

# STUDIES OF THYROID FUNCTION AND THE PERIPHERAL METABOLISM OF I<sup>131</sup>-LABELED THYROXINE IN PATIENTS WITH TREATED GRAVES' DISEASE<sup>1, 2</sup>

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(Submitted for publication May 26, 1958; accepted July 24, 1958)

Recent studies have revealed an abnormality in the peripheral metabolism of thyroxine in patients with active untreated Graves' disease (1). In such patients, an abnormally large fraction of the extrathyroidal pool of thyroxine is degraded daily.

Other authors have also observed an accelerated fractional turnover of thyroxine in patients with untreated Graves' disease (2, 3) and have postulated that the rate of turnover of the hormone might be a function of its concentration in the blood (2). Alternatively, accelerated turnover of thyroxine might merely reflect the general speeding of metabolic processes which occurs during the hyperthyroid state. The present studies were designed to differentiate between these and other possibilities. Rates of turnover of thyroxine have been assessed in patients in whom the thyrotoxicity had been corrected by appropriate therapy, as well as in nonthyrotoxic subjects who were made hypermetabolic by the administration of thyroid hormone. A preliminary report of the data obtained has been published in abstract form (4). It has been shown that the induction of thyrotoxicosis medicamentosa does result in an augmentation of the fractional rate of turnover of thyroxine. However, it has also been demonstrated that accelerated fractional turnover of thyroxine frequently persists in patients with Graves' disease, despite

prolonged correction of the hypermetabolic state, and despite reduction of the protein-bound iodine (PBI) to normal or subnormal values. It is apparent, therefore, that the increase in the fractional rate of thyroxine turnover which occurs in patients with Graves' disease cannot be entirely ascribed to either the concentration of circulating thyroid hormone or the immediate metabolic status of the patient.

## PATIENT MATERIAL

(Table I). Forty determinations of the rate of turnover of thyroxine were made in 23 patients with treated Graves' disease. With but 7 exceptions, all patients had been followed continuously by the authors since the diagnosis of Graves' disease had first been made.

Twenty-two tests were performed in 15 patients who were being treated with propylthiouracil or methylmercaptoimidazole. Duration of symptomatic remission ranged from 2 to 26 months and averaged 12 months. In four instances, tests were performed following 1 month in which antithyroid medication was supplemented by the administration of Lugol's solution, 10 drops, three times daily. Seven additional tests were performed in six of these subjects from 4 to 14 months following withdrawal of propylthiouracil. In these subjects, symptomatic remission had been effected for 17 to 39 months.

Nine tests were performed in seven subjects who had been treated by subtotal thyroidectomy from 7 months to 10 years earlier. One of these patients (No. 12) had previously been studied while receiving antithyroid therapy.

Measurements of the rate of turnover of thyroxine were also made in one patient treated with Lugol's solution and another patient treated with radioactive iodine, five and six years earlier, respectively.

Values were compared with those obtained in normal subjects and in patients with untreated myxedema or thyrotoxicosis. Previously reported data for subjects in these categories (1) have been supplemented by the study of additional patients.

In order to ascertain the reproducibility of the measurement, two determinations of thyroxine turnover were secured in six normal subjects at intervals of three to

<sup>1</sup> This investigation was supported in part by Research Grant No. A-267 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, and in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-412.

<sup>2</sup> Presented in part at the 1955 meeting of the American Society for Clinical Investigation, May 2, 1955, Atlantic City, N. J.

eight months. Values of thyroxine half-time did not vary by more than 0.3 day.

### Methods

**Thyroxine turnover.** The distribution and rate of turnover of thyroxine were measured by a method described previously (1). Briefly, thyroxine (4 to 5  $\mu\text{g.}$ ), labeled with 30  $\mu\text{c.}$  of  $\text{I}^{131}$  was injected intravenously and total daily urinary collections as well as daily specimens of plasma were obtained for the ensuing 9 to 12 days. The virtual volume of distribution of thyroxine was calculated daily. After complete equilibration of the injected isotope, as evidenced by constancy of its volume of distribution, the thyroxine half-time was estimated from the slope of the curve depicting the concentration of the radioactivity in the serum. The fractional rate of turnover of thyroxine was calculated as the quotient,  $0.693/\text{half-time (day)}$ . Daily degradation of hormonal iodine (daily degradation rate) was calculated as the product,  $\text{PBI } (\mu\text{g. per L.}) \times \text{volume of distribution (liters)} \times \text{fractional turnover rate (per cent per day)}$ .

In several instances, in order to avoid economic hardship to the patient, studies were performed on an outpatient basis. In such cases, urinary collections were not made. Fractional rates of turnover of thyroxine were estimated from the slope of the time-concentration curve of radiothyroxine in plasma, employing only data obtained subsequent to the first 48 hours following injection of the tracer. The volume of distribution of thyroxine was estimated, using the isotope dilution principle, by extrapolation of this portion of the curve to zero-time (the time of injection).

It had previously been noted, in patients with untreated Graves' disease, that accelerated fractional turnover of thyroxine rapidly liberated radioiodide which was quickly accumulated by the thyroid gland and released as labeled hormone. This recycled moiety produced an apparent retardation of the rate of disappearance from the plasma of the initially injected radiothyroxine (1). This difficulty could be circumvented if thyroidal reutilization of liberated radioiodide were completely inhibited by antithyroid drugs. Accordingly, all patients in the present studies were given 30 mg. of methylmercaptoimidazole every six hours during the testing procedure. By direct counting over the neck, this dosage of drug was shown to prevent appreciable thyroidal accumulation of the radioiodine liberated by the degradation of labeled thyroxine.

