and concentrations of ^{125}I-T_3 newly formed from 5'-monodeiodination of tracer ^{125}I-T_4 were determined by a chromatographic system using Sephadex G-25 (Superfine, Pharmacia, Uppsala, Sweden) as previously described (12). This method allows quantitative recovery and complete separation of labeled T_4 and T_3 from their labeled metabolites.

Binding studies

Measurement of thyroxine binding to TBG. TBG was separated from other T_4-binding proteins by adsorption of the serum glycoprotein fraction on concanavalin A-Sepharose 4B (Con A-Sepharose) (17, 18). 100 μl of T_4-free serum (diluted 1:20 with phosphate-buffered saline [PBS]) was added to several test tubes containing 200 μl of a slurry of Con A-Sepharose previously washed and diluted (1:1) with PBS (0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4). The mixture was incubated for 30 min at room temperature with frequent mixing, and then allowed to settle. The supernatant was discarded and the gel sediment was washed twice with 1 ml of PBS. The gel was then reacted with 1 ml of a variable amount of unlabeled T_4 and ^{125}I-T_4 in a water bath at 37°C for 1 h, with frequent mixing. The mixture was allowed to settle for another hour, then the supernatant was carefully aspirated and the sediment was counted in a gamma counter. Binding data were analyzed according to Scatchard (19) after correction for nonspecific binding.

Measurement of T_4 binding to albumin. Serum albumin was separated from other T_4-binding proteins by affinity chromatography on Cibacron Blue F3GA immobilized on agarose (Blue Sepharose) (20), after removal of TBG by adsorption on Con A-Sepharose. Briefly, 100 μl of whole serum was applied to a microcolumn of Con A-Sepharose (2 × 0.9 cm) equilibrated with PBS, and eluted with 2 ml of buffer. The eluate was then applied to a column of Blue Sepharose (2 × 0.9 cm), and the albumin was eluted with 1.5 M NaCl in buffer. The albumin fraction was diluted 1:5 with PBS to lower its salt content. Binding of T_4 to albumin was assessed by a modification of the competitive binding technique employing Sephadex G-25 (21). 100 μl of the diluted albumin fraction was added to several test tubes each containing 200 mg of Sephadex G-25 (medium) preequilibrated with PBS. A variable amount of a solution of unlabeled T_4, tracer ^{125}I-T_4, and PBS were added to the tubes to a final volume of 1.5 ml (1.0 ml in the excluded volume) and allowed to equilibrate for 1 h at 37°C with frequent mixing, and then, after settling for another hour, 200 μl of the supernatant was counted. Separate experiments were performed in the same range of T_4 concentrations but without serum proteins to estimate T_4 binding by Sephadex. The calculation of the albumin-bound T_4 was performed according to the original procedure (21), and the binding data were analyzed by the Scatchard method.

Kinetic studies

Noncompartmental approach. Standard noncompartmental (NC) formulas (22–24) were used to analyze the plasma disappearance curves of ^{125}I-T_4 and of ^{131}I-T_3. The following turnover and distribution parameters were computed for both T_4 and T_3 kinetics: plasma (or metabolic) clearance rate (MCR), initial distribution volume (IDV), total distribution volume (TDV), production rate (PR), total extrathyroidal pool (Q_t), and fractional catabolic rate (FCR). FCR = MCR/TDV = PR/Q_t.

The values of the areas and first-order moments of the disappearance curves to be used in NC formulas were computed starting from intercepts and slopes of multiexponential functions (sums of three exponentials), fitted (by least squares) on the experimental plasma ^{125}I-T_4 and ^{131}I-T_3 data.

The plasma appearance curve of ^{125}I-T_3 generated in vivo from the injected ^{125}I-T_4 was used to estimate the T_4 to T_3 conversion ratio (CR) using a convolution approach previously described (12, 25, 26). The peripheral production rate of T_3 was then obtained as the product of the T_4 production rate by the T_4 to T_3 CR (after correction for the T_3/T_4 molecular weight ratio); the thyroidal T_3 secretion rate (T_3 SR) was obtained as the difference between the total T_3 production rate (T_3 PR) and the peripheral T_3 production rate.

Multicompartmental approach. The assumptions and limitations of the NC approach have been previously discussed in detail (26, 27). In brief, the NC approach relies on the assumption that both the production and the disposition of the tracer take place in the central (plasma) pool. This assumption is somewhat in contrast with available physiological information on thyroid hormones; in fact, both T_3 and T_4 are known to be metabolized in peripheral tissues where a large fraction of T_3 is produced from T_4 conversion. Therefore, a multicompartmental (MC) model was also used to analyze the same kinetic data. It consists of two mammillary systems, each having a central, a fast, and a slowly exchanging pool (see Fig. 1). The model is essentially that proposed by Di Stefano et al. (28) to which reference is made for tentative anatomical identification of the compartments (28). This MC model is physiologically more meaningful because it takes into proper account the peripheral degradation of T_3 and T_4, and the peripheral production of T_3. Information may also be obtained regarding the subdivision of the extravascular hormone pool into its fast and slowly exchanging segments. It can be predicted from the theory that the values for thyroidal secretion rates of both T_4 and T_3 and for their initial distribution pools are identical whether computed by NC or MC analysis. In contrast, the peripheral production rate of T_3 and the extravascular pools of both T_4 and T_3 are underestimated by NC analysis.

A limitation of MC analysis is that neither the three-compartment model of T_4 nor that of T_3 can be uniquely identified from the respective plasma disappearance curves of tracer hormones. To overcome this difficulty, we used the quasi-identification approach put forward by Di Stefano et al. (28), whereby the model parameters are defined through intervals (bounds) derived from inequalities written on the basis of the model equations. In particular, we used the midpoint of the intervals defined by the inequalities reported by Di Stephano (28) for k_{21} (T_4 system) and k_{54} (T_3 system, see Fig. 1). Starting from slopes and intercepts of multiexponential functions best fitted on the experimental plasma disappearance curves of labeled T_4, we obtained narrow ranges for k_{21} (mean 3.0%, range 1.6–4.6%), very similar to those found in T_4 kinetic studies in rats (28). Consequently, these interval estimates were judged suitable to identify the three-pool model for T_4 in humans. The same approach applied to T_3 disappearance curves of our normal subjects produced relatively large ranges for k_{54}, (mean 15.8%, range 8.1–42.5%) in contrast with the data reported by Di Stefano et al. for T_3 kinetics in rats (29). For this reason, this approach was not considered to be sufficiently accurate to describe T_3 kinetics in humans (30). Moreover, the same approach does not yield reasonable interval estimates for the rates of T_3 production from T_4 conversion, because the parameters k_{52} and k_{63} cannot be estimated individually but only in the combinations (k_{02} + k_{52}) and (k_{03} + k_{63}), which are the cumulative outputs from the peripheral T_4 pools. In fact, the conversion ratio, computed in cardiac patients according to this method, was determined within rather large bounds (67% on average) (30).

