

THE THYROID HORMONE-PLASMA PROTEIN COMPLEX IN MAN. I. DIFFERENCES IN DIFFERENT STATES OF THYROID FUNCTION^{1,2}

By MILTON W. HAMOLSKY, HASKELL E. ELLISON, AND
A. STONE FREEDBERG³

(From the Department of Medicine, Harvard Medical School, the Medical Research Department of the Yamins Research Laboratories and the Medical Service, Beth Israel Hospital, Boston, Mass.)

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The present consensus characterizes thyrotoxicosis,⁴ a disease of unknown etiology, by the quantitative overproduction and release by the thyroid of excessive amounts of normal thyroid hormone (1-4). According to this concept, the distinctive abnormal signs, symptoms, and laboratory findings of hypermetabolism in patients with diffuse toxic goiter are attributed to the effects of increased amounts of the normal hormone upon peripheral tissue metabolism. Plummer (5), among others, proposed an alternate theory, based chiefly upon clinical observations, which ascribed various manifestations of the disease to the effects of 1) an excess of normal hormone, and 2) a *qualitatively* abnormal hormone, which he suggested was abnormal because of incomplete iodination. In contrast, he suggested that the abnormality in patients with toxic *nodular* goiter was due solely to an excess of normal thyroid hormone. Salter and Johnston (6) concluded, on the basis of acetone precipitation of acidified sera, that the plasma thyroid hormone in hyperthyroidism differed from thyroxine added *in vitro* to normal and hypothyroid sera to levels of protein-bound iodine comparable

to those observed in hyperthyroidism. Means (1), however, found no biochemical evidence to support Plummer's two-product theory of dysthyroidism. Recent studies (2, 7-10), using more precise isotopic and chromatographic methods, have, moreover, failed to indicate any qualitative difference in the hormone circulating in the hyperthyroid as compared to the euthyroid state.

This report deals with three series of experiments employing biological test systems (infusion into dogs and *in vitro* rat diaphragm "uptake" studies) to investigate the question of the qualitative identity of the thyroid hormone-plasma protein complex in euthyroid and hyperthyroid states in man. The consistent differences observed are not adequately explained by present concepts and indicate 1) a qualitative difference in the nature of the hormone-protein complex in the hyperthyroid as compared to the euthyroid states, or 2) a differential effect of a plasma factor(s) on the disappearance from the plasma or tissue incorporation of the hormone in these two states.

EXPERIMENTAL METHODS AND RESULTS

Series 1—Infusion of endogenously I-131-labelled human thyroid hormone—Rate of disappearance in dog

A. Methods. Data identifying the donors and the plasma infused are presented in Table I. Eight euthyroid patients (two with thyroid carcinoma and six with angina pectoris or congestive failure), and ten with typical diffuse toxic goiter served as donors. The euthyroid subjects received for therapeutic purposes 10.2 to 61.9 mc. I-131, which delivered an estimated thyroid radiation of 1,610 to 55,000 R.E.P. (11, 12). The thyrotoxic patients received 4.7 to 15.4 mc. I-131, or 8,500 to 15,300 R.E.P. to their thyroid glands.

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³ Senior Ziskind Fellow, Beth Israel Hospital, Boston, Mass.

⁴ For this discussion, we refer to a disease associated with increased activity of the autonomic nervous system, characterized by diffuse enlargement of the thyroid gland, eye signs, tachycardia, tremor, hypermetabolism. It includes exophthalmic goiter, hyperthyroidism, Graves', Basedow's, or Parry's disease associated with diffuse toxic goiter.