In all instances, determination of the 24 hour thyroidal percentage accumulation of a tracer dose of radioiodine as well as measurements of basal metabolic rate were made within the week prior to the determination of the thyroxine turnover rate. In patients receiving antithyroid drugs, medication was continued in its usual dosage during the measurement of thyroidal radioiodine uptake. Tracer doses of inorganic iodide were suffi-

ciently small (3  $\mu\text{c.}$ ) so that any accumulated  $\text{I}^{131}$  released during the subsequent turnover study would not obscure the true rate of disappearance of injected radioactive thyroxine from the plasma.

**Thyroxine-binding protein.** In eight patients who displayed persistently accelerated turnover of thyroxine, the capacity of the serum's inter-alpha migrating thyroxine-binding protein (TBP) to bind thyroxine was assessed by techniques described in detail elsewhere (5). Sera from these and from normal control subjects were enriched with stable and with  $\text{I}^{131}$ -labeled thyroxine in amounts sufficient to achieve four standard concentrations of added thyroxine over the range from 10 to 160  $\mu\text{g. per cent}$ . Sera were then subjected to electrophoresis in filter paper, using veronal buffer, pH 8.6, ionic strength 0.05. The distribution of radioactivity in the individual electrophoretograms was ascertained in an automatically recording strip-scanner. Scanning patterns were then traced onto hard paper of uniform thickness. The weight of the tracing of the radioactive components associated with albumin and with TBP were determined on an analytical balance, and were expressed as a percentage of the total weight of the tracing. Values from normal subjects and from patients with treated Graves' disease were compared.

**Suppression of thyroidal uptake of  $\text{I}^{131}$ .** On 30 occasions in 21 patients, the ability of exogenous thyroid hormone to suppress thyroidal accumulation of radioiodine was assessed. Three patients were studied before and after the oral administration of 75  $\mu\text{g.}$  of triiodothyronine daily for three weeks. Eighteen patients received desiccated thyroid (U.S.P.) according to the following dosage schedule: following three weeks of an initial dosage of 2 or 3 gr. daily, thyroidal uptake was repeated. If suppression had not been achieved, the dosage was increased by 2 gr. daily during each two week period until suppression was achieved or until a dosage of 8 or 9 gr. daily had been employed. In one patient (No. 13), this regimen was discontinued at a dosage of 5 gr. daily because of severe symptoms of thyrotoxicity. Suppression was considered to have occurred if the 24 hour thyroidal uptake of radioiodine was decreased either to less than 10 per cent of the administered dose or to less than half of its initial value.

In six of the patients in whom suppression was achieved, thyroidal uptakes were allowed to return to control values. During the ensuing three to four weeks, patients were given quantities of inorganic iodine equal to the iodine content of the suppressive dose of desiccated thyroid. Thyroidal uptake was then re-evaluated.

**Induction of thyrotoxicosis medicamentosa (Table II).** The effect of artificially induced thyrotoxicosis on the peripheral turnover of thyroxine was assessed in four patients. One patient with nontoxic nodular goiter was given 10 gr. of desiccated thyroid daily. One patient with treated Graves' disease, one with nontoxic goiter, and one patient with primary amenorrhea were given 200  $\mu\text{g.}$  of triiodothyronine daily. Studies of the peripheral turnover of thyroxine were performed before and at the end of a 60 day treatment period.

<sup>3</sup> Obtained from Abbott Laboratories, Oak Ridge, Tenn.

TABLE I  
Summary of data obtained in patients with treated Graves' disease

Patient	Sex	Age	Rx	Duration of remission (months)	Thyroxine half-time (days)	Protein-bound iodine ( $\mu\text{g. \%}$ )	Organic iodine degradation ( $\mu\text{g./day}$ )	Thyroidal 24-hour $\text{I}^{131}$ uptake (% dose)	Suppression	Basal metabolic rate
Studied during antithyroid therapy										
1	F	33	PTU*	7	5.9	6.0	77	62	—	+ 3
			PTU	14	5.2	5.2	64	61	Yes	+ 7
2	F	34	PTU & Lugol's	11	5.5	—	—	—	—	- 7
			PTU	17	5.1	5.8	97	80	No	- 1
3	M	43	PTU	8	3.9	1.2	67	74	Yes	+ 3
			PTU & Lugol's	14	4.9	—	—	—	—	+ 5
4	F	35	PTU	5	4.8	2.2	26	55	Yes	+12
5	F	35	PTU	14	5.0	3.5	60	59	Yes	0
6	M	43	PTU	6	4.0	2.4	37	52	Yes	- 5
			PTU	22	4.6	4.0	50	46	Yes	+ 3
7	F	37	MMI†	18	5.2	1.5	22	92	Yes	-27
8	M	38	PTU	26	5.8	4.7	56	43	Yes	+ 4
9	F	47	PTU	2	5.2	5.7	59	56	No	+10
			PTU & Lugol's	4	5.8	—	—	—	—	+ 4
10	F	62	PTU	10	4.5	1.7	23	68	Yes	-15
			PTU & Thyroid	15	3.8	6.3	102	(2)‡	Yes	+18
			PTU & Lugol's	19	3.4	—	—	—	—	+ 6
11	M	15	PTU	6	4.0	3.6	46	89	Yes	+ 3
12	F	40	PTU	6	3.6	4.5	71	40	No	+ 7
13	F	48	PTU	10	6.0	4.4	46	70	No	+ 5
14	F	44	PTU	7	4.6	2.8	40	85	Yes	- 1
15	F	46	MMI	8	4.9	1.9	23	49	Yes	-30
			Mean	12	4.8	3.7	54	64		0
			Std. dev.	6	0.8	1.6	25	16		12
Studied after cessation of antithyroid therapy										
1	F	34	PTU	27	5.2	6.5	85	45	—	+ 7
2	F	35	PTU	29	6.3	6.1	67	34	Yes	+ 8
4	F	36	PTU	17	5.5	5.6	62	70	Yes	+ 3
			PTU	35	7.9	6.2	50	58	Yes	- 1
5	F	37	PTU	39	5.4	5.2	74	53	—	+ 6
11	M	16	PTU	20	4.1	5.0	70	65	Yes	+10
14	F	45	PTU	20	5.8	5.4	68	47	Yes	- 2
			Mean	27	5.7	5.7	68	53		+ 4
			Std. dev.	8	1.2	0.5	11	12		5
Studied after surgical therapy										
12	F	41	—	18	5.0	3.8	57	46	Yes	+ 4
		42	—	31	4.6	4.0	67	24	Yes	- 5
16	M	34	—	7	5.2	5.1	67	53	—	+ 2
		35	—	21	5.8	4.6	57	48	Yes	+ 1
17	F	36	—	10 yrs.	6.2	6.5	73	30	Yes	+ 5
18	F	65	—	5 yrs.	6.6	5.0	47	81	—	+12
19	F	67	—	4-1.5 yrs.§	7.1	6.9	63	—	—	- 4
20	F	53	—	10 yrs.	7.1	3.5	35	22	Yes	-10
21	F	21	—	7 yrs.	6.1	5.0	51	32	Yes	- 5
			Mean	4.7 yrs.	6.0	4.9	57	42		0
			Std. dev.	3.6	0.9	1.2	12	20		7
Miscellaneous therapy										
22	F	24	Lugol's	5	4.8	5.0	43	18	Yes	+ 8
23	F	53	$\text{I}^{131}$	6	5.1	5.6	64	54	Yes	+ 6
			Grand mean	28	5.2	4.5	57	55		+ 1
			Std. dev.	29	1.0	1.4	19	19		9

