A SIMPLE PROCEDURE FOR HIGH-EFFICIENCY RADIOIODINATION OF PROTEINS

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The iodine monochloride method for radioiodination of proteins described by McFarlane in 1958 used radioiodine as the source of label (*1*). Helmkamp *et al* extended this procedure to use sodium radioiodide and to work at high activity levels; however, the efficiency of radioiodination depended on a critical step that required one to rapidly mix $Na^{131}I$ with ICl and jet the mixture into protein (*2*). This insistence on rapidity was still apparent in the improved procedures of McFarlane (*3,4*), of Bale *et al* (*5–7*), of Gill *et al* (*8*), and of Hügli (*9*).

In this paper further modifications are presented. The need for a critical step that requires rapid manipulation has been eliminated. As a means to separating unreacted radioiodide, ion exchange has been compared with dialysis. The removal of H_2O_2, a contaminant of radioiodide solutions that reduces the efficiency of radioiodination at high activity levels (*2*), has been studied. A preliminary report has been made (*10*).

Very recently, McConahey and Dixon (*11*) described a convenient modification of the chloramine-T method of radioiodination of Greenwood, Hunter and Glover (*12*). This method appears to be superior for radioiodinations of less than 1 mg protein. Comparative studies have not yet been made to determine which method is superior for radioiodinations above 1 mg protein nor to evaluate the merits of other procedures (*13–15*).

METHODS AND MATERIALS

Reagents. Stock glycine-buffered iodine monochloride (ICl) 0.01 *N*, *p*H 2.0, was prepared as follows. Potassium iodide (1.110 gm), KIO_3 (0.7134 gm) and 84.2 gm NaCl were dissolved *successively* in approximately 600 ml H_2O. This solution was stirred vigorously and continuously in a fume hood while 10.0 ml of concentrated HCl was added quickly. Five minutes later sufficient 1.0 *N* NaOH (about 99 ml) was added slowly to raise the *p*H up to, but not beyond, 2.0. The solution was boiled vigorously for 1 hr in an open beaker and then cooled. The evaporated water was restituted approximately, and 25 ml of 0.2 M glycine-HCl buffer*, *p*H 2.0, was added. The ICl content of the solution was determined by titrating 5.0 ml against† 0.02 *N* $Na_2S_2O_3$ in the presence of 0.5 ml 10% KI with two drops of 1% soluble starch added as indicator near the end-point. The ICl solution was diluted until 5.0 ml of the $Na_2S_2O_3$ solution was required for the titration. This 0.01 *N* ICl solution was stable when stored frozen in 3 ml aliquots. Thawed aliquots were discarded after 8 hr at 3°. Larger volumes of 0.01 *N* ICl, such as 100 ml, showed no deterioration up to 10 days at 3°. The dilutions of stock ICl required for radioiodinations were prepared in 0.02 M glycine-HCl buffer, stored at 3° and used within 3 hr.

The borate buffer (*2*), *p*H 7.8, was prepared by dissolving 18.7 gm NaCl and 24.74 gm H_3BO_3 in 90 ml 1.0 *N* NaOH and 500 ml water; a slight adjustment of *p*H to 7.8 was made, and water to 1,000 ml was added. The fixing solution F contained 95 ml water, 4.0 ml of 0.1 M $Na_2S_2O_3$ and 0.5 ml of 10% KI. The carrier solution C for determining protein-bound radioiodine contained 95 ml water, 2.0 ml 30% bovine serum albumin (Dade Reagents, Inc.) and 3.0 ml 10% KI. $Na^{131}I$ and $Na^{125}I$ were carrier- and reductant-free (Isoserve, Inc.).

To remove unreacted ^{131}I, we used Dowex I-X4 resin in the chloride form, 50–100 mesh (No. 1903, J. T. Baker Chemical Co.). The resin was suspended in water and layered 7 cm above a wad of glass wool in a 0.7-cm i.d. chromatography tube 27 cm long. The resin was washed rapidly with 30 ml water and kept wet.

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* Made by dissolving 7.507 gm glycine in 101 ml 1.00 *N* HCl and adding H_2O to 500 ml.

† Prepared by diluting 0.1 *N* $Na_2S_2O_3$ (12.41 gm $Na_2S_2O_3$, $5H_2O$/500 ml).