To obtain narrower interval estimates for the parameters of T_3 kinetics, we exploited the experimental data of the appearance curve of labeled T_3 generated in vivo from the conversion of the injected labeled T_4. In our approach, the best fit of this latter experimental curve with

Table I. Mean Clinical and Thyroidal Functional Parameters of Subjects Submitted to the Metabolic Study

Subject	Sex and age	Weight	Body surface	Serum total T_4	Serum free T_4	Serum total T_3	Serum free T_3	Serum rT_3	Serum TBG	Serum TBPA	Serum albumin	TRH test
		kg	*m^2*	*μg/100 ml*	*(% total)*	*ng/100 ml*	*pg/ml*	*ng/100 ml*	*μg/ml*	*mg/100 ml*	*g/100 ml*	
FDH												
1	F 55	66	1.70	17.7	0.0087	134	4.0	35.4	19	32	4.15	N
*2	F 71	59	1.60	19.0	0.0076	142	3.6	39.4	19	35	4.07	N
*3	F 37	52	1.52	18.8	0.0072	157	3.9	28.2	26	26	4.31	N
*4	M 40	94	2.16	15.6	0.0094	159	5.8	30.9	22	37	4.32	Blunted
5	M 65	69	1.77	15.8	0.0082	135	5.2	36.2	23	37	4.43	N
6	M 31	84	2.02	18.3	0.0071	115	5.1	37.5	22	40	4.72	N
6*bis*	M 31	84	2.02	15.3	0.0084	120	5.4	36.4	23	40	5.03	N
‡7	M 44	102	2.20	15.8	0.0075	173	4.9	23.9	15	32	4.06	N
‡8	F 71	75	1.80	16.4	0.0078	134	4.1	32.2	18	34	4.44	N
Mean	51.8	75.1	1.85	17.0	0.0080	141	4.7	33.3	20.8	34.7	4.31	
±SD	14.9	16.1	0.24	1.4	0.0007	18	0.7	4.7	3.1	4.2	0.21	
TBG excess												
§1	F 52	101	2.02	16.6	0.0067	272	2.8	31.3	78	14	4.55	N
§2	F 67	64	1.65	14.3	0.0070	204	3.1	33.0	54	26	3.77	N
3	F 48	66	1.67	16.2	0.0066	163	3.2	23.0	60	25	3.98	N
4	F 49	60	1.50	11.6	0.0085	203	3.0	28.2	50	22	4.01	N
Mean	54.0	72.8	1.71	14.7	0.0072	211	3.0	28.9	60.5	21.7	4.08	
±SD	7.6	16.5	0.19	2.0	0.0007	39	0.1	3.8	10.7	4.7	0.29	
Normals (n = 15)												
Mean	40.2	69.4	1.78	8.1	0.012	121	4.1	24.8	23.4	35.1	4.25	
±SD	12.9	8.4	0.15	1.1	0.002	12	0.7	5.3	5.4	9.5	0.32	

*,‡,§ indicate the family.

the theoretical curve computed on the basis of the complex six-pool model allowed the computation of the products k45 × k52 and k46 × k63. These products are associated with the pathways through which the T_3 generated from T_4 deiodination in the fast or the slow pool reaches the sampling compartment (compartment 4). This additional datum allows computing the thyroidal T_3 secretion rate and to constrain the parameters of T_3 kinetics within narrower bounds. In particular, the T_3 production rate in our normal subjects could be determined within intervals of 6% on the average (range 1.0–8.8%) whereas the T_4 to T_3 conversion ratio was obtained with bounds of 8.1% (range 0.6–17.8%).

The computed kinetic parameters of the complex six-pool model are reported in this paper as mean population values (see Fig. 8); note that, although thyroidal secretion rates of T_4 and T_3 and the masses of central pools of T_4 and T_3 are directly identified, all the other parameters are reported as the midpoints of their computed bounds.

Statistical analysis

Values are given as mean±standard deviation or standard error of the mean. Student's *t* test was used to compare population means.

Results

Binding studies

The diphasic Scatchard plot obtained in the binding studies of albumin in FDH subjects pointed out the occurrence of two classes of binding sites. Affinity constants (K_a) and binding site concentrations calculated from these plots for the high-affinity sites were assumed to represent the affinity and the molar concentration of the abnormal albumin.

The mean values of the measured T_4 affinity constant of albumin and TBG binding sites in FDH and in high-TBG subjects are reported in Table II. The T_4 K_a of abnormal albumin in FDH subjects ($4.4\pm1.5 \times 10^6$ liters/mol) was 12.5 times higher than that of normal albumin, but still much lower (~ 1,000-fold) than that of TBG ($4.3\pm0.8 \times 10^9$ liters/mol). The observed values measured for T_4 K_a of TBG and serum albumin are in good agreement with those measured by this and other techniques previously reported (31–33). Tracer ^{125}I-T_4 distribution among carrier proteins in normals and FDH subjects is depicted in Fig. 2. In FDH the percentage of T_4 carried by albumin (41.2±6.3%) was significantly increased and, consequently, the percentage of T_4 carried by TBG was significantly decreased ($P < 0.001$). These data are in good agreement with those reported previously (4, 10).

Kinetic studies

The mean plasma curves obtained in the three study groups for ^{125}I-T_4 and ^{131}I-T_3 are depicted in Fig. 3 together with the corresponding fitting functions. It can be seen that the disappearance curve of labeled T_4 of high-TBG subjects is slower than that of the controls, whereas the curve of FDH subjects is intermediate between the two. The plasma T_3 disappearance curves of normals and FDH subjects are virtually superimposable, whereas high-TBG subjects exhibit a remarkably slower decay rate. Fig. 4 shows the plasma appearance curves, in normals and FDH subjects, of ^{125}I-T_3 newly formed in peripheral tissues (by 5′-monodeiodination of labeled T_4) together with the corresponding fitting functions computed by convolution analysis. The appearance curve of ^{125}I-T_3 in FDH subjects is clearly delayed in comparison to that observed in the controls.

