of glutamic acid from glucose, and hence from carbohydrates, may be appreciable.

Aspartic acid accounts for about one-third of the activity in the hydrolysate. The specific activity in aspartic acid is considerably greater than that of glutamic acid. This suggests that, though a small amount of aspartic acid may be synthesized from glucose through glutamic acid, the major portion is formed directly.

SUMMARY

1. The metabolism of uniformly ^{14}C-labelled glutamic acid during the germination of green gram seeds has been investigated. A very rapid catabolism has been noted, along with strong presumptive evidence for the synthesis of glutamic acid during germination.

2. The major portion of the radioactivity of the glutamic acid metabolized is recovered in the carbon dioxide given off during germination.

3. Aspartic acid and asparagine are important products of glutamic acid degradation.

4. A slight conversion of glutamic acid into arginine and into proline has been noted.

5. By the use of ^{14}C-labelled glucose, evidence for the synthesis of glutamic acid from carbohydrates during germination has been noted.

6. The rapid degradation and the extensive synthesis of glutamic acid during germination clearly indicate its high metabolic activity in germinating seeds.

One of us (V.M.S.) is indebted to the Government of India for the award of a Senior Research Scholarship during the tenure of which these investigations were carried out.

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Labelling of Plasma Proteins with Radioactive Iodine

By A. S. McFARLANE

National Institute for Medical Research. Mill Hill, London, N.W. 7

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The substitution of 5–10 atoms of iodine in the molecule of serum albumin takes place predominantly in the tyrosine ring and is associated with little or no alteration to the physical properties of the proteins (Hughes & Straessle, 1950). The same authors showed that at higher combining ratios histidine is iodinated and oxidative destruction of cystine, tryptophan and other residues occurs along with the appearance of abnormal sedimentation and electrophoretic characteristics of the protein. Horse, human and bovine albumin lose their ability to crystallize, become to a large extent insoluble on dialysis, and cease to have the specific immunological configuration of albumins (Shahrokh, 1943).

With only 5–10 atoms of iodine bound iodinated albumins become antigenic in the sense that they react with antisera to similarly iodinated albumins of other species. The fact that they do not give rise to precipitable antibodies when injected into the same species (Shahrokh, 1943) does not guarantee, however, that they will behave in the host precisely like the uniodinated host albumin, e.g. so far as partition between plasma and lymph or ultimate metabolic fate is concerned. Before such partially iodinated homologous plasma proteins can be used as reliable *in vivo* tracers it is essential to show that the animal cannot select them for special treatment from a population of its own uniodinated plasma protein molecules. This has been attempted by various indirect methods.

Iodinated homologous normal globulins have been injected along with antibody globulin and the behaviour of the isotope was compared with that of the immunological labels (Melcher & Masouredis, 1951; Dixon, Talmage, Maurer & Deichmiller, 1952). This method is confined to globulins, of course, and presupposes that normal and antibody globulins are treated in the same way, a matter which may not always be assumed (cf. Humphrey & McFarlane, 1954). Another approach has been to study the elimination of iodinated heterologous proteins. Thus Sterling (1951) has given minute amounts of iodinated human albumin to rabbits along with thousand-fold greater amounts of the same uniodinated protein. Precipitin titres which reflect predominantly the elimination of the unlabelled molecules decline in the same general way as the ^{131}I specific activities. The technique has numerous difficulties, which are discussed in detail by Gitlin, Latta, Batchelor & Janeway (1951). These authors conclude that the use of isotopically labelled proteins to study the behaviour of antigens *in vivo* and vice versa, is complicated by the heterogeneity of most labelled antigen preparations. In any case, the assumption that heterologous and homologous proteins will be treated in the same way is not permissible, at least for periods longer than 6–10 days, depending on the protein and on the species of animal used (Talmage, Dixon, Bukantz & Dammin, 1951), and longer periods of observation are required to obtain true exponential elimination rates (cf. Dixon *et al.* 1952).

An alternative method of much wider scope is now available, namely the injection of iodinated protein along with molecules of the same protein which have been labelled biosynthetically with another isotope (^{14}C, ^{15}N or ^{35}S). Labelled plasma proteins have recently been prepared by feeding high-specific activity ^{14}C-amino acids to rabbits (Dovey, Holloway, Piha, Humphrey & McFarlane, 1954) and these proteins are eminently suitable for this purpose if the assumption is made that they are not distinguishable by the recipient animal from its own unlabelled proteins. Present knowledge suggests that this is a reasonable assumption provided that the labelled proteins are not mishandled during the process of transference. This paper describes a series of iodinations by different procedures and briefly compares the *in vivo* behaviour of carbon-labelled proteins with various iodine-labelled ones.

