Determination of Triiodothyronine Concentration in Human Serum

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ABSTRACT A simplified method has been described for the measurement of triiodothyronine (T3) in human serum. The sensitivity was sufficient for determinations on hypothyroid as well as normal and thyrotoxic sera. The values obtained have been in reasonable agreement with a double isotope derivative assay.

The normal T3 concentration in human serum approximates 0.2 μg/100 ml; the mean $\pm$SD of 31 normal sera was 220 $\pm$27 ng/100 ml. Elevations were observed in sera from 40 patients with thyrotoxicosis (752 $\pm$282 ng/100 ml), and diminutions were found in sera from 10 hypothyroid patients (98 $\pm$48 ng/100 ml).

In rare instances thyrotoxicosis may be due to elevated serum T3 with normal thyroxine (T4) concentration. The incidence of this condition remains to be determined.

In approximately half the cases with low serum T4 after ^{131}I therapy, the eumetabolic state may be maintained by normal or elevated T3 concentration.

From these data and kinetic studies indicating a rapid turnover it may be inferred that T3 rather than T4 may be the more important hormone in health and in disease.

INTRODUCTION

Since its discovery (1) by Gross and Pitt-Rivers in 1952, triiodothyronine (T3) has received attention because of its greater potency than thyroxine (2, 3). T3 has been shown to have a rather short biologic half-time in the circulation (4), which has been ascribed at least in part to its weak binding by TBG and by albumin, and its lack of significant binding by TBPA (5–8).[1]

The precise role of T3 in thyroid hormone metabolism has awaited clarification (8, 9) pending agreement on the concentration of the hormone in human serum. The early reports of Pind (10) and Maclagan, Bowden, and Wilkinson (11) as well as several more recent contributions (12–22) may be viewed as generally compatible with the findings of the present paper. The method presented is considered sufficiently simple, sensitive, and precise to be made available for widespread clinical use. The results have been verified by a double isotope derivative assay. The findings lead to the inference that T3 may indeed be the more important secretory product of the thyroid gland, rather than representing a "second" thyroid hormone.

METHODS

The fundamental approach to the measurement of T3 in human serum consisted of the following steps: (*a*) removal of the thyroid hormones from human serum with the aid of small columns of cation exchange resin; (*b*) separation of T3 from T4 completely by descending paper chromatography with an improved solvent system; and (*c*) quantitation of the eluted T3 by displacement methodology.

The serum, usually stored in the frozen state, was thawed and labeled with a tracer amount of triiodothyronine-^{125}I (usually < 0.02 μg/100 ml). A small aliquot was retained to determine recovery. To each resin column was added 5 ml of serum. The use of eight columns simultaneously was found convenient. It was customary to use one or two columns for a normal serum pool as an over-all check on the method, and another column for the same serum enriched with a known quantity such as 800 ng T3/100 ml.

The use of resin columns had been suggested by the work of Lewallen (23). The cation exchange resin (24) employed

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Received for publication 12 December 1968 and in revised form 21 February 1969.

[1] Abbreviations used include: 3,5,3'-L-triiodothyronine, T3; thyroxine, T4; thyroxine-binding globulin, TBG; and thyroxine-binding prealbumin, TBPA.

was Dowex AG 50 W-X2 (H^+ form), 100–200 mesh, purchased from Bio-Rad Laboratories, Richmond, Calif., catalogue No. 43025. A single sedimentation in water was sufficient for removal of fine particles.

The columns were 250 × 12 mm bottom inside diameter (Chromaflex column No. 1, catalogue No. K-42223, Kontes Glass Co., Vineland, N. J.) with inside diameter of chimney approximating 13 mm.

A 1:2 slurry of resin was maintained in aqueous suspension with a magnetic stirrer, and transferred to the column with an inverted 10 ml pipet. The columns were packed to a height of 8.0 cm, then activated by washing with 15 ml 1 N HCl which caused shrinkage. This was followed by 30 ml of water which caused swelling back to the original height (24). A porous polyethylene disc (Bel-Art Products, Pequannock, N. J.) 12.5 mm in diameter, was added to each column and tamped down onto the top of the resin bed with a broad stirring rod through a fluid head of 2 cm.

The 5 ml of labeled serum was added to each column, followed by 0.15 M ammonium acetate, pH 8.5. After the void volume of 5.0 ml of clear fluid the next 5 ml of eluate looked like serum and contained approximately 10–15% of the initial labeled T3, followed by clear fluid. A volume of 70 ml of ammonium acetate solution was used to wash the column free of most of the protein and lipids.

