Effect of Thyroid-Suppressive Doses of Triiodothyronine on Thyroxine Turnover and on the Free Thyroxine Fraction

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ABSTRACT The relationship between free thyroxine concentration and thyroxine turnover was studied during thyroid suppression with triiodothyronine. Although there was some increase in the proportion of serum thyroxine bound to thyroxine-binding globulin, the ratio of ultrafilterable to protein-bound hormone was not significantly affected. The fractional disappearance rate of thyroxine increased from an average control value of 11.47%/day to 14.72%/day. Because of contraction of the thyroxine distribution space the clearance of thyroxine was less markedly affected, increasing from 1.37 to 1.56 liters/day. Since the ratio of thyroxine turnover to free thyroxine concentration, i.e., the free thyroxine clearance, increased proportionately (4.79–5.55 liters $\times 10^3$/day) we conclude that triiodothyronine stimulates thyroxine clearance by a mechanism that is independent of effects on free thyroxine concentration.

INTRODUCTION

The hypothesis advanced by Recant and Riggs (1), that free thyroxine concentration is a determinant of thyroxine turnover rate, is now supported by a considerable body of evidence (2–5). However, as Riggs points out, the observed second order relationship between serum thyroxine concentration and thyroxine turnover which seems to support the free thyroxine hypothesis would also result if thyroxine stimulated metabolic processes which are responsible for its own catabolism (6). This would be consistent with the effect of thyroxine on the metabolism of other substances, e.g., insulin (7), cortisol (8, 9), and albumin (10, 11). Unfortunately, since the hormonal activity of thyroxine is generally proportional to free thyroxine concentration, a mass law effect of free thyroxine concentration on turnover is difficult to distinguish from an effect of hormonal action. To differentiate these effects it seemed advantageous to vary independently the concentration of free thyroxine and the metabolic activity of the hormone. With this purpose we undertook to investigate the relationship between free thyroxine concentration and thyroxine turnover during triiodothyronine suppression of thyroxine secretion. We anticipated that thyroid suppressive doses of triiodothyronine (12) would decrease free thyroxine concentration by decreasing total serum thyroxine concentration without diminishing the concentration of unoccupied thyroxine-binding sites. Since the hormonal action of triiodothyronine is similar to that of thyroxine, the metabolic effects of thyroid hormone would be maintained or possibly increased despite a decreased free thyroxine concentration.

METHODS

Studies were performed on six medical residents. None of the subjects had a history of thyroid disease, thyroid

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enlargement, or other clinical evidence of abnormal thyroid function. L-3,5,3′-Triiodothyronine [1] was administered in a dose of 25 μg t.i.d. Thyroxine turnover and binding studies were performed during a control period and again during the 3rd wk of triiodothyronine treatment. The initial studies were done on subjects M. O., K. E., and N. O. Subsequently, studies on S. C., M. C., and L. Y. were carried out. Different batches of thyroxine-^{131}I [2] were used to determine thyroxine binding and turnover in the two groups.

Serum thyroxine binding. The ratio of free to protein-bound thyroxine in serum, the free thyroxine fraction, FTF, was determined by ultrafiltration of serum containing predialyzed thyroxine-^{131}I (13). Because of its low value this fraction is most conveniently expressed as a percentage.

$$\text{FTF} = \frac{\text{ultrafiltrate thyroxine-}^{131}\text{I cpm}}{\text{serum protein-bound thyroxine-}^{131}\text{I cpm}} \times 100. \quad (1)$$

Free thyroxine concentration, $[T_4]$, is the product of FTF and the serum protein-bound thyroxine concentration, $[T_4 \sim P]$.

$$[T_4] = \text{FTF} \times [T_4 \sim P]. \quad (2)$$

Thyroxine binding to individual serum proteins was assessed by conventional paper electrophoresis of sera containing thyroxine-^{131}I in Tris-maleate buffer at pH 8.6 (14). After electrophoresis, ^{131}I activity on the paper strips was determined by an integrating, gas flow chromatogram scanner. The paper was then stained to identify serum proteins. Activity associated with each binding protein was expressed as a percentage of the total activity on the strip.

