

## A Procedure for the Determination of Total Iodine in Radioactive Iodide Preparations of High Specific Activity<sup>1, 2</sup>

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When  $^{131}\text{I}$  is produced by neutron-induced fission of  $^{235}\text{U}$  or by thermal activation of natural tellurium, it is never in a strict sense carrier-free, since stable  $^{127}\text{I}$  and long-lived  $^{129}\text{I}$  are also formed. We have found the total iodine in  $^{131}\text{I}$  samples received soon after processing to be two-to-three times the  $^{131}\text{I}$  present (1). This ratio increases then as the  $^{131}\text{I}$  undergoes further radioactive decay. The importance of the total iodine in  $^{131}\text{I}$  preparations, used to prepare  $^{131}\text{I}$ -labeled proteins of high specific radioactivity but of a low degree of iodination, has sometimes not been recognized<sup>3</sup>. Its significance depends on the method used to incorporate  $^{131}\text{I}$  into the protein. If all of the iodide used, both that present in the  $^{131}\text{I}$  preparation and that which may be added as a carrier, is treated with an oxidizing agent such as nitrous acid (2) or chloramine T (3) to produce positive iodine for the purpose of iodination, then the percentage of  $^{131}\text{I}$ -radioactivity in the iodinated product is a measure of the percentage of the total iodine introduced

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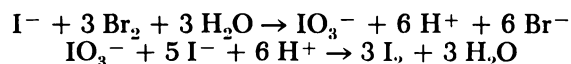
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2. This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

3. Tellurium highly enriched in  $^{130}\text{Te}$  ( $^{128}\text{Te}$ , 04%;  $^{128}\text{Te}$ , 3.6%;  $^{130}\text{Te}$ , 96%) is available at the Oak Ridge National Laboratory (personal communication from J. H. Gillette, Superintendent, Isotope Division, Union Carbide Nuclear Company, ONRL). Activation of this material should yield  $^{131}\text{I}$  of such relatively high purity that the amounts of  $^{127}\text{I}$  and  $^{129}\text{I}$  present per 100 mC of  $^{131}\text{I}$  will become significant in the high specific activity labeling of proteins with  $^{131}\text{I}$  only after a decay period of several half-lives.

into the protein. However, the average degree to which molecules, in a given amount of protein, have been iodinated can be determined only if the total amount of iodine used is known. When  $^{131}\text{I}$  is introduced by isotopic exchange into the iodinating agent, iodine monochloride (4), the average extent to which protein molecules are iodinated is determined by the amount of iodine monochloride, and is independent of the iodide content of the  $^{131}\text{I}$  preparation. The efficiency of  $^{131}\text{I}$  incorporation in the protein, however, is adversely affected by the quantity of iodide present.

The total iodine content of small samples of  $^{131}\text{I}$  shipments (0.1 ml or less) may be conveniently determined by Winkler's method for iodide (5) modified to make analytical use of the radioactivity of these samples. In this method iodide is oxidized to iodate by bromine in a slightly acidic solution, and the excess bromine is removed by boiling the solution<sup>4</sup>. Addition of an excess of iodide liberates elementary iodine, which is then titrated against thiosulfate:



Raben (7) suggested the use of a tracer amount of radioactive iodide, in place of titration with thiosulfate, for determining the liberated iodine. In his procedure 20-50 times the stoichiometric amount of iodide solution, containing a tracer quantity of  $^{131}\text{I}$ , is added to the acidic solution of the iodate, and the free iodine is then extracted with carbon tetrachloride. The amount of iodine in the extract is given by the radioactivity of the carbon tetrachloride solution (counts/sec) divided by the specific activity (counts/sec/ $\mu\text{g I}$ ) of the iodide solution used to reduce the iodate. Four iodide determinations are reported, of which only one was on a sample containing less than 1  $\mu\text{g}$  of iodide ion. No details of the bromine oxidation procedure are given.

