THE EXCHANGEABLE THYROID HORMONAL POOL. I. ITS MAGNITUDE AND RATE OF TURNOVER IN VARIOUS THYROID STATES IN MAN

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Research Article

THE EXCHANGEABLE THYROID HORMONAL POOL. I. ITS MAGNITUDE AND RATE OF TURNOVER IN VARIOUS THYROID STATES IN MAN [1,2]

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Recent reviews (1, 2) have summarized the significant advances in the elucidation of certain aspects of thyroid physiology resulting from studies utilizing radioactive iodine and improved chemical methods for the determination of iodine. Quantitative data defining the size of the thyroid hormonal pool and the rate and amount of "peripheral utilization" or turnover of the thyroid hormone within this pool are, however, fragmentary (1).

Estimates based on the thyroxine requirements in man at various basal metabolic levels indicate that approximately 10 mgm. of thyroxine are stored in the human body (3). Values obtained by various methods for the daily thyroid hormone turnover in man are listed in Table I. Most recently, Riggs (1) has estimated that the half-life of thyroid hormone in tissues is 11.9 days.

This report is concerned with the experimental determination of the magnitude and turnover of the exchangeable thyroid hormonal pool in man based on the rates of disappearance from the circulation of endogenously labelled (I^{131}) human thyroid hormone after its infusion into patients with normal and abnormal thyroid function.

MATERIALS AND METHODS

Seven patients with the classical symptoms, signs, and laboratory findings of thyrotoxicosis served as donors of endogenously labelled thyroid hormone. Each donor received 10.3 to 23.1 millicuries of I^{131} for therapeutic purposes. The dosage was calculated according to a previously described method (12) and delivered 5 to 15,000 R.E.P. to the donors' thyroid glands. Two to six days later blood was withdrawn from each donor under sterile precautions into acid citrate solution. After centrifugation for 30 minutes at 3,000 R.P.M., the plasma was separated under sterile conditions. Analysis by trichloracetic acid precipitation (13) and after dialysis showed 100 per cent protein-binding in five of the seven donors; the plasma iodine of the remaining two donors was 80 and 84 per cent protein-bound. Within an hour after withdrawal, the plasma was infused intravenously [4] into the recipients—4 euthyroid patients, 2 with thyrotoxicosis, and 1 with spontaneous myxedema. The recipient's plasma was analyzed repeatedly at intervals for 23 to 70 hours after infusion for total and protein-bound I^{131} according to methods previously described (13).

The data were subjected to standard graphic analysis, as indicated in Figure 1. Counts per minute per cc. of plasma were plotted as ordinate on semi-logarithmic paper against time following infusion as abscissa. The resultant curve could be resolved into straight line components, (1) an initial steep slope which could be mathematically resolved into several components, the first one of which is shown as an example in Figure 1, and (2) a "slower" exponential component from which the half-times (t^{1}) of the disappearance of the infused material from the circulation were determined. The turnover was calculated from the value for half-time of the second or slow component and the equation for exponential decay, $A = Aoe^{-kt}$,[5] using the formula:

$$\frac{\text{Turnover rate}}{\text{per cent per day}} = \frac{\ln 2}{t^{1}} = \frac{0.693}{t^{1}}.$$

The specific activity after infusion of the thyroid hormone was determined by division of the counts per cc. plasma at zero time as obtained by extrapolation from the slow component by the recipient's protein-bound iodine concentration in micrograms per cc.; protein-bound

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[4] The total body radiation dosage was calculated from the data of Marinelli, Quimby and Hine (14) and Marinelli (15) according to the method of Sterling (16). The total radiation delivered by the infused I^{131} ranged from .093 to .014 R.E.P.

[5] $A = I^{131}$ tagged hormone at any time.
Ao = tagged hormone at zero time.
t = time (days).
k = fraction of hormone pool turned over per day.