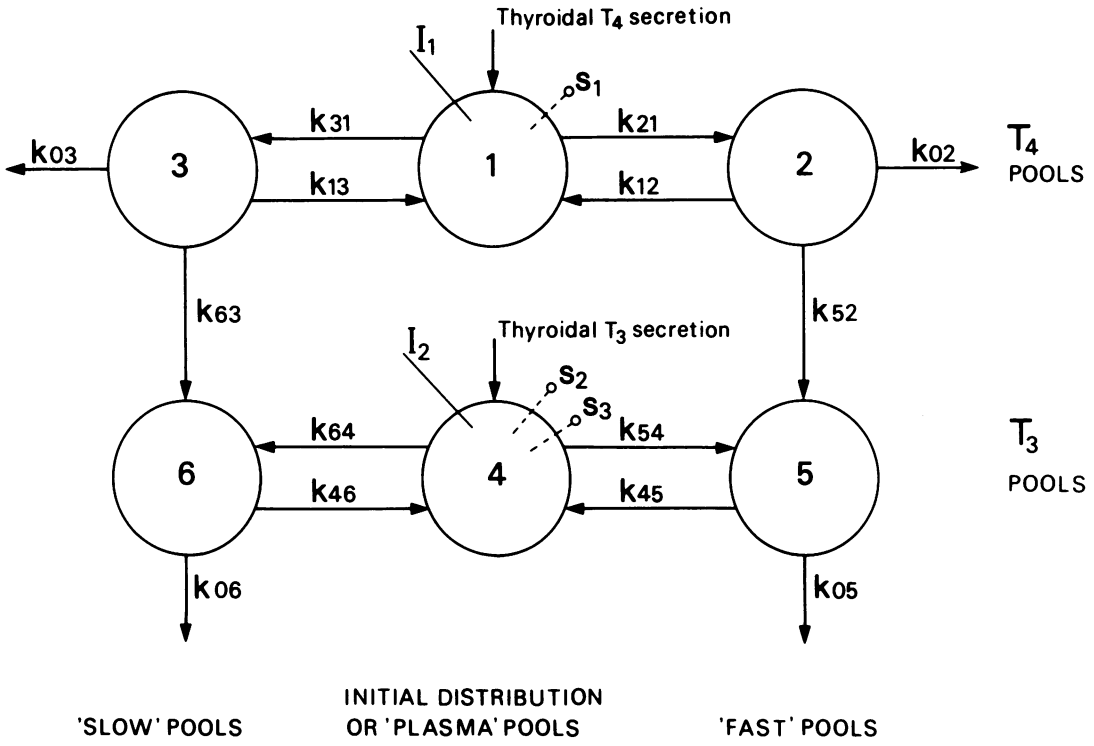

Figure 1. Complex six-compartment model of thyroid hormone kinetics: both T_4 and T_3 systems are modeled by three-compartment model interconnected by pathways relative to the conversion of T_4 into T_3 (fractional rates k52 and k63). Also indicated in the scheme are the simultaneous tracer inputs (I_1, pulse injection of ^{125}I-T_4; I_2, pulse injection of ^{131}I-T_3) and the sampling sites of the three experimentally measured curves (S_1, plasma concentration of ^{125}I-T_4; S_2, plasma concentration of ^{131}I-T_3; S_3, plasma concentration of ^{125}I-T_3 generated in vivo).

Table II. Measured Affinity Constants (Ka) of Serum Carrier Proteins for Thyroxine (37°C, pH 7.4; Ionic Strength 0.15 M)

Subjects	K_a TBG	K_a HSA	K_a aHSA*	aHSA
	liters/mol	*liters/mol*	*liters/mol*	*% HSA‡*
FDH (n = 8)				
Mean	4.3×10^9	3.5×10^5	4.4×10^6	20.4
±SD	0.8	1.5	1.5	2.6
TBG excess (n = 4)				
Mean	3.9×10^9	4.5×10^5	—	—
±SD	1.2	1.8		

* aHSA, serum albumin with "abnormal site"
‡ % aHSA, abnormal serum albumin as percentage of total serum albumin.

NONCOMPARTMENTAL ANALYSIS (TABLE III)

FDH subjects. Mean values of total T_4 and total T_3 serum concentrations in unextracted sera (17.0±1.4 μg/100 ml and 141±18 ng/100 ml, respectively) were virtually identical to those measured after ethanol-butanol extraction when corrected for extraction recovery of 64.5±0.75% and 84.3±0.4%, respectively. This finding is in agreement with the data previously reported by Stockigt et al. (3).

T_4 MCR (0.47±0.12 liters/d per m^2) was significantly reduced in comparison to normal subjects (0.68±0.12 liters/d per m^2, $P < 0.001$). T_4 PR, on the other hand (77.7±13.6 μg/d per m^2), was significantly increased ($P < 0.001$), because total circulating T_4 was increased more than its MCR was reduced. It is noteworthy that the total serum T_4 concentrations of our FDH patients varied over a wider range (15.3–19 μg/100 ml) than previously reported (1, 10). Moreover, a highly significant, inverse correlation was observed between T_4 MCR and total T_4 concentration ($r = 0.96$; $P < 0.001$, Fig. 5). To provide a better comparison between our findings and those of Henneman et al. (1) and of Mendel and Cavalieri (10), our FDH patients were subdivided according to whether their T_4 concentrations were lower (subgroup A) or higher (subgroup B) than 17 μg/100 ml. In the high-T_4 subgroup (B) (cases 1, 2, 3, and 6; total T_4 = 18.5±0.56 μg/100 ml, Table III), T_4 PR (63.3±3.31 μg/d per m^2) was slightly but not significantly increased in comparison to the control group (Fig. 6). In contrast, T_4 PR (89.5±3.56 μg/d per m^2) was significantly increased (by 66%, $P < 0.001$) in FDH subjects with lower total T_4 serum concentration (cases 4, 5, 6*bis*, 7, and 8; total T_4 = 15.8±0.37 μg/100 ml, subgroup A).

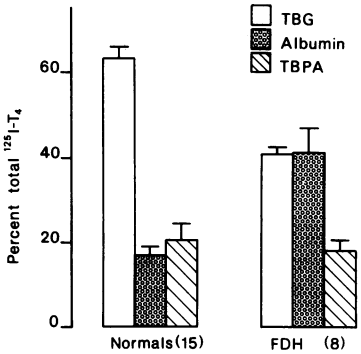

Figure 2. Distribution of ^{125}I-T_4 among carrier proteins in serum from 15 euthyroid controls and 8 patients with familial dysalbuminemic hyperthyroxinemia. TBPA, thyroxine-binding prealbumin. Values are means; bars denote standard deviation.

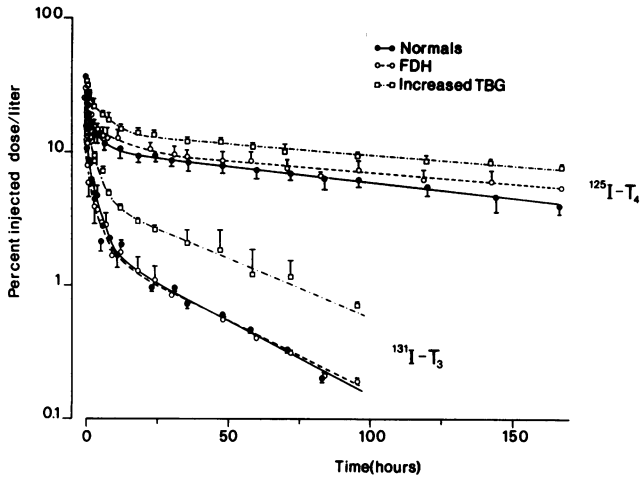

Figure 3. Mean plasma disappearance curves for ^{125}I-T_4 and ^{131}I-T_3 for the three groups of subjects submitted to the kinetic studies. The curves are represented in a logarithmic scale together with the respective multiexponential fitting functions. The vertical bars represent standard deviation of each average value.

Both T_4 IDV (1.73±0.15 liters/m^2) and T_4 TDV (4.98±1.08 liters/m^2) were similar in FDH and control subjects (Table III). Consequently, total T_4 extrathyroidal body pool (Q_t) was significantly increased (852±233 μg/m^2, $P < 0.001$) in comparison with normal (438±80 μg/m^2). T_4 FCR (10.0±3.6%/d) was slightly but significantly reduced ($P < 0.05$) in the FDH subjects as a whole, with subgroup B (6.9±2.2%, $P < 0.001$) contributing most of the change (subgroup A = 12.4±2.5%/d). The T_4 to T_3 conversion ratio (CR) was slightly but significantly ($P < 0.05$) reduced (21.0±3.8% vs. 26.1±5.9%). This was due to subgroup A (19.8±4.1%; $P < 0.05$) rather than subgroup B (23.1±2.3%). T_3 MCR was not different from the control subjects. Consequently, the observed increment in total T_3 PR (23.7%) can all be ascribed to the elevation of total serum T_3 levels. Although still within the normal range, total T_3 in patients (141±18 ng/100 ml) was significantly higher than in controls (121±12 ng/100 ml, $P < 0.001$), in accord with the findings reported by Ruiz et al. (4). The increase in T_3 PR was larger (32%) in subgroup

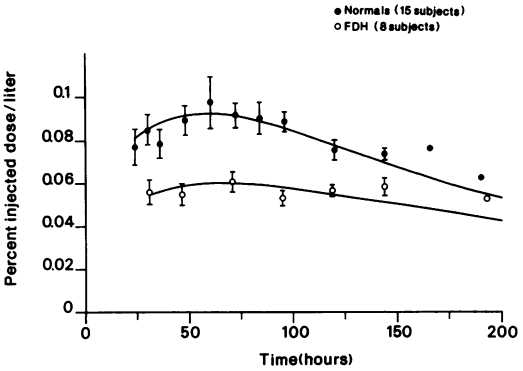

Figure 4. Mean plasma appearance curves of in vivo generated ^{125}I-T_3 from ^{125}I-T_4 together with the respective fitting functions computed by convolution approach (see data analysis), obtained in normal subjects (*upper curve*) and in patients with familial dysalbuminemic hyperthyroxinemia (*lower curve*). The bars represent the standard error of each mean.