The subjects of this study received five drops of Lugol's solution twice daily to minimize thyroidal uptake of ^{131}I. Serum iodothyronines were therefore separated from iodide by a resin column before measurement of iodine content.[3] The method used does not distinguish between thyroxine and triiodothyronine.

Thyroxine metabolism. Thyroxine turnover was studied by following the decrease of serum radioactivity after the intravenous injection of a tracer dose of thyroxine-^{131}I (15). Thyroxine-^{131}I was diluted to 10 μc/ml in 5% human serum albumin on arrival and injected within 24 hr. The dose for each study was approximately 40 μc. A linear regression equation for the logarithm of serum radioactivity was obtained by the method of least squares. We have used the notation of Rall, Robbins, and Lewallen, (5) with some modification, in the turnover studies. In order to avoid possible bias introduced by graphic analysis of the regression line, values for the half-time of disappearance, $t_{\frac{1}{2}}$, fractional disappearance rate, k, and the thyroxine distribution space, V_{ST_4}, were obtained directly from the regression equation. (See Appendix.)

Serum thyroxine clearance, C_{ST_4}, was calculated as follows:

$$C_{ST_4} = k \times V_{ST_4}. \quad (3)$$

Thyroxine turnover, ρ, is defined by the following equation:

$$\rho = k \times V_{ST_4} \times [ST_4], \quad (4)$$

where $[ST_4]$ is the serum thyroxine concentration.

In order to evaluate the relationship between thyroxine turnover and free thyroxine concentration the ratio $\frac{\rho}{[T_4]}$ was calculated. The dimensions of this ratio are volume/time. The ratio is by definition the clearance of free thyroxine, C_{T_4}.

From equations 2 and 4:

$$C_{T_4} = \frac{\rho}{[T_4]} = \frac{k \times V_{ST_4} \times [ST_4]}{\text{FTF} \times [T_4 \sim P]}. \quad (5)$$

By definition:

$$[ST_4] = [T_4 \sim P] + [T_4]. \quad (6)$$

However, $[T_4]$ is only $0.026 \pm 0.004\%$ of $[T_4 \sim P]$ (13) and, within the limits of accuracy of the studies reported here, $[ST_4]$ does not differ significantly from $[T_4 \sim P]$. Therefore,

$$C_{T_4} = \frac{k \times V_{ST_4}}{\text{FTF}}, \quad (7)$$

or from equation 3:

$$C_{T_4} = \frac{C_{ST_4}}{\text{FTF}}. \quad (8)$$

Statistical analysis of data was performed according to methods described by Snedecor (16).

RESULTS

Except for one subject, M. I., who complained of insomnia and irritability, there was no evidence of increased thyroid hormone effect during triiodothyronine administration. However, basal meta-

TABLE I

Depression of Serum Iodothyronine-Iodine Concentration by Triiodothyronine Administration

Subject		Iodothyronine-iodine μg/100 ml serum
S. C.	Control	4.4
	Triiodothyronine	2.3
M. C.	Control	5.4
	Triiodothyronine	2.6
L. Y.	Control	4.3
	Triiodothyronine	2.4

[1] Cytomel, Smith Kline & French Laboratories, Philadelphia, Pa.

[2] Abbott Laboratories, North Chicago, Ill.

[3] Thyroxine by column. Bio-Science Laboratories, Van Nuys, Calif.

TABLE II

Effect of Triiodothyronine on the Binding of Serum Thyroxine*

Subject		Endogenous ST_4				+130 μg thyroxine/100 ml serum
		FTF	TBG	Alb	TBPA	TBG
		%	%	%	%	%
M. O.	Control	0.029				
	Triiodothyronine	0.029				
K. E.	Control	0.024	59.26	26.85	13.89	16.15
	Triiodothyronine	0.026	71.63	19.85	8.51	19.83
N. O.	Control	0.034	60.29	21.53	18.18	15.38
	Triiodothyronine	0.030	64.97	27.65	7.37	16.27
S. C.	Control	0.027	50.00	23.94	26.05	19.23
	Triiodothyronine	0.023	56.69	26.75	16.56	14.41
M. C.	Control	0.029	54.05	24.32	21.62	14.37
	Triiodothyronine	0.028	65.38	13.46	21.15	14.10
L. Y.	Control	0.030	42.71	10.42	46.87	13.29
	Triiodothyronine	0.031	48.37	12.64	38.95	14.03
Average	Control	0.029	53.26	21.41	25.32	15.68
	Triiodothyronine	0.028	61.41	20.07	18.51	15.72
		N.S.	$P < 0.01$	N.S.	$P < 0.025$	N.S.

