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with that of over-iodinated or H_2O_2-treated albumins which contain a high proportion of molecules which are rapidly withdrawn from the plasma for special treatment.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.

The experiments described here have been carried out over a period of two years and the author is greatly indebted to numerous colleagues who have helped at different times. In particular, thanks are due to G. H. Bradley, S. Cohen, G. Hodgson (University of Chile, Santiago), J. H. Humphrey, R. C. Holloway and P. R. Purser. I am also grateful to D. J. Perkins who kindly carried out the low-temperature fractionations of rabbit serum, and to Mr D. Hart for constructing numerous trial jet-burettes and for the final model.

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

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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

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iodinated by the same method was by no means constant in the subjects studied. Comparison of the elimination of ^{35}S- and ^{131}I-labelled plasma proteins has led Volwiler *et al.* (1954) to conclude that iodination denatures the protein sufficiently to reduce the life span, a view which is supported by the results of Armstrong, McLeod, Wolter & Kukral (1954) and Armstrong *et al.* (1955), using the same labels.

These findings have created considerable doubt about the validity of metabolic data obtained through the use of iodinated plasma protein. However, the observations of McFarlane (1956) reveal that the fate of labelled protein molecules may be considerably modified by slight alterations in the iodination procedure, and he describes a method which appears to be reproducible and not to change the proteins adversely. In this paper the intra- and extra-vascular distribution of ^{131}I and its elimination from plasma and urine following administration of homologous and autogenous rabbit plasma labelled according to this method will be described. The fate of this iodinated material will be compared with that of ^{14}C-labelled homologous plasma protein transfused from donor rabbits. Finally, the value of this method of iodination in studies of plasma protein metabolism will be assessed.

EXPERIMENTAL

Animals. Male sandylop rabbits, weighing between 2·7 and 3·7 kg., were used throughout. The animals received pelleted diet no. 18 (Bruce & Parkes, 1946) and were separately housed in metabolism cages which permitted the quantitative collection of urine. At least 2 days before injection of iodinated plasma protein and throughout the subsequent period of observation NaI (100 mg./l.) was added to the drinking water.

Preparation of ^{131}I-labelled plasma protein. All iodinations referred to in this work, with the exception of two in Table 2, were carried out by the jet-iodination procedure described by one of us (McFarlane, 1956). A portion (3 ml.) of protein solution containing 150–200 mg. of protein was mixed with 1·5 ml. of a buffer made up by dissolving 0·6 g. of glycine in 8 ml. of 5·85% (w/v) NaCl and adding 2 ml. of N-NaOH. This was done in the cold immediately before jet-mixing with 1 ml. of a 0·05% solution of free iodine containing any desired amount of carrier-free ^{131}I. The free iodine solution was prepared immediately before mixing by adding one drop of N-HCl to a solution containing 0·15 mg. of $NaIO_3$ and 0·40 mg. of NaI. Sodium iodide (1·75%, 0·5 ml.) was then added and the iodinated protein mixture transferred to an Amberlite IR4B column previously buffered with histidine at pH 6·5 and saturated with NaCl. The iodinated protein emerged after a few minutes in a volume of approximately 6·0 ml. and was found to contain less than 1·5% of the total radioactivity in the supernatant after precipitation of proteins with 10% trichloroacetic acid (TCA) in the cold. 20–30% of the radioactivity used was bound to protein and, when whole rabbit plasma was iodinated, the counts were found to be distributed as follows: albumin, 78%; globulin, 16%; fibrinogen, 6%.

Preparation of ^{14}C-labelled plasma protein. Three rabbits, referred to as *A*, *B* and *C* in the text, were labelled with ^{14}C-Chlorella protein obtained from the Radiochemical Centre, Amersham, Bucks. *A* and *B* each received 3 mc by stomach tube and the plasmas were collected at 17–18 hr. Rabbit *A* was normal and *B* had been immunized with type III pneumococci as described in an earlier publication (Dovey, Holloway, Piha, Humphrey & McFarlane, 1954). Rabbit *C* was given intravenously amino acid hydrolysate from 1 mc of chlorella protein by Dr J. H. Humphrey and the plasma was collected at 4 hr. In all cases the plasma proteins were dialysed against a solution of carrier amino acids to get rid of any labelled amino acids present. Carrier amino acids were prepared by hydrolysis of rabbit serum albumin in constant-boiling HCl for 36 hr. at 108°.