Two popular methods of iodinating plasma proteins which differ mainly in details have been used. That of Hughes & Straessle (1950) depends on mixing free iodine in potassium iodide solution with the protein in bicarbonate buffer at pH 9·5–10. That of Francis, Mulligan & Wormall (1951) depends on liberating the iodine by acidifying a mixture of iodine and iodate, and adding the mixture to an ammoniacal solution of the protein. We have also used a third method in which iodine is liberated from iodide by the action of H_2O_2 in the presence of the protein at neutrality. In all three methods unbound iodine was removed by dialysis in the cold against inactive iodide and then against changes of phosphate–saline. Additional features of difference in the three methods concern the number of iodine atoms finally bound to each molecule of protein, the temperature of the reaction mixture and the concentration of protein in it. Arising out of experience with these methods a fourth one is described which is based on jet injection of free iodine into the protein in glycine buffer at pH 9·3, followed by removal of unbound iodine and rapid re-establishment of a less alkaline pH by ion exchange.

A detailed investigation of the behaviour of jet-iodinated rabbit proteins in the rabbit is described in a following paper (Cohen, Holloway, Matthews & McFarlane, 1956).

EXPERIMENTAL

Materials

^{14}C-Albumin and globulin. These were prepared by sodium sulphate fractionation of dialysed plasma taken at 18 hr. from a rabbit which received 3 mc of ^{14}C-*Chlorella* protein by stomach tube. Full details are given elsewhere (Dovey *et al.* 1954). The plasma was dialysed against a solution of carrier amino acids to dilute any labelled amino acids present. The albumin contained some α- and β-globulins, and traces of these were also present in the globulin fraction.

Salt-precipitated albumin and globulin. These were prepared by precipitating globulin from diluted normal rabbit serum by addition of 27% (w/v) Na_2SO_4 to make a final concentration of 18%. The supernatant was dialysed until free of salt and then against 0·65% NaCl buffered to pH 7·5 with phosphate. The globulin precipitate was redissolved and again precipitated at 16% (w/v) Na_2SO_4. The protein was dialysed until salt-free and finally against buffered 0·9% NaCl. It contained traces of α- and β-globulins but no albumin.

Methanol-precipitated albumin. The method of Pillemer & Hutchinson (1945) was used. Normal rabbit serum (2 ml.) and 1 ml. of acetate buffer (72 ml. of M acetic acid + 12 ml. of N-NaOH made up to 1 l.) were mixed and cooled to 0°; 7 ml. of methanol solution (607 ml. of methanol + 393 ml. of water, cooled to 0° and made up to 1 l. with methanol) was added slowly with gentle stirring. During this addition the temperature fell to −5° and the mixture was allowed to stand at this temperature for 0·5 hr. and then centrifuged at −5°. The supernatant was dialysed against several changes of 0·9% M NaCl and then water for 3 days at 0° and then freeze-dried. Electrophoretically the product contained 98% of albumin.

Methods

Fractionation of plasma and serum samples. Heparin plasma samples were diluted with 2 vol. of 0·9% NaCl and adjusted to 10% (w/v) concentration of Na_2SO_4. Fibrino-

gen precipitates were discarded and subsequently plasma and serum samples were treated alike, i.e. globulins were precipitated and reprecipitated at 18% and the first supernatants were taken to contain albumins of sufficient purity. Both fractions had broadly similar electrophoretic compositions to the ^{14}C-albumin and ^{14}C-globulin described above, and no claim for homogeneity is made. For scintillation counting the globulin precipitates were dissolved in water and the albumins were precipitated from their supernatants by addition of trichloroacetic acid (TCA) to make 5% and the precipitates dissolved in the minimum of N-NaOH. When the samples were subsequently to be assayed for radiocarbon the use of TCA was avoided, and both albumins and globulins were heat-coagulated in the presence of dilute acetic acid and washed with water until salt-free.

Isotope assays

^{131}I-Samples. These were counted in solution by scintillation measurement of their γ-radiation in a hollow NaI crystal. The solutions (or measured fractions) were then used for measurement of protein concentration by the biuret method of Gornall, Bardawill & David (1949). Both measurements were accurate to $\pm 2\%$.

^{14}C-Samples. These were converted into CO_2 by combustion and assayed in the gas phase by the technique of Bradley, Holloway & McFarlane (1954). All specific radioactivities of samples were corrected for the total volume of plasma withdrawn before the sample in question (cf. Humphrey & McFarlane, 1954).

Technique of iodination

H_2O_2-Iodinations. ^{14}C-Rabbit-globulin (236 mg.) in 4 ml. of saline buffered to pH 7·5 was used. Carrier-free radioactive iodide (Harwell, IBSI) (6 mc) in 0·70 ml. was added dropwise with stirring, followed by 0·15 ml. of H_2O_2 (British Drug Houses, 100 vol. %) to liberate iodine. In the same way 213 mg. of ^{14}C-albumin in 3 ml. received 2·5 mc of iodine in 0·30 ml., followed by 0·15 ml. of H_2O_2. Both mixtures were then incubated at 37° for 1·5 hr. and dialysed in the cold for 24 hr. against 2 l. of sodium phosphate buffer, pH 7·2, ionic strength 0·02, containing 0·9% NaCl and 0·5% KI and then against changes of the same saline phosphate buffer but without KI until iodide-free. Of the activity used 24% was bound to protein in the case of albumin and slightly less in the case of globulin.