TABLE I
Infusion of endogenously I-131-labelled human plasma into dogs

Experi- ment No.	Donor		Infusion							Recipient		Results		
	Thyroid status*	Dose (mc.)	24-hr. I-131- thyroid uptake (11)	Total R.E.P. to thyroid gland (11, 12) ($\times 10^6$)	R.E.P. β - I-131- 2nd 24 hrs. (16) ($\times 10^6$)	Interval between Rx and infusion (days)	Volume infused (ml.)	Total counts infused ($\times 10^6$)	PBI-131 $\times 100$ Total I-131 (14)	But. I-131 $\times 100$ PBI-131	Weight (Kg.)	Sex	Hours followed	Half-time of disappearance
														(hrs.)
1	Eu. Ca.	61.9	28.6	55.0	4.7	10	30	559	94.5		10.7	M	56	27.0
2	Eu. Ca.	40.9	38.6	42.0	3.1	5	35	930	89.4		5.9	F	30	21.0
3	Eu. Card.	14.6	40.5	34.0	2.0	6	50	150	58.6	96.1	12.7	F	24	34.2
4	Eu. Card.	22.0	24.2	29.0	2.2	6	45	332	51.0	76.0	10.2	M	24	23.0
5	Eu. Card.	15.4	36.4	31.0	2.3	6	50	115	53.8	88.0	13.6	M	46	32.0
6	Eu. Card.	11.1	24.1	28.0	1.0	5	60	44	82.0	88.0	11.4	M	52	33.8
7	Eu. Card.	10.2	44.0	22.0	1.8	6	52	170	75.0	92.0	12.4	M	96	37.6
8	Eu. Card.	11.6	13.2	1.6	0.3	6	35	123	64.5	75.0	6.0	M	45	27.0 Av. = 29.5 \pm 5.5
9	Tx	6.6	81.6	9.6	1.1	3	30	242	82.0	88.8	12.7	M	46	12.0
10	Tx	4.7	77.6	10.0	1.1	5	21	187	95.0		8.6	F	28	12.6
11	Tx	10.8	90.6	12.0	1.0	6	35	966	97.5		6.0	F	24	12.4
12	Tx	7.7	76.5	8.5	0.8	4	40	656	94.6	83.8	13.6	M	51	10.2
13	Tx	9.5	72.6	11.9	1.3	5	50	2,016	90.0	92.0	13.6	M	41	12.0
14	Tx	6.2	59.7	12.4	1.4	5	60	836	100.0	100.0	10.0	F	24	12.0
15	Tx	9.0	60.0	15.3	1.2	5	60	340	97.8	87.8	13.6	F	25	12.0
16	Tx	6.5	64.8	14.6	0.9	5	50	432	102.0	68.0	12.3	M	24	16.8
17	Tx	15.4	61.0	10.7	0.7	6	50	2,093	100.0	89.0	11.8	F	192	15.0
18	Tx	9.3	67.0	9.2	0.9	6	50	502	86.0	83.0	15.9	M	52	17.0 Av. = 13.2 \pm 2.2
19	Nod. Tx	8.1	60.0	12.5	1.4	3	55	659	79.0	100.0	9.6	M	34	21.0
20	Nod. Tx	12.0	58.8	7.0	0.8	7	50	620	100.0	82.0	10.2	F	26	22.0
21	Nod. Tx	9.4	64.6	10.0	0.9	6	50	585	100.0	80.0	11.4	F	31	22.2

* Eu. Ca. = Euthyroid-thyroid carcinoma.
Eu. Card. = Euthyroid-cardiac.
Tx = Diffuse toxic goiter.
Nod. Tx = Nodular toxic goiter.

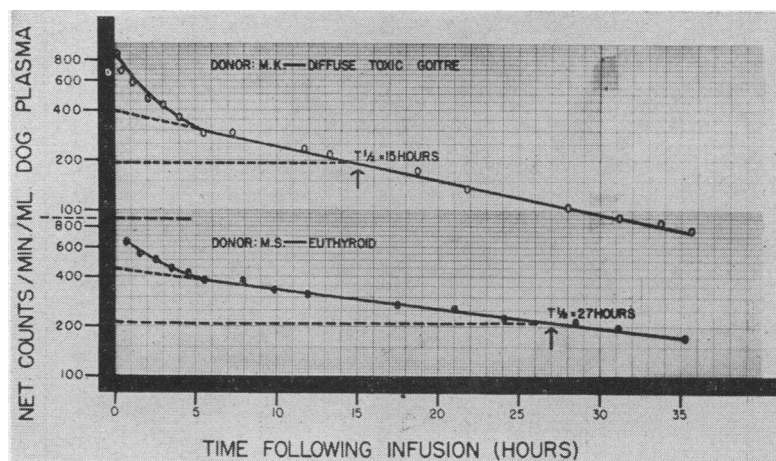


FIG. 1. COMPARISON OF THE EARLY DISAPPEARANCE IN DOGS OF ENDOGENOUSLY I-131-LABELLED THYROID HORMONE FROM A HYPERTHYROID VS. A EUTHYROID SUBJECT

These dosages delivered during the second 24 hours (12) 308 to 4,700 estimated R.E.P. β in the euthyroid group, and 670 to 1,365 R.E.P. β in the hyperthyroid patients. Following I-131 therapy, blood was removed into acid citrate after intervals (5 to 10 days in the euthyroids, 3 to 6 days in the hyperthyroids) shown by previous dialysis, trichloroacetic acid precipitation, and butanol extraction studies to permit maximal incorporation of the I-131 into a plasma protein-bound and butanol-soluble (*i.e.*, thyroxin-like) form. Plasma was separated by centrifugation at 3,000 r.p.m. for 30 minutes. All procedures were carried out under sterile precautions. The butanol extractable fraction of the protein-precipitable I-131 was 76 to 96 per cent in the euthyroids and 68 to 100 per cent in the thyrotoxic.

Each plasma was then infused intravenously into an unanesthetized dog. At frequent intervals for the next 24 to 96 hours, blood was removed by femoral vein puncture from the recipient dog and the plasma radioactivity determined (13). Within 30 minutes following the infusion, the radioactivity of the recipient's plasma was virtually 100 per cent protein-precipitable.

The data were subjected to standard graphic analysis, as previously described (14), and as exemplified in Figure 1. Counts per minute per ml. of plasma were plotted as ordinate on semi-logarithmic paper against time following infusion as abscissa. Each resultant curve was resolved into straight line components—1) an initial

steep slope for six to eight hours which could be resolved mathematically into several components followed by 2) a "slower" exponential component for the next 20 to 40 hours from which the half-times ($t_{1/2}$) of the disappearance of the infused material from the circulation were determined.