\* Propylthiouracil.

† Methyl-mercaptoimidazole.

‡ Value obtained during suppression with desiccated thyroid.

§ Surgery 1.5 years after initiation of antithyroid therapy.

TABLE II  
*The effect of diiodotyrosine and of induced hypermetabolism on the peripheral degradation of thyroxine*

Patient	Diagnosis	Status	Thyroxine half-time	PBI	Thyroxine degradation	BMR
			days	μg. %	μg. I/day	
17	Treated Graves' disease	Control	6.2	6.6	74	+ 3
		Diiodotyrosine*	6.0	7.4	82	+ 6
24	Nontoxic goiter	Control	5.9	5.2	54	-10
		Diiodotyrosine*	6.0	5.3	55	-12
20	Treated Graves' disease	Control	7.1	4.8	42	+ 3
		Diiodotyrosine*	6.8	5.1	47	+ 5
		Triiodothyronine†	5.0	1.7	(21)§	+28
25	Nontoxic goiter	Control	8.0	6.8	58	-15
		Triiodothyronine†	5.1	1.7	(19)	+25
26	Secondary amenorrhea	Control	8.7	6.7	44	+ 5
		Triiodothyronine†	4.5	4.6	(32)§	+31
27	Nontoxic goiter	Control	6.8	6.0	70	+ 3
		Desiccated thyroid‡	5.2	12.3	170	+41

\* Chromatographically pure diiodotyrosine, 500 μg. P.O. daily for 60 days.

† Cytomel®, 200 μg. P.O. daily for 60 days.

‡ Desiccated thyroid, U.S.P., 10 gr. daily for 60 days.

§ Values in patients receiving triiodothyronine, indicated in parentheses, are presumed to be inaccurate, since neither the rate of turnover of triiodothyronine nor its percentage contribution to the PBI were determined.

In addition, studies of thyroxine turnover were performed in two patients with treated Graves' disease and one patient with nontoxic nodular goiter before and after a 60 day treatment period with 500 μg. daily of diiodotyrosine.

*Chemical analysis and radioactive assay.* Concentrations of protein-bound iodine (PBI) were determined by the method of Barker, Humphrey and Soley in sera obtained from each patient at the beginning and the conclusion of the thyroxine turnover study (6).<sup>4</sup> Assays for radioactivity in blood, urine, and thyroid gland were performed as previously described (1).

#### RESULTS (TABLE I)

##### *Patients studied during therapy with antithyroid drugs*

In 22 tests performed in 15 patients, thyroxine half-time averaged  $4.8 \pm 0.8$  days (mean  $\pm$  standard deviation), a value significantly different from the normal of  $6.8 \pm 0.5$  ( $p < 0.001$ ). Only four subjects (Nos. 1, 8, 9, 13) exhibited half-times within the normal range (Figure 1). Duration of remission averaged 12 months in both these subjects and in the subjects with abnormally rapid turnover of thyroxine. Of the six patients who were studied more than once during anti-

thyroid therapy, three demonstrated a decrease in half-time between successive tests, and three demonstrated an increase. Valid estimations of PBI could be obtained during 18 tests. These ranged between 1.2 and 6.3 μg. per cent and averaged  $3.7 \pm 1.6$  (Figure 2). Values less than 3.0 μg. per cent were found in seven instances (mean, 2.0 μg. per cent), and three of these seven patients (Nos. 7, 10, 15) displayed enlarging goiter, subnormal basal metabolic rate (BMR), delayed relaxation of the deep tendon reflexes, and other stigmata of hypometabolism at the time of the measurements. Thyroxine half-times averaged 4.9 days in the three hypometabolic subjects and 4.3 days in the remaining four patients with subnormal PBI's.