The T3 and T4 were then eluted with 7.4 N ammonium hydroxide (concentrated reagent diluted with an equal volume of water). The void volume of 5 ml was discarded and the next 12 ml of clear ammoniacal solution was collected in transparent plastic tubes (No. 2001 tubes, 17 × 100 mm, Falcon Plastics, Los Angeles, Calif.). This solution contained approximately 75–80% of the labeled T3 present in the original serum. The column chromatographic separation of the hormones from serum using eight columns required about 1 hr.

The eluates were placed in a water bath gradually raised from room temperature to 70°–80°C over the course of a half hour to avoid bumping, and dried with a brisk stream of nitrogen, which required 3.5–4 hr. On occasion air was used instead of nitrogen without evident oxidative deiodination. The dried residue contained a brownish material which facilitated transfer to paper strips for chromatography because of its visibility and small losses to the walls of the tubes. The alternative procedure of an anion exchange resin column yielded barely visible residues which, however, caused troublesome adherence of labeled hormone to plastic tubes.

The dried labeled serum extract was transferred onto chromatographic strips by dissolving it in small amounts of methanol-ammonia solution (99 volumes of methanol to 1 volume of 2 N ammonium hydroxide), which usually required three 200-μl washes and agitation with a Vortex mixer. The empty tubes were counted to verify the transfer of most of the tracer.

The material was applied to 2.5 × 58 cm strips of Whatman 3MM chromatography paper with T4-^{131}I tracer added at the origin as a reference marker. Descending chromatography was carried out with the improved solvent system previously described (25); this consists of hexane–tertiary amyl alcohol–2 N ammonia, 1:5:6 (v/v/v).

The system resembles the conventional tertiary amyl alcohol one, but the addition of hexane resulted in more rapid migration and, even more crucial, appreciably improved separation between T3 and T4, which is obviously of utmost importance. Care was required to prevent evaporation of the volatile hexane from the organic phase employed in the troughs. The inner wall of the cylindrical chromatography tank was lined with Whatman 3MM paper which was thoroughly moistened with the aqueous phase before each run. The separation was optimal at 80°–90°F, with an overnight descending run of approximately 15–16 hr.

When a chromatography cabinet was employed instead of a cylindrical tank, it was necessary to use not only paper chimneys for the aqueous and organic phases, but also two sheets of Whatman 3MM paper suspended from troughs containing organic phase and also moistened with it to assure adequate running rates and separation of the hormones. The presence of sufficient gaseous hexane in chromatography chambers appears essential for the more rapid migration and better separation of hormones achieved by this solvent system as opposed to conventional tertiary amyl alcohol.

After the strips were dried, rapid scanning was carried out with a modified Nuclear-Chicago Actigraph III, model 1004. Adequate separation of the T3-^{125}I and T4-^{131}I was evident when the peaks were approximately 8 cm apart (Fig. 1). Lesser separation between the hormones could result in the inclusion of some T4 in the T3 eluates, with falsely elevated values. It had previously been ascertained by separate label studies, as well as experiments with nonradioactive hormones, that no significant amount of T4 was present in the T3 area

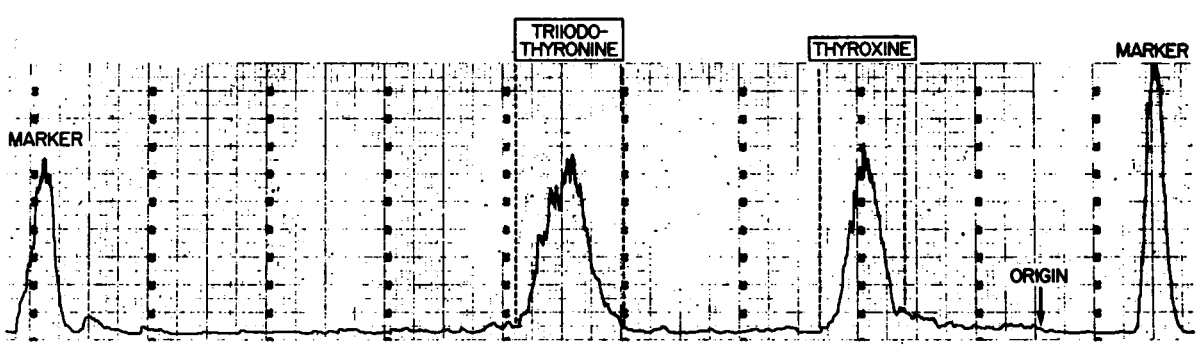

FIGURE 1 Radioactive scan of chromatogram of labeled hormones from serum. The T3 peak of radioactivity is T3-^{125}I added to the original serum, while the T4 peak is T4-^{131}I tracer added at the origin. Hexane–tertiary amyl alcohol–ammonia system, 1:5:6 (v/v/v), descending chromatography for 15 hr.