B. Results. The results are presented in Table I. The half-times of disappearance in dogs of the radioactivity of the plasma obtained from euthyroid donors ranged from 21.0 to 37.6 hours, with an average of 29.5 ± 5.5 hours. In contrast, the half-times of disappearance of radioactivity of the thyrotoxic plasma ranged from 10.2 to 17.0 hours, with an average of 13.2 ± 2.2 hours. The difference is very highly significant statistically (the standard error (S.E.) of the difference between the means is 2.2, and "t" value is 7.41, and "p" is < 0.001).⁵

In preliminary observations, the half-times of disappearance in dogs of radioactivity of plasma from three patients with *nodular* toxic goiter fell within the euthyroid range—21.0, 22.0, and 22.2 hours. (See Table I.)

Series 2—Infusion of in vitro I-131-thyroxin-labelled human plasma—Disappearance rate in dog

A. Methods. Data identifying the donors and the plasma infused are presented in Table II. For each of seven paired experiments, plasma was obtained as described above from one euthy-

⁵ Calculated for unpaired data with unequal variance and seven degrees of freedom.

roid subject and one patient with typical diffuse toxic goiter who had received no anti-thyroid therapy. An approximately equal amount of I-131-l-thyroxine⁶ was added, under sterile precautions, to each plasma in a paired run. This varied from 1.0 to 20.0 $\mu\text{c.}$, representing 0.22 to 14.9 $\mu\text{g.}$, added to 12 to 22 ml. of plasma. After standing at room temperature for the same interval of time, which varied in different experiments, equal volumes of each plasma were infused into unanesthetized dogs. In Experiment No. 7, each dog received morphine sulfate (1.5 mg. per Kg.) 30 minutes before the infusion. The recipients' plasmas were analyzed at frequent intervals for residual radioactivity (see above). Following an initial rapid decrease in plasma radioactivity for six to eight hours, there was a slower exponential decrease for 30 to 48 hours from which the half-times of disappearance were obtained as described above and compared in each paired experiment.

B. *Results.* Data identifying the recipient dogs and the comparison of the disappearance rates of

each paired experiment are presented in Table II. I-131-thyroxine, added to plasma, is almost immediately incorporated within the protein-precipitable fraction. In both the euthyroid and thyrotoxic plasmas infused, the radioactivity of aliquots was, within the limits of the methods, completely trichloroacetic acid-precipitable and butanol-soluble. The euthyroid and hyperthyroid plasmas, therefore, could not be differentiated by these chemical procedures.

In contrast, in the first six experiments, the half-times of disappearance in dogs of the I-131-thyroxine-euthyroid plasmas were 17.0 to 30.2 hours, with an average of 23.5 ± 4.6 hours; those of the I-131-thyroxine-hyperthyroid plasmas were 10.4 to 15.0 hours, with an average of 12.7 ± 1.5 hours. The difference is highly significant—S.E. of the mean of the differences of the paired experiments equals 1.60, "t" equals 6.75, $p < 0.001$. In each paired experiment, the half-time of disappearance of the material obtained from the euthyroid donor was greater than that from the thyrotoxic patient; the ratios (euthyroid to hyperthyroid half-times) ranged from 1.3 to 2.4. In Experiment 7, in which each animal was pre-treated with morphine sulfate, both disappearance

TABLE II
Infusion of in vitro I-131-thyroxine-labelled human plasma into dogs

Expt. No.	Donor		Infusion				Recipient			Results	
	Thyroid status*	24-hr. I-131 uptake	Incubation period	Amount infused	I-131-thyroxine added		Sex	Wt.	Time followed	$t_{1/2}$	Ratio of $t_{1/2}$'s Eu/Tx
		%	(hrs.)	(ml.)	$\mu\text{c.}$	$\mu\text{g.}$		(Kg.)	(hrs.)	(hrs.)	
1	Eu	22.5	$\frac{1}{2}$	12	11.7	5.7	F	12.3	30	18.0	1.5
	Tx	71.0			11.7	5.7	F	12.3	45	12.0	
2	Eu	27.0	22	18	15.1	13.5	M	13.6	44	17.0	1.3
	Tx	75.0			16.7	14.9	M	15.9	44	13.0	
3	Eu	37.5	2	20	5.0	1.5	F	16.4	144	30.2	2.0
	Tx	73.8			5.0	1.5	M	16.8	144	15.0	
4	Eu	21.7	$1\frac{1}{2}$	20	1.0	0.41	M	11.8	48	24.5	2.1
	Tx	80.2			1.4	0.57	M	11.4	48	11.5	
5	Eu	17.0	3	20	3.5	0.22	M	15.0	96	26.5	1.9
	Tx	80.7			5.3	0.32	M	16.4	96	14.0	
6	Eu	18.0	$\frac{3}{4}$	22	2.3	2.3	F	30.9	96	24.6	2.4
	Tx	76.7			3.1	3.1	M	29.5	96	10.4	
7	Eu	37.5	24	12	20.0	6.3	M†	13.6	76	32.5	1.5
	Tx	73.8			15.5	4.9	M†	12.7	76	22.0	

* Eu = Euthyroid.

Tx = Diffuse toxic goiter.

† Each dog received morphine sulphate 1.5 mg. per Kg. 30 minutes before the infusion.

