RADIOCHROMATOGRAPHIC IDENTIFICATION OF THYROXIN IN AN ALPHA GLOBULIN FRACTION OF SERUM SEPARATED BY STARCH ZONE ELECTROPHORESIS [1]

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(Submitted for publication May 7, 1953; accepted October 21, 1953)

Iodine administered orally as NaI^{131} associates with two electrophoretically identifiable serum proteins (1–4). It appears first with the albumin fraction where it is associated with the slower moving component. It is later found in a zone of low protein concentration just ahead of alpha-2 globulin. This behavior was observed in the serum from both euthyroid and hyperthyroid subjects. However, in hyperthyroid subjects the tracer iodine concentrated faster, attained higher levels, and declined more rapidly in this alpha globulin zone (5). This suggested that the hormonal iodine may be associated with this specific protein. The exact nature of this iodine compound is unsettled although the presence of thyroxin and more recently 3:5:3′-L-triiodothyronine (6) has been demonstrated in whole serum.

The purpose of this present paper is to report the radiochromatographic analysis of iodine containing amino acids associated with an alpha globulin fraction separated by starch electrophoresis.

CLINICAL MATERIAL

The four patients selected for this study were seen in the Department of Medicine of the University of Wisconsin. Thyroid function was measured by the determination of the 24-hour uptake of I^{131} [3] as reported previously (7). All were hyperthyroid as judged by their symptoms, physical findings, and elevated I^{131} uptake. Pertinent clinical data are summarized in Table I. In each instance therapeutic quantities of carrier-free I^{131} ranging from 4 to 6 mc. were administered orally. These doses were calculated to administer the same amount of radiation (75 to 100 microcuries per Gm.) to the gland in all subjects. This estimation was based upon previous uptake studies of "tracer" quantities (100 microcuries) of I^{131} and upon the size of the gland.

Upon completion of the diagnostic studies and administration of therapeutic I^{131}, blood samples were drawn on the fourth post-treatment day, placed in dry centrifuge tubes, and the serum separated for starch electrophoresis.

METHODS

Starch electrophoresis

The procedure of Kunkel and Slater (8) was followed in principle. However, a different type of apparatus, illustrated in Figure 1, was used. This consists of a lucite bridge of rectangular cross section connecting two vessels containing 0.1 M veronal buffer pH 8.6. Within the horizontal portion of the bridge a 1.5 by 5.5 by 38 cm. mold of buffer soaked starch is formed. Two ml. of the buffer dialyzed serum was introduced in a line across the starch block 8 cm. from the cathodal end. A period of one hour was allowed for equilibration. The electrophoresis was carried on at 3° C. After electrophoresis for approximately 20 hours at 35 milliamperes the starch block was removed from the bridge, partially dried and cut transversely into 1 cm. segments. The protein of each segment was eluted by shaking twice with 3 ml. of cold physiological saline in a test tube.

Quantitation of the protein of the eluate of each segment was performed by the modified Folin tyrosine reagent (9). When these values are plotted, the resultant curve demonstrates separation of the major protein fractions comparable to that of standard electrophoresis.

Localization of radioactivity in the serum protein fractions was determined by counting a 100 microliter aliquot of each eluate in an end window GM counter.

Chromatography

The eluates of the alpha globulin and the albumin segments containing measurable quantities of I^{131} were each pooled and extracted separately with N-butanol, once with twice the volume and two times with equal volumes. The butanol extracts were concentrated at room temperature to approximately 100 microliters. Colorimetrically identifiable quantities (approximately 50 micrograms) of carrier thyroxin [4] and 3:5:3′-L-triiodothyronine [5] were added to the concentrate which was then chromatographed.

Using a modification of an ascending chromatographic

[1] This work was supported in part by the Wisconsin Alumni Research Foundation.

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[3] Obtained from Isotopes Division, Atomic Energy Commission, Oak Ridge, Tennessee.

[4] Kindly supplied by E. R. Squibb & Sons.

[5] Kindly supplied by Smith, Kline & French Laboratories.

TABLE I

Summary of clinical data of hyperthyroid subjects

	Patient	Age	Sex	Physical findings	I^{131} uptake* at 24 hours %	I^{131} administered *mc.*
1.	J. M.	32	M	Diffuse goiter, est. wt. 40 grams.	53	6
2.	E. S.	21	F	Diffuse goiter, est. wt. 60 grams.	78	4
3.	H. V.	50	F	Diffuse goiter, est. wt. 40 grams.	68	4
4.	M. L.	60	F	Nodular goiter, est. wt. 50 grams.	52	7

* I^{131} uptake expressed as per cent of the dose present in the thyroid at 24 hours.