under these conditions. The actual T4 contamination in the T3 zone is well under 0.5%. The scans of simultaneously run strips containing only labeled tracers were always quite similar.

After the T3 areas were cut out, elution of T3 was most conveniently achieved by allowing the papers to soak for approximately an hour in 5 ml of methanol-ammonia in the Falcon plastic tubes. The paper strips were removed by means of disposable wooden applicator sticks, which allowed squeezing out most of the methanol-ammonia without appreciable loss. The eluates were completely dried in a 50°C water bath with a stream of nitrogen which required 15–20 min.

The dry tubes were then assayed for ^{125}I which permitted calculation of the over-all recovery of T3, usually about 45–60%. The starting material used was always at least 98% T3 by chromatography and electrophoresis; in the event of impurities in excess of 2%, the material was not employed for the present procedure. Accordingly, no correction was made for the quantitatively insignificant amount of contaminant in the original. Whereas it was possible to achieve recoveries of greater than 70% by exerting utmost care, it was considered pointless to expend the additional time, since valid results could be obtained where the final amount of T3 in the tube exceeded 4 ng; this was the case in all but a few sera from hypothyroid subjects, in which circumstance it was advisable to use 10 ml rather than 5 ml of serum at the start.

The displacement technique (26-29) for determination of T3 in the dried eluates was carried out by measuring the resin sponge uptake (Triosorb sponges, Abbott Laboratories, North Chicago, Ill.). The unknown results were compared with a standard curve of triplicate points obtained for T3 additions of zero, 5, 10, 20, 30, and 40 ng/ml of isotope solution. The standard curve on semilogarithmic plot of protein bound tracer (100% minus sponge uptake) against nanograms of T3 was slightly curvilinear, or occasionally a straight line (Fig. 2). Similar data could be obtained using the resin IRA-400 in the formate cycle (30), but resin sponges were preferred to free resin particles because of greater speed and convenience. A potentially advantageous and highly sensitive displacement method has been developed in which dextran-coated charcoal is used.[2] This development may permit a modification of the present method requiring only 1 ml of serum.

The standard isotope solution consisted of tracer triiodothyronine-^{131}I (approximately 0.2 μg/100 ml) added to a serum pool (from clinical laboratory) which had been diluted to 7.5 volumes of serum/100 ml in 0.05 M sodium glycinate buffer, pH 9.6. Stock solutions with the various nonradioactive T3 concentrations in the isotope solution were used to obtain the standard curve with each run, and were prepared weekly to avoid deterioration, which was sometimes detectable within 10 days to 2 weeks. It was observed that the standard curve so obtained did not differ significantly from curves determined by adding nonradioactive T3 in methanol-ammonia to tubes and drying before adding regular isotope solution.

The tubes containing dried eluates were placed in a tray of ice and water, and 1 ml quantities of isotope solution were added. The tubes were agitated by hand to dissolve the dried extract, and an interval of 30 min was allowed for cooling to bath temperature. Resin sponges were added to all standard and unknown tubes, and then the sponges were compressed with plastic plungers (Abbott Laboratories), so that virtually all fluid entered the sponges. The timing was begun with the compression of the sponges, and incubation in the ice water bath was carried out for 60 min. During the course of the incubation each tube was removed from the bath and counted for 1 min to determine total ^{131}I radioactivity. At the end of the 60 min period, the solutions were aspirated with a special plastic plunger aspirator (Abbott Laboratories) which compressed the sponges. Then the sponges were washed with about 10 ml of water and compressed with the plunger before aspiration. The sponges, washed three times, were then assayed for ^{131}I and the resin sponge uptake computed. This percentage subtracted from 100% gave the per cent protein-bound radioactivity which was read off the standard curve done concurrently. From the nanograms of T3 in the individual tube plus the recovery data it was possible to calculate the concentration of triiodothyronine in the serum. Appropriate correction was made for the T3 of the T3-^{125}I "tracer" added to the original serum at the start by subtraction from the total T3 concentration calculated. This correction, often close to the limits of error of the method, could occasionally be significant, especially in hypothyroid sera. An alternative procedure obviated addition of triiodothyronine-^{131}I for the displacement assay by employing the T3-^{125}I already present and assayed for recovery determination. When T3-^{125}I was substituted for T3-^{131}I, the former tracer was added only to standards but not to unknowns, so that a small appropriate adjustment was required for the points of the standard curve due to this increment of T3-^{125}I added to them.

