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Research Article

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Formation of Iodoprotein during the Peripheral Metabolism of 3,5,3′-Triiodo-L-Thyronine-^{125}I in the Euthyroid Man and Rat

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ABSTRACT 3,5,3′-Triiodo-L-thyronine-^{125}I (T3-^{125}I) metabolism was studied in nine euthyroid human subjects on blocking doses of nonradioactive iodide. After the intravenous injection of T3-^{125}I, the fractional disappearance rate of plasma radioactivity progressively decreased with time. Analysis of individual plasma samples by dialysis, electrophoretic, and extraction techniques revealed three radioactive components: T3-^{125}I, iodide-^{125}I, and an unidentified material which was nonextractable in acid butanol (NE^{125}I). Ne^{125}I rose to maximal levels 24–36 hr after injection of T3-^{125}I and then decreased with a fractional rate which approached, after 12–14 days, approximately 0.05 day^{-1} ($t_{\frac{1}{2}} = 14$ days). The plasma T3-^{125}I concentration, obtained by subtraction of iodide-^{125}I and NE^{125}I from the plasma total ^{125}I, declined at a constant fractional rate with time with a $t_{\frac{1}{2}}$ of 1.5 days. Qualitatively similar results were obtained in rats. After 72 hr, 57% of the plasma and 40% of the liver radioactivity was NE^{125}I. Chromatographic purification of the T3-^{125}I before injection did not alter these results. The extrathyroidal origin of NE^{125}I was further demonstrated by similar results in thyroidectomized rats maintained on thyroxine. NE^{125}I from human sera separated from the other radioiodinated substances by ion-exchange chromatography was quantitatively precipitated by trichloracetic acid, not dialyzable, insoluble in $CHCl_3$: CH_3OH, and migrated with albumin during starch-gel electrophoresis. Based on these properties, NE^{125}I was tentatively identified as iodoalbumin. Observations in rats equilibrated with ^{125}I, as well as nonradioactive iodine determinations in human sera before and after acid butanol extraction, indicate that 10–20% of the serum organic iodine is in the form of iodoprotein. Our studies suggest that this moiety may be derived at least in part from the peripheral metabolism of the thyroid hormones.

INTRODUCTION

Most estimates of thyroid hormone turnover depend upon an analysis of the plasma disappearance curve of intravenously injected hormones labeled with radioactive iodine in the outer (β) ring of the thyronine nucleus. It is generally assumed that after an initial period of equilibration, the labeled hormone disappears from the circulation at a constant fractional rate as reflected by a linear time function on a semilogarithmic plot. It was thus of considerable interest that, contrary to expectations from published reports, the disappearance curve of triiodothyronine-^{125}I (T3-^{125}I) was observed to be curvilinear over a period up to 10 days after injection of the tracer. Even after correcting for inorganic ^{125}I we were unable adequately to describe any portion of the curve with a straight line. The possibility was considered that these results might be due to the appearance of a radioiodinated metabolic product of T3-^{125}I with a slow half-time. Accordingly, studies were initiated to analyze the plasma radioactivity after injection of T3-^{125}I. The results of the studies to be described are interpreted to indicate that the curvilinear disappearance curve of injected radioactivity is in fact due to the formation of a circulating iodoprotein. The appearance of

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iodoprotein as a product of the peripheral metabolism of thyroid hormone was considered to be of interest not only with respect to the specific problem of measuring the plasma disappearance rate of T3 but also with respect to broader problems bearing on the cellular metabolism of thyroid hormones.

METHODS

L-Thyroxine (T4), 3,5,3′-triiodo-L-thyronine (T3), and iodide labeled either with ^{125}I or ^{131}I were obtained from Abbott Laboratories, North Chicago, Ill. The iodide was carrier-free, and the specific activity (SA) of the iodothyronine preparations was 30–45 mc/mg. The radiochemical purity of these materials was determined by paper chromatography in tertiary amyl alcohol saturated with 2 N NH_4OH followed by radioautography. The areas of interest were counted in a well-type, 2-channel, Autogamma spectrometer (Packard Instruments Co., Inc., Downers Grove, Ill., series 410E). 3-Monoiodotyrosine-^{125}I ($M^{125}IT$) (SA 30 mc/mg) was synthesized by the iodination of tyrosine by the method of Greenwood, Hunter, and Glover (1).

The human subjects employed for the turnover studies were hospitalized patients with various illnesses whose clinical condition was relatively stable. One was a paid volunteer (Table I). They were free from thyroid disease as determined by history, physical examination, and measurement of thyroxine-iodine–by column chromatography (T4I-by column, BioScience Laboratories, Los Angeles, Calif.). Lugol's solution, 5 drops t.i.d., was administered throughout each study starting from the day preceding the isotope injection in order to limit thyroidal trapping of radioiodide either injected as the isotope under study or released during the metabolism of radioactive thyroid hormones. The adequacy of the thyroid blockade was determined periodically by external scintillation scanning of the neck area along with appropriate neck phantoms containing known fractions of the injected dose. Weighed amounts of the various radioactive preparations (20–75 μc) diluted in 1% human serum albumin or 0.9% NaCl (subjects W.B. and T.B.) were injected through the tubing of a running intravenous infusion of isotonic saline. Heparinized plasma was obtained at appropriate intervals thereafter for determination of radioactivity and for other analyses.

Turnover studies were also performed in male rats of the CD strain, weighing 150–250 g, which were obtained from Charles Rivers Laboratories, Boston, Mass. The animals were maintained at 25°C and fed Wayne Rat Diet (iodine content approximately 1 μg/g of diet). When thyroidectomized rats were used, thyroxine, 3 μg/100 g of body weight, was administered daily by intraperitoneal injection, and calcium gluconate was added to the drinking water (1% solution). All animals received 1 mg NaI/day subcutaneously throughout each turnover study. Weighed solutions of various substances labeled with ^{125}I were injected intravenously through the tail vein under light ether anesthesia. Heparinized blood samples (0.3 ml) were obtained at appropriate intervals by cutting the tail. At the termination of each study the animals were exsanguinated through the abdominal aorta, and the livers and kidneys were excised. Tissues were homogenized in ice-cold saline (1:5, w/v) with a motor-driven homogenizer with a Teflon pestle. Tissue homogenates were then counted and extracted as described below.