Trace iodination procedure for 1–10 mg protein. Except when interfering impurities were present (see results), initial dialysis of the protein solutions was unnecessary. The solutions were prepared in water or in isotonic saline, *p*H 7–8. When necessary, solutions were clarified by centrifugation at 5° for 30 min at 4,000 G.

The protein solution was placed in an open Erlenmeyer flask in a fume hood. Then 1.5 ml borate buffer was added. This was followed by $Na^{131}I$ or $Na^{125}I$ solution*. Thorough manual mixing was begun and maintained while 0.75 ml ICl solution of calculated strength was added dropwise from a pipette; this procedure required 15–30 sec. The reaction was stopped by dropwise addition of 0.3 ml fixing solution F.

If substantial removal of uncombined radioiodide was desired, the mixture was passed through a 1-X4 resin column at 12 drops/min and rinsed through by two additions of 4 ml water or isotonic saline. If less than 3 mg protein was present, dialysis against hemagglutination buffer (Difco), with stirring for 6 hr at 3° and buffer changes every 2 hr, gave somewhat higher recoveries.

The amount of ICl added depended on the desired ratio of moles ICl/mole protein. For iodination of 1 mg of a γG globulin with an assumed molecular weight of 160,000, a ratio of 4 moles ICl/mole protein required 0.75 ml of a 1:300 dilution of stock 0.01 *N* ICl, prepared in a 1:10 dilution of the glycine-HCl buffer. For proteins with different molecular weight, for other mole ratios of ICl/protein or for different amounts of protein, the dilution of stock ICl was varied appropriately, but the volume of the ICl dilution added to the protein was kept constant.

Iodination procedure for above 10 mg protein. To offset the acidity of increased amounts of ICl, the volume of borate added to the protein solution was increased by an amount equal to the volume of 0.001 *N* ICl to be used. The volume of fixing solution F was also increased by the same amount. For instance, if 0.8 ml of 0.0005 *N* ICl were to be added, this corresponded to 0.4 ml of 0.001 *N* ICl; therefore 0.4 ml additional volume of borate buffer and of solution F was used.

Radioiodinations at high activity levels. Radioiodinations at high activity levels were identical with three exceptions: H_2O_2 present in radioiodide solutions usually required inactivation; protective protein was usually added after completion of the iodination reaction, and all operations were done behind lead bricks in a hood.

Whether inactivation of H_2O_2 was necessary depended on the desired conditions of radioiodination. It was more likely to be necessary when the activity of the radioiodide solution was high, the time interval between production in the reactor and delivery to the laboratory was long, and both the quantity of protein to be iodinated and the ratio of moles ICl/mole protein were low. The data in Table 4 and Fig. 4 are adequate to decide whether inactivation of H_2O_2 is likely to be necessary.

H_2O_2 was destroyed by heating for 2 hr at 90° in alkaline solution (*p*H 10–11). For this purpose, 0.15 ml of 0.10 *N* NaOH was added to each 10 ml of radioiodide solution obtained from Isoserve, Inc. Because of evaporation, an aliquot of the radioiodide solution (rather than the entire shipment) was placed in a Pyrex tube inside a lead isotope container, which was partially filled with water. This water was kept close to 90° by standing the lead container in a large Petrie dish filled with boiling water. Extreme care was taken to insure that neither the radioiodide solution nor the water in the Petrie dish dried out during the heating. After 2 hr, the Pyrex tube was placed in ice water, neutralized† and used for radioiodination 3 min later.

After completion of radioiodination, a suitable protective protein could be added to retard the destruction of biological activity (*2*). This could be important when the protein content of the solution was low and when the specific activity of the solution exceeded roughly 0.03 mc/ml. The protein involved and its intended use were of course very relevant (*2*).

Work with 10–15-mc aliquots of ^{131}I was done on a base of 1-in.-thick lead bricks. Several 2-in.-thick lead bricks and 2 lead-glass bricks gave lateral protection. Only the hands and arms were not protected.

Determination of protein-bound radioiodide. A sample (0.5 ml) of radioiodinated protein solution, or a suitable dilution of it‡, was pipetted into counting tube A, followed by 2.5 ml H_2O, and mixed. Another sample (1.0 ml) was mixed with 1.0-ml carrier solution C (see above), followed by 4.0 ml 15% trichloracetic acid. After thorough mixing and centrifugation for 10 min at 700 G, 3.0 ml of supernatant was transferred to counting tube B. The per-

* To couple a constant proportion of radioactive and nonradioactive iodine to protein, the $Na^{131}I$ or $Na^{125}I$ solution should be added to the ICl instead of to the protein solution. This is not recommended for routine use.