To determine the total iodine in  $^{131}\text{I}$  sample of unknown specific activity, a precise amount of inactive iodide must be added to the radioactive iodate formed on bromine oxidation of the analytical sample. For the method to have high sensitivity, the added iodide cannot be in large excess. Analyses of  $^{131}\text{I}$  shipments of high specific activity from the Oak Ridge National Laboratory, and the Union Carbide Nuclear Company at Tuxedo, New York, showed that volumes containing 10-20 mC of radioactivity at the time of receipt (0.1 ml or less) commonly contained considerably less than 1  $\mu\text{g}$  of total iodine. Since the iodate obtained from such samples on oxidation would require stoichiometrically no more than 5.0  $\mu\text{g}$  of iodide, the addition of this quantity of iodide was adopted by us as a standard practice. Instead of extracting the liberated iodine with carbon tetrachloride, we found it more convenient to remove the iodine by volatilization and to determine the amount vaporized by a radioactivity count on the residual solution.

4. Chlorine was originally suggested as the oxidizing agent. However, aqueous solutions of chlorine deteriorate on standing, and after boiling they are still capable of oxidizing iodide (6). Even freshly prepared chlorine water after prolonged heating and aeration with nitrogen will liberate an amount of iodine from iodide which is quite appreciable in a micro-determination. Bromine water does not behave in this manner.

IODIDE DETERMINATIONS AT A TRACER LEVEL OF  $^{131}\text{I}$  ACTIVITY

To establish conditions to be subsequently used in the analysis of  $^{131}\text{I}$  samples of high specific activity, a large number of preliminary runs were made on solutions containing known amounts of inactive iodide and a tracer quantity of iodine-131. Almost without exception, the amounts of iodine found in these runs were less than those known to have been present in the samples analyzed. Occasionally in our earlier experiments a decidedly low value was obtained. There was some evidence that the use of larger volumes of more dilute solutions, in particular of the reagents added after volatilization of the excess bromine, gave lower results than those obtained when smaller volumes of more concentrated solutions were used. In the procedure finally adopted, the total volume of the reaction mixture prior to volatilization of the excess bromine was 0.5 ml. The volumes of the subsequently added potassium iodide ( $= 5.0 \mu\text{g I}$ ) and sulfuric acid were 0.2 ml and 0.1 ml respectively.

For the oxidation of iodide to iodate and the vaporization of the excess bromine, it is usual to make the solution only slightly acidic, since it has been found that the more acidic the solution, the greater is the likelihood of iodic acid being volatilized with the bromine (6). Even at an acidity as low as 0.01 N in  $\text{H}_2\text{SO}_4$  a small loss of iodine may occur. However, any loss in this step is readily determined by radioactivity measurement<sup>1</sup>. Since  $\text{Na}^{131}\text{I}$  shipments from the Oak Ridge National Laboratory are received as solutions with a pH varying from seven to nine, and those from the Union Carbide Nuclear Company at Tuxedo, New York, are in 0.1 N NaOH solution, it was decided to make the reaction mixtures at a tracer level of radioactivity approximately 0.08 N in  $\text{H}_2\text{SO}_4$  in the expectation that the amount of acid required to produce this acidity, when added to the more basic highly radioactive  $\text{Na}^{131}\text{I}$  preparations, would yield solutions which would still be adequately acidic for the oxidation of iodide to iodate and the subsequent volatilization of the excess bromine.

Since boiling the solution to expel the bromine might produce an undesirably high acid concentration, we chose to heat the solution in a boiling water-bath and to aerate it simultaneously with a slow stream of moist, oxygen-free nitrogen for three minutes—a time period which was found to be sufficient to volatilize all free bromine present.