The iodine pools of one group of rats were equilibrated with ^{125}I administered by daily injection. First, their stable iodine pools were depleted by 2-wk feeding of Low Iodine Test Diet, Nutritional Biochemicals Corporation, Cleveland, Ohio (iodine content [Bioscience Laboratories], less than 0.05 μg/g). Then they received daily subcutaneous injections of 12 μg of iodide-^{125}I, SA 0.1 mc/mg, for 70 days.[1] At the end of this period, plasma and tissue samples were obtained and counted and extracted as described below. In addition, whole body homogenates were prepared from three rats. After the animals were killed by cervical fracture the thyroids were removed. The shaved carcass was passed through

[1] The assumption that isotopic equilibration had been attained by this method is based on the observations of Heninger, R. W., F. C. Larson, and E. C. Albright. *J. Clin. Invest.* 1963, **42**: 1761.

TABLE I
Clinical Data

Subject	Sex	Age	Weight	Diagnosis	Turnover study	Plasma thyroxine–iodine
			kg			*μg/100 ml*
W.B.	M	38	57.3	Sarcoidosis	T3	5.4
W.S.	M	24	69.1	Pulmonary tuberculosis	T3	5.1
W.E.	M	26	97.8	Paid volunteer	T3	4.6
T.B.	F	76	91.4	Chronic cholelithiasis	T3	4.1
W.B.	M	62	58.9	Carcinoma of the lung	T3	5.6
R.C.	M	35	49.5	Cardiomyopathy	T3	4.9
L.C.	F	63	70.2	Fracture lumbar vertebra, traumatic	T3	3.3
B.S.	M	67	63.6	Chronic bronchitis	T3	3.7
J.W.	M	56	72.8	Laennec's cirrhosis	T3	4.9
C.B.	M	33	89.2	Pulmonary tuberculosis	T4	5.8
J.N.	M	42	66.4	Pulmonary tuberculosis	T4	4.4
N.Z.	F	62	67.5	Mitral stenosis	Iodide	4.7
P.C.	M	56	63.8	Chronic bronchitis	Iodide	5.6
C.B.	M	67	71.0	Carcinoma of the lung	Iodide	4.3

T3, 3,5,3′-triiodo-L-thyronine; T4, L-thyroxine

a hand meat grinder and then homogenized for 20 min in a large Waring blender in the cold.

Samples of plasma and tissue homogenates were counted in thin-walled plastic tubes to limit self-absorption of the low energy emission of ^{125}I. Known dilutions of the injected dose were counted simultaneously to obviate corrections for physical decay. When ^{125}I and ^{131}I were present in the same tube, the counts observed in the ^{125}I channel were corrected for the contribution of ^{131}I. Sufficient counts were accumulated so that counting errors did not exceed 1%. The per cent of the counts due to iodide was determined by rapid paper electrophoresis (2) or by dialysis. In the latter procedure, 1 ml of plasma or homogenate was dialyzed against 400 ml of phosphate buffer, 0.1 mole/liter, pH 7.4, for 2 hr. The per cent of iodide was calculated as the difference between the sample counting rate before and after dialysis divided by the initial counting rate. Preliminary studies with different isotopes added to plasma or tissue homogenates in vitro had shown that virtually all iodide was removed from the sample by this procedure, while less than 1% of T4 or T3 was lost.

The per cent of nonextractable radioactivity in plasma samples and tissue homogenates was determined by recounting each sample after four extractions with 4 volumes of 1-butanol saturated with 5% HCl. In the M^{125}IT turnover studies 95% ethanol was used as the solvent. In the human turnover experiments ^{131}I-labeled T4, T3, or I^- was added to the plasma in vitro before extraction. The per cent of nonextractable radioactivity of the ^{131}I isotope, generally 1–3%, was subtracted from the per cent of nonextractable ^{125}I to correct for day-to-day variations in the extraction procedure.

The concentration of nonextractable unlabeled iodine was determined in the serum of six human subjects with documented hypothyroidism who had been maintained on synthetic preparations of T4 and T3 for a minimum of 4 months. Protein-bound iodine and T4-iodine (T4I)–by column determinations were performed on a 3 ml portion of each serum. 10-ml samples of each serum were then dialyzed for 2 hr against distilled water. After dialysis the serum was removed from the dialysis bag and divided into two equal portions. One portion was lyophylized, while the other was extracted five times with 4 volumes of 1-butanol saturated with 5% HCl and then lyophylized. Total iodine determinations were then performed on the powders (BioScience Laboratories). In these studies doubly glass distilled, deionized water was employed for all procedures (resistance $= 1.2 \times 10^6$ ohms, iodine content = less than 0.05 μg/100 ml).

For the separation of the nonextractable radioactivity of two human sera from the other radioactive constituents 25-ml portions of sera were fractionated on Dowex 1-X2, 200–400 mesh ion-exchange resin by the method of Lewallen (3). The optical density at 280 mμ for various fractions of the eluates was determined on a Beckman DU-2 spectrophotometer. The distribution of radioactivity in starch-gel electrophoretograms was measured by methods previously described (4).

RESULTS

Turnover studies in human subjects. The disappearance of plasma radioactivity after the injection of T3-^{125}I into nine human subjects is depicted in Figs. 1 and 2. A curvilinear function of the log of the plasma radioactivity against time was observed in each subject during the first 7–10 days of study so that a straight line indicating a single exponential function

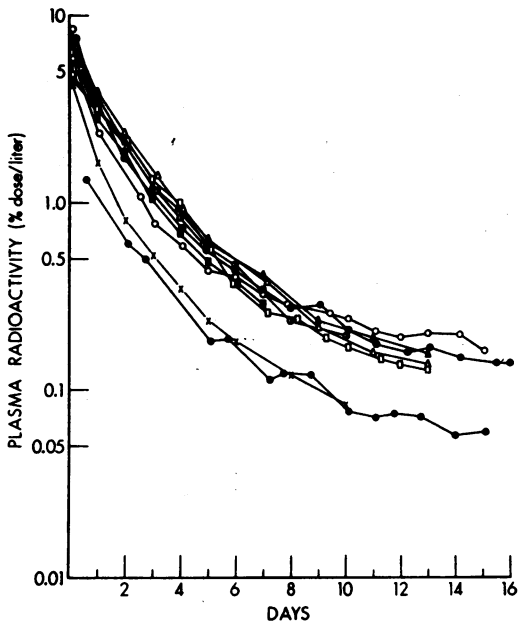

FIGURE 1 Individual curves for the disappearance of plasma radioactivity after injection of 3,5,3'-triiodo-L-thyronine-^{125}I (T3-^{125}I) into nine human subjects.