It should be mentioned that the specific activity was quite constant across the T3 peak of paper chromatograms, as judged by studies in which the faster and slower portions of the T3 area were compared. In the improved chromatographic system employed, tetraiodothyroacetic acid ("Tetrac") runs approximately midway between the T3 and T4 zones. Although we have apparently detected this compound in minute amounts in human serum under certain circumstances, it has no appreciable effect in the displacement assay employed (work in progress in collaboration with Ingbar

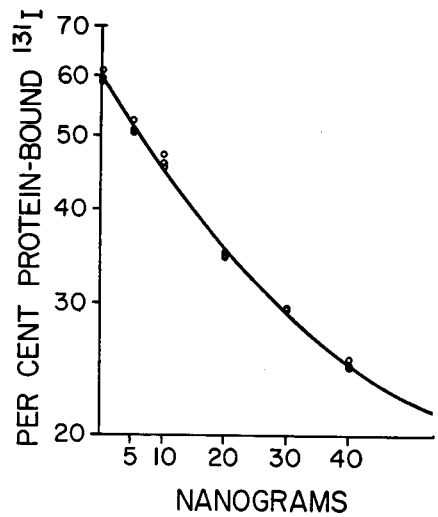

FIGURE 2 Displacement curve. Semilogarithmic plot. The points are obtained by subtracting the T3 resin sponge uptake values from 100%. The standard curve obtained was slightly curvilinear or occasionally a straight line.

[2] Fisher, Delbert A. Personal communication.

and colleagues). No other interfering compounds were detected. Other areas of the paper strip were customarily run as "baseline" controls or "paper blanks," and gave negligible values in the assay.

Double isotope derivative assay

In this procedure triiodothyronine-^{131}I rather than ^{125}I was added to 10-ml samples of serum. The hormones were separated as described through the step of elution of T3 from the paper strips. The residues were redissolved in methanol-ammonia and transferred to 3-ml glass-stoppered centrifuge tubes (acetylation vessels). The glass stoppers were removed and the solvent was evaporated by a stream of nitrogen. The tubes were then placed in a vacuum desiccator for 2–3 hr to eliminate all possible moisture.

Redistilled anhydrous pyridine (20–30 μl) was added to each tube to redissolve the residue followed by 5–20 μl of ^{3}H-labeled acetic anhydride (20% v/v in benzene) with a specific activity of 0.6–1 c/mmole.

The amounts of acetic anhydride-^{3}H were chosen to give a final molar ratio of acetic anhydride/T3 of 200:1. Previous experiments had established that such a ratio was necessary to obtain 85–90% conversion of the hormone to its acetyl derivative.

The tubes were then tightly closed with their ground glass stoppers and placed in an oven at 45°C for 15 hr. The acetylation reaction was stopped by adding to each tube 50 μl of methanol and incubating for an additional 30 min.

At the end of the incubation the contents of the tubes were evaporated under vacuum. Anhydrous benzene (50 μl) was then added and the tubes were again evaporated. This operation was repeated five times.

The final dry residue was transferred with 20 ml of ethyl acetate to a separatory funnel. The solution was then washed three times each with 5% hydrochloric acid, water, and finally 10% sodium bicarbonate. After each washing the aqueous phase was separated from the ethyl acetate layer and discarded. The ethyl acetate solution was then dehydrated with sodium sulfate.

The ethyl acetate was finally evaporated at 40°C by a stream of nitrogen, and the residue was resuspended in 100 μl of ethanol–2 N ammonia (9:1) and chromatographed on paper in the hexane–tertiary amyl alcohol–ammonia descending system. The T3 diacetate-^{3}H was located in each chromatogram by scanning. Standard T3 diacetate-^{3}H chromatographed in parallel with the samples served as a check. The derivative had a higher R_f than T3, running slightly behind the solvent front.