Within the entire group, daily degradation of organic iodine averaged  $54 \pm 25$  μg. This value is not significantly different from the normal mean ( $55 \pm 12$ ). Among the seven patients with subnormal PBI's the three hypometabolic individuals displayed degradation rates averaging 22 μg. of iodine per day. A fourth patient (No. 4) demonstrated no metabolic symptoms despite a daily degradation rate of 26 μg. of hormonal iodine. In the remaining three patients with low PBI's degradation ranged between 37 and 67 μg. of iodine

<sup>4</sup> Performed by Bio-Science Laboratories, 2231 South Carmelina Avenue, Los Angeles, Calif.

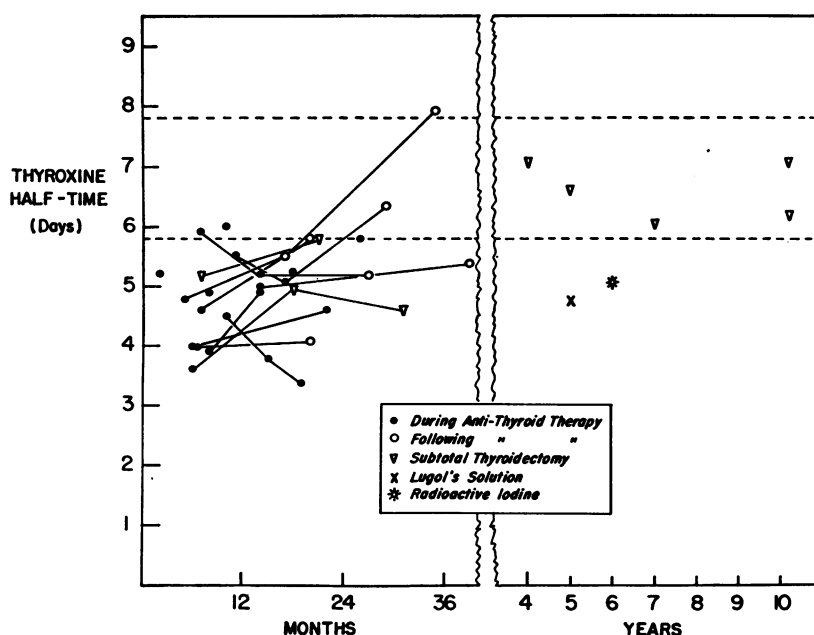


FIG. 1. THE RELATIONSHIP BETWEEN THE DURATION OF SYMPTOMATIC REMISSION AND THE RATE OF TURNOVER OF THYROXINE PERIPHERALLY

Dotted lines represent the normal mean thyroxine half-time  $\pm 2$  standard deviations, based on observations in 30 normal subjects of all ages.

per day, despite the reduction in the concentration of circulating thyroid hormone.

Values for the 24 hour thyroidal uptake of  $I^{131}$  averaged  $64 \pm 16$  per cent for the entire group, and only four values of less than 50 per cent were noted. The latter value is considered to be the upper limit of normal in this laboratory. Thyroidal uptakes in the seven patients with subnormal PBI's averaged 68 per cent. In the four instances in which it was assessed, no demonstrable discharge of thyroidal radioactivity followed the oral administration of 1.0 Gm. of potassium thiocyanate.

Suppression of thyroidal uptake by exogenous hormone was assessed 17 times in 15 patients, and was achieved in 13 instances. In 3 of the 4 instances in which suppression was not achieved, subnormal values for thyroxine half-time were present. Except in 1 patient (No. 3) in whom 5 gr. was required, the daily dose of desiccated thyroid necessary to effectuate suppression ranged between 2 and 4 gr. In 6 patients, administration of inorganic iodine in doses equivalent to the iodine content of the suppressive dose of hormone failed to alter the thyroidal uptake of  $I^{131}$ .

#### *Patients studied after completion of antithyroid therapy*

Subnormal values of thyroxine half-time were found in four tests in four of six patients. In the other three tests, including one test performed in a patient whose previously determined half-time had been short (No. 4), normal values were seen. In four of the six patients, thyroxine half-time was longer than it had been while the patient was receiving antithyroid medication. PBI's were normal in all subjects, as were values for the daily degradation of organic iodine. Thyroidal uptakes of radioiodine greater than 50 per cent were found in three of six subjects, and were not necessarily associated with abnormally rapid peripheral turnover of thyroxine. Thyroidal uptake of radioiodine was suppressible in all instances.

#### *Patients studied after surgical therapy*

Values of the thyroxine half-time in the normal range were found in six of nine tests performed in the seven patients in this category. In the three tests in which abnormal values were