When an acidic solution containing a fraction of a microgram of iodine as iodate, 5  $\mu\text{g}$  of iodine as iodide, and a tracer amount of  $^{131}\text{I}$  is heated with nitrogen aeration in boiling water for five minutes, the percentage of the liberated iodine lost through vaporization depends on the acidity of the solution. The loss was found to be practically negligible when the acidity was 0.02 N in  $\text{H}_2\text{SO}_4$ . With increasing acid concentration it increased until it reached a maximum at an

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<sup>1</sup>A probably not very significant part of the decrease in radioactivity noted at this stage of the procedure may result from (a) a trace of the solution being left on the tip of the capillary through which nitrogen is introduced during the heating of the solution, and (b) a trace being deposited as a spray in the test tube above the level of highest registering efficiency of the counter.

acidity of 0.4 - 0.5 N in  $\text{H}_2\text{SO}_4$ . Although the amount of iodine volatilized was always less than that theoretically formed, it was found to be a constant percentage (93%) of the calculated value under the conditions of the procedure described below. The fact, that no further loss occurred on more prolonged heating or at higher acidities, confirmed our earlier finding that under the conditions used to volatilize the free iodine, no significant amount of the excess iodide undergoes oxidation or is lost by vaporization as hydrogen iodide.

#### REAGENTS

The distilled water used in preparing solutions of reagents was twice redistilled, first from alkaline permanganate and then from potassium hydroxide.

*Potassium iodide solutions.* The stock solution of potassium iodide had a concentration of 49.46  $\mu\text{g}$  I/ml, as determined by Winkler's method.<sup>1</sup> This solution was then diluted to yield (a) a series of solutions having concentrations varying from 0.10  $\mu\text{g}$  to 0.90  $\mu\text{g}$  I/0.2 ml (to be referred to as the potassium iodide test solutions), and (b) a solution with a concentration of 5.0  $\mu\text{g}$  I/0.2 ml (to be referred to as the potassium iodide reagent solution).

*Solution of bromine in 0.2 N  $\text{H}_2\text{SO}_4$ .* Liquid bromine containing a tracer quantity of  $^{131}\text{I}$  was extracted with 1 N NaOH solution and then with water until no radioactivity appeared in the aqueous extract (8). Bromine vapor expelled by a current of water-washed air from an aqueous solution of the purified bromine was introduced into 50 ml of 0.2 N  $\text{H}_2\text{SO}_4$  until the solution had a deep yellow color<sup>2</sup>.

*Tracer  $^{131}\text{I}$  solution.* A small volume of a highly diluted Oak Ridge  $^{131}\text{I}$  shipment was further diluted with 0.01 N KOH to give a solution with a radioactivity of 0.5 -1.0  $\mu\text{C}/\text{ml}$ .

Nitrogen used for aeration was passed through Fieser's solution (9), lead acetate solution, and distilled water.

#### PROCEDURE

To 0.2 ml of a potassium iodide test solution (corresponding to 0.10 - 0.90  $\mu\text{g}$  I depending on the solution used) in a 13  $\times$  100 mm test tube was added 0.1 ml of tracer  $^{131}\text{I}$  solution. After the radioactivity had been determined in a well-type sodium iodide scintillation counter, 0.2 ml of the bromine solution in 0.2 N  $\text{H}_2\text{SO}_4$  was introduced, and the mixture allowed to stand for three minutes. The

<sup>1</sup>Bromine vapor was used to oxidize iodide to iodate. The thiosulfate was standardized against potassium iodate.

<sup>2</sup>A solution which has stood several hours or longer may be preferable to a freshly prepared one, since freshly prepared bromine solutions in 0.01-0.02 N  $\text{H}_2\text{SO}_4$ , after being heated and aerated with nitrogen for five minutes, were found to be still capable of liberating  $\text{I}_2$  on addition of potassium iodide and more acid, whereas solutions which had stood for a day prior to heating and aeration failed to do so. On the other hand, a freshly prepared bromine solution in 0.5 N  $\text{H}_2\text{SO}_4$ , when similarly heated and aerated and then treated with potassium iodide solution containing a tracer quantity of  $^{131}\text{I}$ , lost no radioactivity on further heating and aeration.