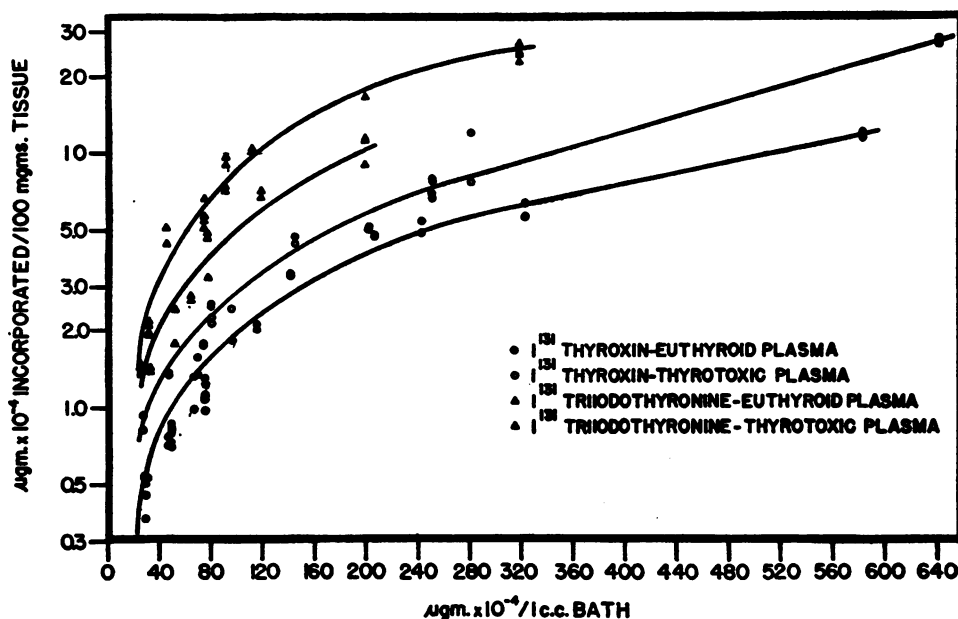


FIG. 2. FIVE HOUR *In Vitro* "UPTAKE" BY RAT DIAPHRAGM OF LABELLED THYROID HORMONE COMPONENTS FROM EUTHYROID AND HYPERTHYROID PLASMAS

Each symbol represents the average of two to four runs with a given plasma.

rates were slower than in untreated animals in the respective groups, but again the disappearance of the euthyroid-labelled plasma was distinctly slower than the hyperthyroid with a ratio similar to that of untreated animals.

Series 3—In vitro I-131-thyroxin- or I-131-triiodothyronine-labelled human plasma—Uptake by rat diaphragm

A. Methods. Varying amounts of I-131-l-thyroxin (I-131-l-THY) or I-131-l-triiodothyronine (I-131-l-TRI) were added to plasmas obtained as described above from euthyroid and untreated thyrotoxic patients. Three-ml. aliquots were then incubated with shaking at 37°C. under 95 per cent O₂-5 per cent CO₂ atmospheres in stoppered 10-ml. Erlenmeyer flasks for one-half to two hours. Female Sprague-Dawley rats, weighing 150 to 250 grams, fed on stock laboratory diet, were sacrificed by a sharp blow on the head and exsanguinated via a thoracic incision. The hemidiaphragms were rapidly excised, weighed on a Roller-Smith torsion balance, and placed in a labelled plasma aliquot. When paired experiments were performed, one hemidiaphragm was placed in the labelled euthyroid plasma, the other in the

thyrotoxic plasma. The flasks were then shaken at 37°C. for varying periods, usually five hours, with replenishment of the 95 per cent O₂-5 per cent CO₂ atmosphere at one or two hourly intervals. Following this period, each hemidiaphragm was removed, blotted, washed twice, each time for 20 seconds in 20 ml. 0.85 per cent NaCl solution, with interval and final blotting. The radioactive content was then determined by one of two methods: 1) a direct count of each hemidiaphragm in a well-type scintillation counter, or 2) by dissolving the tissue in hot 2N NaOH, drying an aliquot, and counting with conventional end window G-M tubes (13). From the specific activity of the radioactive solutions, the "uptake" from the euthyroid and hyperthyroid plasmas was calculated as μg. incorporated per 100 mg. wet tissue. The percentage "uptake" per 100 mg. wet tissue was also determined by comparison with the radioactivity of the bath solutions before addition of the hemidiaphragm. Each step was performed at least in duplicate.