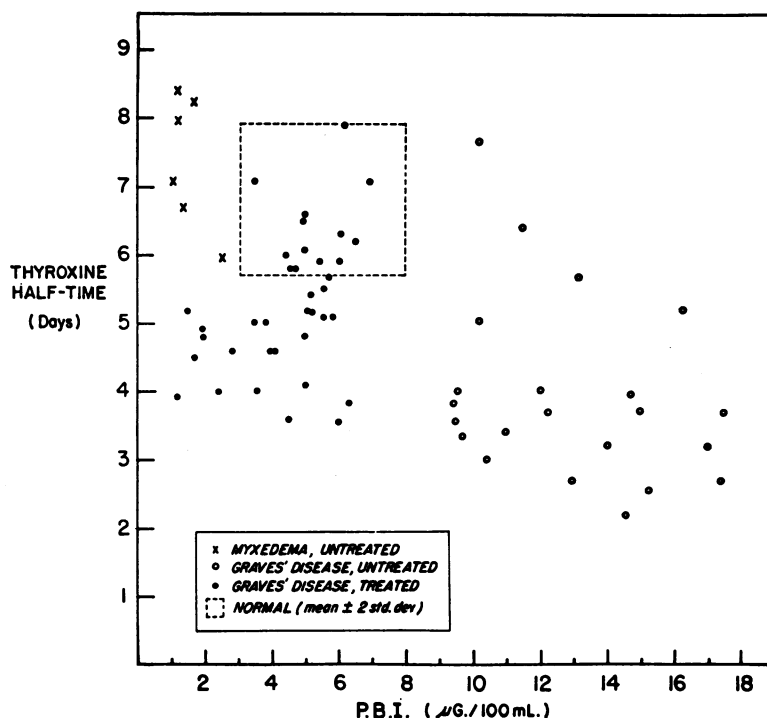


FIG. 2. THE RELATIONSHIP BETWEEN THE RATE OF TURNOVER OF THYROXINE AND THE CONCENTRATION OF CIRCULATING THYROID HORMONE IN PATIENTS WITH DIVERSE STATES OF THYROIDAL FUNCTION

Values in normal group based on observations in 30 normal subjects.

found, the duration of remission averaged 19 months. In the six instances in which normal values were found, the duration of remission averaged 6.3 years. PBI's averaged  $4.9 \pm 1.2$   $\mu\text{g.}$  per cent for the entire group and 4.1  $\mu\text{g.}$  per cent in the three instances in which the turnover of thyroxine was rapid. Thyroidal uptake of radioiodine averaged 41 per cent at 24 hours in patients with rapid turnover of thyroxine and 33 per cent in patients whose thyroxine half-times were normal. Total daily degradation of organic iodine was normal in all subjects, and the thyroidal uptake of  $\text{I}^{131}$  could be suppressed by exogenous hormone in all instances.

#### *Patients studied following miscellaneous therapy*

One patient treated five years earlier with Lugol's solution as well as another patient treated six years earlier with radioactive iodine displayed accelerated peripheral turnover of thyroxine. PBI's, total daily degradation of hormone, and metabolic status were normal in both.

#### *Effects of thyrotoxicosis medicamentosa (Table II)*

Significant alterations in the kinetics of the metabolism of thyroxine were not seen in three patients given large doses of diiodotyrosine for periods of two months. On the other hand, significant acceleration of fractional turnover rate was induced in four patients made mildly thyrotoxic by the administration of either triiodothyronine or desiccated thyroid. Mean thyroxine half-time in this group decreased from 7.6 to 5.0 days. Administration of exogenous hormone was associated with a diminution in PBI in the three patients given triiodothyronine and a marked increase in PBI in the patient given desiccated thyroid. Daily degradation of organic iodine increased in the latter patient. In the patients who received triiodothyronine, calculations of the total quantity of organic iodine degraded daily could not validly be made, since neither the fraction of the PBI contributed by triiodothyronine nor its rate of turnover was known.

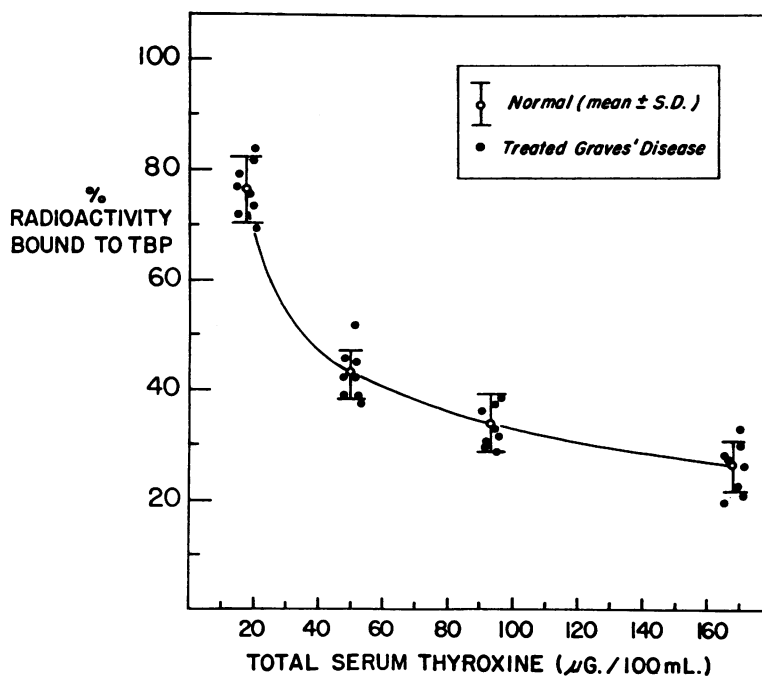


FIG. 3. BINDING OF THYROXINE BY TBP DURING ELECTROPHORESIS IN VERONAL BUFFER OF SERA OBTAINED FROM NORMAL SUBJECTS AND FROM PATIENTS WITH TREATED GRAVES' DISEASE

Concentrations of thyroxine in serum achieved by enrichment of serum with both stable and  $I^{131}$ -labeled hormone.

#### Thyroxine-binding by TBP

In eight patients with treated thyrotoxicosis who displayed persistent acceleration of thyroxine turnover, no abnormality of thyroxine-binding by TBP could be demonstrated when sera enriched with both stable and radioactive thyroxine were electrophoresed in veronal buffer, pH 8.6, ionic strength 0.05 (Figure 3).

Under similar electrophoretic conditions, thyroxine-binding by TBP was normal or increased in the sera of the three patients rendered hypermetabolic by the administration of triiodothyronine.

