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The Determination of Protein Bound Iodine-131 In Thyroid Gland Homogenates

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A number of methods have been described for estimating the fraction of radioiodine bound to protein in thyroid gland homogenates. In a recent study Nagataki and Ingbar found that values obtained by filter-paper electrophoresis for inorganic iodide-131 were uniformly lower than those obtained by paper chromatography, dialysis, acid precipitation and iodate oxidation (1). Similarly, Taurog found higher values for inorganic iodide-131 when chromatography was compared with electrophoresis (2). As these studies were performed under circumstances in which the fraction of thyroidal iodide-131 was small, it was of interest to compare the separation of organic from inorganic ^{131}I by electrophoresis and chromatography over the wide range of values encountered in *in vitro* incubations.

MATERIALS AND METHODS

Thyroid glands from male rats weighing 240-300 gms were incubated as whole and as sliced lobes in KRP buffer containing ^{131}I and supplemented with KI at levels between 1×10^{-4} and 1×10^{-6}M. In some experiments, flasks containing 20 mu/ml TSH (Thytropar, Armour Pharmaceutical Company, Kankakee, Ill.) were employed for incubations. After two or three hours, the thyroids were removed from the incubation media, rinsed briefly and then homogenized in veronal buffer, supplemented with 1×10^{-3}M thiouracil (3). Electrophoresis of the homogenate was performed in a Beckman-Spinco cell with tris-maleate buffer, pH 8.6 (1). Separation of iodide was achieved with the application of 200 V for 75 minutes. Carrier iodide was revealed with 0.2 per cent palladium chloride. Portions of the strips corresponding to radioiodide and protein bound ^{131}I (PBI-131) were cut and counted in a well-scintillation counter. Chromatograms were developed in an ascending fashion in butanol:acetic acid:water (78:5:17) for 20 hours. The paper was transferred to chromatography jars as soon as the point of application was dry. All chromatograms were scanned in an automatic strip-scanning counter. The partition of ^{131}I between iodide and protein-bound form was quantitated by planimetry of the counting record. In those instances in which the fraction of ^{131}I corresponding to iodide was very low or very high, appropriate portions of the chromatograms were cut out and counted in a well counter.

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RESULTS

Electrophoresis and chromatography were carried out in 101 thyroid homogenate samples. The results of this comparative study are presented in Figure 1. In this illustration the value for the PBI-131 of each homogenate sample as determined by chromatography has been plotted against the value obtained by electrophoresis. In confirmation of the results of others, values for PBI-131 by electrophoresis were slightly higher than those observed in chromatograms of paired samples. This difference is more evident at the lower range of PBI-131 values. Although there is some spread of the PBI-131 values in the mid-range, correlation of results between the two methods is good.

DISCUSSION

In previous studies filter-paper electrophoresis gave lower values than chromatography for the determination of the ^{131}I fraction in thyroid gland homogenates. This difference may have been due, in part, to the process of spontaneous deiodination of the homogenate that Taurog observed, when transfer of filter paper to chromatography jar was delayed (1). Although the values for iodide-131 in the present study were slightly lower, and conversely the values for PBI-131 slightly higher when electrophoresis was compared to chromatography, the overall correlation suggests that chromatography is an adequate method to use over the wide range of values encountered in *in vitro* studies. It might be argued that another solvent system, such as butanol:dioxane:ammonia, with its greater mobility for iodide, would have been a better choice for this study. Halmi and Pitt-Rivers have reported difficulty in the separation of inorganic iodide and protein-bound radioactivity in this chromatographic system (4). In the present investigation, a comparison was made of the value for PBI-131 determined by chromatography in butanol:dioxane:ammonia and butanol:acetic acid:water. It was found that in 15 homogenate preparations, the PBI-131 was uniformly lower in chromatograms developed in butanol:dioxane:ammonia, and because of this, further experiments with this system were abandoned.

Many of the chromatograms were analyzed by planimetry of the counting record of a strip-scanning counter. Where the results obtained by this method were compared with those obtained by counting sections of the chromatogram in a well-counter, there was no significant difference. As a matter of convenience, when the fraction of ^{131}I on the chromatogram as iodide was either very high or very low, appropriate portions of the chromatogram were counted in a well-counter. However, Shimoda and Greer have found that by manipulation of the scaling factor of the chromatogram scanner, areas as small as 0.1 per cent of the total radioactivity on the strip can be estimated with accuracy (5).

SUMMARY

A comparison has been made between filter-paper electrophoresis and chromatography in an acid butanol system for the determination of ^{131}I bound to protein in rat thyroid homogenates. Correlation between the two methods over a

wide range of values was quite good (r = .973). However, electrophoresis may be preferred by some investigators because it requires less time. Chromatography of thyroid extracts in butanol:dioxane:ammonia was not very satisfactory since the value for ^{131}PBI was uniformly lower when compared to chromatograms developed in acid butanol.

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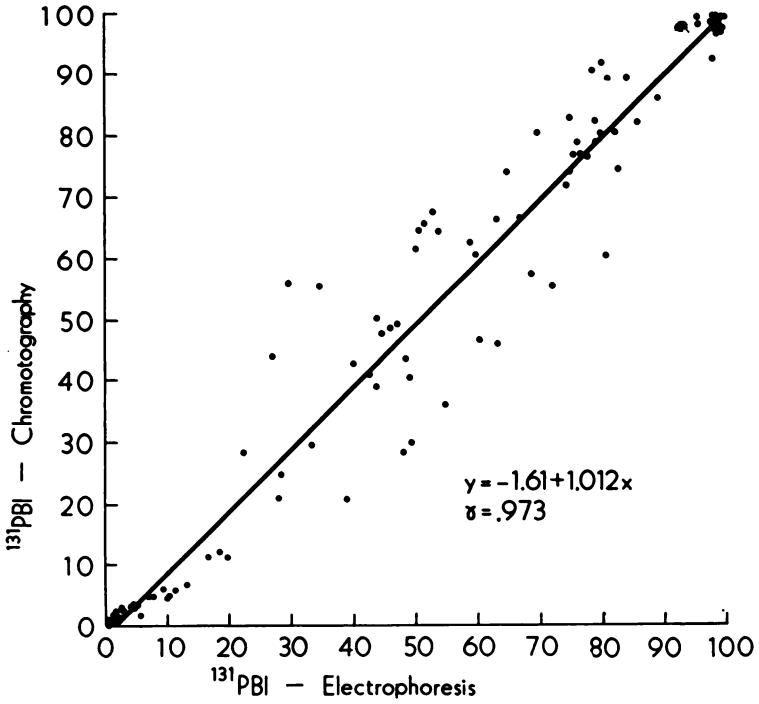

Fig. 1. Results for the determination of the fraction of thyroidal ^{131}I bound to protein in 101 homogenate samples are presented in this illustration. The value obtained by electrophoresis for each homogenate sample has been plotted against that obtained by chromatography. The correlation between the two methods is highly significant.

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