Table III. Metabolism of T_4 and T_3 Measured by Noncompartmental Approach in Subjects with FDH, in Subjects with Inherited TBG Excess and in Normals

Case no.	Serum total T_4	T_4 MCR	T4 IDV	T4 TDV	T4 PR	T4 Q_1	T4 FCR	Serum total T_3	T_3 MCR	T_3 IDV	T_3 TDV	T_3 PR	T_3 Q_1	T_3 FCR	T_3 SR	T_4 to T_3
	μg/100 ml	*liters/d per m²*	*liters/m²*	*liters/m²*	*μg/d per m²*	*μg/m²*	*%/d*	*ng/100 ml*	*liters/d per m²*	*liters/m²*	*liters/m²*	*μg/d per m²*	*μg/m²*	*%/d*	*μg/d per m²*	*CR %*
FDH																
*1	17.7	0.340	1.72	5.18	60.3	918	6.5	134	11.4	2.92	16.0	15.1	21.4	71.2	3.2	22.4
*2	19.0	0.333	1.96	7.34	63.4	1395	4.6	142	11.3	2.56	13.9	17.4	19.7	81.3	5.3	20.6
*3	18.8	0.321	1.65	5.41	60.3	1017	6.0	157	12.0	2.05	18.6	19.0	29.2	64.5	5.2	26.1
‡4	15.6	0.550	1.70	4.93	86.1	769	11.3	159	18.9	3.12	19.4	30.1	30.8	97.4	10.0	27.4
‡5	15.8	0.587	1.87	4.96	92.5	784	11.8	135	16.3	3.10	26.6	22.1	35.9	61.3	7.1	18.4
*6	18.3	0.372	1.41	3.52	68.4	644	10.6	115								
‡6*bis*	15.3	0.615	1.74	3.55	94.0	543	17.3	120	12.5	2.35	16.8	15.0	20.1	74.4	0.4	17.7
‡7	15.8	0.571	1.72	5.47	90.2	864	10.3	173	12.2	2.50	13.8	21.6	23.9	88.4	5.2	20.1
‡8	16.4	0.516	1.84	4.48	84.8	737	11.5	134	9.5	2.40	10.5	14.1	15.5	90.5	0.2	15.5
Mean	17.0§	0.467§	1.73	4.98	77.7§	852§	10.0‖	141§	13.0	2.62	17.0	19.3‖	24.8‖	78.6	4.6	21.0‖
±SD	1.4	0.12	0.15	1.08	13.6	233	3.6	18	2.9	0.36	4.5	5.0	6.4	12.1	3.1	3.8
TBG excess																
1	16.6	0.287	1.74	3.84	47.8	638	7.5	272	4.8	2.08	8.6	13.0	23.4	55.8	3.9	22.8
2	14.3	0.364	1.50	3.88	51.9	553	9.4	204	7.0	2.19	10.6	15.4	21.6	66.0	4.6	22.1
3	16.2	0.273	1.33	3.39	44.2	549	8.1	163	6.8	1.93	8.1	11.1	13.2	83.7	2.5	23.3
4	11.6	0.406	1.39	4.65	47.1	539	8.7	203	4.9	1.72	9.0	9.9	18.2	54.4	2.1	20.0
Mean	14.7§	0.332§	1.49	3.94¶	47.8	570¶	8.4§	211§	5.9§	1.98¶	9.1§	12.3	19.1	70.0	3.3	22.1
±SD	2.0	0.05	0.16	0.45	2.8	40	0.7	39	1.0	0.18	0.9	2.1	3.9	11.7	1.0	1.3
Normals (n = 15)																
Mean	8.1	0.682	1.61	5.48	54.7	438	12.5	121	12.9	2.62	16.1	15.6	19.5	76.9	3.7	26.1
±SD	1.1	0.12	0.19	0.78	9.5	80	1.7	12	2.1	0.41	3.1	2.9	4.1	23.9	2.3	5.9

* Subgroup B. ‡ Subgroup A. Different from controls: §$P < 0.001$; ‖$P < 0.05$; ¶$P < 0.01$.

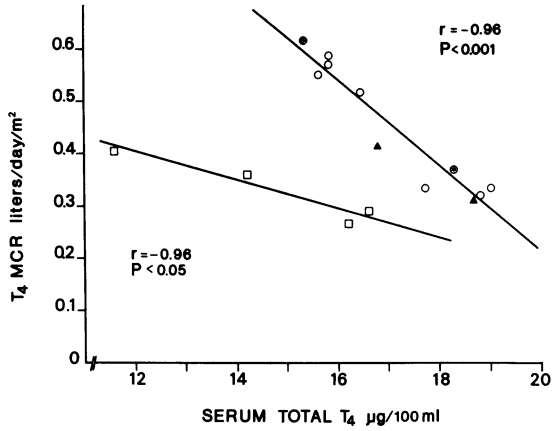

Figure 5. Upper plot: correlation observed between T_4 MCR and total serum T_4 concentration in patients with familial dysalbuminemic hyperthyroxinemia of the present study (*open circles*) and in the two patients (*solid triangles*) studied by Hennemann et al. (1). Symbols with * refers to subject 6 studied at two different times. Affinity constant (K_a) of abnormal albumin binding sites (aHSA), for T_4: case 6, 4 $\times 10^6$ liters/mol; case 6*bis*, 1.8 $\times 10^6$ liters/mol. The correlation holds also when our data are normalized by body weight as those reported by Mendel and Cavalieri (10) ($r = 0.87$; $P < 0.001$). *Lower plot:* correlation between T_4 MCR and total serum T_4 concentration in subjects with TBG excess (*open squares*). All values are corrected to body surface.

A than in subgroup B (10.1%). The thyroidal T_3 secretion rate, in percent of total T_3 PR (23.8±11.8%), as well as the IDV and TDV of T_3, all were similar to those of the control subjects. The T_3 Q_t increased by 27.2%, ($P < 0.05$).