TABLE I

Quantitative estimations of daily thyroid hormone turnover in man

Method	Author and Reference	Estimate
Replacement requirement of athyreotics	Plummer and Boothby (4)	300 μgm. *dl*-thyroxine
	Thompson, McLellan, Thompson and Dickie (5)	325 μgm. *dl*-thyroxine (116 μgm. hormonal I₂)
	Rosenblum (6)	180 μgm./sq.M as *l*-thyroxine
	Means (7)	100 mgm. U.S.P. thyroid
	Riggs (1)	85–90 μgm. *l*-thyroxine
Mathematical analyses	Stanbury and associates (8)	45+ μgm. hormonal I₂ 83 μgm. hormonal I₂ 195 μgm. hormonal I₂
	Stanley (9)	240 μgm. hormonal I₂
	Salter, DeVisscher, McAdams and Rosenblum (10)	180–320 μgm./sq.M as *dl*-thyroxine
Disappearance rate of I¹³¹ labelled hormone *a*) obtained from euthyroids *b*) obtained from thyrotoxics	Tubiana (11) Hamolsky, Freedberg, Kurland and Wolsky	250 μgm. hormonal I₂ 119±11 μgm. hormonal I₂

iodine determinations were carried out by a modification of Barker's method.[6] The exchangeable thyroid hormone pool was then estimated by the principle of isotope dilution,

$$viz. \frac{\text{no. of counts injected}}{\text{specific activity cts. per μgm.}}.$$ Thyroid hormone turnover (μgm. per day) was computed from the value for the exchangeable thyroid hormone pool and the per cent turnover per day. Measurement of the circulating hormone pool was made, by a similar method, utilizing data from the initial rapid component of the curve. The magnitude of the extravascular pool was approximated by subtracting the value for the circulating pool from that of the total exchangeable pool.

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[6] These measurements were carried out at the PBI Laboratory of the Massachusetts General Hospital.

RESULTS AND INTERPRETATION

The identifying data concerning the nature of the material infused, the subjects of the experiments, and the results are presented in Table II. In each instance there was an initial rapid fall in radioactivity, lasting four to five hours. This was considered to result from the distribution of labelled hormone throughout the "exchangeable pool." This period also included the disappearance of the small fraction of infused non-protein I¹³¹ since measurements at intervals greater than one hour following infusion revealed the radioactivity to be virtually 100 per cent protein-bound. The early
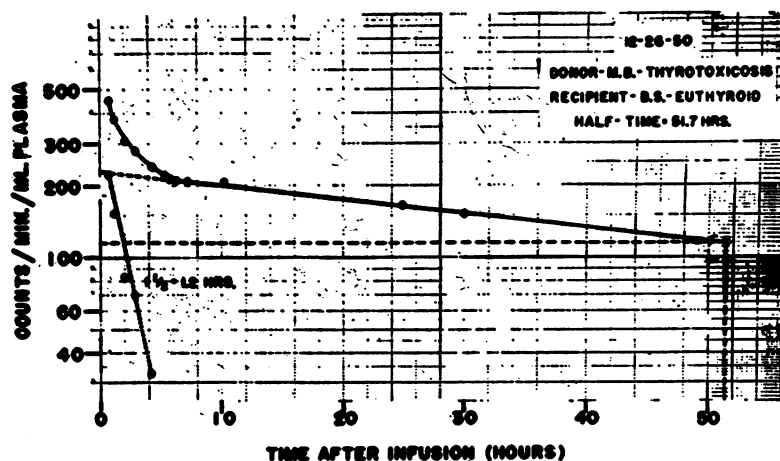

FIG. 1. DISAPPEARANCE OF RADIOACTIVITY FOLLOWING INFUSION OF LABELLED (I¹³¹) HUMAN THYROID HORMONE (CASE 4)