† NaOH for inactivation of H_2O_2 was neutralized by adding 0.15 ml 0.01 *N* HCl/ml radioiodide solution. ^{131}I fresh from the reactor contains approximately 500 mc/ml 0.1 *N* NaOH and is diluted with distilled water before shipment to the laboratory. NaOH present is also neutralized by adding a corresponding amount of 0.01 *N* HCl.

‡ For iodinations with 10 mc ^{131}I, a 1:10,000 dilution in water that contained 1 ml carrier solution C/100 ml was used to avoid saturating the scintillation counter.

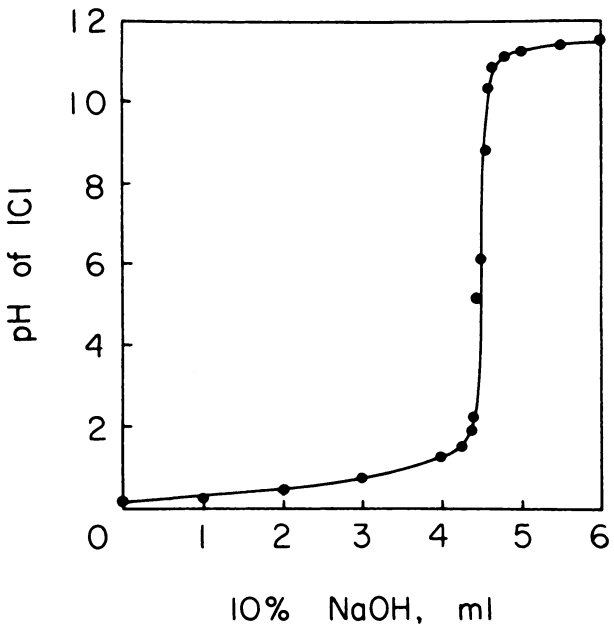

FIG. 1. Neutralization of 10 ml of 0.02 M ICl, pH 0.3, as used by Helmkamp et al (2), by 10% NaOH.

cent of free radioiodide was 100 (counts B/counts A). The percent of protein-bound radioiodide was 100 minus the percent of free radioiodide.

RESULTS

Trace iodinations. When a solution of ICl (about *p*H 0.3), prepared as described by Helmkamp *et al* (*2*), is neutralized with NaOH, a sharp titration curve is obtained (Fig. 1). Neutralization to physiologic *p*H requires a relatively large amount of alkali or borate buffer. This requirement is sharply reduced when the *p*H of the ICl solution is raised to 2.0 (*3,4*) although the partially neutralized reagent is less stable (Fig. 2).

When an ICl solution is suddenly raised in *p*H from 2.0 to 7.8, ICl is destroyed very rapidly (Fig. 3). The characteristic yellow color of ICl changes to brown, presumably because molecular iodine is liberated which gradually escapes into the atmosphere. This finding (Fig. 3) explains why in previous procedures (*2–9*) ICl had to be added rapidly to the protein solution once the ICl had been neutralized.

In this study, we eliminated the need for rapid addition by placing the *p*H 7.8 borate buffer in the protein solution and by adding ICl at *p*H 2.0, at which it is sufficiently stable. This change raised the efficiency of radioiodination and minimized the escape of radioiodine into the atmosphere. It was also preferable to mix the radioiodide solution with the protein before adding ICl.

We investigated the effect of various experimental conditions on the efficiency of radioiodination of 10 mg human gamma globulin (Table 1). The efficiency of labeling increased as more ICl was used (flasks 1–5). Magnetic rather than manual stirring was permissible (flask 6). No difference was observed when the reaction was performed in an open rather than in a closed Erlenmeyer flask (flask 7). Adding $Na^{131}I$ to the protein rather than to the ICl solution was preferable (flask 8). The $Na_2S_2O_3$ fixing solution F could be added immediately after the ICl solution (flask 9), which indicated that the iodination reaction was fast.

With quantities of bovine gamma globulin that varied from 1 to 1,000 mg, satisfactory efficiencies of iodination were obtained (Table 2). In another experiment with 1–10 mg portions of bovine gamma globulin, a reduction in reaction volume from 10 to 3 ml increased the efficiency of radioiodination only about 5%. Experiments on the removal of free ^{131}I from radioiodinated protein showed that either passage through a column of Dowex 1-X4 resin or dialysis was very effective; dialysis gave slightly higher recoveries of protein-bound ^{131}I for amounts of protein under about 3 mg (Table 3).