To study the effect of the serum protein-bound iodine level, chromatographically pure sodium-l-thyroxin or l-triiodothyronine was added, in varying amounts, to euthyroid plasma, incubated at

TABLE III

In vitro rat diaphragm uptake of I-131-l-thyroxine and I-131-l-triiodothyronine from human plasma

Thyroid status	Diaphragm uptake (%/100 mg.)	
	I-131-thyroxine	I-131-triiodothyronine
Euthyroid (20)	0.68 ± 0.11*	1.79 ± 0.15
Diffuse toxic goiter (15)	1.05 ± 0.08	2.64 ± 0.5
Nodular toxic goiter (8)	0.69 (range, 0.58–0.8)	1.8 (range, 1.3–2.4)

* Standard deviation.

room temperature (23 to 25°C.) or at 37°C. for 20 minutes to 24 hours, following which the rat diaphragm uptake of I-131-THY or I-131-TRI was determined, as above, and compared to control uptakes in unenriched plasma. Forty-seven such comparisons were made, in duplicate to quadruplicate, at enriched PBI levels of 9.0 to 20 $\mu\text{g. per cent.}$

B. *Results.* The absolute tissue radioactive content increased with increasing bath concentrations (Figure 2). The percentage "uptake," however, varied within a limited range over a wide range of bath concentrations. In 164 duplicate determinations, using euthyroid plasmas from 123 subjects, labelled by the addition of 15 to 585×10^{-4} $\mu\text{g. I-131-l-THY per ml.}$, five hour "uptakes" ranged from 0.4 to 0.9 per cent per 100 mg. diaphragm, averaged 0.68 ± 0.13 per cent. In one determination, the uptake was 1.2 per cent. Similarly, in 140 runs, using euthyroid plasma from 109 subjects labelled by the addition of 12 to 475×10^{-4} $\mu\text{g. I-131-l-TRI per ml.}$, "uptakes" ranged from 1.2 to 2.5 per cent per 100 mg. tissue, averaged 1.74 ± 0.27 per cent.

TABLE IV

Effect of addition of l-thyroxine (stable) to plasma on in vitro rat diaphragm uptake of I-131-l-thyroxine

Plasma PBI	Diaphragm uptake
$\mu\text{g./100 ml.}$	%/100 mg.
7.5 (control)	0.91; 0.91; 0.86
10.7	0.89; 0.91; 0.84
12.0	0.81; 0.76; 0.84
14.3	0.80; 0.85; 0.85

In 24 of 26 paired experiments with I-131-THY bath concentrations of 30 to 500×10^{-4} $\mu\text{g. per ml.}$, the ratios of "uptakes" (hyperthyroid)/euthyroid, at the same bath concentration, ranged from 1.3 to 2.3 with an average of 1.7; in the remaining two runs, the ratio was 1.1. In 25 paired experiments with I-131-TRI (46 to 217×10^{-4} $\mu\text{g. per ml.}$), the ratios ranged from 1.3 to 2.3, with an average of 1.9. In one run, the ratio was 1.0. In preliminary experiments, the "uptakes" from eight *nodular* toxic goiter plasmas were in the euthyroid range (Table III).

The prior addition of chromatographically pure stable l-thyroxine or stable l-triiodothyronine to euthyroid plasmas to yield protein-bound iodine plasma concentrations comparable to those of the hyperthyroid state generally failed to increase significantly the subsequent percentage radioactive "uptake" by the rat diaphragm. A typical experiment is shown in Table IV. The various combinations of stable components added and subsequent radioactive "uptakes" are summarized in Table V. Thus, in 30 of 32 comparisons in which PBI levels were increased up to 18.4 $\mu\text{g. per cent}$ by the addition of l-thyroxine, there was no significant increased per cent uptake. An increase to

TABLE V

The effect of prior enrichment of euthyroid plasma on diaphragm "uptake"

Stable component added	PBI levels attained	Radioactive label	No. of runs	Effect on % "uptake"/100 mg. tissue
THY	$\mu\text{g./100 ml.}$ 8.6–17.4	I-131-THY	15	None
THY	10.4–18.4	I-131-TRI	15	None
	17.4		1	Increased from 2.0 to 3.1%
	15.8		1	Increased from 2.1 to 2.7%
TRI	8.0–15.0	I-131-THY	6	None
TRI	8.0–15.0	I-131-TRI	8	None
	15.2		1	Increased from 2.1 to 2.8%

hyperthyroid "uptake" levels of I-131 TRI was noted in two instances at PBI levels of 15.8 and 17.4 $\mu\text{g.}$ per cent. In correlative studies, not included in Table V, increased uptakes were consistently obtained at PBI levels above 25 $\mu\text{g.}$ per cent. The lowest level at which an increase was consistently effected was not determined. Quantitative data on triiodothyronine plasma levels in hyperthyroidism are not definitive. In 14 of 15 runs with PBI levels increased up to 15 $\mu\text{g.}$ per cent by the addition of stable triiodothyronine, there was no significant increased per cent "uptake" of I-131-THY or I-131-TRI. In one experiment, an increase in PBI from 5.0 to 15.2 $\mu\text{g.}$ per cent resulted in an increased "uptake" from 2.1 to 2.8 per cent.

DISCUSSION

The results of the three series of experiments show striking and consistent differences—1) thyroid hormone from patients with diffuse toxic goiter, labelled either endogenously by prior I-131 administration, or *in vitro* by the addition of I-131-tagged-thyroxin, and infused into dogs, disappeared more rapidly from the circulation than similarly labelled thyroid hormone from euthyroid subjects, and 2) greater amounts of radioactivity were incorporated *in vitro* by rat diaphragm from thyrotoxic plasma labelled *in vitro* with I-131-thyroxin or I-131-triiodothyronine than from similarly tagged euthyroid plasma, or from euthyroid plasma artificially enriched to hyperthyroid PBI levels.