tube was then inserted to within 1-2 cm of its top in boiling water, and a slow stream of purified moist nitrogen was bubbled into the solution through a fine capillary suspended in a glass cap which could be readily lowered over the top of the test tube. The cap was provided with a side-arm through which the radioactive vapor issuing from the test tube was aspirated into a filter flask containing a sodium hydroxide solution (Fig. 1). After three minutes the tube was removed from the bath, and the radioactivity of the solution was redetermined. Thereupon 0.2 ml of the potassium iodide reagent solution ( $= 5.0 \mu\text{g I}$ ) and 0.1 ml of 2.5 N  $\text{H}_2\text{SO}_4$  were added. The mixture was heated and aerated again for three minutes, and the residual radioactivity in the tube was then measured. The results of the 22 runs made by this procedure are given in Table I. Since, irrespective of the amount of iodine taken, only 92-94% of the  $\text{I}_2$  theoretically formed was volatilized in these runs, the correction factor 100/93 has been applied in calculating the iodine content of the highly radioactive  $^{131}\text{I}$  samples subsequently analyzed.

A verified explanation can not be given for the failure to obtain volatilization of the theoretical amount of iodine. Two series of runs were carried out in which iodine, formed in acidic solution from known amounts of iodate and iodide, was volatilized under conditions similar to those of the runs listed in Table I. In the first series, mixtures of  $^{131}\text{I}$ -labeled iodate and inactive iodide were used; in the second series, the iodide was labeled and the iodate was inactive. As

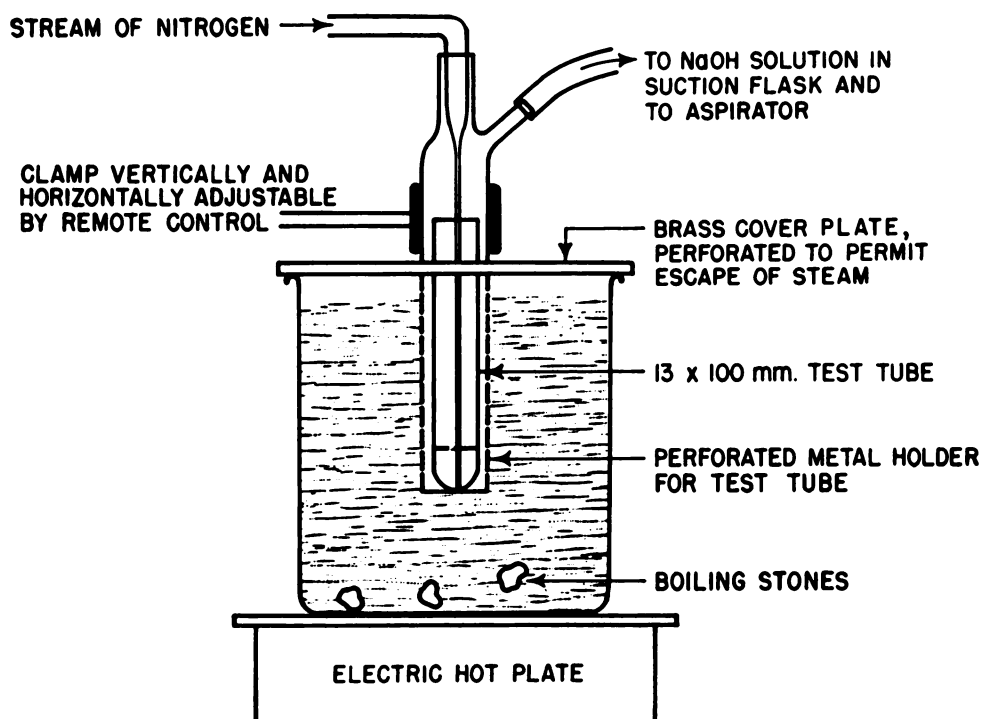


Fig. 1. Heating and aeration apparatus.

shown in Table II, the amount of  $I_2$  which could be volatilized was the same in both series — 92-94% of that theoretically formed. The fact that this percentage was not significantly altered by the addition of bromide ion demonstrates that the incomplete volatilization of the theoretical amount of  $I_2$  in the runs in Table I cannot be attributed to a reduction of some of the iodate ion by bromide ion on the addition of more sulfuric acid.