Various proteins can be iodinated with this technique (Table 4). Extended dialysis resulted in improved iodination efficiency for human serum albumin, human gamma globulin and trypsin, but no improvement for bovine serum albumin. These results suggest the presence of inhibitory contaminants derived from the procedures used to purify these proteins.

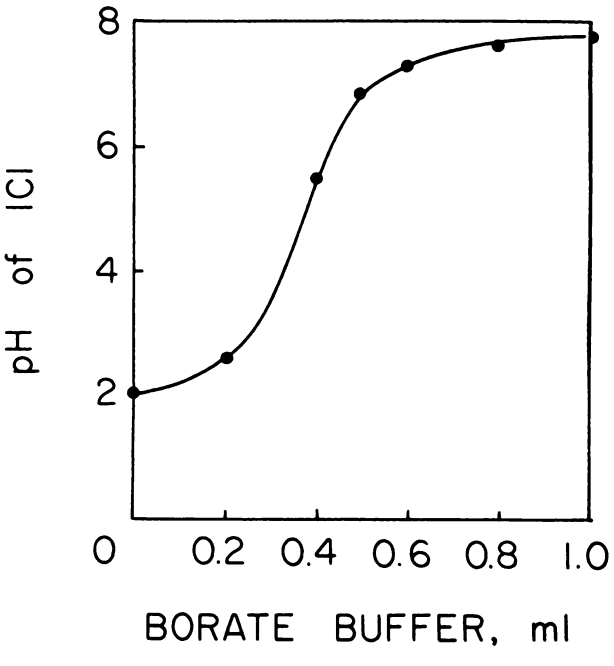

FIG. 2. Neutralization of 1.0 ml of 0.01 M ICl, pH 2.0, as used in this study, by 0.2 M borate buffer, pH 7.8.

High-activity iodinations. H_2O_2 strongly inhibits iodination. When 10 mg rather than 1 mg bovine gamma globulin was iodinated, 10 times as much ICl was used and 10-times-higher quantities of H_2O_2 were needed to give equivalent inhibition (Fig. 4). Thus, the inhibition caused by H_2O_2 is completely dependent on the quantity of ICl (and, therefore, also on the quantity of protein and the ratio moles ICl/mole protein) used in the iodination.

The H_2O_2 content of 10–15-mc aliquots of radioiodide solutions was determined by potentiometric titration (*11*) against 0.001 M $K_3Fe(CN)_6$ with platinum and calomel electrodes (Table 5). A stepwise increase in μg H_2O_2/mc ^{131}I during storage of the isotope at high concentration has long been suspected but not previously substantiated (Table 5); therefore the isotope should be delivered as soon as possible after production in the reactor. The limited data available suggested that this is not true for ^{125}I samples delivered up to 60 days after production because variability between different commercial lots is a more important factor.

TABLE 1. EFFECT OF EXPERIMENTAL CONDITIONS ON EFFICIENCY OF RADIOIODINATION*

Flask number	Description of experiment	Moles ICl/mole γ-globulin	^{131}I incorporated into protein (%)
1	Variation in ICl	1	63.7
2	Variation in ICl	2	74.7
3	Variation in ICl	4	85.8
4	Variation in ICl	8	90.8
5	Variation in ICl	16	92.3
6	Magnetic stirring	4	88.1
7	Reaction in open Erlenmeyer flask	4	87.8
8	$Na^{131}I$ and ICl added together	4	76.1
9	$Na_2S_2O_3$ added at once after ICl	4	88.0

* Radioiodination of 10-mg human gamma globulin. Mean results of two experiments.

TABLE 2. EFFICIENCY OF TRACE IODINATION*

Bovine γ-globulin (mg)	^{131}I incorporated into protein (%)
1	72.3
2	77.8
4	82.6
10	86.2
100	88.9
1,000	89.2

* At 4 moles ICl/mole gamma globulin. Mean results of two determinations.

Using sodium sulfite to destroy H_2O_2 in ^{131}I solutions (*2*) has three disadvantages. First, Na_2SO_2 inhibits iodinations and must be completely destroyed. Second, and most important, atmospheric contamination with ^{131}I is negligible when the prescribed rate of air flow (*2*) is maintained through the solution at 100°; however, a slight increase in flow rate leads to a *serious loss* of ^{131}I into the atmosphere. Finally, highly radioactive solutions should be manipulated as little as possible. Use of catalase to destroy H_2O_2 (*7*) requires one to add an extraneous protein, although in minute amount.