Bicarbonate iodination. This followed the general procedure of Hughes & Straessle (1950). To 200 mg. of salt-precipitated globulin in 2 ml. of sodium phosphate buffer, pH 7·2, ionic strength 0·02, containing 0·9% NaCl was added 0·3 ml. of a carbonate–bicarbonate buffer prepared by dissolving 10·6 g. of Na_2CO_3 and 2·8 g. of $NaHCO_3$ in 50 ml. of warm water (final pH 9·9). To 15·4 mc of carrier-free radio-iodide in 0·76 ml. of water was added 0·1 ml. of a solution containing 0·54 mg. of KI and 0·54 mg. of KIO_3, followed by 1 drop of N-HCl. This liberated approximately 0·5 mg. of free iodine, and the suspension was then added dropwise with vigorous stirring to the globulin solution. A further dropwise addition of 0·5 mg. of carrier iodine was then made and the mixture incubated at 37° for 2 hr. Dialysis followed in the cold as described for H_2O_2-iodination. Of the activity used 18% was bound to globulin. A small precipitate was discarded after dialysis.

Ammoniacal iodination. This was carried out as described by Francis *et al.* (1951). Rabbit oxalate plasma (2 ml.) containing 100 mg. of protein received 0·4 ml. of 5N-NH_4OH. To 500 μc of carrier-free radio-iodide in 0·04 ml. of water was added 1·2 ml. of a solution containing 2 mg. of KI and 1 mg. of KIO_3 followed by 2 drops of N-HCl to liberate 1·85 mg. of iodine. The suspension was then added dropwise with vigorous stirring to the protein solution. After 30 min. the pH was brought back to 7·5 by careful addition of acetic acid and the solution was then dialysed as described above. Again a small precipitate was discarded after dialysis. Of the activity used 25% was bound to albumin and 6% to globulin. Assuming that carrier iodine distributes itself in the ratio of the activities, this means that albumin was iodinated at 3 and globulin at 4–5 atoms per molecule.

Jet iodination

Solutions. To 3 ml. of oxalate plasma or protein solution containing approximately 200 mg. of protein was added 1·5 ml. of a glycine buffer prepared by dissolving 0·6 g. of glycine in 8 ml. of 5·85% (w/v) sodium chloride and adding 2·0 ml. of N-NaOH (pH 9·3). To any desired amount of carrier-free ^{131}I-iodide was added 0·1 ml. of a solution containing 0·40 mg. of NaI and 0·15 mg. of $NaIO_3$ and the volume made up to 1·0 ml. with water. Immediately before jet mixing the iodine was liberated by adding one drop of N-HCl and the suspension quickly sucked into the smaller of the two burettes (cf. Fig. 1).

Burette system. This consists of a 2 ml. and a 10 ml. graduated Pyrex burette joined together through concentric delivery jets. The diameters of the jets given in Fig. 1 were arrived at by trial so that the inner one with 10 lb. positive pressure delivered 2 ml. of water in 10 sec. while the outer one with 5 lb. negative pressure required 1–2 sec. more to suck up 10 ml. of water. In addition, it was necessary to find by trial (using a coloured solution in the smaller burette and water in the bottle under the jet) the maximum permissible gap between the tip of the burette and the bottom of the bottle such that jet mixing at these pressures was confined entirely to the microchamber inside and immediately under the tip of the burette. If the gap is too large coloured solution escapes into the water in the bottle and if it is too small the passage of water up into the large burette is impeded. Once this gap was established for a particular burette mechanical arrangements were easily made so that the bottle could be replaced reproducibly.

Amberlite column. Amberlite IR4B anion-exchange resin was prepared in bulk by washing with N-NaOH, followed by water, and was then stored in N-HCl. The column volume was 1·4 ml. and height 6 cm. and contained approximately 1·5 g. (dry wt.) of resin supported on a loose plug of glass wool. Approx. 500 ml. of 0·9% NaCl was sucked through the resin, followed by 20 ml. of a histidine buffer prepared by dissolving 2 g. of histidine monohydrochloride in 95 ml. of water and adding 9·2 ml. of N-NaOH (pH 6·5). Finally, at least 100 ml. of 0·9% NaCl was passed through.