#### DISCUSSION

The majority of patients with untreated Graves' disease display an increase in the fractional rate of turnover of thyroxine in peripheral tissues (1-3). The present studies, intended to elucidate the factors responsible for this abnormality, have revealed that in a large number of patients, increase in fractional turnover rate may persist in greater or lesser degree for long periods following allevi-

ation of the thyrotoxic state.<sup>5</sup> For the entire group of treated patients studied, thyroxine half-times averaged  $5.2 \pm 1.0$  days, a value significantly different ( $p < 0.001$ ) from the normal mean of 6.8 days (based on studies in 30 normal subjects). However, mean values of the PBI, daily degradation of organic iodine, and BMR were all within the normal range. Mean duration of remission averaged 28 months.

In order to obtain these measurements thyroidal reaccumulation of  $I^{131}$  was blocked by the administration of methyl-mercaptoimidazole. It seems reasonable to enquire whether this antithyroid agent might not be at least partially responsible for the augmentation of the rate of thyroxine turnover observed. This, however, seems unlikely. Since these studies were begun, all patients, in-

<sup>5</sup> Data concerning the turnover of thyroxine in patients with treated Graves' disease, somewhat at variance with the present findings, have recently been reported by Sterling (7). Since the data were presented in abstract form, attempts to reconcile differences in results must await fuller exposition of patient material and experimental method.

cluding normal individuals and patients with myxedema or nontoxic goiter, have been given comparable doses of antithyroid drug during determination of thyroxine degradation rate. In 21 normal subjects studied on this regimen, no difference in the rate of turnover of thyroxine could be discerned from values previously reported in 9 normal subjects studied without thyroidal blockade (1). Thus the effect of methyl-mercaptoimidazole, if any, must be selectively exerted in the population with treated Graves' disease. One pharmacological factor, however, requires further inquiry. In three of four patients studied with and without supplementation of antithyroid medication by Lugol's solution, thyroxine half-times were longer when iodine was concomitantly administered. These observations are perhaps correlated with the reported inhibition by iodide of the deiodination of thyroxine *in vitro* (8, 9). Although acute administration of iodides has not altered the half-time of thyroxine in nonthyrotoxic subjects (3), studies are now in progress to ascertain the effects, if any, of iodide on the turnover of thyroxine in patients with treated Graves' disease.

In the present studies, evaluation of the duration of accelerated turnover of thyroxine is rendered difficult by the variation in the mode of therapy employed. Thus, the majority of patients studied more than 3 years after treatment had undergone subtotal thyroidectomy, and displayed a normal rate of thyroxine turnover. On the other hand, the majority of patients studied during the first few years after onset of treatment had been treated with antithyroid drugs and displayed an abnormally rapid rate of thyroxine turnover. Despite the preponderance of abnormal values in patients treated with antithyroid drugs, the data do not support the supposition that the abnormally rapid turnover of thyroxine was somehow conditioned by these agents. Four of six patients continued to demonstrate the abnormality during sustained remission following cessation of antithyroid therapy, and abnormal values were found in the two patients treated with stable or radioactive iodine. Furthermore, among patients treated surgically, abnormally rapid turnover of thyroxine was observed in those studies performed less than three years after operation. These data suggest that time, rather than mode of therapy,

is the more important variable with regard to restoration of normal values.

Although the fractional turnover of thyroxine was abnormally rapid in the present group of patients with treated Graves' disease, it was nevertheless significantly ( $p < 0.001$ ) slower than that found in patients with active thyrotoxicity (mean half-time  $3.9 \pm 1.1$  days, based on values obtained in 22 patients). In addition, in five of six patients studied prior to and during treatment, a reduction in the fractional turnover rate was noted after restoration of the eumetabolic state. Finally, the present studies have indicated that thyrotoxicosis medicamentosa can increase the fractional turnover of thyroxine. Thus, it seems likely that a portion of the acceleration of thyroxine turnover noted in patients with untreated Graves' disease results directly from the thyrotoxicosis or some concomitant thereof. However, the residual abnormality in the metabolism of thyroxine, noted in many patients in the present study, remained to be explained. No correlation was found between the presence or absence of increased fractional turnover of thyroxine and the age of the patient, or the presence of exophthalmos or of other associated endocrine or constitutional abnormalities. An explanation was further sought among those factors which have been suggested as possible determinants of the peripheral turnover of thyroxine. These have been extensively considered in an earlier communication (10), and will be considered here in relation to the present findings.

In serum, thyroxine is bound to specific transport proteins, one of which migrates during paper electrophoresis at pH 8.6 in the zone between the  $\alpha_1$  and  $\alpha_2$  globulins (11). This moiety has been termed the thyroxine-binding protein of plasma (TBP). Recently another highly potent thyroxine-binding protein has been noted in serum, having an anodal electrophoretic mobility greater than that of albumin (pre-albumin) (12). It has been suggested that the distribution and rate of turnover of thyroxine might be governed by the metabolism of the proteins to which it is bound, and it was initially suggested that the accelerated turnover of proteins and other metabolites which occurs in patients with Graves' disease might lead to acceleration of the turnover of the hormone (1). Although highly purified preparations of TBP and pre-albumin are now available (13), it has not as



yet been possible to ascertain whether the turnover of these proteins remains abnormally rapid in patients with treated Graves' disease. In any event, accelerated turnover of protein resulting from hypermetabolism, *per se*, would seem to be excluded as a contributory factor in the treated patient, who may continue to demonstrate rapid turnover of thyroxine despite reduction of the metabolic rate to normal or subnormal values.