The preferred method for inactivation of H_2O_2 in this study was heat treatment at alkaline *p*H (Fig. 5).* Unfortunately, the reaction did not go entirely to completion, even after extended time periods. The

* The extinction coefficient of H_2O_2 in neutral solution was 110 at 220 mμ.

TABLE 3. REMOVAL OF FREE ^{131}I FROM RADIOIODINATED BOVINE GAMMA GLOBULIN (BGG)

Radioiodinated BGG (mg)	Content of free ^{131}I (%)	Recovery of protein-bound ^{131}I after		Free ^{131}I after	
		Ion exchange* (%)	Dialysis† (%)	Ion exchange* (%)	Dialysis† (%)
0.7	25.9	60.8	75.4	0.9	0.8
1.7	19.9	68.7	90.0	0.8	0.9
3.3	17.4	79.3	91.1‡	0.9‡	1.1‡
8.7	15.1	83.4	87.6‡	1.0	1.0‡
87	13.2	89.8	—	1.2	—
892	10.8	98.6	—	1.5	—

* 1-X4 column 0.7 cm i.d., 7 cm long.
† Dialysis against 40 volumes hemagglutination buffer (Difco) with stirring for 6 hr at 3° and buffer changes every 2 hr.
‡ Single values. Other results are mean of 2 values.

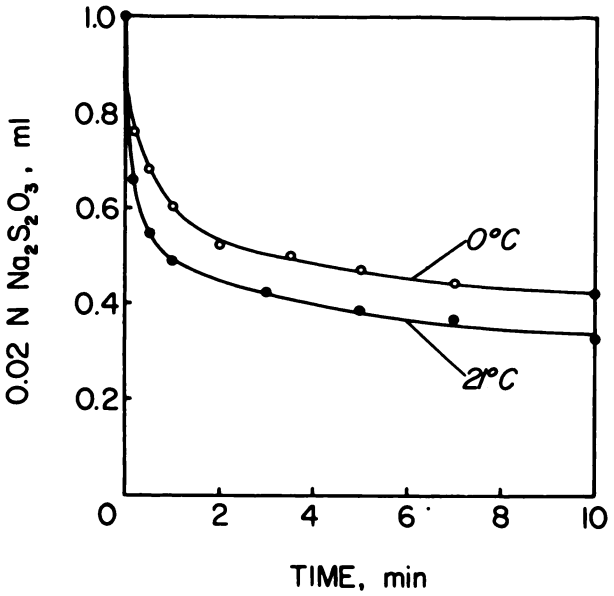

FIG. 3. Destruction of 2 ml 0.02 N ICl when brought from low pH to pH 7.75 by sudden addition of 0.2 M borate buffer, pH 7.8, at time zero. Ordinate: 0.02 N $Na_2S_2O_3$ required to titrate ICl plus free I_2 present after addition of borate buffer.

TABLE 4. RADIOIODINATION OF VARIOUS PROTEINS*

Protein	Moles ICl added/mole protein	^{131}I incorporated into 10-mg protein: Before dialysis (%)	^{131}I incorporated into 10-mg protein: After dialysis (%)
Trypsin, cryst	0.6	6.7	11.2
Pepsin, cryst	0.9	67.4	91.6
Soy bean trypsin inhibitor, cryst	0.6	77.3	—
Human serum albumin, cryst	1.7	54.7	77.1
Human α-globulin	5.0	49.3	70.9
Bovine serum albumin, cryst	1.7	29.7	28.2
Bovine γ-globulin	4.0	90.0	93.1

* Mean results of two experiments.

reaction was catalyzed by colloidal MnO_2, but the disadvantages of the addition of an extraneous chemical outweighed its slight benefit.

Experiments on the iodinating 1-mg protein with 10 mc ^{131}I indicated that alkaline inactivation for 2 hr at 90° was optimal. Nevertheless, results were only moderately successful for iodinations of less than 3 mg protein (Table 6). No difficulties were encountered at these high-activity levels in removing free ^{131}I by passing it through Dowex 1-X4 resin.