ST_4, serum thyroxine concentration; FTF, free thyroxine fraction; TBG, thyroxine-binding globulin; alb, albumin; TBPA, thyroxine-binding prealbumin.

* 25 μg t.i.d. for 3 wk.

bolic rate was not measured, and a change in metabolic rate cannot be excluded. Triiodothyronine decreased serum iodothyronine-iodine concentration in three subjects in whom it was measured (Table I).

The results of thyroxine-binding studies are shown in Table II. There was no consistent change in FTF during triiodothyronine treatment. Since serum protein-bound thyroxine concentration could not be determined in the presence of exogenous triiodothyronine, $[T_4]$ was not estimated. However, on the basis of the depression of iodothyronine-iodine concentration in this and previous studies (17), it can be assumed that thyroxine concentration was decreased by triiodothyronine administration. In the absence of a change in FTF this assumption leads to the conclusion that $[T_4]$ was also decreased.

Electrophoretic analysis of serum thyroxine binding was done in five of the six subjects. In each of these subjects the percentage of total serum thyroxine bound to thyroxine-binding globulin (TBG) increased during triiodothyronine administration. After enrichment with 130 μg of thyroxine per 100 ml, there was no longer a significant difference in the percentage of serum thyroxine bound to TBG before and during triiodothyronine treatment. Thyroxine binding to TBG at this level of serum thyroxine concentration is considered to represent the TBG binding capacity (3). If we assume that the initial concentration of serum thyroxine was within normal limits in these subjects, the contribution of endogenous thyroxine to the calculated binding capacity would be only about 5%. Calculated on the basis of exogenous thyroxine alone, i.e., per cent serum thyroxine-^{131}I bound to TBG × 130 μg of thyroxine per 100 ml, the average TBG binding capacity was 20 μg of thyroxine per 100 ml both before and during triiodothyronine administration. This value is within the normal range for this method.

The increased proportion of thyroxine bound to

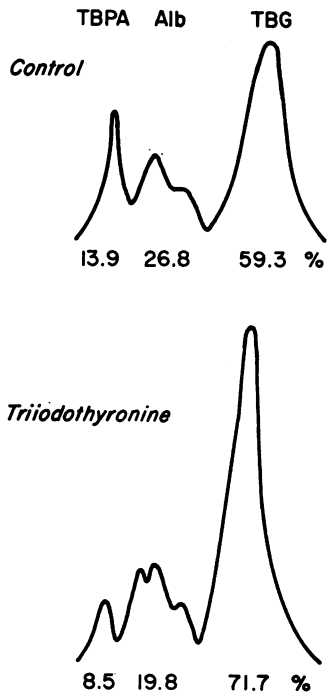

FIGURE 1 Distribution of thyroxine-^{131}I in serum of subject K. E. *TBG,* thyroxine-binding globulin; *Alb,* albumin; *TBPA,* thyroxine-binding prealbumin.

TBG during triiodothyronine suppression was associated with decreased binding of thyroxine-^{131}I to thyroxine-binding prealbumin (TBPA). Control levels of binding to TBPA were less than those reported by Ingbar and Freinkel, who used the same method (3). This discrepancy was probably due to trailing of prealbumin activity into the albumin zone in the present study. TBG and albumin were well separated in all electrophoretic analyses (Fig. 1). TBPA capacity was not measured.