TABLE II

Infusion of human I¹³¹-labelled thyroid hormone

	Donor*							Recipient								
			Interval between I¹³¹ therapy and withdrawal *days*				Serum protein-bound iodine *μgm.%*	I¹³¹ labelled hormone infused			Observed half-time *hrs.* (Slow phase)	Calculated turnover time (Half-time ×1.44)	Turnover rate *%/day*	Exchang. thyr. pool *μgm.*	Circulating thyr. pool *μgm.*	Turnover thyr. horm. *μgm./day*
Case No.	Dose *mc.*	Radiation delivered to thyroid (12) *R.E.P.*		Clinical thyroid status	Wt. *kgm.*	Hematocrit *per cent*		Volume of plasma *cc.*	Total *cts./min.*	Per cent of protein bound						
1	10.6	8,040	2	Euthyroid	61.5	36	5.8	53	447,320	80	64.5	93	25.7	432	191	111
2	10.3	11,200	5	Euthyroid	66.4	40	3.9	56	1,067,000	94	50.2	72.3	33.2	323	134	107.3
3	23.1	4,965	4	Euthyroid	65.9	40	4.7	70	2,620,000	81	56.5	81.4	29.5	418	193	123.4
4	12.1	11,600	5	Euthyroid	52.2	38	5.6	50	1,725,000	95	51.7	74.4	32.2	422	176	136
Average											55.7±5.6	80.3±8.1	30.2±2.9	399±44	174±23	119±11
5	19.3	14,300	4	Thyrotox.	46.5	39	17.3	55	3,220,000	97	39.5	56.9	42.0	1142	557	480
6	9.1	5,500	6	Thyrotox.	55.9	38	11.7	70	1,771,000	96	34.0	48.9	48.8	648	280	317
7	12.9	15,250	4	Myxedema	64.6	40	1.0	48	1,350,000	96	41.2	59.4	34.3	65	23	26.2

* Each donor had thyrotoxicosis.

component was followed by a slower exponential decrease in radioactivity which has been attributed to the replacement within the exchangeable pool of the I¹³¹ labelled hormone by non-labelled hormone, *i.e.* the turnover of the hormone.

The half-times of the euthyroid patients were greater (50.2–64.5 hours) than those of the thyrotoxic recipients (39.5 and 34.0 hours) and the turnover rates, therefore, correspondingly less (25.7–33.2 per cent per day vs. 42 per cent and 49 per cent per day). The half-time in the myxedematous patient (41.2 hours) was shorter than that observed in the euthyroid patients. The exchangeable thyroid hormonal pool and the daily turnover of thyroid hormone were 1.5 to three times greater in the thyrotoxic patients than in the four euthyroid subjects (Table II). Values in the four euthyroid patients were in close agreement; the exchangeable pool ranged from 323 to 432 μgm. and the daily turnover from 107 to 136 μgm. Corresponding values for the patient with myxedema were strikingly less, namely 65 μgm. and 26.2 μgm./day.

DISCUSSION

The method used in the present study is based on the generally accepted concept that administered I¹³¹ is taken up by the thyroid gland, incorporated therein into the thyroid hormone complex and released into the circulation, thus serving as a source of endogenously labelled hormone. Thyrotoxic patients were selected as donors for the present study because in previous studies virtually complete protein-binding has been demonstrated after therapeutic doses of I¹³¹. Related studies in this laboratory on several patients with thyrotoxicosis who received therapeutic doses of 5 to 20 millicuries have uniformly revealed that the protein precipitable plasma radioactivity at the time intervals used in this study is virtually all butanol soluble, *i.e.*, thyroxine-like (17). Using chromatographic and isotopic methods, Rosenberg (18) found, 2 to 10 days after administering 10 to 15 millicuries to 6 thyrotoxic patients, that practically the total organic iodine plasma radioactivity was in the thyroxin fraction which was considered to represent the circulating thyroid hormone. We have assumed, therefore, that the radioactivity of the in-

fused plasma in our cases represented natural thyroid hormone labelled with I^{131}.

Recent studies by Gross and Pitt-Rivers show clearly that the blood organic iodine labelled by I^{131} consists principally of thyroxine with smaller amounts of at least one other compound, probably triiodothyronine (19). Other questions concerning the stability of thyroid hormone endogenously labelled by I^{131}, as well as the biologic identity of thyrotoxic and euthyroid thyroid hormone (generally considered to be identical), are under investigation.