The escape of ^{131}I into the atmosphere during iodinations with 10 mc ^{131}I was monitored by using a completely enclosed system. A gentle air stream flushed the system continuously and was bubbled through a wash bottle that contained 0.04 *N* $Na_2S_2O_3$. In three experiments, the total quantity of ^{131}I trapped by the $Na_2S_2O_3$ solution did not exceed 0.001% of the ^{131}I added for the radioiodination.

In vivo elimination of radiolabeled γG globulin. Marked differences in biological activity and rate of elimination have been observed for proteins iodinated at different levels of atoms iodine/mole protein (*8,11,13,17,18*). The main purpose of our *in vivo* experiments was to test whether equilibration

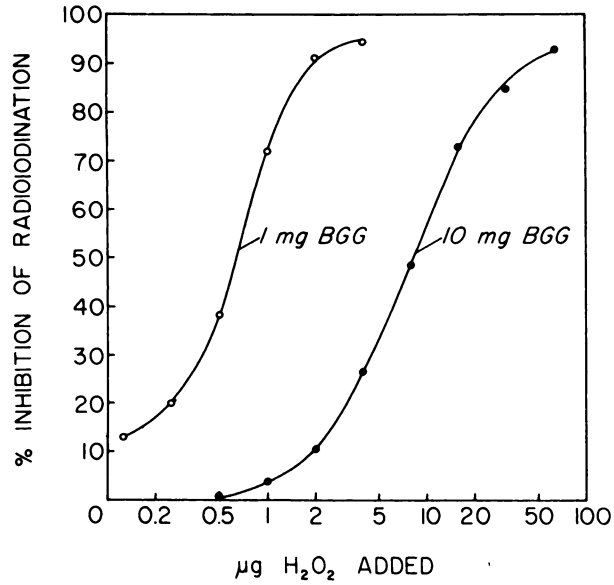

FIG. 4. H_2O_2 strongly inhibits iodination. Figure shows inhibition by added H_2O_2 of radioiodination of bovine gamma globulin (BGG) at 4 moles ICl/mole BGG.

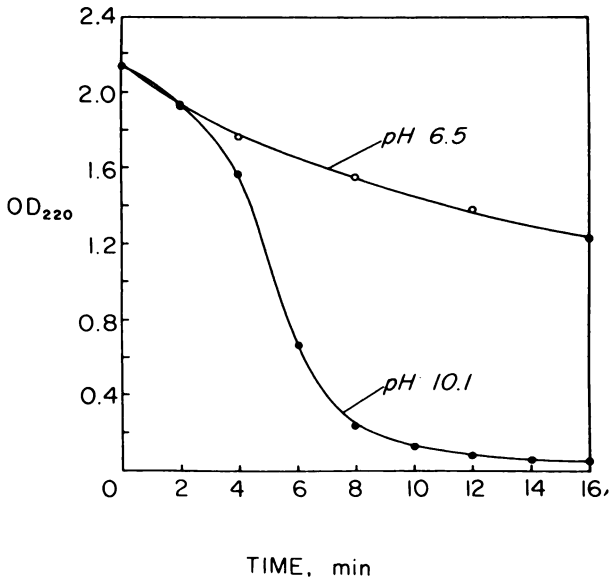

FIG. 5. Destruction of 0.075% H_2O_2 in aqueous solution at 90°. Concentration of H_2O_2 is proportional to its optical density (OD) at 220 mμ.

TABLE 5. H_2O_2 CONTENT OF HIGHLY ACTIVE RADIOIODIDE SOLUTIONS

Sample	Radio-iodide	Concentration as		Time interval		H_2O_2 content	
		Produced in reactor (mc/ml)	Delivered to laboratory (mc/ml)	Production-delivery (days)	Delivery-testing (days)	On receipt (μg/ml)	17 days later (μg/ml)
1	^{131}I	506	5.3	1	1	0.74	0.50
2	^{131}I	591	2.8	8	1	1.10	0.96
3	^{131}I	475	1.4	15	1	2.2	1.74
4	^{125}I	104	1.0	48	0	0.36	—

of radioactive and nonradioactive iodine was essential before one added the iodinating agent (ICl) to protein, or whether the radioiodide solution could be added to the protein rather than to the ICl solution. The latter procedure gave a slightly higher iodination efficiency, but did not assure that a constant proportion of radioactive-to-nonradioactive iodine was coupled to protein. For proteins equally radiolabeled by these two different procedures, any discrepancies would be revealed by more rapid elimination of proteins more heavily labeled with nonradioactive iodine. A second purpose was to compare the rate of elimination of γG globulin radiolabeled by the "jet" (*2,5–7*) and by the present procedure. Finally, the question of conformity with previous data obtained *in vivo* would be answered.