The  $^{131}I$ -labeled iodate used in the runs recorded in Table II was prepared by taking advantage of the fact that iodate can readily be eluted from a column

TABLE I  
IODIDE DETERMINATIONS AT TRACER LEVEL OF  $^{131}I$ -ACTIVITY

Volume of each solution prior to the first heating and  $N_2$ -aeration period: 0.5 ml (= 0.2 ml KI test solution + 0.1 ml of tracer  $^{131}I$  solution + 0.2 ml of solution of  $Br_2$  in 0.2 N  $H_2SO_4$ ). First heating and aeration period: 3 minutes. Subsequent additions: 0.2 ml KI reagent solution + 0.1 ml 2.5 N  $H_2SO_4$ . Second heating and aeration period: 3 minutes.

<i>Amt of <math>I^-</math> taken as KI</i> $\mu g$	<i>Amt of <math>I^-</math> found</i> $\mu g$	<i>Ave amt of <math>I^-</math> found</i> $\mu g$	<i>Ave ratio: <math>I^-</math> found <math>I^-</math> taken</i>
0.1	0.087, 0.10, 0.095 0.090, 0.0935	0.093	0.93
0.2	0.185, 0.18, 0.19	0.185	0.93
0.35	0.33, 0.33	0.33	0.94
0.5	0.46, 0.47	0.46	0.92
0.6	0.55, 0.56	0.555	0.925
0.7	0.64, 0.66, 0.64	0.65	0.93
0.8	0.73, 0.77	0.75	0.94
0.9	0.84, 0.82, 0.81	0.83	0.925
		Average	0.93

The loss of radioactivity during the first heating period was 1.2 — 4.9 per cent (average: 3.1 per cent). Data on a typical run are given below:

Iodide in analytical sample:	0.35 $\mu g$
Radioactivity measurements:	
Initial:	37757 counts/min
After volatilization of excess $Br_2$ :	36566
After volatilization of $I_2$ formed:	23436

Iodine found:

$$\left(5.0 + 0.35 \times \frac{36566}{37757}\right) \times \left(1 - \frac{23436}{36566}\right) \times \frac{37757}{36566} \times \frac{1}{6} = 0.33 \mu g$$

of Dowex 1-X4 resin by saline, while iodide is retained. To 0.4 ml of potassium iodide reagent solution ( $= 10 \mu\text{g I}$ ) was added 0.2 ml of  $^{131}\text{I}$  tracer solution. The iodide was then converted into iodate by the addition of 0.4 ml of a solution of bromine in 0.3 N  $\text{H}_2\text{SO}_4$ . The excess bromine was volatilized by heating the mixture in boiling water and simultaneously aerating it with nitrogen for three minutes. The solution was then passed through Dowex 1-X4 resin contained in a 2 ml syringe. The column was rinsed with distilled water until no longer acidic, and the iodate eluted with 15 ml of saline. The recovery of the radioactivity in the eluate was 98.5 per cent.

TABLE II

EFFICIENCY OF VOLATILIZATION OF  $\text{I}_2$  FORMED FROM  $\text{HIO}_3$  AND  $\text{HI}$  IN THE ABSENCE AND IN THE PRESENCE OF  $\text{Br}^-$ . CONDITIONS OF VOLATILIZATION SIMILAR TO THOSE OF THE RUNS IN TABLE I.