The T3 diacetate-^{3}H was eluted from the chromatogram into counting vials with ethanol–2 N ammonia (9:1). The solvent was evaporated and 10 ml of phosphor solution (0.4% 2,5-diphenyloxazole (PPO) and 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in toluene) was added. ^{131}I and ^{3}H contents of vials were measured simultaneously in a Packard Tri-Carb liquid scintillation spectrometer.

T3 content was calculated from the following equation:

$$\frac{Ii}{If} \times \frac{H}{Sc} \times \text{M.W.} \times \frac{100}{10\ \text{ml}} = \text{ng of T3 per 100 ml}$$

where 10 ml = volume of serum analyzed; Ii = ^{131}I cpm added to sample; If = ^{131}I cpm in counting vials; H = ^{3}H cpm in counting vials; Sc = specific activity of T3 diacetate (cpm ^{3}H/ mμmole); and M.W. = molecular weight of T3 (651).

Materials

T3-^{125}I and T3-^{131}I in 50% propylene glycol solution with 0.2% cysteine added, were obtained from Abbott Laboratories and tested for purity by paper chromatography and by electrophoresis. Triosorb resin sponges were generously supplied by Abbott Laboratories.

For the double isotope derivative assay, acetic anhydride-^{3}H of high specific activity (0.6–1 c/mmole) was obtained from International Chemical and Nuclear Corporation (ICN), City of Industry, Calif.

Thyroxine and T3 were purchased from Mann Research Laboratories, Inc., New York. All chemicals used in the procedure were certified reagent grade and were obtained from commercial sources.

Clinical material

Normal. 31 normal sera were obtained. Four were from healthy volunteers and 27 normal sera were obtained from a commercial source.

Thyrotoxicosis. 40 patients with thyrotoxicosis, 20 untreated and 20 in relapse, were available from the Medical Service of the Veterans Administration Hospital, Harlem Hospital, or the Thyroid Clinic of The Presbyterian Hospital. All the diagnoses were verified by 24 hr thyroidal radioiodine uptake and determination of serum PBI and/or thyroxine iodine by column, as well as other tests in some instances. All were considered cases of toxic diffuse goiter. In addition, seven sera were obtained from patients with normal serum thyroxine and/or PBI values, who were considered to have possible "T3 thyrotoxicosis" from their clinical pictures. (See Results and Discussion sections.)

Hypothyroidism. 10 hypothyroid patients were also available from the same sources. The diagnoses were verified by 24 hr radioiodine uptake and determination of serum PBI and/or thyroxine iodine by column and other indices of thyroid function. Seven of the ten subjects had spontaneous idiopathic myxedema or hypothyroidism, one had subacute thyroiditis with elevated antithyroglobulin antibodies, and two had hypopituitarism.

Low serum thyroxine after treatment of thyrotoxicosis. 18 sera were obtained from consecutive patients whose sera had shown abnormally low PBI and/or thyroxine iodine values in follow-up tests. 14 were subjects who had received

TABLE I

Comparison of Two Methods for T3 Determination

Status	Serum	Displacement	DIDA*
		ng/100 ml	
Normal	A4334	240	284
	A4335	251	237
	A4337	168	156
	A4345	231	171
Hyperthyroid	E. T.	463	510
	L. H.	1728	1560
	L. H.	1845	2013
	D. B.	1172	1120
	V. S.	389	363
	J. K.	493	403

* DIDA signifies double isotope derivative assay.

^{131}I therapy 2 or more yr earlier, two were on antithyroid drug therapy, and two had previous thyroidectomies.

RESULTS

Comparison of the results obtained by the displacement method with the values obtained by double isotope derivative analysis is shown in Table I. In the 10 sera examined no systematic difference between the two methods is evident. The reasonable correspondence was considered to afford confirmation of the displacement method by the double isotope derivative analysis. Serum A4345 was examined a second time after the addition of 811 ng/100 ml of nonradioactive T3, with a recovery of 103% of the expected value.

The results in the various sera are presented in Table II and Fig. 3. The normal T3 concentration in human serum was found to approximate 0.2 μg/100 ml. The mean ±SD of the 31 normal sera was 220 ±27 ng/100 ml, expressed as T3, not T3 iodine.

All sera from patients with thyrotoxicosis had elevated values, with the highest occurring in previously untreated patients.