Rabbit γG globulin was prepared by modifying a batch adsorption method (*19*), using DEAE-cellulose equilibrated with 0.01 M phosphate buffer of *p*H 8.0. In a single iodination experiment 90 μc $Na^{131}I$ were mixed with an amount of ICl calculated to give a desired level of atoms iodine/mole globulin while 15 μc $Na^{125}I$ were mixed with the globulin solution. Then iodination was accomplished by pipetting the ICl-$Na^{131}I$ mixture into the protein-$Na^{125}I$ mixture so that 10 mg globulin was simultaneously iodinated with radioiodide equilibrated ($Na^{131}I$) and not equilibrated ($Na^{125}I$) with the nonradioactive iodination reagent (ICl). When 0.3, 2.5, 3, 5, 8 and 10 moles ICl/mole globulin were used for the radioiodination, 0.12, 2.0, 2.6 4.6, 7.2 and 9.2 atoms iodine/mole globulin, respectively, were actually incorporated. Except at the lowest level of ICl where the incorporation of $Na^{131}I$ was 21% lower than that of $Na^{125}I$, the incorporations of $Na^{131}I$ and $Na^{125}I$ agreed within 0.5%. Identical procedures were used in all iodinations except at 2.5 moles ICl/mole globulin when the iodination with $Na^{131}I$ followed the "jet" procedure (*2,6–8*) and was done with a different solution of globulin than the iodination

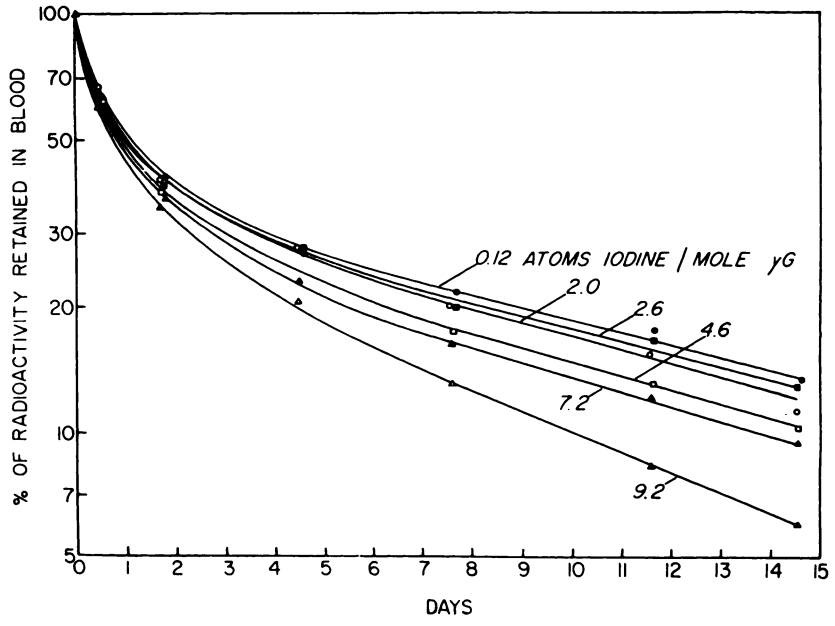

FIG. 6. Elimination of rabbit γG globulin, labeled with different levels of atoms iodine/mole γG globulin, from blood of rabbits.

TABLE 6. HIGH-ACTIVITY RADIOIODINATION OF BOVINE GAMMA GLOBULIN (BGG)*

BGG (mg)	Efficiency of iodination†		After passage through 1-X4 column	
	Before (%)	After (%)	Recovery of BGG-^{131}I (%)	Free ^{131}I (%)
1	6.2	40.4	87.8	4.2
3	30.6	70.2	86.4	1.5
10	67.2	88.6	87.3	1.6

* With 10-mc ^{131}I at 4 moles ICl/mole BGG.
† Before and after inactivation of H_2O_2.

with Na^{125}I; the two protein solutions were then mixed.

The radioiodinated globulins were dialyzed against buffered saline to remove all but 2% of free radioiodide, and each batch was injected intravenously into 2 or 3 rabbits. Samples of 1.5 to 2 ml heparinized blood were withdrawn 21 min after injection (time zero) and at suitable intervals thereafter (Fig. 6). Counts of ^{131}I and ^{125}I were made on duplicate samples of 0.5 ml blood, expressed as a percent of the counts for samples drawn at time zero and averaged for each group of rabbits.