Procedure. After the free iodine solution had been gently sucked up into the smaller burette the tip of the burette was rinsed with water and the bottle containing the protein solution placed in position. With reservoir pressures adjusted to +10 and −5 lb./sq.in. the two burette taps were turned in one operation so that pressure and suction were applied simultaneously. The yellow iodine colour

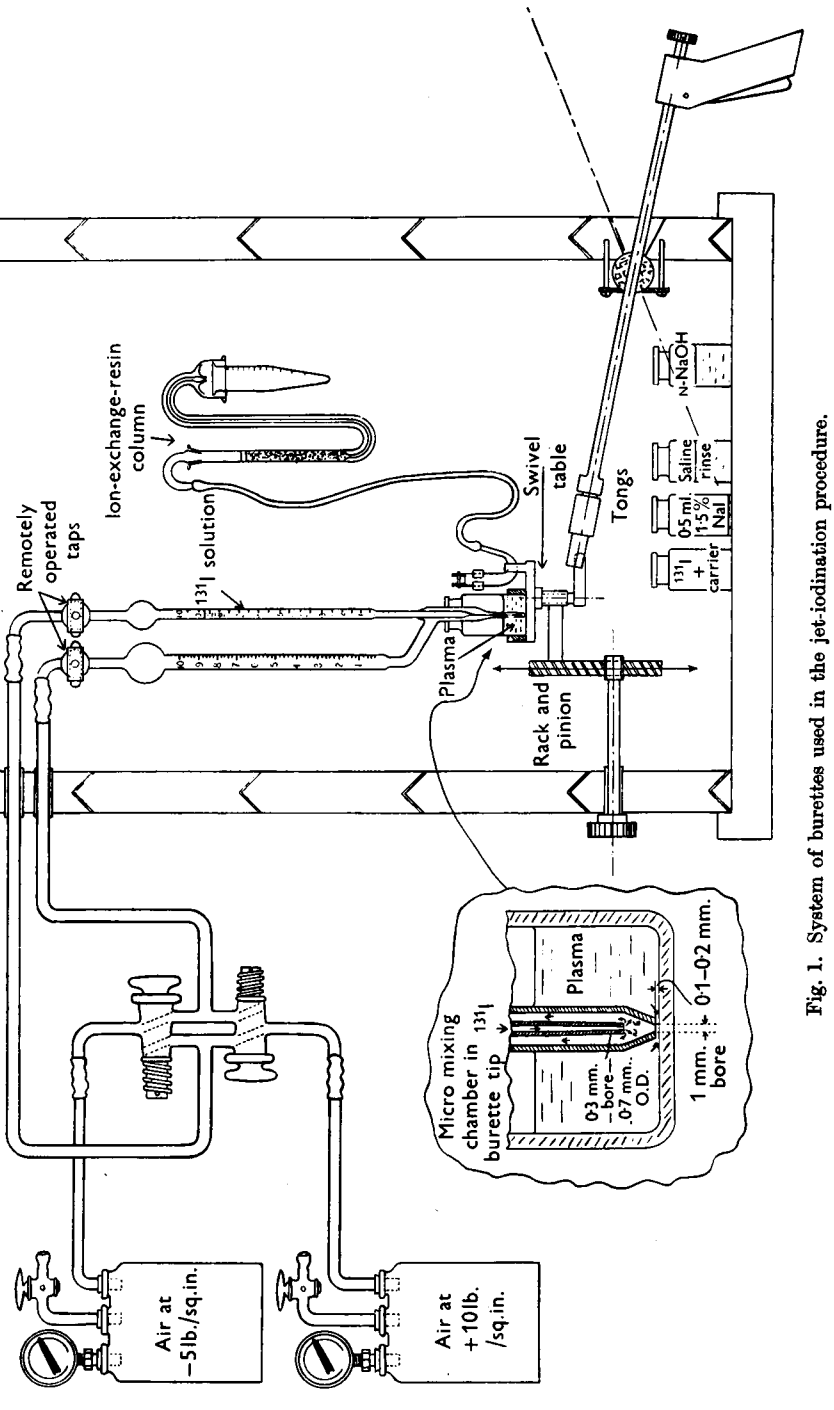

Fig. 1. System of burettes used in the jet-iodination procedure.

disappeared in the tip of the burette as both solutions were transferred in the course of 10 sec. to the larger burette. The iodine burette emptied just before the last of the protein solution left the bottle. The taps were quickly closed again in one operation, some air being unavoidably sucked into the larger burette. The bottle was now changed for one containing 0·5 ml. of a 1·75% (w/v) solution of NaI and the iodinated protein solution discharged into this and then sucked back up into the burette. With the help of the glass and rubber connector shown the protein solution was now transferred drop by drop to the resin column through which it passed as a relatively sharp boundary. In the first 6 ml. to be collected 90% of the protein was recovered. The effluent had a pH of 6·5–7·0 and was sufficiently near to isotonicity to be ready for intravenous injection. With rabbit albumin, globulin and whole plasma 25–30% of the activity used was bound to protein. Less than 1·5% of the activity in the final solution was present in the supernatant after precipitating the proteins with 5% TCA in the cold.