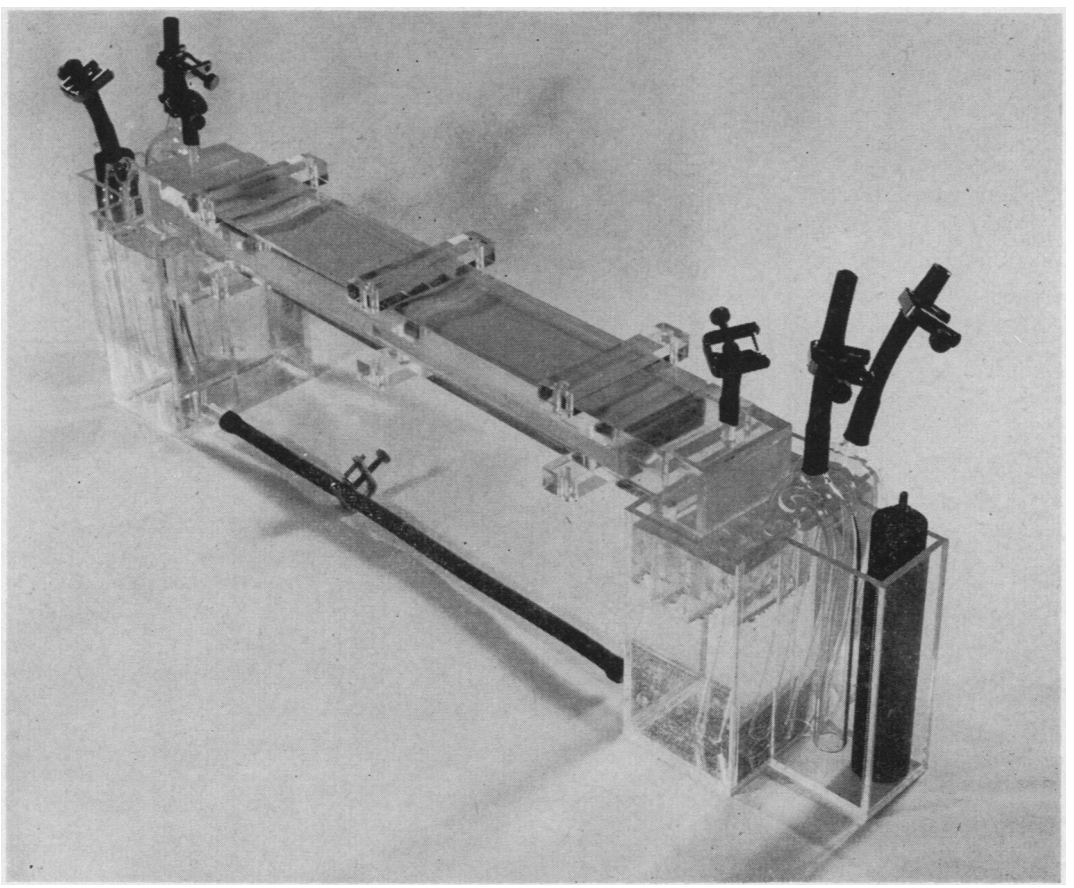

FIG. 1. LUCITE APPARATUS FOR STARCH ELECTROPHORESIS

method described by Kowkabany and Cassidy (10), it was found that triiodothyronine and thyroxin could be separated by single dimension chromatography in both collidine-water and butanol-dioxane-ammonia systems (11). Whatman No. 3MM filter paper was cut into a tapered form which proved helpful in maintaining discrete spots, thereby aiding materially the separation of the two amino acids. The validity of this method can be seen in Figure 2 which illustrates the separation of I^{131} labelled triiodothyronine[6] and thyroxin[6] in both solvent systems.

Following development of the chromatogram and the color reaction, the paper strips were cut transversely into 1 cm. segments, and the radioactivity localized.

[6] Obtained from Abbott Laboratories, Oak Ridge, Tennessee.