During triiodothyronine administration the fractional disappearance rate of thyroxine, k, increased in each subject (Fig. 2). Fig. 3 shows the reciprocal relationship between k and the thyroxine distribution space, V_{ST_4}. Since thyroxine clearance, C_{ST_4}, is the product of k and V_{ST_4}, it reflects the opposing tendencies of changes in these factors. Consequently, the increase in C_{ST_4} was relatively smaller than the increase in k. The effects of k and V_{ST_4} on C_{ST_4} are illustrated by the relation of control and treatment points to isobars of constant C_{ST_4} in Fig. 3. The clearance of free thyroxine, C_{T_4}, was increased in five of the six subjects.

TABLE III

*Thyroxine Turnover Studies before the Administration of Triiodothyronine**

Subject		V_{ST_4}	k	C_{ST_4}	C_{T_4}
		liters	*%/day*	*liters/day*	*liters* $\times 10^3$*/day*
M. O.	Control	12.90	9.94	1.28	4.41
	Triiodothyronine	9.56	15.26	1.46	5.03
K. E.	Control	12.12	11.21	1.36	5.66
	Triiodothyronine	10.83	13.46	1.46	5.62
N. O.	Control	10.49	14.17	1.49	4.38
	Triiodothyronine	10.41	15.23	1.59	5.30
S. C.	Control	11.83	11.83	1.40	5.19
	Triiodothyronine	10.05	14.53	1.46	6.08
M. C.	Control	11.82	9.90	1.17	4.03
	Triiodothyronine	10.08	14.71	1.48	5.29
L. Y.	Control	12.87	11.77	1.52	5.06
	Triiodothyronine	12.52	15.16	1.90	5.94
Average	Control	12.01	11.47	1.37	4.79
	Triiodothyronine	10.57	14.72	1.56	5.55
		$P < 0.05$	$P < 0.005$	$P < 0.025$	$P < 0.01$

V_{ST_4}, thyroxine distribution space; k, fractional disappearance rate; C_{ST_4}, serum thyroxine clearance; C_{T_4}, free thyroxine clearance.

* 25 μg t.i.d. for 3 wk.