Animal experiments. Male sandylop rabbits (2·5–3·5 kg.), bred at the National Institute for Medical Research, were used. In all cases the animals received NaI in the drinking water (100 mg./l.) for 3 days before the start of the experiment and throughout its course. Injections were made directly into the marginal ear vein, usually not more than 3 ml. of an isotonic neutral protein solution containing approx. 100 μc. The first blood samples (2–3 ml.) were taken at 2 min. from the opposite ear into heparin, and subsequent samples at 1–3-day intervals.

In the experiments with bicarbonate-iodinated globulin rabbit 87 received 2·0 mc and the control rabbit (86) 200 μc. After 65 hr. 10 ml. of blood were taken from 87 and also from a normal rabbit (88) and each sample was injected into the other animal.

RESULTS

The curves for ^{14}C-albumin and -globulin in Fig. 2*a* are typical of many which have been obtained and which are assumed to represent the behaviour of the rabbits own unlabelled proteins. More detailed consideration of these curves is given in the next paper (Cohen *et al.* 1956). The intercept at 0 hr. obtained by extrapolating the linear portion of each curve represents a hypothetical specific activity of the injected labelled plasma protein, assuming it to have been instantaneously diluted by all unlabelled protein of the same kind in the plasma and extracellular fluid of the recipient. This value, expressed as an inverse fraction of the initial specific activity, is defined as the distribution ratio, and is taken to represent the ratio of the mass of the particular protein in intra- and extravascular compartments to the circulating mass of the same protein (cf. Sterling, 1951).

The specific activities of the plasma proteins decayed with almost perfect exponential linearity over the period 3–26 days. The half-life of albumin (190 hr.) is equivalent to an elimination (or replacement) rate of 8·8% per day, and of globulin (148 hr.) to a replacement rate of 11·2% per day. Theoretically the distribution ratio and the elimination rate should be sufficient to define the curve, excepting its precise course in the first 2–3 days during which time lymph/plasma equilibrium is being established. In practice, as will be explained later, a third parameter has been found useful, the S_{100} value, i.e. the protein specific

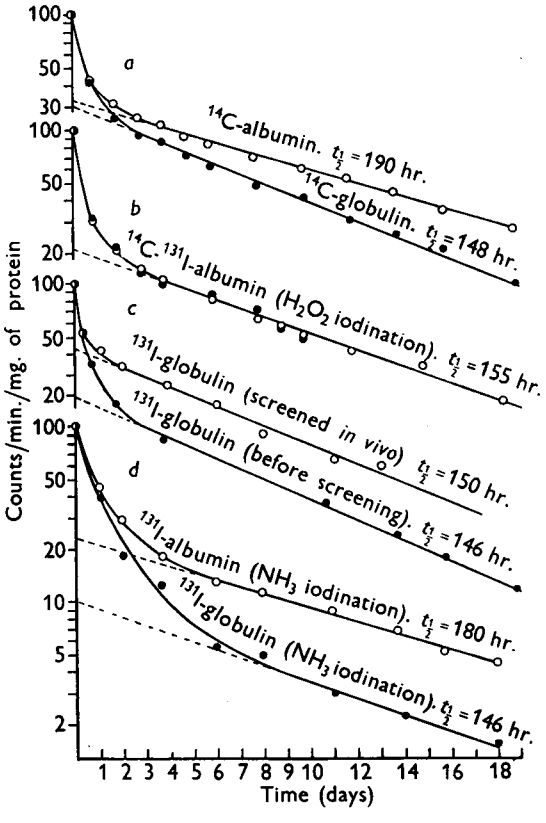

Fig. 2. Behaviour of ^{14}C- and ^{131}I-proteins labelled by various procedures and injected into rabbits.

activity in the plasma at an arbitrary time (100 hr.) expressed as a percentage of the initial value. For rabbit ^{14}C-albumin in this experiment the S_{100} value is 28·5% and for globulin 22% (cf. Table 1).

A notable change is apparent in the behaviour of the same ^{14}C-proteins after iodination in the presence of H_2O_2 (Fig. 2*b*). The two labels behave in the same way as is to be expected of isotopes firmly bound to the same molecules, and the protein half-lives are not significantly altered. The distribution ratios, however, are much larger (5·5 and 4·4) and the S_{100} values are only 12–13% (cf. Table 1). It is evident that the second animal has recognized about half the iodinated protein as altered and has removed it from its plasma in the

Table 1. *Behaviour of various* ^{14}C- *and* ^{131}I-*labelled proteins after injection into rabbits*

For calculation of half-life and definition of distribution ratio and S_{100} see text.