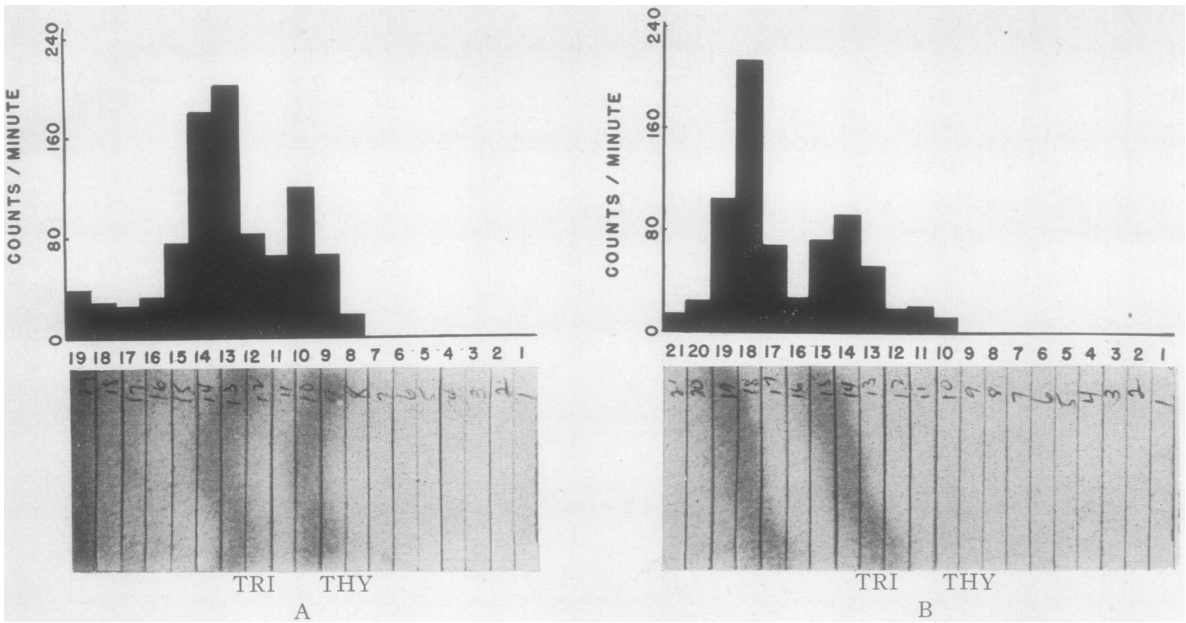

FIG. 2. DISTRIBUTION OF RADIOACTIVITY ON THE CHROMATOGRAMS OF BUTANOL EXTRACTS OF SERUM TO WHICH RADIOTHYROXIN AND RADIOTRIIODOTHYRONINE WERE ADDED

A—collidine-water developer. B—butanol-dioxane-ammonia developer. Position of the amino acid is indicated by legend: THY—thyroxin, TRI—triiodothyronine.

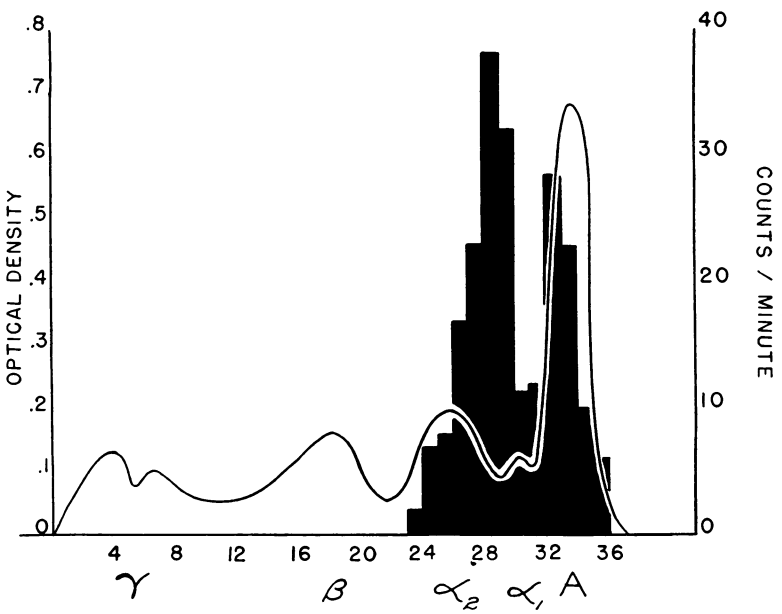

FIG. 3. DISTRIBUTION OF RADIOACTIVITY IN SERUM PROTEIN FRACTIONS SEPARATED BY STARCH ELECTROPHORESIS

Bar graph represents radioactivity in eluates of 1 cm. segments of starch block. Linear graph represents protein determination in eluates of corresponding segments.

