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ISOLATION AND QUANTITATIVE ESTIMATION OF IODIDE, THYROXINE, AND SUBSTANCES RELATED TO THYROXINE BY MEANS OF AN ANION EXCHANGE RESIN*

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In previous years extensive work has been done dealing with the isolation of thyroxine and similar substances, especially in thyroid hydrolysates and plasma. (Taurog, Tong, and Chaikoff in the United States; Harrington, Gross, and Pitt-Rivers in England; Leblond in Canada; Roche, Michel, and Lissitsky in France; and many others.) All of these workers have been using paper chromatography with various solvents, and their studies have enabled them to fix the different steps of the synthesis of thyroxine. But paper chromatography is not quantitative, and a precise estimation of these substances is difficult.

Recently B. M. Dobyns and S. R. Barry[1] succeeded in separating and determining the amount of different substances contained in thyroid hydrolysates on a starch chromatographic column. They found inorganic iodide, thyroxine, monoiodotyrosine, diiodotyrosine, and two unknown compounds. Their method signifies real progress over the previous ones, paper chromatography, as the quantitative estimation is made possible.

We have been searching for a method permitting an easy, simple, and rapid estimation of inorganic iodine, protein bound iodine, and of each of the amino-acids of the thyroglobuline molecule.

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*** "In Memoriam".

The liquids to be investigated, either plasma or thyroid hydrolysates, from animals previously injected with tracer doses of I^{131}, are poured directly on a column of the anion exchange resin. The dimensions of the column are 6 inches high and 1/2 inch diameter. (The resin used was Dowex 2* of particle size 200-400 mesh. This resin, supplied in the chloride form, is regenerated before use with carbonate free 2N sodium hydroxide until chloride free, then washed with carbonate free distilled water until the alkalinity of the eluate falls below pH 8.)

When equilibrium is attained on the column, elution is performed with 0.2N HCl at a rate of 40 ml./hour, and effluent (total volume 500 ml.) is collected in fractions of 1.5 ml. by an automatic device. The radioactivity of each fraction is measured with an automatic counter.

We found that all the mineral iodine remained fixed on the column and is not eluated by HCl N/10.

Since one of us (R. W. D.) prepared I^{131} tagged monoiodotyrosine, diiodotyrosine, thyroxine, and triiodothyronine, these substances were mixed with inactive thyroid hydrolysate or plasma and used to calibrate the ion exchange column. Under eluting conditions specified above, the locations of the peaks are shown in Table I. The results show the average of ten elutions of each substance labeled with I^{131}.

Table I

Location of elution peaks

	Location of peak, ml. eluting fluid.	Extreme limits of bands, ml.
Thyroxine	30 ± 3	22 - 40
Monoidotyrosine	55 ± 3	45 - 76
Diiodotyrosine	125 ± 2	91 - 180

* K. G. Scott and W. A. Reilly (2) used this resin early in 1953 for a clinical test in plasma. This resin was made by the Dow Chemical Company, Midland, Michigan.

Next, blood plasma or thyroid hydrolysates from rabbits that had previously received a tracer dose of I^{131} were poured on the column. Peaks corresponding to thyroxine, monoiodotyrosine, and diiodotyrosine were found along with a fourth peak which is now being identified.

This ion exchange method combined with counting procedures allows us to separate and measure active fractions of mineral and protein bound iodine and of other substances of importance in thyroid metabolism. A detailed paper will follow.

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