These results are not adequately explained by prevailing concepts and indicate 1) a difference in the qualitative nature of the thyroid hormone-protein complex in hyperthyroidism as compared to the euthyroid state, or 2) the presence of a factor(s) in the plasma of the hyperthyroid individual differing qualitatively or quantitatively in its effect(s) on the disappearance from the circulation, or on tissue uptake, of the labelled hormone moiety.

Series 1 was based on the generally accepted concept that administered I-131 is taken up by the thyroid gland, incorporated therein into the thyroid hormone, which is released to circulate as a protein-bound hormone complex. It has been shown (8, 9, 15, 16) that the administration of very large doses of I-131 results in the release

into the circulation of an abnormal I-131-labelled product, probably thyroglobulin, which is protein-precipitable but not butanol-soluble. Since it was therapeutically necessary to administer larger I-131 doses to our euthyroid subjects than to the patients with hyperthyroidism, the possibility had to be considered that the slower disappearance rate of the euthyroid plasma radioactivity in our studies was due to its content of an abnormally large, I-131-tagged thyroglobulin-like compound. However, the I-131 dosages used and the R.E.P. delivered to the donors' glands were, with the exception of Case 1, Series 1, below the range, that is, 3,600 R.E.P. β or more during the second 24 hours, found by Robbins, Rall, Becker, and Rawson (16) to be associated with the release of significant amounts of a thyroglobulin-like material into the circulation. Tubiana (17) found the butanol solubility of serum radioactivity of euthyroid patients receiving 50 to 100 mc. to be comparable to that following tracer doses.

The butanol solubility of the plasma was determined in six of the eight euthyroid subjects of Series 1. There was complete overlapping of these values with those of the thyrotoxic plasmas. Therefore, no significant amounts of a thyroglobulin-like fraction appear to have been released from the thyroid glands by the large dosages employed in our euthyroid group as compared to the thyrotoxic group. That the release of such a substance is probably not an important factor in the differences observed is further indicated by the consistent comparative results in Series 2 and 3 which are independent of I-131 radiation effect upon the donor's thyroid gland.

The possibility has been considered that the increased rate of disappearance or tissue "uptake" of the labelled thyrotoxic plasma might be due, in part, to its greater content of an unlabelled protein-bound iodine moiety, *i.e.*, a greater "saturation" of the specific binding plasma protein(s) by thyroxin or triiodothyronine with a resultant "spill-over" of the labelled component onto another plasma protein (*e.g.*, albumin) with a less firm binding capacity. Gross and Leblond (18) have shown, in the rat, that with greatly increasing dosage of infused I-131-thyroxin, from 10 to 2,000 times the estimated physiological range, there was a decrease in the percentage of the dose remaining in the plasma at given time intervals with a cor-

responding increase in the proportion found in the liver. Our results in Series 3 similarly indicate a positive correlation between the plasma concentrations of the labelled hormone component and the resultant absolute *in vitro* tissue uptake thereof.

Several recent studies have dealt with the problem of the binding capacity for thyroxin of serum, more specifically its thyroxin-binding protein ("TBP") or globulin ("TBG"). Albright, Larson, and Deiss (19) found this binding capacity to vary inversely with thyroid function, the hypothyroid binding more and the hyperthyroid less thyroxin than normal. The relationship between thyroxin added and that bound by "TBG" was roughly linear at concentrations of 0.05 to 0.4 $\mu\text{g.}$ per ml., then reached a plateau. "Saturation" of "TBG" was reached at concentrations of approximately 0.15 to 0.2 $\mu\text{g.}$ per ml. in the hyperthyroid as compared to 0.25 $\mu\text{g.}$ per ml. in the euthyroid subject. There was distinct overlapping of the amounts bound by the euthyroid and hyperthyroid "TBG" at concentrations up to 0.2 $\mu\text{g.}$ per ml. Robbins and Rall (20), in contrast, found that the concentration of thyroxin in α -globulin did not reach a plateau but continued to increase with increasing addition of thyroxin. The per cent thyroxin bound to alpha globulin at serum thyroxin concentrations of 0.059 to 0.12 $\mu\text{g.}$ per ml. varied in an apparently random fashion between limits of about 60 and 80 per cent. After a critical evaluation of the significant limitations of the methods, they estimated that saturation of "TBP" occurs at serum thyroxin levels at least two to three times normal. They were not certain whether the values for thyroxin binding differed significantly in a single hyperthyroid serum from those obtained with normals. In a subsequent study using a method of reverse-flow zone electrophoresis, Robbins (21) found the thyroxin-binding capacity of the binding sites for thyroxin in the α -globulin in seven normal subjects to range from 0.16 to 0.25 $\mu\text{g.}$ thyroxin per ml. serum, with a mean of 0.19. Freinkel, Dowling, and Ingbar (22) found that in all sera from hyper-, hypo-, and euthyroid subjects a small percentage of radioactivity migrated with albumin even at the lowest concentrations of thyroxin, and this progressively increased as the concentration of added thyroxin was augmented. They concluded, however, that because "TBP" had not been isolated,