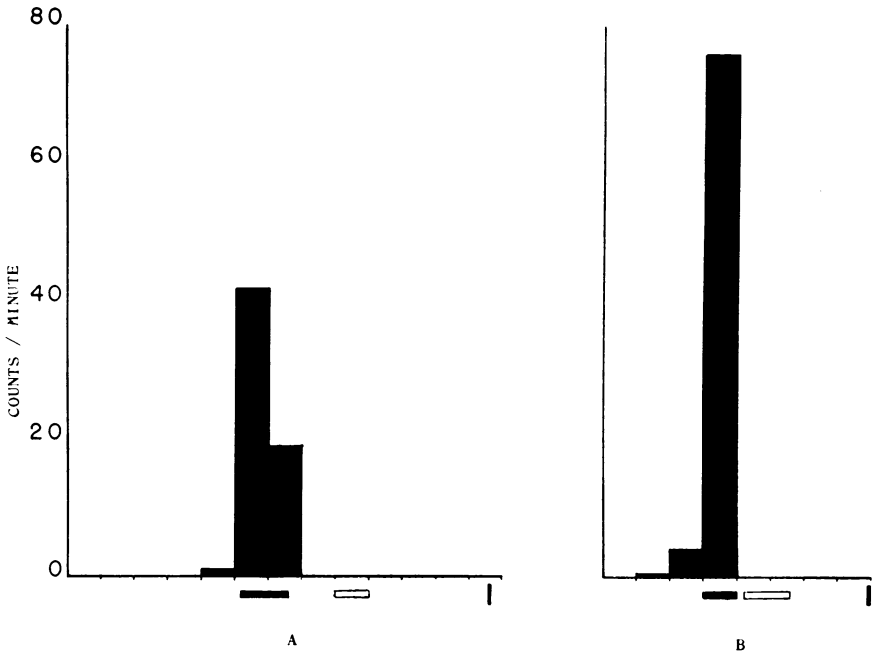

FIG. 4. GRAPH OF RADIOCHROMATOGRAM OF BUTANOL EXTRACT OF ALPHA GLOBULIN FRACTION OF SERUM OF HYPERTHYROID PATIENT

A—in butanol-dioxane-ammonia developer. B—in collidine-water developer. The developer front is indicated by the solid vertical bar below the abscissa. Horizontal solid bar represents thyroxin, open bar 3 : 5 : 3′-L-triiodothyronine.

RESULTS

The distribution of the radioactivity in the eluates of the segments of the starch block together with the protein quantitation is seen in Figure 3. As reported previously, with paper electrophoresis there are two peaks of radioactivity, one, in the slower albumin zone and the other just ahead of alpha-2 globulin.

The radioactivity of the alpha-globulin eluate was wholly extractible with butanol. On the other hand, no significant radioactivity could be extracted from the albumin eluate. The radiochromatography of the butanol extract of the alpha-globulin eluate in one representative patient is illustrated in Figure 4. An exact correlation with thyroxin is evident in both solvent systems. No correlation of radioactivity with carrier triiodothyronine on the fourth day was seen in either solvent system in the four patients studied.

DISCUSSION

It has been generally believed that thyroxin is the active hormone secreted by the thyroid gland. More recently 3 : 5 : 3′-L-triiodothyronine has been demonstrated in plasma (6), but its exact relationship to thyroid physiology is a matter of speculation. Studies on the localization of orally administered I^{131} in serum protein fractions have led to the concept that hormonal iodine is specifically associated with an alpha globulin (5). The present study indicates that the alpha globulin I^{131} is, (*1*) entirely extracted with butanol, and (*2*) can be identified as thyroxin by paper radio-chromatography. The albumin-bound I^{131} is not butanol extractible under the same conditions and is assumed not to contain thyroxin.

The failure to demonstrate triiodothyronine in alpha-2 globulin is not meant to exclude its presence in serum. This amino acid is known to have a rapid turnover and to disappear quickly from the intravascular compartment (12). This may be related to the difference in protein binding of this amino acid, as it has been shown *in vitro* (13) that triiodothyronine is less firmly bound than thyroxin to the alpha globulin. However, these data indicate that the iodine-containing compound in alpha-2 globulin is thyroxin at a time when the

radioactivity of serum is at a maximum following oral administration of I^{131}.

SUMMARY AND CONCLUSIONS

Four hyperthyroid subjects were given therapeutic quanties of I^{131}. After a period of four days, the serum proteins were separated by starch zone electrophoresis. Two peaks of radioactivity appeared, one in the slowest moving albumin and the other in an area of low protein concentration just ahead of the alpha-2 globulin. Saline eluates of these zones were extracted with butanol. The radioactivity of the alpha zone was wholly extractible and was identified as thyroxin by paper chromatography. The albumin radio-activity was not extractible with butanol under these same conditions.

ACKNOWLEDGMENT

The authors wish to acknowledge the invaluable assistance of Miss Sue Ames, medical technician.

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