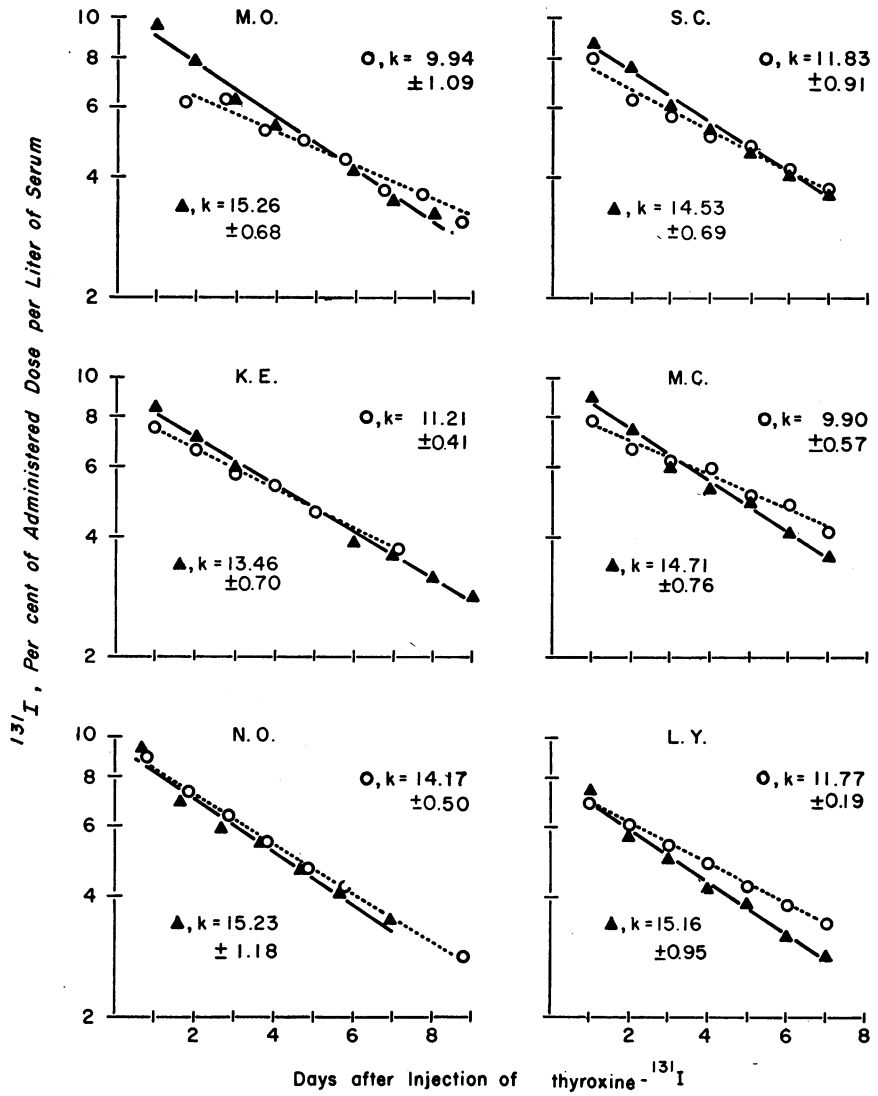

FIGURE 2 Thyroxine-^{131}I disappearance. O----O are control observation; ▲——▲ are observations during triiodothyronine administration. k = fractional disappearance rate ± standard deviation.

The relative magnitude of the average increase in C_{T_4} was slightly but not significantly greater than that of the increase in C_{ST_4} (Table III).

DISCUSSION

The FTF is determined by the concentration (and association constants) of unoccupied thyroxine-binding sites (18). Since the unoccupied binding sites of each class considerably outnumber those to which thyroxine is bound (3), changes in serum iodothyronine concentration result in numerically equal but proportionately much smaller changes in the concentration of unoccupied binding sites and correspondingly small changes in FTF. Therefore, it is not surprising that no significant decrease in FTF was observed with the decrease in iodothyronine-iodine concentration during triiodothyronine administration.[4] These

[4] Iodothyronine concentration could not be calculated from serum iodothyronine-iodine, since the relative contributions of thyroxine and triiodothyronine were unknown. However, if one assumes 85% absorption of the administered triiodothyronine and a triiodothyronine clearance of 16 liters/day (19), the contribution of ex-

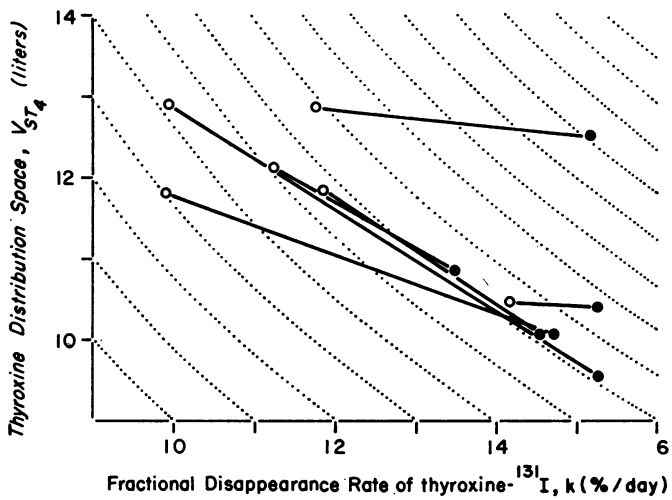

FIGURE 3 The effect of triiodothyronine on the thyroxine distribution space, V_{ST_4}, and the fractional disappearance rate of ^{131}I-thyroxine, k. ○ = control. ● = observations during triiodothyronine administration. Dotted lines represent isobars of constant clearance, C_{ST_4}.