Riggs (14), as well as Berson and Yalow (2), have suggested that the quantity of thyroxine degraded daily might vary linearly with the square of the PBI. No explanation for this relationship was apparent. More recently, however, Robbins and Rall have offered the following suggestion (15). These authors have hypothesized that in the plasma, thyroxine exists in equilibrium between the protein-bound and the free or unbound state. According to their calculations, values of free thyroxine vary inversely with the thyroxine-binding capacity of TBP and directly with the total concentration of hormone in the plasma. It was calculated that as the PBI increased, a progressively greater fraction of the plasma's hormone was present in the free state. Inspection of their data reveals that the calculated concentration of free thyroxine varies roughly with the square of the PBI. It is not surprising, therefore, that, employing the data of Berson and Yalow, Robbins and Rall found a linear relationship between the daily degradation of hormone and the concentration of free thyroxine.

Implicit in these considerations is the conclusion that the fractional rate of turnover of the total plasma hormone (the function measured in the present study) must vary with the concentration of free thyroxine as conditioned by *a*) the total concentration of hormone and *b*) the binding activity of TBP. In the present study, however, abnormally rapid fractional turnover of thyroxine has been found in patients whose PBI's were in the normal or the myxedematous range. Furthermore, since the sera of such patients demonstrated normal binding activity of TBP, calculated values of free thyroxine were normal or decreased. Thus, though the foregoing general relationships concerning thyroxine turnover may pertain in subjects with treated Graves' disease, they must at least be quantitatively altered, and the operation of other factors must be invoked.

Additional evidence in this regard is provided by the present patients made hypermetabolic with triiodothyronine. In these patients, PBI diminished and the binding capacity of TBP was unchanged. Here, too, calculated concentrations of free thyroxine were diminished, but the fractional rate of turnover of thyroxine was increased.

It is thus apparent that quantitative alterations in the interaction between thyroxine and TBP cannot account for the increased fractional rate of turnover of thyroxine found in some patients with treated Graves' disease. The role of interactions between thyroxine and pre-albumin has not as yet been evaluated. Preliminary studies indicate that the thyroxine-binding capacity of pre-albumin is markedly diminished during active thyrotoxicosis. Since binding of thyroxine by pre-albumin appears to be a critical determinant of the cellular uptake of thyroxine in *in vitro* systems, especially at concentrations of hormone in the thyrotoxic range (13), the reported increase in the cellular uptake of thyroxine *in vitro* from the sera of thyrotoxic patients (16, 17) may be merely a manifestation of altered binding by pre-albumin. Unfortunately, few data are available concerning cellular uptake of thyroxine from the serum of patients with treated Graves' disease, nor has it been possible as yet to assess the thyroxine-binding capacity of pre-albumin in a significant number of patients in this category.

Thus, the accelerated turnover of thyroxine found in some patients with treated Graves' disease cannot presently be correlated with any of the extracellular factors which have previously been suggested as possible determinants of the rate of turnover of the hormone. Attention may then turn to the possible role of factors within the cell itself. As has been suggested elsewhere (10), alterations in the activity of those cellular components which bind thyroxine might be expected to augment the rate of removal of hormone from the extracellular fluid, and, if subsequent steps in the metabolism of hormone were not rate-limiting, might result in increased fractional rate of turnover of hormone. At present, however, techniques are not available for assessing this variable.

Enzymes capable of degrading thyroxine have been demonstrated in a variety of tissues by a number of investigators (8, 9, 18). Larson, Tomita and Albright have noted an increase in

the thyroxine-deiodinating activity of renal tissue of rats made hypermetabolic by the administration of thyroxine, and have suggested that this may represent an adaptive augmentation of enzyme activity, consequent upon an increased delivery of substrate (19). A similar sequence could occur in patients with active Graves' disease, and could be responsible, at least in part, for the accelerated turnover of thyroxine found in untreated patients. However, currently available data provide no basis for prediction of how long such adaptive increases in the deiodination of thyroxine might persist following alleviation of the hyperthyroxinosis. Persistence for as long as five or six years, the duration of the most persistent abnormalities in turnover noted in the present study, seems highly unlikely. The present data further indicate that if adaptive increases in deiodination do occur, they must be relatively substrate specific, since administration of large quantities of diiodotyrosine for periods of two months failed to alter the rate of turnover of thyroxine. The acceleration of thyroxine turnover found in patients made hypermetabolic with triiodothyronine might be construed as indicating that the two hormones share common deiodinating pathways. Alternatively, it seems possible that in these patients, as in rats given large doses of thyroxine, acceleration of hormonal degradation resulted from hypermetabolism, *per se*.

Thus, it is difficult to ascribe the increased fractional turnover of thyroxine found in some patients with treated Graves' disease to any of the several mechanisms which have already been suggested as possible regulators of the peripheral turnover of the hormone. However, other possibilities remain to be considered. Among these, consideration must be given to the thyroid gland itself. In the present series, many, though not all of the patients who demonstrated abnormally large fractional rates of turnover of thyroxine also displayed 24 hour thyroidal uptakes of  $I^{131}$  well above the normal range. This was most commonly found in patients receiving antithyroid drugs. Administration of small quantities of iodine failed to diminish the increased uptakes, indicating that iodine deficiency was not responsible for the augmentation of uptake. In the several instances in which KSCN was administered, only a small fraction of the accumulated iodine could be dis-