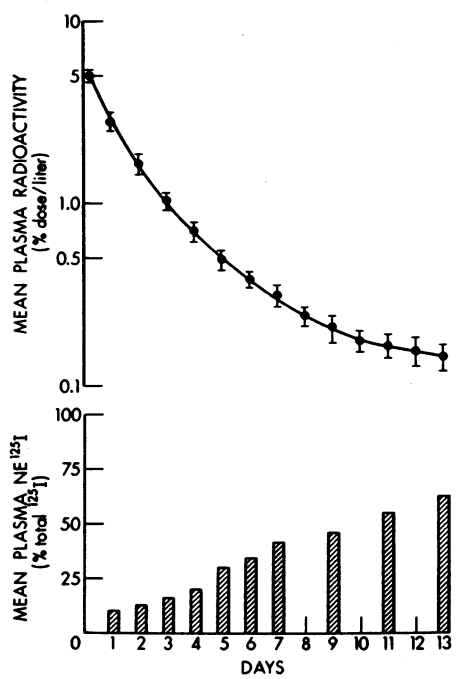

FIGURE 2 Plasma total ^{125}I and nonextractable ^{125}I (NE^{125}I). The data points in the upper panel represent the mean ±SE of the plasma total radioactivity for nine human subjects after injection of T3-^{125}I. The bars in the lower panel show the average per cent of NE^{125}I in the plasma of three subjects (L.C., B.S., and J.W.).

for the disappearance rate of this hormone could not be drawn through any series of data points. The adequacy of thyroidal block during these studies was assessed in three subjects (L.C., B.S., and J.W.) by measurement of thyroidal radioactivity using external scintillation scanning. Multiple determinations during the first 10 days after injection of T3-^{125}I revealed a thyroidal uptake always less than 2.0% of the injected dose.

The nature of the radioactivity in each plasma sample from three subjects, L.C., B.S., and L.W. was then examined. The per cent of iodide-^{125}I (^{125}I$^-$) and nonextractable ^{125}I (NE^{125}I) was determined directly. The per cent dose/liter of plasma T3-^{125}I was assumed to represent the difference between the plasma total ^{125}I and the contribution of NE^{125}I and ^{125}I$^-$.

The most striking observation resulting from these analyses was the presence of an increasing fraction of NE^{125}I with time in each of the patients studied (Fig. 2). Representing 8.3% of the plasma ^{125}I at 24 hr, the average per cent of NE^{125}I increased to 30.9% and 45.7% after 5 and 9 days respectively. Observations were extended to 36 and 44 days in J.W. and B.S. In these subjects, the per cent of NE^{125}I achieved an apparent plateau value of about 70% at 14–16 days. As will be pointed out below the per cent of NE^{125}I determined after acid butanol extraction may be underestimated by 15–25%, since NE^{125}I isolated by ion-exchange chromatography is partially extracted by this solvent. The partial extraction of NE^{125}I by acid butanol may account for the apparent failure to achieve a higher percentage of NE^{125}I in J.W. and B.S.

The three components of the plasma T3-^{125}I disappearance curve from a representative subject are shown in Fig. 3. Both ^{125}I$^-$ and NE^{125}I rose to their maximal values 24–36 hr after T3-^{125}I was injected. Plasma ^{125}I$^-$ concentration then declined at a relatively constant fractional rate, parallel to the calculated T3-^{125}I curve. The disappearance rate of plasma NE^{125}I was slower than T3-^{125}I and ^{125}I$^-$ and, after 10–14 days, approached that of the plasma total ^{125}I.

The terminal portion (16–44 days) of the plasma disappearance curves of B.S. and J. W. was linear with slopes (k) of 0.058 and 0.046 day^{-1} ($t_{\frac{1}{2}}$ = 12 and 15 days respectively). If it is assumed that ^{125}I$^-$ derived from the metabolism of NE^{125}I is excreted solely in the urine, the volume of distribution (V_d) of NE^{125}I can be estimated by the following relationship:

$$V_d = \frac{\text{urine } ^{125}\text{I per 24 hr}}{k \text{ (mean plasma } ^{125}\text{I)}}$$

V_d for NE^{125}I was 8.0 liters in J.W. and 7.6 liters in B.S.

The calculated T3-^{125}I concentration decreased at a constant fractional rate during the first 7 days of the study, but, thereafter, the data points were above that

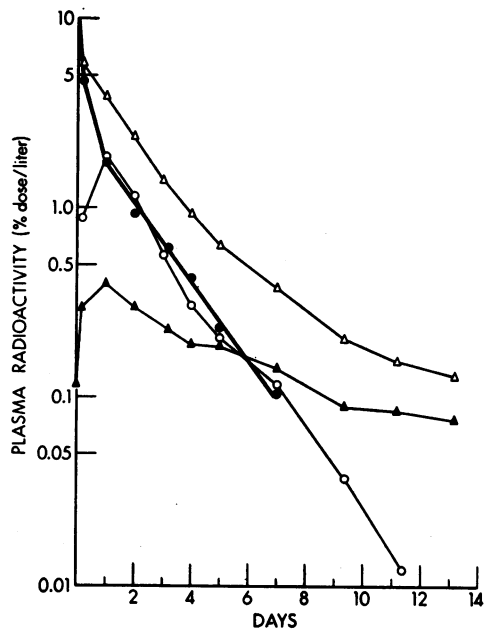

FIGURE 3 Plasma total radioactivity and its components after injection of T3-^{125}I into B.S. The individual curves represent the plasma total radioactivty (△——△), NE^{125}I (▲——▲, T3-^{125}I (●——●), and ^{125}I$^-$ (○——○).

anticipated by a forward extrapolation of the curve. This may be due in part to a 15–25% underestimation of NE^{125}I by acid butanol extraction as well as to errors inherent in cumulative subtraction. The presence of T3 conjugates was not evaluated. If the per cent of NE^{125}I is adjusted upwards by 20%, then the calculated T3-^{125}I concentration falls precisely on the extrapolated curve through 11 days, at which time it constitutes less than 10% of the plasma radioactivity. The mean distribution volume for the calculated T3-^{125}I curves determined by 0-time extrapolation was 38.1 liters. The mean T3-^{125}I fractional turnover rate in three subjects was 0.473 day^{-1} ($t_{\frac{1}{2}}$ = 1.47 days).

To determine whether the formation of NE^{125}I was uniquely related to the metabolism of T3, similar analyses were carried out in plasma after injection of T4-^{125}I and ^{125}I$^-$. After the first 48 hr, the rate of disappearance of T4-^{125}I over 50 days was linear on a semilog plot. The absolute concentration of NE^{125}I during T4-^{125}I metabolism was one-tenth that observed during T3-^{125}I metabolism after a comparable number of half-lives. After 50 days (approximately eight half-lives) NE^{125}I represented 11.1 and 8.7% of the plasma radioactivity in J.N. and C.B., respectively.

The plasma disappearance rate of injected ^{125}I$^-$ was linear (semilog plot) in three subjects (Fig. 4). Extraction of plasma obtained during these studies failed to reveal any NE^{125}I.