The differences in the percent of ^{131}I and ^{125}I that remained in blood at the time of each sample was computed for each group of rabbits. The sums of these differences for the groups of rabbits injected with 0.12, 2.0, 4.6, 7.2 and 9.2 atoms iodine/mole globulin were, respectively, −5.7%, −2.8%, +4.2%, +3.0%, +1.3% and −0.8%. These results show no significant differences in the rate of elimination of γG globulin radiolabeled by any of the methods compared.

The rate of elimination of the radiolabeled globulin (Fig. 6) was in good agreement with previous results (*18*).

DISCUSSION

Because the present method is primarily intended for radioiodination of antibodies, we should review the ill effects of iodination on their biologic function and metabolism. Johnson, Day and Pressman have reported that for antibodies that require only one site for activity (such as localizing, binding or hemolytic antibodies), iodination below 2 atoms/molecule does not affect antibody activity; localizing activity begins to decrease when the number of iodine atoms increases beyond 2.5 per molecule (*17*). Gill, Papermaster and Mowbray (*8*) found no difference in the rate of elimination of synthetic polypeptides from the serum of rabbits, whether they are radioiodinated by the McFarlane or the Talmadge (*13*) method. The modification of the chloramine-T method described by McConahey and Dixon has the fundamental advantage that no carrier iodine is needed and therefore fractional numbers of iodine atoms/mole globulin can be used. However, the presence of excess chloramine-T can cause denaturation and must be avoided if the iodinated proteins are not to be eliminated faster than when iodinated by other methods. In agreement with previous data (*17*), the rate of elimination of globulin iodinated in this study was not increased substantially beyond that of minimally radioiodinated globulin until the labeling exceeded 2.6 atoms iodine/mole (Fig. 6). While the average content of the minimally labeled globulin was 0.12 atoms iodine/mole globulin, each radiolabeled molecule contained at least 1 atom of radioiodine, although presumably seldom more.

The ratios of moles ICl/mole gamma globulin stated in this study depended on assuming a molecular weight of 160,000. Except in the *in vivo* experiment, globulins other than γG were present, and the true mean molecular weights were higher. Consequently, the true molar ratios were lower and the iodination efficiencies higher than stated.

The efficiency of iodination for 3–10-mg gamma globulin by the present procedure (Table 2) was 20–30% higher than the efficiency obtained by the Helmkamp modification of the McFarlane procedure (Table 1 of ref. *2*) when identical quantities of gamma globulin and ICl were used. Iodinations above 3 mg protein consistently gave good results without special precautions, and many iodinations could be performed in 1 day. For iodinations under 3 mg protein, the amount of ICl used was proportionately minute, and scrupulously clean glassware and fresh reagents were necessary to obtain high iodination efficiencies. For iodinations under 1 mg protein, the chloramine-T method (*11*) seems greatly superior to the present method.

What is the reason for the variation in iodination efficiencies of different proteins (Table 4)? The most reactive groups present on serum proteins such as albumin are free sulfhydryl (SH) groups (*20*). The content of free SH groups of serum albumin and gamma globulin are, respectively, 0.3–0.75 and 0.2–0.3/molecule (*20–23*). This difference in content of free SH groups is more marked on a unit weight basis. Human and bovine serum albumin have similar contents of free SH groups, but contain, respectively, 0.70 and 5.91 groups of cysteine/mole (*24*); if some of the SH groups of cysteine residues become unmasked during storage or dialysis, the free SH group content of bovine serum albumin

would be higher. Since ICl iodinates tyrosine but not cysteine residues (7), these data are in accord with the possibility that free SH groups can inactivate ICl. If this explanation is correct, proteins that contain a relatively high amount of free SH groups cannot be iodinated with high efficiencies by the ICl method.

SUMMARY

The iodine monochloride method of McFarlane has been adapted to permit labeling efficiencies of 70–90% for quantities of gamma globulin varying from 1 to 1,000 mg. The procedure is simple and rapid for radioiodinations at tracer levels; at high activity levels, hydrogen peroxide is first removed from the radioiodide solution by heat treatment at alkaline *p*H. There is no theoretical limit to the quantity of protein that can be iodinated at one time.

ACKNOWLEDGMENT

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