DETERMINATION OF HORMONAL RADIOIODINE IN SERUM WITH A CATION EXCHANGE RESIN

Jan D. Wiener and Peter Roos

Academisch Ziekenhuis der Vrije Universiteit, Amsterdam, The Netherlands

Since its introduction by Clark *et al* (*1*) and Silver and Fieber (*2*), the measurement of protein-bound radioiodine in the serum (PBI*), generally performed one or more days after a tracer dose, has proved to be a useful test of thyroid function, especially in the diagnosis of hyperthyroidism.

In the original procedure, the PBI* is precipitated with trichloroacetic acid (TCA), washed several times with TCA and dissolved in alkali. Although a certain proportion of radioiodide (I*) is coprecipitated and not removed by successive TCA washings (*3,4*), this disadavantage can be overcome by adding large amounts of stable iodide carrier (*4*). Even so, the procedure remains relatively time-consuming.

Several simpler procedures have been proposed in which I* and PBI* are separated by an anion exchange resin—used either batchwise (*5*) or in columns (*3,6,7*)—or by dialysis (*8*). The method presented in this paper is an adaptation of a technique developed for the chemical assay of serum hormonal iodine (*9*); the method has since proved its value in over 3,000 determinations. It is very simple and gives very good separation of I* and PBI*. Because protein-like compounds are not included, we prefer to speak of radioactive iodoamino acids (IAA*) rather than PBI*.

The use of the method to determine the early (3-hr) conversion ratio will be reported elsewhere.

METHOD

Glass columns, 10 mm i.d. and about 12 cm long, are fitted with a plug of glass wool. A suspension of Dowex 50W × 2, 200–400 mesh, H^+-form, is poured into the columns to a depth (when settled) of about 20 mm. The columns are washed with about 8 ml 1 *N* HCl (column filled up) and then with 8 ml water.

Two milliliters of serum are allowed to run through a column. The column is washed with 8 ml water. One milliliter of 5 *N* ammonia is then added,* and the eluate is discarded. A counting tube is placed under the column, 4 ml ammonia are run through the column and the eluate is collected in the tube.

The eluate is counted in a well-type scintillation counter. So that it can be compared with a 2-ml standard or serum sample, the result is corrected for volume difference.

After use, the resin is washed with 4 ml ammonia to remove traces of radioiodine, and regenerated by washing with 4 ml water, 8 ml 1 *N* HCl and 8 ml water. The same resin can be used 100 or more times; columns have been in regular use for over 1 year without detectable changes of the results. The columns do not rapidly dry up. If they are not in use for several days, they are kept in distilled water.

RESULTS

The method has been developed with the aid of serum obtained from patients who had received a therapeutic dose of radioiodine. Results of a representative experiment are shown in Table 1. Five 2-ml aliquots of serum were pipetted into columns, and the effluent was collected in counting tubes. Four consecutive 4-ml water washings were collected. The IAA* was then eluted with 1, 2, 2, 1, and 1 ml portions of 5 *N* ammonia, and the fractions were collected separately. All samples were counted for 20 min (100-keV window, 3-in. scintillation crystal, background around 750 counts in 20 min). The results were corrected for volume differences. Total counts of all tubes from one column averaged 1.069,820 (standard deviation = 2.839). The effluent serum and the first and second 4-ml water washings together contained 7.59% (s.d. 0.44%) of this activity; the third and fourth washings together con-

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For reprints contact: Jan D. Wiener, Academisch Ziekenhuis der Vrije Universitat, Dept. of Medicine, 1117 De Boelelaan, Amsterdam 11, The Netherlands.

* Because this volume depends on the dead volume of the column, it should be determined separately in each laboratory.

TABLE 1. FRACTIONATION OF RADIOACTIVITY IN SERUM OBTAINED 4 DAYS AFTER 16 MC ^{131}I GIVEN FOR HYPERTHYROIDISM

Sample	% total radioactivity	
	Mean	Range
Effluent serum	3.30	2.90 hr– 3.54
1 hr water wash	4.21	3.88 hr– 4.92
2nd water wash	0.08	0.05 hr– 0.11
3rd water wash	0.03	0.03 hr– 0.04
4th water wash	0.02	0.02 hr– 0.03
1 hr ammonia (1 ml)	0.07	0.02 hr– 0.41
2nd ammonia (2 ml)	73.16	69.77 hr–76.06
3rd ammonia (2 ml)	16.41	13.97 hr–19.00
4th ammonia (2 ml)	1.82	1.59 hr– 2.36
5th ammonia (1 ml)	0.90	0.64 hr– 1.13

tained 0.07% or less. The second through fifth milliliters of the ammonia eluate contained 89.57% of the total activity; the standard deviation amounted to only 0.61%, indicating a very high precision. It has been shown previously (*9*) that the adsorption of iodoamino acids on the resin is virtually complete.

In another experiment, radioiodide was added to nonradioactive serum. Ten 2-ml aliquots were then processed as described under "Method." The second through fifth milliliter of the ammonia eluate were collected together; counting results were corrected for the volume difference. An average of 0.86% (s.d. 0.11%) of the added I* was recovered.

Parallel analyses with the conventional TCA-precipitation technique and the new method were then done in 102 samples of serum from 41 patients sent for thyroid-function tests with ^{131}I. Blood was drawn 2, 24 and/or 72 hr after the ingestion of 15 μc ^{131}I. All samples were counted for 20 min. Because about 3% of the I* in the samples was retained in the TCA precipitate after washing, TCA values were corrected by deducting 3% of the difference between the total and IAA activity in samples in which the activity was chiefly or exclusively I*; i.e., in all 2-hr samples and in later samples from patients with hypothyroidism, impaired renal function or cardiac insufficiency. The results are shown in Table 2. With the possible exception of the PBI*–IAA* differences, the values were not normally distributed; ranges rather than standard deviations are therefore given. The mean PBI*–IAA* difference was —0.001%/liter (s.d. 0.032%/liter) in the high-I* group and 0.005%/liter (s.d. 0.045%/liter) in the low-I* group. The difference exceeded 0.10%/liter in only one sample.