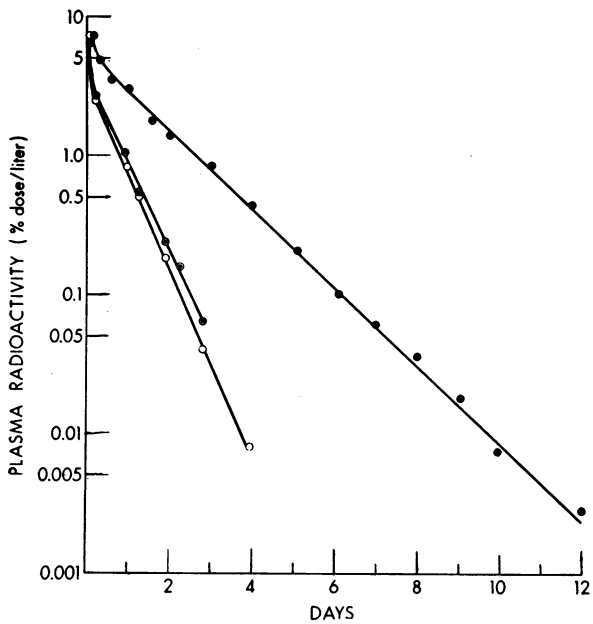

FIGURE 4 Disappearance of plasma radioactivity after injection of $^{125}I^-$ into three human subjects. The slow disappearance rate in subject N.Z. (0.622 day^{-1}) (upper curve) was due to mild congestive heart failure and unsuspected renal disease. In this subject, the renal clearance of iodide was 4.2 ml/min, and the endogenous creatinine clearance was also reduced to 45 ml/min.

Turnover studies in rats. In order to develop an experimental animal model for the generation of $NE^{125}I$, turnover studies of labeled T3, T4, iodide, and MIT were performed in rats. The rate of disappearance of these compounds decreased with time. Acid butanol extraction of each plasma sample in the T3 study showed an increasing fraction of $NE^{125}I$ with time. In the terminal plasma sample, $NE^{125}I$ constituted 56.9% of the total plasma radioactivity (Table II). Although $M^{125}IT$ and $^{125}I^-$ disappeared from the plasma at a greater fractional rate than T3-^{125}I, little $NE^{125}I$ was demonstrated during their metabolism. Thus, at approximately the same time after injection of the isotopes, the plasma $NE^{125}I$ concentration generated from T3-^{125}I was 10 times that from $M^{125}IT$ and 40 times that from $^{125}I^-$. Because of its slower fractional rate of metabolism, the plasma disappearance of T4-^{125}I was studied for 216 hr. Thyroxine-^{125}I disappearance rate appeared constant for the first 120 hr after injection but then decreased. The plasma $NE^{125}I$ concentration observed after T4-^{125}I was appreciably less than after T3-^{125}I.

After the plasma disappearance studies were completed, the livers and kidneys from these rats were examined for $NE^{125}I$ (Table II). The concentration of $NE^{125}I$ was greater in the tissues than in the plasma for all labeled compounds under study.

In order to evaluate the possible role of the thyroid gland in the generation of $NE^{125}I$ the peripheral metabolism of T3-^{125}I was studied in thyroidectomized rats maintained euthyroid by the daily injection of nonradioactive thyroxine (3 μg/100 g of body weight). The fractional rate of disappearance of T3-^{125}I decreased with time over 6 days (Fig. 5) with the mean curve comparable to the previous study with intact animals. The per cent of $NE^{125}I$ in each plasma sample was also similar to the above study.

Studies were also performed to evaluate the possibility that the nonextractable radioactivity might be an impurity in the commercially prepared labeled compounds which were employed for these investigations. A mixture of commercial T3-^{131}I and T3-^{125}I and a mixture of commercial T3-^{131}I and chromatographically purified T3-^{125}I were prepared. The radiochromatographic distribution of the individual isotopic preparations is shown in Table III. Each mixture was injected into two rats and the disappearance of plasma radioactivity for both isotopes as well as the per cent of $NE^{125}I$ determined over 10 days. Curves from representative rats injected with each mixture (Fig. 6) demonstrate no consistent difference between the plasma concentrations of each isotope within the two mixtures. Furthermore, no differences between the two isotopes were observed in the per cent of non-

TABLE II

*Nonextractable ^{125}I ($NE^{125}I$) in Plasma, Liver, and Kidney after Metabolism of T3-^{125}I, $^{125}I^-$, $M^{125}IT$, and T4-^{125}I in Rats**

Compound	Time	Plasma ^{125}I,	$NE^{125}I$			
			Plasma		Liver	Kidney
	hr	*% dose/ml*	*% total ^{125}I*	*% dose/ml*	*% dose/g*	*% dose/g*
T3-^{125}I	71	0.0084	56.9	0.0048	0.0157	0.0198
$^{125}I^-$	72	0.0003	33.3	0.0001	0.0006	0.0005
$M^{125}IT$	70	0.0028	14.3	0.0004	0.0009	0.0021
T4-^{125}I	216	0.0014	28.2	0.0004	0.0028	—

* T3-^{125}I, 3,5,3′-triiodo-L-thyronine-^{125}I; $M^{125}IT$, 3-monoiodotyrosine-^{125}I; T4-^{125}I, L-thyroxine-^{125}I; $NE^{125}I$, nonextractable ^{125}I. Figures represent the average for groups of two to four animals.

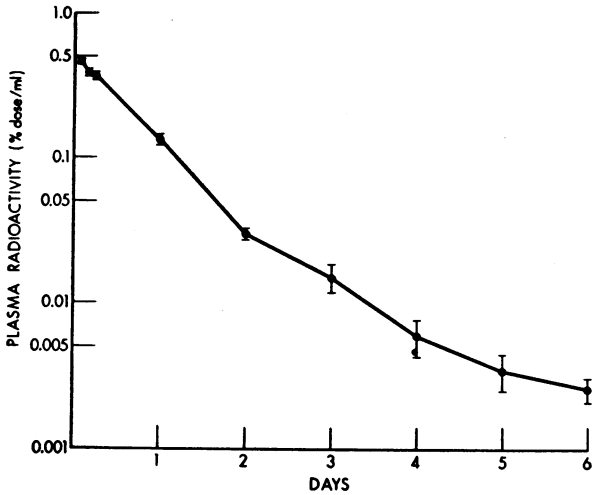

FIGURE 5 Disappearance of plasma radioactivity after injection of T3-^{125}I into thyroidectomized rats. Animals were maintained euthyroid by daily injections of thyroxine (3 μg/100 g, body weight) and were also given NaI, 1 mg/day, by subcutaneous injection. Data are plotted as mean ±SE for four rats.

extractable radioactivity in the individual plasma samples. Approximately 60–80% of the plasma radioactivity was nonextractable in the last 5 days of these studies.