<i>Series 1</i>					
<i>Initial Vol of Solution (ml)</i>	<i>Amt of <math>^{127, 131}\text{I}</math> Taken as <math>\text{KIO}_3</math> (<math>\mu\text{g}</math>)</i>	<i>Amt of I Taken as KI (<math>\mu\text{g}</math>)</i>	<i>Initial Acidity (<math>\text{H}_2\text{SO}_4</math>)</i>	<i><math>\text{I}_2</math> Volatilized <math>\times 0.167</math> (<math>\mu\text{g}</math>)</i>	<i>Per Cent of Theoretically Formed <math>\text{I}_2</math> Volatilized</i>
0.6	0.334 <sup>a</sup>	5.0	0.4 N	0.307	0.922
0.5	0.454 <sup>a</sup>	5.0	0.5 N	0.409	0.924

*Series 2*

Initial volume of solution in each run: 0.5 ml. Initial acidity:  $\approx 0.5$  N in  $\text{H}_2\text{SO}_4$ .

<i>Amt of I Taken as <math>\text{KIO}_3</math> (<math>\mu\text{g}</math>)</i>	<i>Amt of I Taken as <math>^{127, 131}\text{KI}</math> (<math>\mu\text{g}</math>)</i>	<i>Amt of <math>\text{Br}^-</math> Added (<math>\mu\text{g}</math>)</i>	<i><math>\text{I}_2</math> Volatilized <math>\times 0.167</math> (<math>\mu\text{g}</math>)</i>	<i>Per Cent of Theoretically Formed <math>\text{I}_2</math> Volatilized</i>
0.25	4.95	0	0.235	0.94
0.25	4.95	5	0.236	0.94
0.25	4.95	20	0.232	0.93
0.50	4.95	0	0.470	0.94
0.50	4.95	20	0.475	0.95
0.50 <sup>b</sup>	4.95	0	0.470	0.94

a. Amounts calculated on the basis of the radioactivities of the samples of eluate taken.

b. In this run the iodate was treated with 0.2 ml of bromine solution in 0.2 N  $\text{H}_2\text{SO}_4$ , and the subsequent procedure was the same as that of the iodide determinations listed in Table I.

DETERMINATION OF THE TOTAL IODINE IN Na<sup>131</sup>I SAMPLES OF  
HIGH SPECIFIC ACTIVITY

The procedure followed in these determinations was similar to that developed at a tracer level of <sup>131</sup>I activity except that customary precautions were taken to minimize the danger of radiation exposure. The volume of the analytical sample was increased by the addition of distilled water so that the total volume, after the addition of 0.2 ml of the bromine solution in 0.2 N H<sub>2</sub>SO<sub>4</sub>, was approximately 0.5 ml.<sup>1</sup> In column 5 of Table III are recorded the results of the analysis of nine <sup>131</sup>I shipments of high specific activity, eight of which were prepared by fission of <sup>235</sup>U (Oak Ridge National Laboratory), and one by thermal neutron activation of natural tellurium (Union Carbide Nuclear Company, Tuxedo, New York).

TABLE III

TOTAL IODINE FOUND IN Na<sup>131</sup>I PREPARATIONS OF HIGH SPECIFIC ACTIVITY.  
<sup>131</sup>I-IODINATION EFFICIENCIES OBTAINED WITH THESE PREPARATIONS ON IODI-  
NATION OF 4 mg  $\gamma$ -GLOBULIN WITH 0.1  $\mu$ MOLE OF ICl.

Ship- ment	Activity (Date of Shipment) of			Iodine Found $\mu$ g/100 mC (Date of Shipment)	<sup>131</sup> I-Iodination Efficiency	
	Prepara- tion mC/ml	Iodination Sample mC	Analytical Sample mC		Found Percentage	Calc Percentage
1	449	284	35.7	2.2	45.9	44.5
2	246	269	25.1	4.0	44.4	36.3 <sup>a</sup>
3	191	248	18.0	3.0	44.6	44.8
4	100	93	9.2	5.6	46.7	46.4
5	549	264	12.7	3.3	39.6	38.8
5A <sup>b</sup>	90	124	1.08 - 4.32	18.3 $\pm$ 2.5	23.4	23.7 $\pm$ 2.1
6	303	332	30.9	2.3	43.7	41.4
7	189	47	10.6	2.5	59.5	60.1
8 <sup>c</sup>	192	288	6.9	12.4	15.3	17.2

a. No check analysis was carried out.