Rabbit proteins injected	Method of labelling	Atoms of iodine bound/ mole of protein	Additional treatment	Recipient rabbit no.	Half-life (hr.)	Distribution ratio	S_{100} (%)	Reference
^{14}C-Albumin and ^{14}C-globulin	Feeding ^{14}C-amino acids	—	Dialysis	59	190 148	3·2 3·3	26 21	Fig. 2*a*
^{14}C-^{131}I-Albumin and ^{14}C-^{131}I-globulin ^{14}C-^{131}I-Albumin and ^{14}C-^{131}I-globulin	Separate iodination of ^{14}C-albumin and -globulin, using H_2O_2 at pH 7·5	0·5	Mixtures incubated 1·5 hr. at 37° and dialysed 4 days	75 76	145 130 155 115	5·5 4·4 4·85 3·0	12·0 13·5 13·4 18·0	— — Fig. 2*b* —
^{131}I-Globulin	Iodination in bicarbonate buffer at pH 10	1	Dialysis 3 days	86 87	146 130	5·2 5·1	12·3 11·7	Fig. 2*c* —
Plasma at 3rd day from rabbit 87	—	—	—	88	150	2·34	24·3	Fig. 2*c*
^{131}I-Albumin and ^{131}I-globulin	Oxalate plasma in N-NH_4OH	3 5	Dialysis 3 days	130	180 146	4·2 9·9	16·6 6·9	Fig. 2*d*
^{131}I-Albumin and ^{131}I-globulin	Jet-iodination of oxalate plasma in glycine buffer at pH 9·3	0·5 0·5	Carrier NaI and amberlite column	97	202 144	3·1 3·2	28·5 22	Fig. 3*a*
Human ^{131}I-albumin and ^{131}I-globulin	Jet-iodination of oxalate plasma in glycine buffer at pH 9·3	1 0·5–1	Carrier NaI and amberlite column	99	170 —	2·65 —	24·5 21	Fig. 3*b* —
^{131}I-Albumin and ^{131}I-globulin	Jet-iodination of oxalate plasma in glycine buffer at pH 9·3	6 6	Carrier NaI and amberlite column	139	168 98	7·7 5·3	9·0 9·0	Fig. 3*c*
^{131}I-Albumin (prepared by salt fractionation)	Jet-iodination in glycine buffer at pH 9·3	0·5 0·5 0·5	Carrier NaI and amberlite column	89 90 91	220 215 210	2·8 2·95 2·5	25·5 24·5 29	— Fig. 3*d* —
^{131}I-Albumin (prepared by cold methanol fractionation)	Jet-iodination in glycine buffer at pH 9·3	0·5 0·5 0·5	Carrier NaI and amberlite column	146 147 148	147 162 147	2·05 3·05 2·65	28 25 28	— Fig. 3*d* —

first 3–4 days. This applied both to albumin and globulin. The labelled molecules which remain after this time are also eliminated more rapidly than the uniodinated ^{14}C-molecules. With ammoniacal iodination (Fig. 2*d*) the effect was also apparent in both but was more marked in the case of globulin (S_{100}, 6·9 %). Iodination in bicarbonate buffer at pH 10·0 was carried out on globulin alone with results (Fig. 2*c* and Table 1) which showed almost half of this protein to be changed. Unusually high

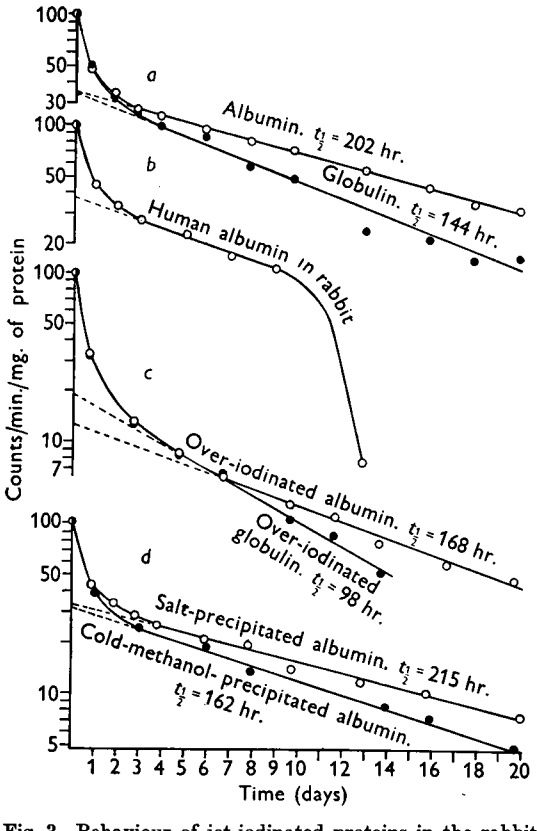

Fig. 3. Behaviour of jet-iodinated proteins in the rabbit.

radioactivity was given to this globulin (but maintaining the same low molar ratio of iodine to protein) so that it was possible to transfer a small amount of plasma from the recipient animal at 2·5 days to a second recipient and to follow the plasma activities in the latter for another 11 days. The curve obtained (Fig. 2*c*) shows the grossly altered part of the labelled protein to have been effectively screened out by the first rabbit.