A direct interaction between T3-^{125}I and the plasma proteins resulting in the formation of significant quantities of iodoprotein was excluded by experiments in which T3-^{125}I was added to human or rat sera in vitro. After incubation for 3 days at 37°C the T3-^{125}I was still completely extractable. Furthermore, careful ion-exchange column chromatographic studies of the preparations of T3-^{125}I in 1% albumin just before injection into human subjects or rats failed to reveal any iodoprotein.

Quantitation of nonextractable iodine in human plasma and in rats. When isotopic equilibration of iodine pools had been achieved after 70 daily injections of ^{125}I, the per cent of iodide (assayed by dialysis) and the per cent of nonextractable iodine were determined in the liver and plasma of five rats and in the whole body homogenates of three rats (Table IV). Iodide-^{125}I or T4-^{131}I were added to all samples in vitro before dialysis or acid butanol extraction as an internal control. Mean NE^{125}I was 6.8, 6.5, and 4.8% of the total iodine in the liver, plasma, and whole body homogenates, respectively. Since iodide constituted a large and variable fraction of the total iodine in these tissues, NE^{125}I was also calculated as the per cent of the organic iodine. On this basis, mean NE^{125}I was 8.4% for the livers, 10.9% for the plasmas, and 11.3% for the whole body homogenates.

The amount of NEI in human sera was determined in six subjects with well-documented hypothyroidism who had been treated with either T4 alone or with various

TABLE III

Distribution of Radioactivity in Different Preparations of Radioactive 3,5,3′-Triiodo-L-thyronine (T3)

Chromatographic area	Per cent of total radioactivity on chromatogram		
	Commercial preparations		
	T3-^{131}I	T3-^{125}I	Purified T3-^{125}I
Origin	0.1	0.2	0.1
Iodide	1.0	3.4	0.5
T4	2.0	2.0	0.7
T3	95.6	93.3	97.7
Solvent front	1.3	1.1	1.0

doses of a combination of synthetic T4 and T3 (Table V). The mean PBI was 5.1 μg/100 ml, and the mean T4I-by column was 4.5 μg/100 ml. After dialysis and lyophilization, the total iodine content of the sera was 4.5 μg/100 ml. When dialyzed sera were exhaustively extracted with acid butanol and then lyophilized, the iodine content of the residues was equivalent to 1.0 μg/100 ml of serum. By the use of tracer quantities of ^{125}I$^-$ and T4-^{131}I added to the sera before dialysis it was determined that 97–99% of iodide but virtually no T4 was removed by dialysis, and that 97–98.5% of the T4 was subsequently extracted. Thus, the serum NEI in these

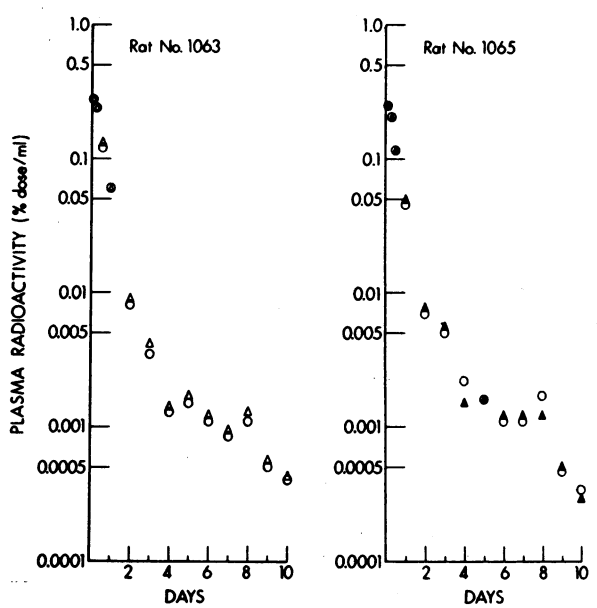

FIGURE 6 Disappearance of different preparations of radioactive T3 in rats. Rat No. 1063 was injected with commercial preparations of T3-^{131}I (○) and T3-^{125}I (△). Rat No. 1065 received the same preparation of T3-^{131}I together with chromatographically purified T3-^{125}I (▲). All animals were given NaI, 1 mg/day, by subcutaneous injection throughout the study.

TABLE IV

*Determination of Various Iodine Pools in Rat Livers, Plasma, and Whole Body Homogenates after Isotopic Equilibration**

Rat	Tissue	Total iodine	Iodide	Nonextractable iodine	Extractable iodine
1135	Liver	17.1	3.2	1.4	12.5
1136		12.0	1.9	0.5	9.6
1137		16.2	3.1	1.1	12.0
1139		18.4	4.9	1.4	12.1
1140		12.6	1.7	0.9	10.0
1135	Plasma	34.5	14.3	3.2	17.0
1136		30.5	10.2	1.4	18.9
1137		31.1	15.7	1.7	13.7
1139		36.8	17.0	2.7	17.1
1140		26.8	6.3	1.5	19.0
1141	Whole body homogenate	6460	3730	300	2430
1142		5670	3360	310	2000
1143		6620	3710	280	2630

* Data are expressed as nanograms per gram of liver, nanograms per milliliter of plasma, and total nanograms for the whole body homogenates.

functionally athyreotic subjects constituted 11.8% of the PBI when calculated as the difference between the PBI and the T4I-by column and 22.0% of the serum organic iodine as measured by direct iodine analysis of the serum residues after extraction.

Partial purification and identification of NE^{131}I. Large samples of plasma (60 ml) were obtained from subjects B.S. and J.W. 96 hr after the injection of T3-^{125}I. After the pH was adjusted to 9.0, 25-ml portions were percolated through Dowex 1-X2 ion-exchange resin columns which were then washed with 0.1 M ammonium acetate, pH 9.0. A peak of radioactivity paralleling the elution of plasma proteins (OD, 280 mμ) was observed in each case (Fig. 7). Less than 1% of the T3-^{131}I or $^{131}I^-$ added to the plasma before column chromatography appeared in the pH 9.0 eluate, but 28.4% and 30.6% of the applied ^{125}I counts were recovered in this peak. These findings agreed closely with the per cent of NE^{125}I in the same plasma samples determined independently by acid butanol extraction.

Separate fractions from the column eluates (pH 9.0) were pooled. Radioactivity was quantitatively precipitated with 5% trichloroacetic acid (TCA) and was not dialyzable. An average of 19% of the radioactivity was soluble in acid butanol and 4% was soluble in $CHCl_3$: CH_3OH, 2:1. Starch-gel electrophoretograms from 1-ml portions of the column eluates showed that the radioactivity was associated with albumin (Fig. 8).