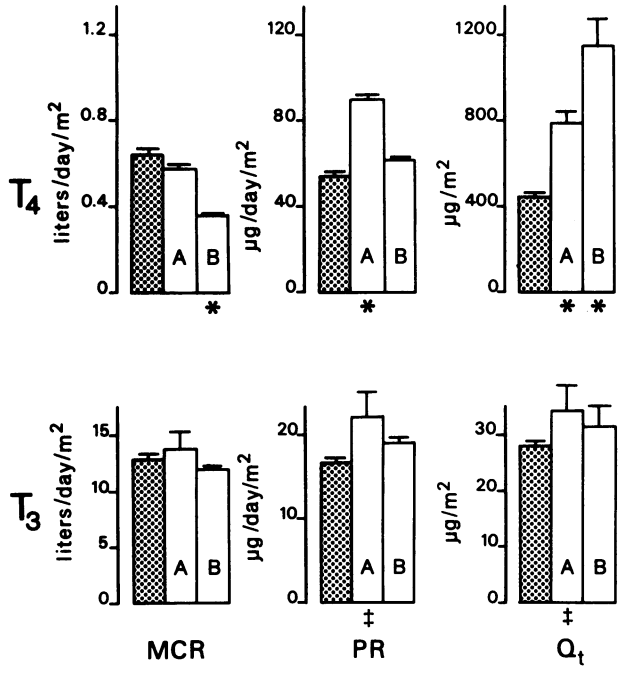

Figure 6. Comparison of MCR, PR, and Q_t values respectively for T_4 (*upper panel*) and T_3 (*lower panel*) for normal subjects and FDH subjects with relatively higher total T_4 serum concentration (18.5±0.56 µg/100 ml, subgroup B) and with relatively lower total T_4 serum concentration (15.8±0.37 µg/100 ml, subgroup A). Values are means; the bars denote standard deviation, * indicates $P < 0.001$, ‡ indicates $P < 0.05$ (by unpaired t test).

Subjects with inherited TBG elevation. Both T_4 and T_3 MCR were markedly decreased ($P < 0.001$), by 51.3% and 54.3% respectively, in comparison with the controls (Table III). T_4 and T_3 PR were not different from normal, because the rise in serum hormone concentrations fully compensated the decrease in MCR. A significant inverse correlation was observed between T_4 MCR and total T_4 serum concentration ($r = 0.96$; $P < 0.05$, Fig. 5). It is worth noting that the decrease of T_4 MCR is associated with a consensual increment of both total serum T_4 and serum TBG concentrations. T_4 IDV was slightly lower than normal whereas T_3 IDV was significantly reduced, ($P < 0.01$). T_4 and T_3 TDV were both significantly decreased (by 28.1%, $P < 0.01$, and 43.5%, $P < 0.001$, respectively). T_4 Q_t was increased ($P < 0.01$) because the decrease in TDV did not counterbalance the increase in serum T_4 concentration, and T_3 Q_t was not different in comparison with the control group. The T_4 to T_3 conversion ratio (22.1±1.3%) and thyroidal T_3 secretion in percent of total T_3 PR (26.8%) were not significantly different from the normal values. T_4 FCR was significantly reduced ($P < 0.001$) whereas T_3 FCR was only slightly decreased.

Finally, a significant inverse correlation was found to exist between the T_4 to T_3 conversion ratio and T_4 PR ($r = 0.41$; $P < 0.05$) in the whole of the study subjects. This correlation became stronger when the data from nine hypothyroid patients previously studied by us (34) were included (Fig. 7).

MULTICOMPARTMENTAL ANALYSIS

Normal subjects. The mean values of production rates, pool masses, and exchange fluxes of the T_4 and T_3 systems, computed according to the six-compartment model, are reported in Fig. 8 for the 15 control subjects.

T_4 Q_t (448±82 µg/m²) was found to be only minimally (2.2%) underestimated by the NC approach. The fractions of total T_4 Q_t in the initial distribution (or plasma) pool and in the fast and slowly exchanging tissue pools, were 28.8%, 28.8%, and 42.4%, respectively; these figures are similar to those reported by Di Stefano et al. in rats (28). T_3 PR resulted to be 16.9±3.3 µg/d per m², and was 7.7% underestimated by the NC approach; the T_4 PR metabolized by routes different from T_3 generation was 38.9±9.5 µg/d per m². T_4 to T_3 CR was 29.2±6.5% and, because

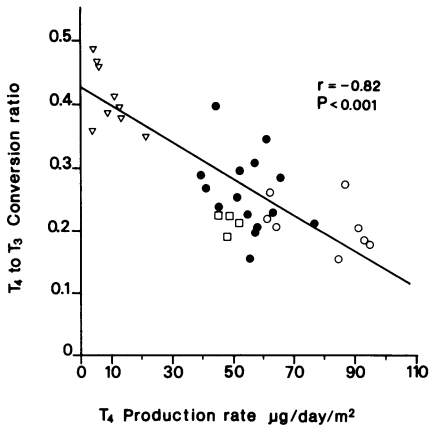

Figure 7. Inverse correlation observed in the whole series of subjects of the present study and in nine hypothyroid patients, previously reported (34) between the conversion ratio values and T_4 daily production rates. (*Solid circles*) Normal subjects, (*open circles*) FDH subjects, (*open squares*) subjects with TBG excess, (*inverted triangles*) hypothyroid subjects.

Normals (15)