could not be measured directly, and had not been characterized as one or several proteins or binding-sites, more quantitative analysis was not justified. In further studies (23), these authors found that the *in vitro* "uptake" of radiothyroxin by slices of sheep, lambs, calves or beef heart, liver and kidney was dependent upon 1) the amount of tissue, 2) the amount of binding protein in the media, and 3) the concentration of thyroxin (stable plus labelled) in the media. Crispell, Kahana, and Hyer (24) found that the *in vitro* "uptake" of I-131-THY by red blood cells was increased by the addition of very large amounts of stable thyroxin (50 to 150 $\mu\text{g.}$) or triiodothyronine (100 $\mu\text{g.}$) to 2.0 ml. unwashed red blood cells. We have found (25) that prior addition of stable THY or TRI to whole blood increases the *in vitro* "uptake" of I-131-THY or I-131-TRI by the erythrocytes. This effect, however, increases with increasing time of prior incubation of the blood with the stable component and may not, therefore, be due solely to saturation of the binding protein(s), which effect is not time dependent.

Thus, precise quantitative relationships cannot be made at this time. Although it appears that available binding sites can be saturated at sufficiently high PBI levels, failure to obtain increased per cent *in vitro* "uptakes" by the enrichment of plasmas to PBI levels found clinically in hyperthyroidism lead us to conclude that our results are not adequately explained on this basis.

Furthermore, between our two groups in Series 1, there was distinct overlapping of the calculated specific activities of the infused material because of the varying concentrations of plasma radioactivity and amounts of plasma infused (Table I). It was estimated that the amounts of protein-bound iodine complex infused ranged from 1.8 to 3.6 $\mu\text{g.}$ in the euthyroid group as compared to 3.2 to 9.0 $\mu\text{g.}$ in the thyrotoxic group. There was no apparent correlation within either group between the estimated amount of the stable plus labelled protein-bound iodine complex in the infusion and the observed disappearance rate in the recipient.

Further evidence was obtained in Series 2 and 3 that the observed difference in disappearance rates between euthyroid and thyrotoxic plasma in Series 1 was not due solely to differences in the respective amounts of protein-bound iodine compounds. Despite the wide ranges of $\mu\text{g.}$ and $\mu\text{c.}$ of

added radioactive materials and the resultant wide ranges of specific activities of the infusions and baths in Series 2 and 3, the disappearance rates of radioactivity and the tissue "uptake" of radioactivity were consistently greater with thyrotoxic than with euthyroid plasmas under comparable experimental conditions. Total "uptake" of thyroxine (labelled plus unlabelled) from thyrotoxic plasma was more than twice that from labelled euthyroid plasmas containing comparable total amounts of thyroxine, calculated with the assumptions that the average PBI of euthyroids is 6.0 $\mu\text{g.}$ per cent, that of the hyperthyroids is 15.0 $\mu\text{g.}$ per cent and that these values represent chiefly thyroxine. Similar estimates of total triiodothyronine "uptake" cannot be made at present because of lack of data quantitating plasma triiodothyronine levels.

The results of the studies in nodular toxic goiter, although preliminary, are of interest. Although these patients could not be differentiated clinically or by standard laboratory methods from patients with diffuse toxic goiter, the disappearance rate of endogenously I-131-labelled thyroid hormone (three patients) and the tissue "uptake" of exogenously labelled plasma (eight patients) fell within the euthyroid range. Such results are consistent with the hypothesis that there is an overproduction of the normal thyroid hormone in certain hyperthyroid patients with a nodular goiter.

Available data are not sufficient to permit precise definition, in qualitative or quantitative terms, of the body compartment(s) involved in our infusion experiments. Extension of these studies to investigate the actual turnover of the infused component requires the establishment of conditions of equilibrium. In certain experiments in Series 1 and 2, the recipient's plasma radioactivity, beyond 48 hours, decreased at a third, slower exponential rate. Since the thyroid gland was not blocked in our experiments, it is possible that this is due, in part, to the addition to the plasma of "new" hormone, endogenously labelled in the dog's thyroid by the I-131 liberated in the catabolism of the infused, labelled hormone. Because this added hormone is not distinguishable by methods of this study from the infused test hormone, we have limited this analysis of infusion studies to the first 48 hours. Our results, therefore, are not to be interpreted in terms of the ultimate metabolism of the

infused hormone, as has been done by Levy, Kelly, Cooper, and Jefferies (26) who demonstrated, in prolonged infusion studies, that the ultimate turnover was dependent upon the metabolic state of the recipient rather than that of the donor.