b. Shipment 5A came from the same Oak Ridge Laboratory batch as shipment 5 but was sent 21 days later. It was obtained specifically to include in our runs an <sup>131</sup>I sample with a high iodide content per 100 mC. Four analyses were carried out on this shipment.

c. Shipment 8 with its high iodide content per 100 mC was sent to us by mistake in place of a more recently processed preparation.

<sup>1</sup>If the <sup>131</sup>I shipment, prior to its analysis and use in the iodination of protein, was diluted by the addition of a basic buffer solution, the analytical sample may have required more than 0.2 ml of the bromine solution to produce an acid reaction.



As has been pointed out, when  $^{131}\text{I}$ -labeled proteins are prepared by the use of iodine monochloride into which  $^{131}\text{I}$  has been introduced by isotopic exchange, the efficiency of  $^{131}\text{I}$  incorporation in the protein decreases with increasing iodide content of the  $^{131}\text{I}$  sample. It was of interest to determine how closely the  $^{131}\text{I}$ -iodination efficiencies, achieved in our laboratory with the  $^{131}\text{I}$  preparations listed in Table III, could have been predicted on the basis of our analyses of their iodine content. The iodination procedure followed was that described in another report from this laboratory (1). The  $^{131}\text{I}$  preparation, previously treated with a small amount of catalase to destroy hydrogen peroxide, was added to a borate-buffered solution of 4 mg of purified rabbit antibody to human fibrinogen, and a sodium chloride solution containing 0.1  $\mu\text{mole}$  of iodine monochloride was vigorously jetted into the mixture. After addition of some protective protein, the solution was passed through a Dowex 1-X4 resin column, which was then rinsed with more of the protective protein solution. The results are given in column 6 of Table III, where the term  $^{131}\text{I}$ -iodination efficiency refers to the percentage of the total radioactivity found in the effluent from the resin column. This is somewhat less than that actually introduced into the protein, since a small per cent of the iodinated protein is retained on the resin.

If protein is iodinated by this procedure in the presence of only a tracer quantity of  $^{131}\text{I}$ , the  $^{131}\text{I}$ -iodination efficiency, as defined above, represents also the amount of iodine attached to the protein in the effluent, expressed as a percentage of the amount of iodine in the iodine monochloride used. This tracer level efficiency was about 65 per cent, when normal rabbit gamma globulin (4 mg) was iodinated with iodine monochloride (0.1  $\mu\text{mole}$ ) in the way in which the high level iodinations in Table III were performed. The calculated  $^{131}\text{I}$ -iodination efficiencies in column 7 of Table III are based on this value.

#### SUMMARY

A method, based on radioactivity measurements, is described for determining total iodine in highly radioactive iodide samples. In  $^{131}\text{I}$ -labeling of protein or other substances, by procedures in which  $^{131}\text{I}$  as iodide is oxidized to positive iodine, this analysis permits calculation of the amount of iodine incorporated in the labeled product. When the iodinating agent is iodine monochloride, into which  $^{131}\text{I}$  is introduced by isotopic exchange, this analysis makes it possible to predict the fraction of the  $^{131}\text{I}$  used that will be in the iodinated substance.

#### ACKNOWLEDGMENT

We wish to express our appreciation to Miss Mary Jane Izzo for her assistance in carrying out the iodinations of human fibrinogen antibody preparations with  $^{131}\text{I}$  shipments 2-6, and 8, and to Dr. Irving Spar and Miss Ruth Goodland for providing us with their data on the iodination with  $^{131}\text{I}$  shipment 7.

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