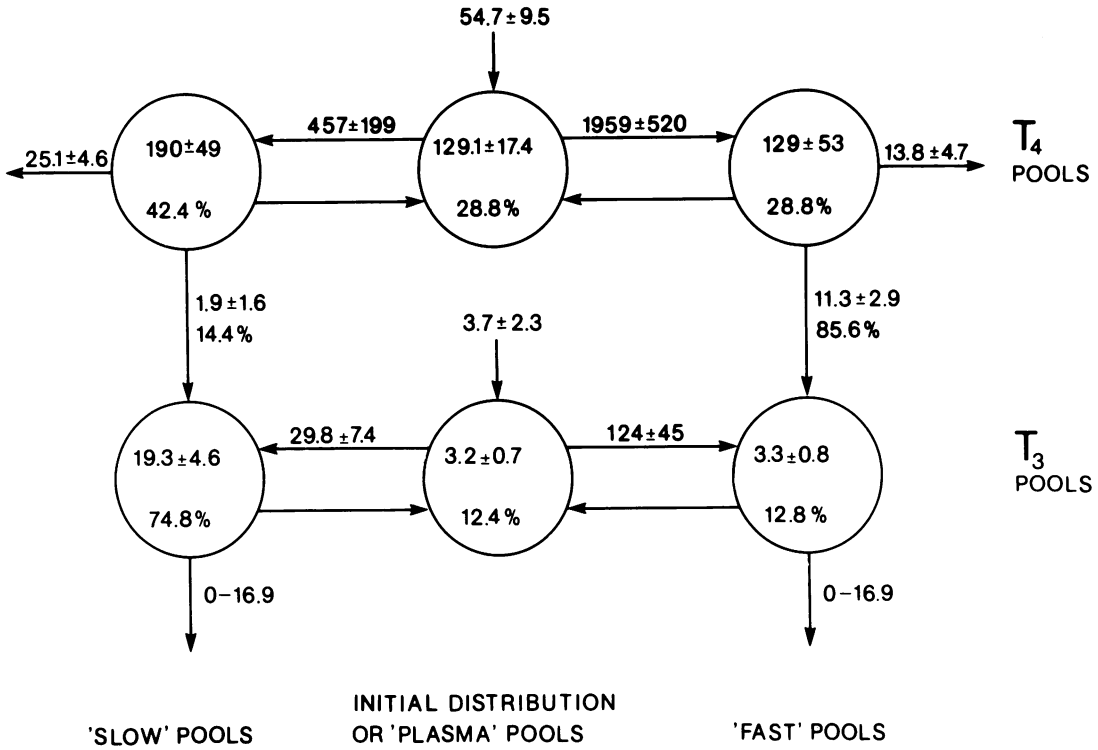

Figure 8. Mean results from multicompartmental analysis of T_4 and T_3 kinetics in normals. The figure shows mean values (±standard deviation) of masses (figures within circles in $\mu g/m^2$) of initial distribution compartment and of the fast and the slowly exchanging compartments, both for T_4 and T_3. The same values are also indicated as percentage of the respective total (extrathyroidal) body pools. For T_4 system, we also report (on the respective arrows in $\mu g/d$ per m^2 of T_4), the production rate of T_4, the exchange rates between central and peripheral compartments and the degradation rate of T_4 associated to pathways different from conversion into T_3. For T_3 system we report (on the respective arrows in $\mu g/d$ per m^2 of T_3), the thyroidal secretion, the peripheral production rates from T_4 conversion both in the fast and in the slow peripheral compartments, the exchange rates between central and peripheral compartments and the disposal rate. The disposal rate is reported as an interval on both peripheral pools, in that the analysis does not allow identifying in which of the peripheral T_3 pools the degradation takes place. The following additional kinetic parameters, useful for comparing MC with NC analysis, are easily computed using the data in the figure and the mean plasma concentrations of the hormones (see Table I), according to the following formulas:

$$T_4TDV = T_4Q_t\ (\mu g/m^2)/\text{serum } T_4 \text{ level } (\mu g/\text{liter})$$
$$= (129 + 129 + 190)/81 = 5.53 \text{ liters}/m^2;$$

$$T_3TDV = T_3Q_t\ (\mu g/m^2)/\text{serum } T_3 \text{ level } (\mu g/\text{liter})$$
$$= (3.2 + 3.3 + 19.3)/1.21 = 21.3 \text{ liters}/m^2;$$

$$T_4 \text{ to } T_3CR(\%) = \text{peripheral } T_3 \text{ production} \times (\text{mol wt } T_4/\text{mol wt } T_3)/T_4PR = 13.2 \times 1.2/54.7 = 29.0\%.$$

it refers to the peripheral T_3 production only, it was more underestimated (10.6%) by NC analysis. Total T_3 Q_t was 25.8±5.0 $\mu g/m^2$; NC analysis underestimated this parameter by 24.4%. Percent distribution in the initial, fast, and slow pools was 12.4%, 12.8%, and 74.8%, similar to those reported by Di Stefano et al. in rats (29) and by Hershman et al. in humans (30). It must be noted that 78% of T_3 PR emanates from peripheral conversion of T_4, of which 86% and 14% originate from the fast and slowly exchanging tissue pools, respectively. This is at variance with the peripheral T_3 production in the rat, where the major fraction of the hormone is generated from T_4 in the slowly exchanging pool (29).

FDH subjects. T_4 Q_t was 917±242 $\mu g/m^2$. The underestimation produced by NC analysis was 7%. The proportion of total T_4 Q_t in the plasma pool, fast and slowly exchanging tissue pools were 32.4%, 21.8%, 45.8%, respectively. Total T_3 Q_t was 33.1±9.3 $\mu g/m^2$, higher than the value obtained by NC analysis (by 33.5%). The fractions of total T_3 Q_t in the plasma, fast and slowly exchanging tissue pools (11.5%, 12.1%, and 76.4%) were very similar to the corresponding figures found in normals. T_3 PR was 21.2±5.1 $\mu g/d$ per m^2, significantly greater ($P < 0.05$) than control values (16.9±3.3 $\mu g/d$ per m^2) (Fig. 9). This value was underestimated by NC analysis by 9%. T_4 to T_3 CR was 25.6±2.9%, slightly less than normal (29.2±6.5%); this figure was underestimated by NC analysis by 18%. The mean fraction of T_4 PR metabolized by other routes than T_3 generation was 74%, practically superimposable to the values found in the controls (71%).

In FDH subjects, 78.3% of the T_3 PR emanates from extrathyroidal conversion from T_4; this figure is identical to the corresponding normal value (78.1%). However, of this amount 57.8% and 42.2% come, respectively, from fast and slowly exchanging tissue pools in FDH in comparison with the corresponding values of 85.6% and 14.4% in the controls. The shift

FDH (8)

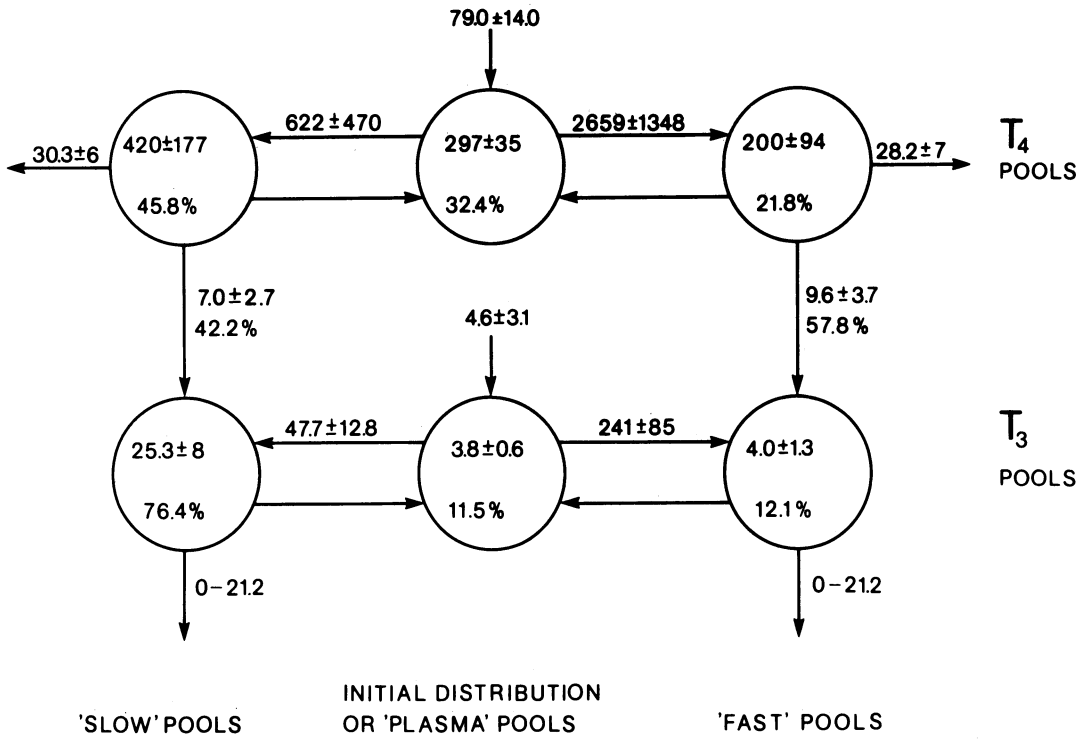

Figure 9. Mean results from MC analysis of T_4 and T_3 kinetics in FDH subjects; see legend of Fig. 8 for explanations. Additional parameters are easily computed using the formulas reported in the legend of Fig. 8: T_4 TDV = 5.39 liters/m^2, T_3 TDV = 23.5 liters/m^2; T_4 to T_3 CR = 25.2%.

of peripheral T_3 production toward the slow compartment ($P < 0.001$) is reflected by the shape of the plasma appearance curve of neogenerated ^{125}I-T_3 (Fig. 4), which appears to lag behind the normal curve. The overall T_4 transfer rate (TR) from plasma, although significantly increased ($P < 0.05$) in absolute terms (3,281±1,267 in respect to normal value of 2,416±496 μg/d per m^2) is significantly reduced ($P < 0.001$) when represented as percent of the plasma pool (1,108 vs. 1,871%/d).