The determination is of little value for varying lengths of time after the administration of iodine-containing preparations. Furthermore the 24-hr IAA* (like the PBI*) proved to be elevated in most euthyroid patients with diminished thyroidal iodine pools, including those with hyperfunctioning autonomous nodules, endocrine ophthalmopathy and ectopic thyroids and those treated surgically or with ^{131}I for hyperthyroidism. Excepting these patients, values of 0.16%/liter or less were obtained in 123 out of 125 consecutive euthyroid patients, and values over 0.16%/liter in 44 out of 49 consecutive hyperthyroid patients. These were unselected patients, including diagnostic problem cases, in whom the final diagnosis was established by two or more physicians on clinical and laboratory data, including stable (chemical) IAA, ^{131}I uptake, absolute iodine uptake, and, when necessary, T-3-suppression tests.

DISCUSSION

With the present method, the recovery of the iodoamino acids is comparable with or higher than that of existing procedures, whereas less than 1% of the iodide is included. This is especially important if the IAA* is determined within a few hours after the dose; the advantages of the early IAA* determination will be discussed in a forthcoming paper. The precision of the method is very good. Because the same resin columns can be used many times, the

TABLE 2.* COMPARISON OF PBI* AND IAA* IN 122 SERUM SAMPLES OBTAINED AFTER TRACER DOSES OF ^{131}I

	No. of samples	% dose/liter				
		Total activity	PBI*	PBI*$_{corr}$†	IAA*	PBI*$_{corr}$–IAA*†
High radioiodide	39	2.20 (0.22–6.41)	0.097 (0.03–0.24)	0.030 (—0.06–0.15)	0.032 (—0.01–0.14)	—0.001 (—0.09–0.08)
Low radioiodide	63	0.335 (0.02–2.38)	0.253 (0.00–2.17)		0.248 (0.00–2.37)	0.005 (—0.20–0.07)

* Mean values, with ranges in parentheses, are given in % dose/liter serum.

† PBI* was corrected in samples with high radioiodide only (see text); in other samples, PBI*$_{corr}$ did not appreciably differ from PBI*, and the latter was used for the calculation of the difference (last column).

method is less time-consuming than TCA, anion exchange resin and dialysis procedures (*1,3–8*).

The present procedure can easily be modified to measure only thyroxine and triiodothyronine (hormonal iodine, HI). Washing with borate buffer *p*H 8.5 removes possible iodotyrosines from the resin columns without appreciably eluting the HI (*9*). In the present study, four 4-ml buffer washings removed no more than 1% of the HI*. The combined determination of IAA* and HI* will be found useful in the study of inborn errors of iodine metabolism, especially deiodinase deficiency. If, on the other hand, the measurement of protein-like radioactivity is desirable, the effluent serum plus water washings can be treated with an anion exchange resin such as Dowex 1 to remove radioiodide (*10*). Protein-like radioactivity is enhanced in some cases of thyroid cancer, Hashimoto's thyroiditis, simple goiter and inborn errors of iodine metabolism.

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REFERENCES

1. CLARK, D. E., MOE, R. H. AND ADAMS, E. E.: The rate of conversion of administered inorganic radioactive iodine into protein-bound iodine of plasma as an aid in the evaluation of thyroid function. *Surgery* **26**:331, 1949.

2. SILVER, S. AND FIEBER, M. H.: Blood levels of I-131 after tracer doses in the diagnosis of hyperthyroidism. *Proc. Soc. Exp. Biol. Med.* **75**:570, 1950.

3. ZIEVE, L., VOGEL, W. C. AND SCHULTZ, A. L.: Determination of protein-bound radioiodine with an anion exchange resin. *J. Lab. Clin. Med.* **47**:663, 1956.

4. CHRISTENSEN, L. K.: Determination of protein-bound radioactive iodine, *Scand. J. Clin. Lab. Invest.* **10**:211, 1958.

5. SCOTT, K. G. AND REILLY, W. A.: Use of anionic exchange resin for the determination of protein-bound I^{131} in human plasma. *Metabolism* **3**:506, 1954.

6. FIELDS, T., KINNORY, D. S., KAPLAN, E., OESTER, Y. T. AND BOWSER, E. N.: The determination of protein-bound iodine^{131} with anion exchange resin column. *J. Lab. Clin. Med.* **47**:333, 1956.

7. DOWBEN, R. M.: Modified resin method for determination of radioiodine conversion ratio. *J. Lab. Clin. Med.* **55**:132, 1960.

8. IMARISIO, J. J., KOTLOWSKI, B. B. AND IMPERATO, A. A.: Dialysis method for PBI^{131} determination compared with TCA and resin column techniques. *J. Lab. Clin. Med.* **60**:526, 1962.

9. BACKER, E. T., POSTMES, T. J. AND WIENER, J. D.: Determination of thyroid hormones in serum by means of a cation exchange resin and chloric acid digestion. *Clin. Chim. Acta* **15**:77, 1967.

10. WIENER, J. D. AND LINDEBOOM, G. A.: The possible occurrence of two inborn errors of iodine metabolism in one patient. *Acta Endocrinol.* **47**:385, 1964.