findings are consistent with those of Levy, Marshall, and McGuire who showed that triiodothyronine administration does not affect triiodothyronine-^{131}I resin uptake (20). Before the development of techniques for the direct measurement of free thyroxine, Ingbar and Freinkel showed that triiodothyronine in doses (200 μg q.d. × 60 days) sufficient to produce thyrotoxicosis medicamentosa and an accelerated fractional disappearance of thyroxine-^{131}I did not affect the electrophoretic distribution of thyroxine or the binding capacity of TBG (21). Unfortunately these studies were done in Veronal buffer which, as Ingbar subsequently demonstrated, inhibits prealbumin binding of thyroxine (22). If, as occurs in Graves' disease, prealbumin-binding capacity was decreased in these patients FTF would have been increased despite a normal TBG capacity. At the lower doses of triiodothyronine used in the present study and with electrophoresis in Tris-maleate buffer, the percentage of thyroxine bound to TBG increased. This increase did not appear to be due to an increase in the total concentration of TBG since, at a high concentration of added cold thyroxine, binding patterns were similar in control sera and during triiodothyronine treatment. It is possible that increased binding to TBG occurred as a result of decreased prealbumin binding capacity but this seems unlikely since FTF was not increased. The increased percentage of serum thyroxine bound to TBG during triiodothyronine seems most consistent with the known inverse relationship between the percentage of serum thyroxine bound to TBG and total serum thyroxine concentration (2). In contrast to the insensitivity of the FTF to small changes in thyroxine concentration, Ingbar and Freinkel have shown that the addition to serum of as little as 2.0 μg of thyroxine per 100 ml (or 1.3 μg of thyroxine iodine) decreases the fraction of serum thyroxine bound to TBG (3).

The average fractional disappearance rate (k = 11.47%) in these subjects during the control period is similar to that observed by Gregerman, Gaffney, and Shock (23) in the same age group. Previous studies of thyroxine turnover and binding have shown that k usually varies inversely with serum thyroxine-binding affinity. Thus where binding affinity is increased as in myxedema, estrogen treatment, and idiopathic hyperTBGemia, k is decreased. Conversely, where serum-binding affinity is decreased as in thyrotoxicosis, salicylate treatment, and hypoTBGemia, k increases (3, 5). In the present study k increased during triiodothyronine administration whereas the free thyroxine fraction and its reciprocal, serum thyroxine-binding affinity, remained unaltered. It is reasonable to conclude that the more rapid k during triiodothyronine treatment was not due to an increased proportion of free thyroxine in serum. However, it is possible that part of the increase in k resulted from the decreased V_{ST_4}. This hypothesis would imply that contraction of V_{ST_4} increased the proportion of exchangeable thyroxine in the

ogenous triiodothyronine to serum iodothyronine-iodine would be only 0.2 μg/100 ml. If this value is correct the decrease in iodothyronine concentration would be almost proportional to the decrease in iodothyronine-iodine concentration.

subcompartment of V_{ST_4} from which thyroxine is actively removed. An advantage of calculating clearance rather than fractional disappearance rate is that the former is unaffected by reciprocal changes in volume of distribution and fractional disappearance rate. If the decreased V_{ST_4} were sufficient explanation for the acceleration of k by triiodothyronine, $k \times V_{ST_4}$, or C_{ST_4} would be constant. The increase in C_{ST_4} shows that the acceleration of k is out of proportion to, and therefore not entirely explained by, the contraction of V_{ST_4}. It also appears that increased clearance contributes to the depression of serum thyroxine concentration during triiodothyronine administration.

During the control period V_{ST_4} averaged 14.6% of body weight. This value is in reasonably good agreement with the mean value of 13.4% of body weight obtained by Rall and coworkers (5) in their review of thyroxine turnover studies from a number of laboratories.[5] The contraction of V_{ST_4} during triiodothyronine administration is difficult to explain. It seems unlikely that it was due to increased competition for the exchangeable thyroxine by serum proteins, since the total binding affinity of these proteins (as measured by the FTF) was not significantly increased. The decrease in V_{ST_4} may be related to redistribution of thyroxine among the serum thyroxine-binding proteins. However, this hypothesis is subject to the criticism that V_{ST_4} is not consistently affected in clinical conditions where the electrophoretic distribution of thyroxine is markedly altered. Possibly triiodothyronine decreased the binding affinity of cellular sites, but this is entirely speculative.