The occurrence of thyrotoxicosis apparently due to elevated T3 in the serum with normal T4 was found in five serum samples sent from other cities and not included in Table II or Fig. 3, as well as in two patients (G. S. and M. S.) observed and followed clinically by the senior author. The values for serum T3 concentration, were as follows:

C. L. (recurrence about two decades after thyroidectomy for Graves' disease) 561 ng/100 ml

D. H. (clinical recurrence of thyrotoxicosis 1 yr after ^{131}I therapy) 534 ng/100 ml

TABLE II

T3 Concentration in Human Serum

Condition	No.	Mean ±SD
		ng/100 ml
Thyrotoxicosis, all cases including relapse	40	752 ±282
Untreated thyrotoxicosis	20	865 ±321
Normal	31	220 ±27
Spontaneous hypothyroidism	10	98 ±48
Low T4 after treatment of thyrotoxicosis	18	215 ±90
Low T4 after ^{131}I therapy	14	220 ±93

The category of "Low T4 after ^{131}I therapy" consists of 14 of the 18 sera listed as "Low T4 after treatment of thyrotoxicosis." The remaining four represented two from patients after propylthiouracil therapy and two after surgery (cf. text and Fig. 3).

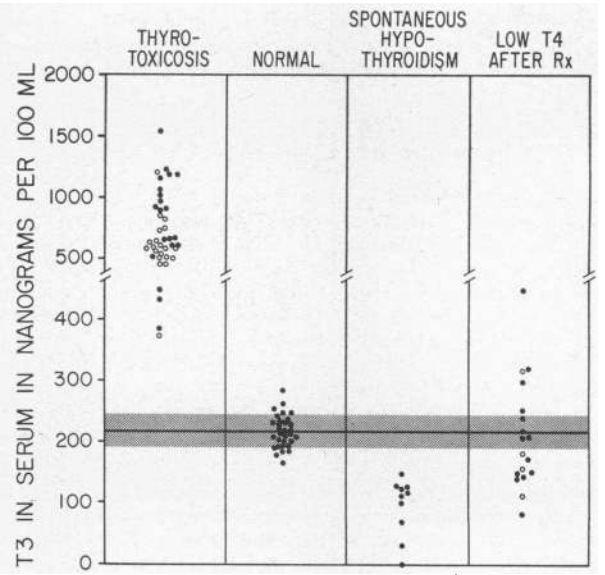

FIGURE 3 T3 concentrations in human sera. The break in ordinate scale from 500 up permitted plotting of the elevated values in thyrotoxic sera. The horizontal line is the normal mean, and the cross-hatched area represents ±1 SD. The filled circles represent untreated thyrotoxicosis. The open circles represent sera from thyrotoxic patients in relapse. The filled circles under "Low T4 after Rx" represent sera from patients who had received ^{131}I therapy for thyrotoxicosis. The four open circles represent other treatment, as follows, from the top down: propylthiouracil, thyroidectomy, propylthiouracil, and thyroidectomy.

S. F. (toxic nodular goiter) 442 ng/100 ml

R. H. (toxic diffuse goiter with exophthalmos) 1332 ng/100 ml

H. P. (toxic nodular goiter with unilateral hyperfunctioning nodules) 643 ng/100 ml

G. S. (clinical recurrence of thyrotoxicosis 1 yr after ^{131}I therapy for toxic nodular goiter) 1016 ng/100 ml

M. S. (solitary hyperfunctioning nodule, toxic nodular goiter) 1563 ng/100 ml

The last three patients are to be included in a clinical report in preparation (Sterling, Refetoff, and Selenkow).

The values in spontaneous hypothyroidism were low, the mean approximating half the normal mean (Table II and Fig. 3).

The sera from patients with low serum thyroxine after treatment of thyrotoxicosis yielded some unexpected findings. The mean value in these 18 sera (215 ±90 ng/100 ml) or in the 14 sera from those who had received ^{131}I therapy (220 ±93 ng/100 ml) did not differ significantly from the normal control sera (Table II and

TABLE III

Effect of Therapy upon T3 Concentration in Thyrotoxicosis

Patient	Before therapy	After therapy
	ng/100 ml	
E. T.	463	287
R. C.	604	264
L. H.	1554	315
R. M.	515	212
L. R.	1034	378

Fig. 3). From inspection of Fig. 3 it is evident that at least half, and probably at least 11 of the 18 values should be considered normal or elevated. Indeed, these patients had no clear objective clinical manifestations of hypothyroidism, despite which several had been given desiccated thyroid replacement therapy in the past, which had been discontinued because of lack of evidence of clinical benefit notwithstanding rise in PBI or serum thyroxine concentration. The clinical impression of euthyroid status was supported by photomotogram or basal metabolic rate determination in several instances.