TABLE V

Iodine Determinations in the Sera of Hypothyroid Patients Treated with Synthetic L-*Thyroxine* (*T4*) *and 3,5,3'-Triiodo-*L-*Thyronine* (T3)*

Patient	Medication		Duration of treatment	Iodine concentrations *ug–100 ml serum*			
	T_4	T_3		PBI	T4I by column	After dialysis†	After dialysis and extraction§
	ug/day		*months*				
A.M.	120	30	9	5.3	5.1	3.6	0.6
M.H.	60	15	10	5.0	4.4	5.2	1.9
J.L.	100	45	10	6.2	6.2	5.5	1.7
F.S.	75	—	6	4.1	3.5	4.2	0.3
A.H.	120	30	12	5.6	4.6	5.3	0.2
R.T.	60	15	4	4.1	3.3	3.3	1.2
Mean				5.1	4.5	4.5	1.0

* All iodine determinations performed by BioScience Laboratories, Los Angeles, Calif.

† Total iodine concentration measured in lyophilized sera after dialysis.

§ Total iodine concentration measured in lyophilized sera after dialysis and extensive extraction with 1-butanol saturated with 5% HCl.

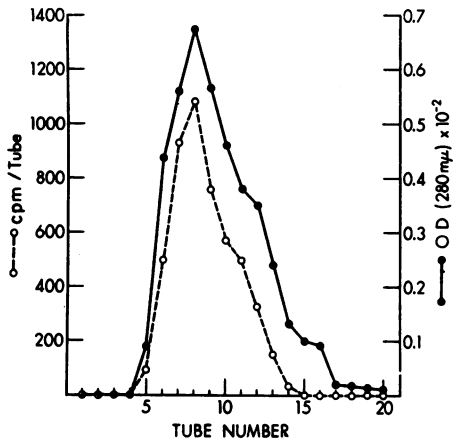

FIGURE 7 Elution pattern of radioactivity and protein (OD, 280 mμ) from a Dowex 1-X2 ion-exchange column. 25 ml of serum from subject B.S. obtained 96 hr after injection of T3-^{125}I was applied to the column. The column was equilibrated and eluted with 0.1 M ammonium acetate, pH 9.0.

DISCUSSION

The present study has demonstrated the formation of an iodinated substance which is insoluble in acid butanol (NE^{125}I) after injection of T3-^{125}I into human subjects and rats. Since the thyroid gland was blocked by the administration of large doses of nonradioactive iodide, it is evident that NE^{125}I originates from an extrathyroidal source. This conclusion is strengthened by the occurrence of NE^{125}I in the plasma of thyroidectomized rats after injection of T3-^{125}I and of butanol-insoluble iodine in the sera of hypothyroid human subjects treated with synthetic T4 and T3. Based on its behavior during column chromatography, dialysis, TCA precipitation, lipid extraction, and starch-gel electrophoresis, the iodinated substance has tentatively been identified as iodoalbumin. This component rises to its peak concentration 24–36 hr after the injection of T3-^{125}I, a time at which it constitutes 10–15% of the plasma radioactivity.

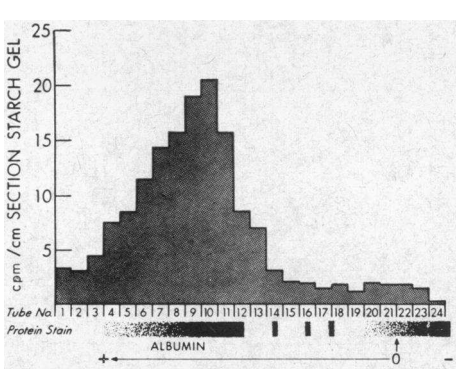

FIGURE 8 Distribution of the column eluate radioactivity of subject J.W. among the plasma proteins separated by starch-gel electrophoresis. 0 = origin, + = anode, — = cathode.

Thereafter, its concentration falls at a rate which becomes asymptotic with the disappearance rate of plasma total ^{125}I. In the two subjects in whom the plasma disappearance of T3-^{125}I was studied for 36 and 44 days, the NE^{125}I constituted the major portion of the plasma radioactivity after 8–10 days. The terminal slope of these curves and the calculated volume of distribution are consistent with the expected values for injected iodinated albumin (5). These isotopic data, therefore, support the tentative chemical identification of the NE^{125}I as iodoalbumin. In separate studies Brown-Grant (6) observed a decreasing disappearance rate of plasma radioactivity and a relative increase in plasma TCA-precipitable radioactivity after injection of T3-^{131}I into guinea pigs. These findings prompted him to suggest the possibility of iodoprotein formation.

The production of NE^{125}I during the metabolism of T3 considerably complicates the interpretation of the T3 disappearance curve when observations are limited to the plasma total or TCA-precipitable radioactivity. The rapid decrease in plasma radioactivity during the first 2 days after injection of labeled T3 represents, primarily, the effects of T3 distribution and metabolism. Although the rate of T3 metabolism remains constant thereafter, the portion of the disappearance curve between days 2–4 is further influenced by the production, distribution, and metabolism of iodide and NE^{125}I. The continually decreasing rate of disappearance of plasma radioactivity reflects increasingly the metabolism of NE^{125}I. Since T3 distribution is probably incomplete until 24 hr after intravenous injection, it is apparent that no portion of the plasma disappearance curve reliably represents the exclusive effects of T3 metabolism. These considerations probably account for the large variations in T3 turnover rate (26.2–72.2% day^{-1}) previously reported (7–13). Since the disappearance of plasma radioactivity after T3-^{125}I is curvilinear, a straight line function drawn through several arbitrarily selected data points in the early portion of a study may misrepresent the true disappearance rate of T3. In future studies, the contribution of T3-^{125}I to the total radioactivity in each plasma sample should be assessed so that the T3 disappearance curve can be plotted and analyzed directly. Analysis of the calculated T3 curves in three subjects in the present study results in an average T3 distribution volume of 38.1 liters and an average plasma disappearance rate of 0.473 day^{-1}. However, since precise information concerning T3 distribution kinetics and metabolism in man is unavailable, the conventional 0-time extrapolation method for the determination of distribution volume may not be entirely reliable. Furthermore, the T3 radioactivity, measured as extractable organic iodine in this study, may be overestimated since no efforts were made to quantitate other

potentially extractable T3 metabolites such as T3 sulfate or 3,3′-diiodothyronine sulfate (14, 15). In any case, the product of the T3 distribution volume and turnover rate observed here and the mean normal plasma T3 iodine concentration of 1.9 μg/liter reported by Nauman, Nauman, and Werner (16) yields a degradation rate for T3 iodine of 34 μg/day. Since the metabolic potency of T3 is three to four times greater than T4 which has a mean degradation rate (T4I) of 60 μg day^{-1} (17), it is probable that T3 is responsible for more than one-half of the calorigenic activity of the thyroid hormones in human beings.