The hypothesis that free thyroxine is available to cellular sites that remove thyroxine from the exchangeable thyroxine pool implies that, *ceteris paribus*, changes in the concentration of free thyroxine will result in similar changes in thyroxine turnover. Effects on thyroxine binding and turnover in a variety of situations have been at least qualitatively consistent with this hypothesis (5). However, a number of observations suggest that thyroxine turnover may change without proportional changes in binding. The persistence of an altered fractional thyroxine turnover rate after treatment of thyrotoxicosis (21) or hypothyroidism (27), the age-related decrease in turnover rate (23, 28), and the early postoperative changes in thyroxine turnover (29), seem to be due to factors other than altered binding. With the development of techniques for measuring the ratio of free to bound thyroxine directly, it is possible to relate free thyroxine concentration and thyroxine turnover quantitatively. The most direct way of presenting this relationship is as the ratio of thyroxine turnover to free thyroxine concentration, i.e., the free thyroxine clearance, C_{T_4} (equation 5). To the extent that changes in thyroxine turnover are proportional to changes in $[T_4]$, C_{T_4} will remain constant. The increase in C_{T_4} during triiodothyronine treatment can be interpreted as an increase in the activity of systems removing thyroxine from the free thyroxine pool either directly or secondarily by affecting a pool with which free thyroxine is in equilibrium. This increase is independent of effects on $[T_4]$ itself. The effect of triiodothyronine on C_{ST_4} is accounted for by the increase in C_{T_4} and therefore can also be considered independent of changes in $[T_4]$.

These findings indicate that, at the doses used, triiodothyronine stimulates the fractional disappearance and clearance of serum thyroxine by mechanisms which are distinct from mass law effects of free thyroxine concentration. The general similarity between the metabolic effects of triiodothyronine and thyroxine suggests that thyroxine has a similar stimulatory effect. Although 75 μg of triiodothyronine per day has been considered a replacement dose (5) and did not produce overt thyrotoxicosis in our subjects, there is evidence that this dose has metabolic effects at least during short-term therapy (30). It seems likely that triiodothyronine stimulates cellular processes by which thyroxine is removed from the exchangeable pool. This stimulation may well be part of a general stimulation of metabolic rate and would be consistent with the suggestion by Sterling and

[5] However, the calculated values of V_{ST_4} must be accepted with some reservations. Whereas most estimates of V_{ST_4} have been obtained by extrapolation of the regression line to injection time, this method is subject to the criticism that it assumes that the fractional rate at which thyroxine-^{131}I is removed from the exchangeable thyroxine pool during equilibration is the same as k at equilibrium. Another source of error is the presence in the tracer of rapidly cleared radioactive contaminants which presumably lead to overestimation of V_{ST_4} (24). Serum iodide-^{131}I derived from thyroxine-^{131}I leads to underestimation of V_{ST_4}, but, because of the rapid clearance of iodide, this is no more than 1 or 2% and can be disregarded (25, 26).

Chodos that cellular metabolic rate has a major role in regulating thyroxine turnover (31).

APPENDIX

A regression equation of form $y = mx + \log b$ was derived from the turnover data by the method of least squares.

y = log per cent of thyroxine-^{131}I dose per liter of serum.
x = days after thyroxine-^{131}I injection.
m and b are constants.

$$t_{\frac{1}{2}} = \frac{\log 2}{-m}.$$

$$k = \frac{\ln 2}{t_{\frac{1}{2}}}.$$

Since $\frac{\ln 2}{\log 2} = 2.3$,

$$k = -2.3m.$$

$$V_{ST_4} = \frac{1 \text{ liter}}{b}.$$

ADDENDUM

Since this manuscript was submitted for publication, Nicoloff and Dowling (32) have demonstrated that the fractional deiodination rate of labeled thyroxine is slowest immediately after injection and gradually increases as the tracer equilibrates in the exchangeable thyroxine pool. Similar results were obtained independently in this laboratory.[6] The slower deiodination of tracer thyroxine during equilibration leads to an overestimation of the zero time intercept, (b), by the extrapolation method and a consequent understimation of V_{ST_4}. Acceleration of k would exaggerate this effect and was probably at least partially responsible for the otherwise unexplained contraction of V_{ST_4} during triiodothyronine administration. The calculated increase in C_{ST_4} and C_{T_4} during triiodothyronine administration should therefore be regarded as minimal estimates. The actual increase in these clearances was probably more nearly proportional to the increase in k.

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