An illustrative case is that of M. S. who was originally seen with toxic recurrent goiter 21 yr after subtotal thyroidectomy. His thyroidal uptake of ^{131}I was 54% in 24 hr, his serum PBI was 11.2 μg/100 ml, and his basal metabolic rate was + 36. After radioactive iodine therapy he became euthyroid clinically and remained so over the course of 8 yr, but the serum PBI values gradually declined. At the time of study of serum T3, the PBI and thyroxine iodine were both 2.7 μg/100 ml and the basal metabolic rate determinations were + 5 and − 3. The patient appeared entirely normal. The serum T3 concentration was 244 ng/100 ml. For 1 month the patient was given oral T3 in dosage of 25 μg three times daily which he took regularly with no subjective or objective change. The basal metabolic rate remained normal, and no change was evident on withdrawal of the T3 tablets. The serum T3 1 month after cessation of T3 tablets was 264 ng/100 ml, and the patient remained asymptomatic with persistently low serum PBI and thyroxine concentrations.

The reduction of elevated serum T3 concentrations in thyrotoxicosis after therapy is illustrated in Table III. The values after successful treatment had declined to or toward the normal range. All five subjects were considered approximately normal at the time of the second T3 determination, based upon clinical appearance and serum thyroxine or PBI concentration. All had received or were receiving antithyroid drugs and R. C., L. H., and R. M. had also received one or more doses of ^{131}I therapy.

DISCUSSION

The present method was considered sufficiently simple for clinical application. The results of double isotope derivative assay afford corroboration.

The report of Pind in 1957 presented T3 concentrations in sera from normal subjects and euthyroid schizophrenic subjects with figures in remarkably close agreement with the present findings. Thus Pind's normal mean value of 0.14 ±0.04 μg/100 ml of T3 iodine, multiplied by the factor 1.71 to convert T3 iodine to T3, gives a figure of 239 ±68 ng T3/100 ml. (*cf.* present mean ±SD of 220 ±27 ng/100 ml).

The other report in 1957 by Maclagan was notable in recording two instances of thyrotoxicosis associated with elevated T3 but normal T4 in the serum. The paper failed to comment specifically on "T3 thyrotoxicosis," except by implication. The method presented, however, was admittedly "too time-consuming for routine use," and may not be sufficiently sensitive for quantitation of any but elevated T3 concentrations.

Other reports, earlier and later, have employed comparison of radioactivity within T3 and T4 areas of chromatograms of serum obtained at one or more intervals after the administration of ^{131}I (12–15, 18). While important information was gained from such studies, the figures do not necessarily reflect the relative concentrations of the two hormones in the circulation, owing to differences in their specific activities. Such divergencies of specific activities of T3 and T4 were evident at varying intervals after radioiodine administration, and may be attributed to the differences in metabolic turnover of the two hormones, different rates of equilibration with their peripheral pools and, indeed, possibly even different specific activities at the time of secretion from the thyroid gland.

During the last several years, a number of reports have shown T3 as the major or apparently the only hormone in the sera of euthyroid or hyperthyroid patients with various thyroid disorders (31–35). The reports were all based upon study of sera after administration of ^{131}I, except that of Werner, Row, and Radichevich (32) who employed both chemical and radioactive assay to demonstrate probable T3 formation and release in a patient with nontoxic nodular goiter.

Despite inadequacies of the methods for measuring T3 then available, these reports all tended to afford support for Maclagan's earlier finding (11) of thyrotoxicosis due to elevation of T3 alone.

A detailed account of a method for measuring T3 in human serum has recently been reported by Nauman, Nauman, and Werner (22). Unfortunately this procedure yields spuriously elevated values, due to the formation of a methyl ester of thyroxine during methanol-

chloroform extraction of the serum.[8] In all of the usual chromatographic systems this T4 methyl ester (which would be unlabeled in the Nauman procedure) has a running rate rather similar to that of labeled T3. Consequently the ester is included in "T3" eluates and causes falsely elevated values in displacement measurements. In work to be reported separately[8] we have found that alcoholic extractions of serum by methyl, ethyl, butyl, and amyl alcohols give rise to esters of T4 and T3, in accordance with previous observations (11, 36). Such ester formation caused interference in earlier phases of the present work, and led to the adoption of resin columns to remove the hormones from serum.