High-TBG subjects. T_4 and T_3 Q_t were 596±37 and 25.0±6.3 μg/m^2, respectively (Fig. 10). These values were higher than those obtained by NC analysis (by 4.6% and 31%). For both T_4 and T_3 there was an increase in the amount present in the plasma pools, either in absolute terms (220±46 and 4.2±1 μg/m^2) and in percentage (respectively, 36.9% and 16.8%) in comparison with the normal situation. Overall T_4 TR from plasma (1,937±379 μg/d per m^2), although not different from normal in absolute terms, was significantly reduced ($P < 0.001$) in percent terms (880 vs. 1,871%/d). The mean fraction of T_4 PR metabolized by other pathways than T_3 generation was 75.9%, similar to the value found in the controls and in FDH subjects. Respectively, 74% and 26% of T_3 generated in peripheral tissues from T_4 derived from fast and slowly exchanging tissue pools.

Discussion

The present study was undertaken to investigate the specific role of serum carrier proteins of two different thyroid hormones, characterized respectively by the highest affinity (TBG) and by the highest capacity (albumin), in the availability of T_4 and T_3 to peripheral tissues. In vivo T_4 and T_3 kinetic studies were carried out in normal subjects and in individuals with congenital thyroid hormone carrier protein defects, characterized by excessive total serum T_4 concentrations and by normal free T_4 levels. In high TBG and FDH, euthyroid hyperthyroxinemia is due to an increase in TBG serum concentration in the former, and to the presence of abnormal albumin binding sites in the latter. The methods we used allow simultaneous in vivo assessment of T_4 and T_3 kinetics. In particular, the overall T_4 into T_3 conversion ratio, peripheral T_3 production, and consequently, thyroidal T_3 secretion rate can be directly measured. In addition, the amount of T_4 metabolized by other routes than T_3 generation (deiodination to rT_3, conjugation, decarboxylation, deamination, urinary and fecal excretion) can be estimated. The multicompartmental approach provides the partition of T_4 and T_3 extrathyroidal body pools among the plasma, slowly and fast exchanging tissue pools, and the relative contribution of slowly and fast exchanging compartments to peripheral T_3 neogenesis.

Because MCR is defined as the ratio between disposal rate and plasma concentration, it can be considered as an index of the availability of the hormone for disposal and, in the case of T_4, for its conversion into T_3. It was thus found that the availability of T_4 is markedly reduced in subjects with congenital elevation of TBG; their higher T_4 levels ensure a normal disposal rate of T_4. In that the conversion ratio of T_4 into T_3 is unchanged, peripheral T_3 production remains in the normal range. The reduced T_4 availability in this condition is confirmed by the significant increase ($P < 0.001$) of the initial distribution (or plasma) pool (36.9% of the total pool vs. 28.8% of the controls) and also by the changes in T_4 TR from plasma and in T_4 FCR, which are reduced by 53% and 32.8%, respectively. A close reduction of T_4 FCR in congenital TBG elevation has been reported also by Nicoloff et al. (35).

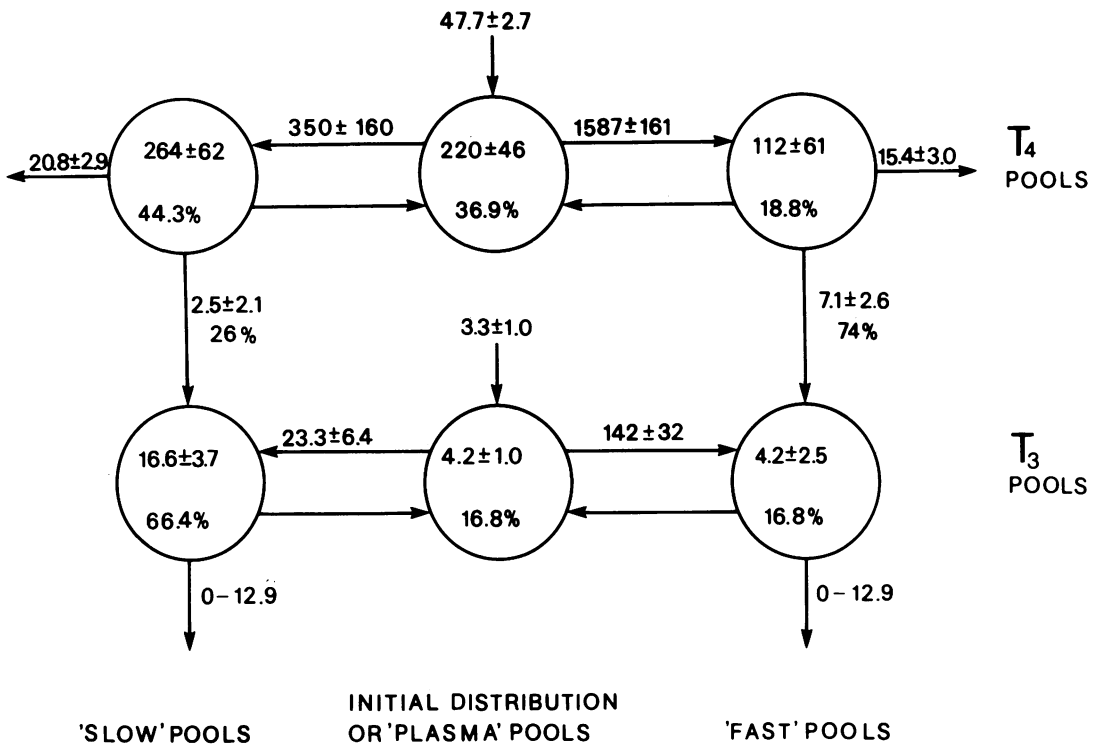

Figure 10. Mean results from MC analysis of T_4 and T_3 kinetics in subjects with congenital TBG excess; see legend of Fig. 8 for explanations. Additional kinetic parameters are easily computed using the formulas reported in the legend of Fig. 8: T_4 TDV = 4.05 liters/m^2, T_3 TDV = 11.8 liters/m^2, T_4 to T_3 CR = 24.2%.

The reduction of T_4 MCR in FDH subjects was less marked than that found in the increased TBG state, indicating that the T_4 carried by abnormal albumin is more available to peripheral tissues than the T_4 carried by TBG. Accordingly, both T_4 TR and T_4 FCR were diminished less in FDH (40% and 20%, respectively) than in TBG subjects (53% and 32.8%, respectively) in comparison with the control group. Furthermore, the higher T_4 availability to peripheral tissues in FDH subjects is confirmed by their normal TDV values whereas in high-TBG subjects TDV was significantly decreased and actually superimposable on the distribution volume of TBG itself (36). TDV values in our FDH cases were similar to those reported by Hennemann et al. (1) and Mendel and Cavalieri (10), who, however, calculated TDV values in the control subjects higher than those obtained by other authors (35, 37, 38) and by us.

Our findings are apparently in contrast with in vivo observations of Mendel and Cavalieri (10) and Hennemann et al. (1) in humans and of Cefalu et al. (11) in rat liver. These authors argue against a special role of albumin in FDH in T_4 transport. Concerning the experimental study, the question arises whether the rat liver can serve as a valid model for studying overall T_4 availability to peripheral tissues in humans. For the studies in human beings, this discrepancy could stem from differences in the methods used for the in vivo kinetic evaluation or from differences in the subjects studied. The former possibility is unlikely because the results obtained in the present study in the subjects with increased TBG and in the control group are in agreement with those reported previously (35, 37, 38).