The general validity of the principles of specific activity and isotope dilution as utilized herein has been discussed by Zilversmit, Entenman, Fishler, and Chaikoff (20) and others (16, 21). Taurog, Chaikoff and Entenman (22) based their estimation of the turnover rate of protein-bound iodine in dogs on a study of the disappearance rate of pooled radioactive rat plasma (10 to 12 rats) for the first six hours following its infusion into dogs. In the course of studies on the fate of radioactive *l*-thyroxine in humans, Myant and Pochin (23) infused into 3 euthyroid patients heparinized plasma obtained from thyrotoxic patients three days after receiving up to 10 millicuries I^{131}. The plasma radioactivity was shown to have the same degree of butanol extractability as following tracer doses. They concluded "that natural thyroxine leaves the circulation more slowly than sodium thyroxine." This is in agreement with our observations regarding the early (four to six hours) portion of the curve of disappearance. Their published results (23) do not permit comparison with our data on the later component of the curve of disappearance which has been considered to represent turnover of thyroid hormone. In studies independent of those reported here, Tubiana (11) infused into 6 euthyroid subjects dialysed plasma from euthyroid humans previously treated with 50 to 100 millicuries I^{131}. The disappearance rate was followed from 6 to 48 hours. In good agreement with the observations reported here, Tubiana obtained turnover (renewal) times of 29 to 50 hours. By assuming a value of 10 μgm. per 100 cc. for plasma protein-bound iodine, Tubiana estimated an average daily hormone secretion of 250 μgm. in euthyroid subjects. Utilizing determined serum protein-bound iodine levels, the average value obtained in euthyroid subjects in

the present study was 119 ± 11 μgm. per day. These results are in substantial agreement with the estimates obtained by other methods (Table I).

The size of the exchangeable pool as measured in this study is smaller than the approximation of the total body pool estimated by other methods. It is possible that the slow component represents a maximal turnover rate and that extension of the observations beyond 70 hours would demonstrate a slower component. Studies in dogs have shown an increase in serum radioactivity from 24 to 48 hours after infusion of labelled hormone attributed to the addition of hormone newly labelled by I^{131} released from the infused material. The formation, endogenously, of new I^{131} labelled hormone in the recipient and its recirculation is not believed to be a significant contributing factor to the estimate of the exchangeable pool by the method reported here since thyroid I^{131} uptake was found to be extremely low during the period of observation. It is important to emphasize that this method measures the over-all utilization within a readily exchangeable compartment. In view of the demonstrated heterogeneity of the labelled thyroid hormone complex, the possibility of variations in individual tissue exchangeability and utilization to explain the observed difference in pool size cannot be excluded.

The observation of a more rapid hormone turnover in the two patients with thyrotoxicosis is consistent with the generally held opinion of an increased metabolic turnover in this disease. It raises the interesting speculation, however, that thyrotoxicosis may be characterized not only by an increased thyroid output and body pool of hormone but also by a peripheral metabolic aberration important in the pathologic physiology of the disease. Further studies are necessary to elucidate this possibility.

SUMMARY

1. The turnover rate of human thyroid hormone has been studied by following the disappearance of plasma I^{131} obtained from thyrotoxic patients who had received 10.3 to 23.1 millicuries of radioactive iodine and infused into 4 euthyroid patients, 2 with thyrotoxicosis and 1 with myxedema.

2. The second or slow exponential decrease in radioactivity has been considered to represent the replacement of tagged hormone or its turnover.

3. The half-times in euthyroid subjects were 50.2 to 64.5 hours, in contrast to 39.5 and 34.0 hours in the patients with thyrotoxicosis and 41.2 hours in the myxedemic patient. The turnover rates were 25.7–33.2 per cent per day in the euthyroids vs. 42 and 49 per cent per day in the thyrotoxics and 34 per cent per day in the myxedematous patient.

4. The exchangeable and circulating hormonal pools have been estimated by the principle of isotope dilution and from the former the daily turnover has been calculated.

5. The exchangeable hormone pool (μgm.) averaged 399 ± 44 in the 4 euthyroid subjects vs. 1142 and 648 in the thyrotoxic patients and 65 in the myxedematous patient.

6. The daily turnover (μgm. per day) averaged 119 ± 11 in the 4 euthyroid subjects vs. 480 and 317 in the thyrotoxic patients and 26 in the myxedematous patient.

7. The method of study used permits delineation of another parameter of thyroid function; its application to varied aspects of thyroid physiology and pathology appears warranted.

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