Fig. 3*a* shows specific activities of albumin and globulin obtained by salt-precipitation of serial plasma samples from a rabbit which received jet-iodinated normal rabbit plasma. Numerous experiments of this kind have been done on normal rabbits (Cohen *et al.* 1956), and analysis of the results shows a variation of ± 20 % both for ^{131}I and ^{14}C proteins. Within the limits of this variation it can be said that iodinated and ^{14}C-labelled albumins and globulins behave in the same general way. If there are significant differences they are in the direction of slightly longer half-lives and lower distribution ratios for the iodinated proteins. Human albumin iodinated by the same process gave a similar kind of curve for 10–11 days after injection into a rabbit. Subsequently, a sudden disappearance of activity from the plasma occurred analogous to that observed by Talmage *et al.* (1951) and also by Gitlin *et al.* (1951), who injected iodinated bovine γ-globulin and albumin into rabbits. These authors associate the effect with the appearance of circulating antibodies.

In order to study the effect of a higher degree of substitution of iodine in the protein molecule, rabbit albumin and globulin were jet-iodinated at 6 atoms per mole. The result (Fig. 3) leaves no doubt that above a certain level of substitution by this method also iodinated albumins and globulins are for the most part unacceptable. At 100 hr. the plasma protein specific activities were 9 % of the initial values. Finally, specific comparison of jet-iodinated albumins prepared by salt precipitation on the one hand and by low-temperature methanol fractionation on the other showed that, whereas the distribution ratios and 100 hr. specific activities are the same, the elimination of methanol-treated albumin is appreciably faster.

DISCUSSION

Many of the results of these experiments must be regarded mainly as suggestive. This arises from the small number of experiments performed and the degree of individual variation in rabbits, which is considered in greater detail elsewhere (Cohen *et al.* 1956). It would obviously have been much more satisfactory to compare ^{14}C- and ^{131}I-plasma protein behaviour in all cases in the same rabbits, but this was precluded on grounds of expense and additional work involved.

Since the various methods of iodination used here differed from each other in numerous ways the broad results of their comparison unfortunately provide little help in diagnosing which, if any, are the more deleterious steps in a particular procedure. It has been found convenient here to attach descriptive terms to the different methods, e.g. ammoniacal iodination, without knowing or implying that the term relates to any critical agent or step in the procedure. However, one fact which appears to be clear is that the incorporation of as many as 6 atoms of iodine into one molecule of

rabbit albumin or globulin has an adverse effect on the acceptability of these proteins by the rabbit. This contrasts with claims to the effect that proteins labelled to this extent are still useful biological indicators (cf. Francis *et al.* 1951). Since the rejection of a large part of the protein by the recipient animal takes place inside a few days it is unlikely that immunity plays any important part in the process.

The marked effect of iodination at 6 atoms per mole raises the question whether it is feasible to bind iodine to plasma protein molecules at any lower level without in some way affecting the properties of the proteins. While it is considered unlikely that iodinated molecules are ever broken down precisely as are uniodinated ones, the evidence submitted here suggests that iodine-labelled molecules can be prepared in such a way that they are retained as long in the plasma and lymph as unlabelled ones and therefore in a restricted field they are useful biological indicators.

Of the various forms of treatment to which plasma proteins have been subjected in this study none have been observed which diminish their elimination rate, and the view is therefore tentatively held that the longer a labelled protein survives in the blood of an animal the nearer it approximates in behaviour to the animal's own unlabelled proteins.

The jet-iodination process described here appears to provide a useful material. The grounds for using the jet principle is the assumption that iodination is almost instantaneous. This is suggested by the immediate disappearance of the yellow colour of free iodine in contact with alkaline protein solutions and by the fact that more than half the maximum proportion of iodine that it is theoretically possible to bind (50%) is firmly associated with the protein in the few seconds which elapse between mixing and adding the carrier iodide at room temperature. Whether alternative mechanical methods of mixing are adequate has not been investigated. The jet arrangement lends itself to remote control, to the maintenance of cleanliness and sterility, and one such remotely operated jet or burette is desirable in any case for dispensing radio-iodine in the quantities normally handled for this purpose.

The use of pH 9·3 was indicated largely by the results of Hughes & Straessle (1950) which show that at lower pH values the efficiency of iodination is reduced and a higher proportion of the iodine is used for side reactions. Above pH 9·5 efficiency again falls because of appreciable reaction of iodine with hydroxyl ions to form I^- and IO_3^-, but the important consideration here appears to be the onset of alkaline denaturation. A major consideration in designing the process has been to limit the period at this pH to a matter of minutes. In addition, since denaturation proceeds faster in more dilute protein solutions, the protein concentration at mixing has been kept at a minimum value of 3 g./100 ml. Since it is equally undesirable to allow the pH to become acid the amberlite column has been stabilized with a histidine buffer at pH 6·5 at the suggestion of Dr A. J. P. Martin.