The highly significant, inverse correlation ($r = 0.96$; $P < 0.001$) observed between T_4 MCR and the serum T_4 concentration suggests the existence in FDH of a variability in the affinity and/or capacity of the abnormal albumin-binding sites. The correlation holds also when the data of Henneman et al. (1) ($r = -0.96$; $P < 0.001$) and those of Mendel and Cavalieri (10) ($r = -0.87$; $P < 0.001$), who normalized the data by body surface area and body weight respectively, are alternatively included (Fig. 5). It is worth noting that the affinity of the abnormal albumin-binding site for T_4 can vary, as shown by our measurements of the K_a of abnormal albumin and T_4 MCR in the same FDH subject (case 6 and 6*bis*) at different times; the observed reduction of K_a and of serum T_4 in this subject was associated with a relatively larger increase of T_4 MCR and, consequently, of T_4 PR (see Table III and Fig. 5). Although the precise mechanism responsible for this phenomenon is unknown, the explanation could include a ligand–carrier protein interaction involving a conformational change in some of the albumin-binding sites (39, 40). The significant increase in T_4 PR in our FDH subjects can be accounted for by a relatively lower reduction of T_4 MCR in this condition in comparison to that observed in congenital elevation of TBG. In fact, it is remarkable that for identical or similar values of serum T_4 concentration, the subjects with high TBG showed much lower T_4 MCR values than the FDH subjects (Fig. 5; Table III). Therefore, the disagreement of the T_4 PR in the present series with those previously reported (1, 10), can be explained by differences among the subjects studied. In fact, in the subgroup of subjects with relatively higher total serum T_4 concentration (18.5 ± 0.76 μg/100 ml, subgroup B), the increment in T_4 PR over the control value was only

15.3% (not significant), and this figure is similar to the data reported by Hennemann et al. (1) (total T_4 17.7 ± 0.9 μg/100 ml, T_4 PR increase 20%). In contrast, an increased T_4 availability in the FDH subjects with relatively lower total serum T_4 concentrations (T_4 15.8 ± 0.37 μg/100 ml, subgroup A) is supported by the finding of a T_4 MCR value not different from the control value, and of a significant increase in the T_4 production rate (see Fig. 6).

The increase in peripheral T_3 PR in the face of an unchanged T_3 thyroidal secretion, and of a small but significant reduction of T_4 to T_3 conversion confirms the increased T_4 bioavailability. The observed decrease in the overall T_4 to T_3 conversion ratio and the highly significant increase in the amount of T_3 produced in slowly exchanging tissue pools suggest the presence of FDH of a peripheral autoregulatory mechanism attenuating the effect of increased T_4 availability. The significant increment in total serum T_3 can be ascribed to augmented peripheral T_3 PR, rather than to increased T_3 affinity of the abnormal albumin-binding sites, as suggested by Ruiz et al. (4). Our hypothesis is consistent with the finding of normal values of T_3 MCR and FCR in FDH subjects, as opposed to the reduction of these parameters in subjects with congenital elevation of TBG (Table III).

The consensual change of total serum T_4 concentration and overall T_4 to T_3 conversion ($n = 7$, $r = 0.70$) in FDH subjects is at variance with the inverse correlation between these parameters reported by other authors and by us in subjects without alteration of thyroid hormones serum carrier proteins (12, 41, 42). However, the overall T_4 to T_3 conversion ratio appears to bear a closer relationship to T_4 PR than to serum T_4 concentration. This is supported by the tight inverse correlation between T_4 to T_3 conversion and T_4 PR which emerged when all the studies (normals, FDH, and high-TBG subjects, hypothyroid patients) were pooled (Fig. 7). This finding may reflect the main peripheral tissue autoregulatory mechanism for maintaining T_3 production rate and circulating T_3 levels, because T_4 to T_3 conversion only occurs within the cells, where T_4 concentration may be different from that observed in the serum. In FDH the large increase in the amount of T_4 carried by abnormal albumin molecules, and the parallel decrement in the T_4 carried by TBG (Fig. 2), can be viewed as facilitated serum hormone transport into the peripheral tissues. With regard to this, it should be borne in mind that the measured affinity constant for T_4 of the abnormal albumin-binding sites was still ~ 1,000-fold lower than the affinity constant of TBG binding sites (Table II).

The increased bioavailability of T_4 in FDH subjects, contrasted with the situation in congenital elevation of TBG, and particularly their increased T_4 disposal rate suggests an independent, special role of normal albumin for T_4 delivery to the peripheral tissues. This hypothesis is consistent with the finding of a dramatic increase in T_4 MCR and FCR in normal or hypothyroid patients with congenital absence of TBG, where T_4 is carried exclusively by albumin and thyroxine-binding prealbumin (43–46). The lack of correlation between the T_4 disposal rate and its free serum concentration in subjects with inherited absence of TBG, in the face of a normal daily T_4 PR and reduced circulating free T_4 levels (47), can again be explained by the higher availability for entry into the peripheral tissues of T_4 carried by normal albumin as compared with T_4 carried by TBG. This is in contrast to the results reported by Cefalu et al. (11), who observed that FDH albumin, like TBG, delays the uptake of T_4 by rat liver cells. However, they found a significantly greater hepatic T_4 extraction from FDH serum in comparison with normal, but they did not find any difference in the concentration of bioavailable T_4 between FDH and control serum. These data are in agreement with the findings of in vivo kinetic studies in humans of Mendel and Cavalieri (10), who showed that the absolute rate of T_4 flux into the rapidly exchangeable cellular compartment (liver) is normal in FDH. Both authors concluded that FDH albumin, like TBG, sequesters T_4 in the plasma compartment.

In conclusion, the present study indicates that the T_4 bound to FDH albumin-binding sites, although less available than T_4 bound to normal albumin, still is more available to peripheral tissues than T_4 carried by TBG. Our data are in agreement with those of Premachandra et al. (48), who found enhanced rate of red cells and lymphocytes uptake of T_4 from sera of FDH subjects.

In species such as man, the evolutionary development of a specific thyroxine-binding protein (TBG) is associated with a large increase in extracellular thyroid hormone reservoir, directly regulating the circulating levels of T_4 and T_3. On the contrary, the primary role of albumin-mediated hormone transport to the tissues could be based on a mechanism involving ligand–serum carrier protein interaction. In a previous paper, we provided evidence that the hormone-binding protein interaction proceeds through the fast formation of a labile hormone–protein complex that successively rearranges to give the final complex and that the intermediate hormone–protein complex dissociates faster than the final complex. Moreover, the fraction of the T_4 bound in the labile intermediate complex is ~ 30% of the albumin-bound T_4 but only 0.4% of the TBG-bound T_4. This implies that a definite fraction of bound hormone can be released from the carried protein in very short times (39, 49, 50).

The consequence of a very high dissociation rate of a loosely albumin bound hormone is that, if the free hormone is lost from the bloodstream across the capillary walls, a quasi-equilibrium can be restored almost instantaneously through prompt dissociation of the bound hormone in the intermediate complex. In this way, not only is the actual free hormone made available to peripheral tissues but also a substantial part of the albumin-bound hormone can be used even in tissues characterized by a short capillary transit time.

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