The recently described method of Hollander (21) is of interest, but requires gas chromatographic technique which is not generally available. A detailed report of the results is awaited.

The problem of the biologic significance of circulating T3 requires consideration. The present findings support a major physiological role in two specific clinical circumstances. A convincing indication of the importance of the hormone is provided by the maintenance of normal clinical status in patients with normal or elevated T3 but low serum T4 concentration after ^{131}I or other therapy of thyrotoxicosis. This phenomenon of apparently normal metabolism due to T3 secretion in more than half of an unselected series with low serum thyroxine after therapy suggests the need for reevaluation of further patients after ^{131}I treatment.

The occurrence of seven instances of thyrotoxicosis in association with elevated serum T3 concentration despite normal serum thyroxine is also compatible with a significant role of T3 in disease. However, thyrotoxicosis due to elevated T3 alone is most probably a relatively rare clinical phenomenon. The five sera sent from other cities had been considered clinical rarities by the clinicians who sent them. Moreover, most of the present series of 40 sera from patients with hyperthyroidism had been collected before clinical detection of the two observed instances of "T3 thyrotoxicosis," which required diligent search. The actual incidence of this condition remains to be determined.

In the clinical situations noted, T3 in the serum may be a crucial factor. The question of the significance of T3 in normal hormonal economy remains to be considered.

From previous information (4) concerning its rapid turnover, T3 may be supposed to have an appreciable role. The biologic half-time for the turnover of T4 is slightly less than a week, while the T3 half-time is slightly longer than 1 day (4, 8, 37–39). Earlier studies (4, 8, 37–39), including those from this laboratory, have suggested a daily removal rate of about 50–55 μg of T4 *iodine,* equivalent to approximately 80 μg of T4. Despite having a concentration in the serum only about 3% that of T4, T3 appears to have an absolute removal rate almost as great. According to tracer studies in progress (Woeber, Sobel, Ingbar, and Sterling), the extrathyroidal pool may approximate 100 μg T3. With a biologic half-time of 1.3–1.4 days, about half of this pool would be replaced daily. This would, then, signify a quantitative T3 turnover approximating 50 μg/day. Regardless of difficulties entailed in the interpretation of data on kinetics and spaces, where metabolic degradation is rapid relative to distribution, as in the case of T3, it seems quite probable that this hormone has an appreciable quantitative turnover, not far from the estimate of 50 μg/day.

If T3 is at least three or perhaps four times as potent as T4 on a weight basis (39), it would, therefore, follow that about two-thirds of the total metabolic effect in normal human subjects may well be due to T3 metabolism.

The previous observations of increased T3 secretion after iodine deprivation in the rat (40, 41) naturally raise the possibility that such a mechanism might account for the normal to elevated T3 concentrations with low T4 in sera from patients after ^{131}I therapy. Indeed, the most recent work by Greer, Grimm, and Studer (41) employing single pass perfusion of prelabeled rat thyroid glands *in situ* with nonradioactive blood showed T3 secretion at a disproportionately higher rate than T4 in relation to their respective intrathyroidal concentrations in the thyroglobulin molecule. Despite the possible importance of low intrathyroidal iodine, an attractive hypothesis, a further explanation must still be sought according to the recent findings of Braverman and Ingbar (42) indicating preferential thyroid synthesis of T3 in ^{131}I-treated rats, including animals which received loading doses of stable iodide as great as 500 μg. At the present time further work is needed to elucidate the factors governing the concentration of T3 in serum in health and in disease.

ACKNOWLEDGMENTS

Valuable technical advice was given by Doctors Ralph Peterson, David Fukushima, Kenneth Roberts, and Charles G. Lewallen. In addition, the authors enjoyed technical discussions with Doctors Janusz and Alicja Nauman and Ildiko Radichevich, and are grateful for the generous and unflagging support of Dr. Satoshi Hamada. The authors are indebted to Abbott Laboratories for generous supplies of Triosorb sponges.

This work was supported in part by Grants AM 10739-03 and 04 from the U. S. Public Health Service and Grant P-461 from the American Cancer Society.

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