Several alternative methods of liberating radioactive iodine from iodide presented themselves. The evidence here is against the use of H_2O_2 or other oxidizing agents in the presence of plasma proteins on the grounds that they may alter the proteins. Furthermore, a high concentration of iodide is necessary to keep iodine in aqueous solution, and the presence of iodide in the iodination mixture reduces the proportion of radioactivity bound to protein. Thus Latta (1951) used 1·2 ml. of a 0·94 M-I_2 solution in 1·33 M potassium iodide to iodinate 50 g. of globulin to 7·8 atoms of iodine per molecule. To iodinate 100 mg. quantities of protein at fewer atoms per molecule requires the use of volumes of iodine solution too small to handle. In addition, radioactive iodine readily gets into the atmosphere from a concentrated solution of I_2 in iodide. We have found it preferable to prepare a stock solution of sodium iodide and sodium iodate in the proportions required by the equation $5HI + HIO_3 = 3I_2 + 3H_2O$ and to take volumes of it containing iodine equivalent to 3 atoms per mole of the protein to be iodinated. Carrier-free ^{131}I-iodide is added to the stock solution and the iodine liberated by addition of one drop of N-HCl immediately before mixing with the protein solution.

The use of an ion-exchange resin for removing uncombined ^{131}I is not new, and according to Sterling (1951) is the method employed by Abbott Laboratories, North Chicago, Illinois, in the commercial production of iodinated human albumin. If an excess of inactive iodide is added after mixing, not more than 1·5% of the total protein-bound radioactivity appears in the supernatant after cold precipitation with 10% trichloroacetic acid. All anions are exchanged for chloride in passing through the column and the amount of inactive iodide added beforehand may be suitably chosen so that the plasma protein solution emerging from the column is isotonic and ready for intravenous injection. Heparin plasma clots in the column, but oxalate plasma emerges free of oxalate and will not clot unless calcium is added.

The possibility that some surface denaturation takes place in the resin column cannot be excluded, especially since about 5% plasma protein is retained in the resin. The nature of the protein retained has not been investigated. In principle, dialysis in the cold against inactive iodide followed by buffered saline appears to be free of any possible

objections, but is time-consuming and difficult to carry out in sterile conditions. We have not found any evidence that a better product results.

The results given in Table 1 and Fig. 3 suggest that, when proteins are separately iodinated, preliminary contact with alcohol even in the cold should be avoided. Methanol-treated albumins behave identically with salt-treated ones so far as their passage out of the plasma into the extravascular space is concerned, but ultimately appear to be more rapidly broken down. Dixon *et al.* (1952) record a similar effect of contact with methanol in accelerating the elimination rate of iodinated rabbit globulin in the rabbit. This behaviour is in contrast with that of over-iodinated or H_2O_2-treated albumins which contain a high proportion of molecules which are rapidly withdrawn from the plasma for special treatment.

SUMMARY

1. Rabbit plasma proteins have been iodinated with ^{131}I by using several procedures, and their behaviour in the rabbit is compared with that of ^{14}C-labelled plasma proteins.

2. In nearly all cases the iodinated proteins were eliminated more rapidly. Some treatments, and especially those in which iodine was substituted at 6 or more atoms iodine per molecule protein, resulted in a form of denaturation which the recipient animals dealt with promptly by rapidly removing the altered molecules from the plasma.

3. In other cases, including preliminary treatment of albumin with methanol at low temperatures, a more subtle change took place which did not effect the intra/extra-vascular distribution of the protein but accelerated its ultimate metabolic breakdown.

4. A procedure is described for labelling whole plasma or its separated proteins with ^{131}I at 0·5 atom iodine per molecule protein in such a way that albumin and globulin so iodinated behave in the rabbit in the same way as the ^{14}C-labelled proteins.

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Distribution and Elimination of ^{131}I- and ^{14}C-Labelled Plasma Proteins in the Rabbit

BY S. COHEN,* R. C. HOLLOWAY, C. MATTHEWS AND A. S. MCFARLANE
National Institute for Medical Research, Mill Hill, London, N.W. 7

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Radioactive iodine probably constitutes the most readily available and convenient label for plasma proteins. During the past five years it has found increasing application in clinical and experimental studies of plasma protein distribution and metabolism. Recently, however, the view that iodine can be regarded as a reliable plasma protein label in metabolic studies has been seriously questioned. Berson, Yalow, Schreiber & Post (1953) have shown that homologous albumin iodinated by any of three different techniques and administered to human subjects contains several components having different degradation rates. In addition, the rate of elimination of separate batches of albumin

* Nuffield Dominion Fellow from the Department of Physiology, Witwatersrand University, Johannesburg.