Despite this limitation, the differences observed between euthyroid and hyperthyroid plasmas under these varied experimental conditions appear significant. They raise the question of the validity of the concept that the essential abnormality in hyperthyroidism is the overproduction of the normal thyroid hormone. While it had been postulated that the circulating thyroid hormone was wholly a thyroxine-protein compound, the studies of Gross and Pitt-Rivers (2, 27, 28) demonstrating the natural formation and circulation of the calorically potent triiodothyronine, have necessitated a re-evaluation of this concept. We have found in preliminary studies (29) that the disappearance rate of I-131-l-triiodothyronine added to euthyroid plasma and infused into a dog is significantly greater than that of equivalent amounts of I-131-l-thyroxine added to an equal aliquot of the same plasma. Rawson *et al.* (30) have demonstrated, in euthyroid athyreotic humans, that I-131-triiodothyronine disappears from the plasma following intravenous administration at rates 2.4 to 4.8 times as rapidly as I-131-thyroxine. Sterling, Lashof, and Man (31) have obtained comparable results in euthyroid subjects. In Series 3, the rat diaphragm "uptake" of I-131-triiodothyronine was always greater than that of I-131-thyroxine at comparable bath concentrations of both euthyroid and hyperthyroid plasmas. Ratios of "uptake" (I-131-triiodothyronine to I-131-thyroxine) of 1.6 to 4.1, with an average of 3.0, were obtained in 31 runs in baths containing 30 to 340×10^{-4} $\mu\text{g.}$ radioactive material per ml. We have also found (32) that, over a wide range of concentrations and incubation times, the "uptake" by rat diaphragm of I-131-l-triiodothyronine in buffer solutions is consistently greater (2.0 to 4.5 times) than that of I-131-l-thyroxine in comparable buffer solutions. Such findings suggest the possibility in hyperthyroidism of delivery to the tissues of a hormone complex containing relatively greater amounts of triiodothyronine or some other as yet unidentified potent component which enters peripheral tissues more rapidly and in greater amounts than does

thyroxin. This would not explain, however, our observations of greater disappearance rates and tissue incorporation of I-131-thyroxin from thyrotoxic as compared to euthyroid plasma.

Other possibilities which may be of pathogenetic importance in hyperthyroidism are 1) an abnormal linkage, in kind or degree, of the thyroxin or triiodothyronine to a plasma protein component(s), and 2) the effect of a plasma factor(s), either abnormal or present in increased amounts, which increases the rate of utilization of the hormone-protein complex, leading to a compensatory increase in production of the hormone by the thyroid gland to meet the increased demand. Such an effect might be specifically on the utilization of the thyroid hormone, or indirectly mediated. Relevant data on the effects of the infusion of heterologous plasma in animals are scanty. Sanford and Blackford (33) have reported that the injection, in dogs, of thyrotoxic sera or extracts of exophthalmic goiters produced a marked transient drop in blood pressure. Schoenborn (34), however, failed to find any difference in blood pressure response in cats following infusion of extracts of normal and thyrotoxic glands. Preliminary attempts to measure the blood pressure response in our unanesthetized dogs have been unsuccessful. Further studies are necessary to clarify these possibilities.

Experience with the methods used in this study has not been sufficient to permit evaluation of their possible use for diagnostic purposes. Extensive experience with tests of each single parameter of thyroid function has yielded overlapping results when applied to the broad spectrum of various levels of thyroid activity. The methods used in this study, however, do permit investigation of important parameters of thyroid function, concerning which pertinent data are meager. It is believed that their extension and integration with other methods in the over-all study of thyroid physiology and pathology are clearly indicated.

SUMMARY

1. Three series of experiments were performed to test the generally accepted concept of the biologically qualitative identity of the circulating thy-

roid hormone in the normal and hyperthyroid states.

2. The half-times of disappearance of endogenously I-131-labelled euthyroid human thyroid hormone during the first 24 to 48 hours following infusion into the dog ranged from 21.0 to 37.6 hours with an average of 29.5 ± 5.5 hours. In comparable experiments using labelled hormone from patients with diffuse toxic goiter, the half-times of disappearance ranged from 10.2 to 17.0 hours, with an average of 13.2 ± 2.2 hours. The difference was statistically very highly significant.

3. The half-times of disappearance in the dog of *in vitro*-labelled I-131-thyroxin euthyroid plasma ranged from 17.0 to 30.2 hours, with an average of 23.5 ± 4.6 hours; those for similarly labelled thyrotoxic plasma ranged from 10.4 to 15.0 hours with an average of 12.7 ± 1.5 hours. The difference was statistically highly significant.

4. Radioactivity of *in vitro*-labelled I-131-thyroxin-thyrotoxic plasma and I-131-triiodothyronine-thyrotoxic plasma was incorporated by the rat diaphragm *in vitro* in amounts averaging 1.7 and 1.9 times that of I-131-l-thyroxin-euthyroid plasma and I-131-triiodothyronine-euthyroid plasma, respectively.

5. The prior enrichment of euthyroid plasma by the addition of l-thyroxine or l-triiodothyronine to hyperthyroid PBI levels failed to increase the subsequent *in vitro* percentage radioactive "uptake" by the rat diaphragm to hyperthyroid levels.

6. In preliminary studies in patients with *nodular* toxic goiter, the half-times of disappearance of endogenously I-131-labelled thyroid hormone and *in vitro* rat diaphragm "uptakes" of I-131-l-thyroxin and I-131-l-triiodothyronine fell within the euthyroid range.

7. The results are not adequately explained by the prevailing concept that thyrotoxicosis with diffuse toxic goiter is caused solely by an excess of the normal thyroid hormone. They indicate 1) a qualitative difference in the nature of the circulating hormone-protein complex in hyperthyroidism as compared to the euthyroid state, or 2) a differential effect of a plasma factor(s) on the "peripheral utilization" of thyroid hormone in the two thyroid states.

8. Certain other theoretical implications of these concepts are discussed.

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