charged from the gland. Thus, it may be concluded that despite the antithyroid drugs, large quantities of iodine were being incorporated into organic moieties. Moreover, by direct counting over the neck, the rate of release of iodine from the thyroid gland of these patients was found to be rapid. Therefore, these organic moieties were being lost from the gland at a rapid rate. Measurements of protein-bound radioiodine in the plasma were not made. It is thus not possible to be certain whether the rapid disappearance of radioactivity from the gland represented intra-thyroidal deiodination or secretion into the circulation. However, the normal values for PBI and BMR which were observed in these instances would suggest that if such material were indeed reaching the circulation, it was rapidly removed and had little calorogenic potency. Iodinated tyrosyl derivatives, such as diiodotyrosine (DIT) and monoiodotyrosine (MIT) could fulfill these criteria. Recent studies indicate that when organic-binding of iodine in the thyroid glands of rats is blocked by increasing doses of propylthiouracil, there occurs a progressive diminution and ultimately a complete disappearance of  $I^{131}$ -labeled thyronines, associated with a progressive reduction in the DIT/MIT ratio, until MIT becomes the sole radioiodinated compound demonstrable (20). Thus, it seems possible that MIT, DIT, or both, may be released from highly active, partially blocked glands, in which the rate of proteolysis and release may exceed the capacity of glandular dehalogenase. In an earlier study, evidence was presented which suggested that compounds of this type may be released from the thyroid gland of the patient with Graves' disease, even prior to the institution of therapy (1), and subsequently iodotyrosines were demonstrated chromatographically in the serum of patients with Graves' disease (21). It is conceivable that such iodinated compounds might stimulate peripheral deiodinating mechanisms. The present negative results with DIT would not support this hypothesis. Nonetheless, it remains possible that other, as yet untested compounds, capable of augmenting the peripheral turnover of thyroxine, may be released from the gland of the treated patient.

Consideration must also be given to the possibility that the thyroid gland itself contributes to the degradation of thyroxine in the patient with

treated Graves' disease. In small animals, exogenous thyroxine does not penetrate the thyroid gland, unless antithyroid agents, such as propylthiouracil are concomitantly administered (22). The patients in the present series were all given blocking doses of Tapazole® during study periods. It is therefore possible that a portion of the administered  $I^{131}$ -labeled thyroxine did penetrate the thyroid gland in these patients, although appreciable thyroidal radioactivity could not be demonstrated by direct counting. Whether thyroxine, once having penetrated the gland, would be metabolized is entirely unknown. Although mechanisms for the intrathyroidal deiodination of thyroxine have not been demonstrated in the normal gland, their possible presence in the gland of the patient with Graves' disease has not been excluded.

Thus, the thyroid gland could contribute to the peripheral degradation of thyroxine, either by secreting a product which stimulates deiodination or by itself degrading the hormone. In an effort to evaluate these possibilities, the effects of prolonged thyroidal suppression on the peripheral degradation of thyroxine are now under investigation.

The possibility remains that the abnormally rapid peripheral turnover of thyroxine in some way reflects the activity of the disease process *per se*. Persistence of the abnormality in the treated state might then reflect the continued operation of underlying pathogenetic mechanisms. It is of interest in this regard that of the 11 patients whose last recorded value of the thyroxine half-time was in the abnormal range, 2 patients have died of unrelated causes, and 3 (Nos. 1, 5, 7) have experienced recurrence of active thyrotoxicity. Of the 12 patients whose last recorded values were within the normal range, none are known to have relapsed clinically. Additional evidence in this regard is perhaps afforded by the observation that accelerated turnover of thyroxine is found in a significant proportion of relatives of patients with Graves' disease, who are not themselves and apparently have never been clinically thyrotoxic (23).

Another abnormality of iodine metabolism has been thought to reflect activity of the morbid process in Graves' disease. This is the failure of exogenous thyroid hormone, even in large doses, to suppress the thyroidal uptake of radioactive iodine

(24, 25). This abnormality, like the increased fractional turnover of thyroxine peripherally which has been described above, has been reported by Werner to persist for long periods after alleviation of active thyrotoxicity (26). Moreover, like the abnormality of peripheral turnover, it frequently abates several years after treatment. In the present studies, no correlation was found between the presence or absence of thyroidal suppression by exogenous hormone and the presence of accelerated fractional turnover of thyroxine. The present data indicate that thyroidal suppression can commonly be achieved while patients are receiving antithyroid therapy. In patients treated by other means, and in patients observed following cessation of antithyroid therapy, the incidence of resistance to suppression in the present series seems less than that reported by Werner (26), and more consistent with the data of Morgans, Oldham and Trotter (27). It seems quite clear, at any rate, that the currently described abnormality is not of sufficient magnitude to account for resistance to suppression by large doses of exogenous hormone, based on increased disposal of the administered material. The precise relationship of the two abnormalities to each other and to the underlying pathogenesis of Graves' disease remains to be resolved in the future.

#### SUMMARY

1. Studies of the distribution and rate of peripheral degradation of exogenous  $I^{131}$ -labeled thyroxine have been performed in 23 patients with Graves' disease, following restoration of a eumetabolic status.

2. Among these patients, many displayed a persistence of increased fractional rate of turnover of the hormone in peripheral tissues for long periods after induction of symptomatic remission.

3. Although induction of thyrotoxicosis medicamentosa by the administration of either desiccated thyroid or triiodothyronine has been shown to increase the fractional rate of turnover of thyroxine, hypermetabolism, *per se*, cannot account for the increase noted in the patients with treated Graves' disease.

4. Analysis of several other factors which might influence the peripheral turnover of thyroxine has failed to reveal the cause of the abnormality in these patients.

## ACKNOWLEDGMENTS

The authors are indebted to Mary Frances Chapin, Barbara Fine, Katherine Tomikawa, and Nancy Warr for their valuable technical assistance.

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