Since iodoalbumin can be produced in the intact rat after radioiodide injection (18), a reaction between albumin and radioiodide released during the metabolism of T3-^{125}I was considered as a possible source of the NE^{125}I. The disappearance of radioiodide in three human subjects was linear, however, and extraction studies failed to demonstrate iodoprotein formation. Although some NE^{125}I was observed in rat plasma after ^{125}I$^-$ injection, the concentration was only 1/40th of that observed after T3-^{125}I. These experiments, therefore, indicate that NE^{125}I is not derived from plasma inorganic iodide. Since iodide introduced into the plasma does not readily penetrate cells (19), the ^{125}I$^-$ turnover experiments do not exclude the possibility that intracellular protein is iodinated by intracellular iodide released during hormonal metabolism. Available evidence favors this pathway. Galton and Ingbar (20) and Nagakawa and Ruegamer (21) have demonstrated transiodination of protein during metabolism of T4 and T3 by liver homogenates in vitro. In other experiments we have confirmed this observation and have further demonstrated that the kinetics of hepatic microsomal iodination closely parallel the deiodination of T4 and T3 by this subcellular fraction.[2] When radioiodide was employed as the substrate in this microsomal system no iodination occurred. During the metabolism of T3-^{125}I in vivo, therefore, it is possible that the plasma NE^{125}I may be generated by iodination of the nascent protein while still attached to the microsome during the course of deiodination of T3.

In order to elucidate the biochemical nature of the translocation of radioiodine from thyroid hormones to proteins, the iodine atoms and possibly other parts of the hormone molecule which enter this pathway must be identified. The commercial radioactive preparations employed in this investigation were labeled by the iodine-exchange method which, it is generally held, exclusively labels iodine in the β-ring (22). Plaskett (23) and Wynn and Gibbs (24), however, have reported that a large fraction of α-ring iodine but very little β-ring iodine becomes fixed to proteins during incubation of liver extracts and microsomes with T4 and T3. Moreover, a significant portion of the thyronine nucleus is also linked to cellular proteins in these in vitro systems. Thus, the occurrence of β-ring iodine in plasma iodoproteins after hormonal metabolism as demonstrated in the current study raises the possibility that other portions of the hormone molecule may be present also. Future studies employing radioactive hormone preparations with precise label localization will be of great interest in the resolution of this problem.

The occurrence of butanol-insoluble iodine in normal human plasma has been reported for many years (25, 26). When calculated as the difference between PBI and serum butanol–extractable iodine (BEI) (25, 26) or the PBI and serum thyroxine–by column chromatography (27), approximately 11–22% of the plasma organic iodine can be considered iodoprotein. Similar data have been obtained recently from methods employing column chromatography (28, 29). The present study confirms these observations in rats equilibrated with radioiodine (11% iodoprotein) and in man by direct iodine analyses of the denatured proteins after extensive butanol extraction of the sera of hypothyroid subjects maintained on synthetic T4 and T3 (22% iodoprotein). The latter study is especially significant since it demonstrates that the plasma iodoproteins occur in subjects without functioning thyroid tissue. The demonstration that T3 and T4 metabolism results in iodoprotein formation, therefore, suggests that the circulating iodoproteins in normal human plasma may originate during the peripheral metabolism of the thyroid hormones. In this connection, the increased concentration, in Graves' disease, of a plasma iodoprotein which is indistinguishable from albumin (30, 31) may well result from the increased metabolism of the thyroid hormones in this condition.

Several preliminary studies were performed to determine whether the NE^{125}I was the source of the extractable iodotyrosines which have been reported to constitute up to 20% of the serum organic iodine (32, 33). The NE^{125}I from two subjects (J.W. and B.S.) was extracted by the hot acid butanol method of Mandl and Block (34) (method I) and by the method of West, Chavre, and Wolfe (35). Although both of these methods reportedly extract iodotyrosines from normal human serum (33), only a small portion of the radioactivity from NE^{125}I was removed. Thus, although the data are limited, the iodoprotein described in this study does not appear to be the source of the extractable iodotyrosines.

An estimate of the rate of transformation of hormonal iodine to iodoprotein may be obtained from the available data as follows: employing an average concentration of 55 μg/liter for the plasma organic iodine and an iodoprotein content of 15%, the iodoprotein concentration

[2] Surks, M. I., J. H. Oppenheimer, and H. L. Schwartz. Unpublished observations.

will be 8.3 μg/liter. The product of the iodoprotein concentration, the albumin distribution volume (6 liters), and the degradation rate for albumin (0.046 day^{-1}) represents the degradation rate for iodoprotein iodine, 2.3 μg/day. Assuming reasonable figures for the degradation rates of T4 and T3 iodine, 60 and 35 μg/day, it is evident that under steady-state conditions, approximately 2.5% of hormonal iodine is transferred to proteins per day. A similar estimate is obtained by analysis of the terminal portion of the T3-^{125}I disappearance curves in subjects B.S. and J.W. Using the final values for plasma ^{125}I concentration and the observed slope of the terminal portion of the disappearance curves, we can readily determine the virtual "0-time" NE^{125}I concentration. The product of this concentration and the observed distribution volume for the iodoprotein yields a rough estimate of the total per cent of the T3-^{125}I converted to albumin-^{125}I. This calculation shows that 1.6% and 3.8% dose was converted to iodoprotein for B.S. and J.W. respectively.

Although only a small percentage of peripheral T3 and T4 iodine metabolism may be directed to the formation of the iodoprotein, the reaction which is involved in the covalent linkage of iodine to protein may be of basic physiologic importance. Unpublished data from our laboratory indicate that iodoprotein is formed in subcellular organelles of liver and kidney as well as other tissues. The formation of the circulating iodoproteins may thus represent only a small facet of the general question of iodinated protein formation after iodothyronine metabolism.

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REFERENCES

1. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. *Biochem. J.* **89**: 114.
2. Berson, S. A., and R. S. Yalow. 1957. Radiochemical and radiobiological alterations of I^{131}-labeled proteins in solution. *Ann. N. Y. Acad. Sci.* **70**: 56.
3. Lewallen, C. G. 1963. Chromatographic fractionation of iodinated compounds in biologic fluids. *In* Evaluation of Thyroid and Parathyroid Functions. F. W. Sunderman and F. W. Sunderman, Jr., editors. J. B. Lippincott Co., Philadelphia. 37.
4. Squef, R., M. Martinez, and J. H. Oppenheimer. 1963. Use of thyroxine-displacing drugs in identifying serum thyroxine-binding proteins separated by starch gel electrophoresis. *Proc. Soc. Exp. Biol. Med.* **113**: 837.
5. Beeken, W. L., W. Volwiler, P. D. Goldsworthy, L. E. Garby, W. R. Reynolds, R. Stogsdill, and R. S. Stemler. 1962. Studies of I^{131}-albumin catabolism and distribution in normal young male adults. *J. Clin. Invest.* **41**: 1312.
6. Brown-Grant, K. 1967. Further studies of the metabolism of thyroxine and 3,5,3′-triiodothyronine in the guinea pig. *J. Physiol.* **191**: 167.
7. Sterling, K., J. C. Lashof, and E. B. Man. 1954. Disappearance from serum of I^{131}-labeled L-thyroxine and L-triiodothyronine in euthyroid subjects. *J. Clin. Invest.* **33**: 1031.
8. Hales, I. B., and B. M. Dobyns. 1960. The metabolism of triiodothyronine in Graves' disease. *J. Clin. Endocrinol. Metab.* **20**: 68.
9. Haddad, H. M. 1960. Studies on thyroid hormone metabolism in children. *J. Pediat.* **57**: 391.
10. Kuhl, W. J., I. S. Halper, and R. M. Dowben. 1961. Thyroxine and triiodothyronine turnover studies in dystrophia myotonica. *J. Clin. Endocrinol. Metab.* **21**: 1592.
11. Wiswell, J. G., and V. Coronho. 1962. Disappearance of I^{131}-triiodothyronine from the plasma in the presence of fever. *J. Clin. Endocrinol. Metab.* **22**: 657.
12. Fisher, D. A., and T. H. Oddie. 1964. Whole-body counting of ^{131}I-labeled triiodothyronine. *J. Clin. Endocrinol. Metab.* **24**: 733.
13. Gregerman, R. I., and N. Solomon. 1967. Acceleration of thyroxine and triiodothyronine turnover during bacterial pulmonary infections and fever: implications for the functional state of the thyroid during stress and in senescence. *J. Clin. Endocrinol. Metab.* **27**: 93.
14. Fauvert, R., J. Roche, R. Michel, P. Thiéblemont, and M. Gruson. 1958. Mise en évidence dans le plasma et la bile de l'homme, de l'ester sulfurique de la 3,5,3′-triiodo-L-thyronine. *Rev. Fr. Etud. Clin. Biol.* **3**: 372.
15. Flock, E. V., J. L. Bollman, and J. H. Grindlay. 1960. Conjugates of triiodothyronine and its metabolites. *Endocrinology.* **67**: 419.
16. Nauman, J. A., A. Nauman, and S. C. Werner. 1967. Total and free triiodothyronine in human serum. *J. Clin. Invest.* **46**: 1346.
17. Oddie, T. H., J. H. Meade, Jr., and D. A. Fisher. 1966. An analysis of published data on thryoxine turnover in human subjects. *J. Clin. Endocrinol. Metab.* **26**: 425.
18. Shimaoka, K., and B. D. Thompson. 1965. Radioiodine labeling of serum albumin *in vivo*. *Endocrinology.* **76**: 570.
19. Wallace, G. B., and B. B. Brodie. 1937. The distribution of administered iodide and thiocyanate in comparison with chloride and their relation to body fluids. *J. Pharmacol. Exp. Ther.* **61**: 397.
20. Galton, V. A., and S. H. Ingbar. 1961. The mechanism of protein iodination during the metabolism of thyroid hormones by peripheral tissues. *Endocrinology.* **69**: 30.
21. Nakagawa, S., and W. R. Ruegamer. 1967. Properties of a rat tissue iodothyronine deiodinase and its natural inhibitor. *Biochemistry.* **6**: 1249.
22. Gleason, G. I. 1955. Some notes on the exchange of iodine with thyroxine homologues. *J. Biol. Chem.* **213**: 837.

23. Plaskett, L. G. 1961. Studies on the degradation of thyroid hormones in vitro with compounds labeled in either ring. *Biochem. J.* **78**: 652.
24. Wynn, J., and R. Gibbs. 1962. Thyroxine degradation. II. Products of thyroxine degradation by rat liver microsomes. *J. Biol. Chem.* **237**: 3499.
25. Taurog, A., and I. L. Chaikoff. 1948. The nature of the circulating thyroid hormone. *J. Biol. Chem.* **176**: 639.
26. Man, E. B., D. M. Kydd, and J. P. Peters. 1951. Butanol-extractable iodine of serum. *J. Clin. Invest.* **30**: 531.
27. Pileggi, V. J., N. D. Lee, O. J. Golub, and R. J. Henry. 1961. Determination of iodine compounds in serum. I. Serum thyroxine in the presence of some iodine contaminants. *J. Clin. Endocrinol. Metab.* **21**: 1272.
28. Rall, J. E., and R. A. Conard. 1966. Elevation of the serum protein-bound iodine level in inhabitants of the Marshall Islands. *Amer. J. Med.* **40**: 883.
29. Brown, C. H., J. M. Lowenstein, F. S. Greenspan, and J. Mangum. 1966. Effect of stimulation and suppression of the thyroid gland upon production of iodoprotein in man. *Metabolism.* **15**: 649.
30. Cameron, C., and K. Fletcher. 1959. An iodine compound associated with albumin in the plasma of thyrotoxic subjects. *Nature (London).* **183**: 116.
31. Stanbury, J. B., and M. A. Janssen. 1962. The iodinated albumin-like component of the plasma of thyrotoxic patients. *J. Clin. Endocrinol. Metab.* **22**: 978.
32. Werner, S. C., and R. J. Block. 1959. Discrepancy between the distribution of iodine in human serum when estimated by iodine-^{131} and iodine-^{127}. *Nature (London).* **183**: 406.
33. Weinert, H., H. Masui, I. Radichevich, and S. C. Werner. 1967. Materials indistinguishable from iodotyrosines in normal human serum and human serum albumin. *J. Clin. Invest.* **46**: 1264.
34. Mandl, R. H., and R. J. Block. 1959. Methods for the qualitative, semiquantitative, and quantitative determination of iodoamino acids and of inorganic iodide in iodoprotein digests and human serum. *Arch. Biochem. Biophys.* **81**: 25.
35. West, C. D., V. J. Chavre, and M. Wolfe. 1965. A serum thyroxine method: application in thyroid disease and iodine-treated patients. *J. Clin. Endocrinol. Metab.* **25**: 1189.