Injection of labelled plasma protein. Labelled plasma proteins prepared as described above were injected into the marginal ear veins of rabbits. Between 0·15 and 0·35 g. of plasma protein in 3·0–8·0 ml. was injected. In the combined ^{14}C and ^{131}I experiments the labelled solutions were pooled and administered together. In every instance the washings from syringe, needle and vessel which contained the radioactive solution were collected and suitably diluted for ^{131}I counting. The proportion of the measured dose which had not been injected was ascertained and the amount of administered ^{131}I- (and ^{14}C-)labelled protein estimated by difference.

Blood samples. Two minutes after injection 3–5 ml. of blood were withdrawn from the marginal ear vein into 0·05 ml. of heparin solution (1000 i.u./ml.); similar blood samples were collected at intervals of 1–3 days throughout the period of observation.

Preparation of plasma protein samples for counting

Total plasma protein. After administration of iodinated plasma protein the level of unbound ^{131}I was estimated in several plasma samples either after dialysis or thrice-repeated precipitation with TCA at a final concentration of 10% (w/v). In samples analysed the unbound activity did not exceed 1·5% and was usually less than 1% of the total plasma radioactivity. The error involved in using total plasma activity as a measure of protein activity was therefore regarded as insignificant.

Samples of plasma (0·2–1·0 ml.) were diluted to suitable volume with 0·9% (w/v) NaCl for ^{131}I counting. Plasma protein for ^{14}C analysis was subsequently precipitated from the same solution by heating after addition of 0·1 N acetic acid. The precipitated protein was separated by centrifuging, washed with ethanol–ether (3:1, v/v) and dried before combustion.

Fibrinogen. Samples of plasma (1–3 ml.) were diluted to 5 ml. with 0·9% NaCl. Fibrinogen was twice precipitated by addition of 27% (w/v) Na_2SO_4 to give a concentration of 10%. The second precipitate was dissolved in about 15 ml. of 0·9% NaCl. Approximately 0·05 ml. of 0·5% (w/v) protamine sulphate and 0·05 ml. of 0·5% (w/v) thrombin were added, and clotting was induced by incubation at 37° for 0·5 hr. The fibrin clot was collected on a glass rod and washed in 0·9% NaCl and then in water. Samples for ^{131}I analysis were dissolved in 1 ml. of 1 N-NaOH and made up with water to the required volume for counting. Fibrin

clots for ^{14}C analysis were washed with ethanol–ether (3:1) and dried at 37° before combustion.

Globulin. Globulin was precipitated from the supernatant at 10% Na_2SO_4 by addition of 27% aqueous Na_2SO_4 to give a final concentration of 18% Na_2SO_4. The precipitate was dissolved in 5 ml. of 0·9% NaCl and reprecipitated at 18% Na_2SO_4. This second precipitate was dissolved in a suitable volume of 0·9% NaCl for ^{131}I counting. Globulin for ^{14}C analysis was subsequently precipitated from the solution by heating with 0·1 N acetic acid. The precipitate was washed twice with water, once with ethanol and finally with ethanol–ether (3:1) and dried at 37° before combustion.

Albumin. A portion of the supernatant at 18% Na_2SO_4 was used for ^{131}I counting. Albumin for ^{14}C analysis was precipitated from the remainder of this solution and prepared for combustion as described above for globulin.

The method of sodium sulphate fractionation described above separates rabbit plasma protein into albumin, globulin and fibrinogen fractions which contain approximately 72, 22 and 6% of the total protein respectively. The use here of the terms albumin and globulin does not imply that these fractions are homogeneous. Electrophoretic analysis of the globulins showed them to be predominantly γ-globulin with small amounts of α- and β-globulin and some albumin (± 4% of the total). The albumins contained α- and β-globulins comprising 15–20% of the total fraction.

Protein estimations. These were done on measured fractions of the solutions used for ^{131}I counting according to the biuret method of Gornall, Bardawill & David (1949); readings were made 30 min. after adding the biuret reagent in a Beckman spectrophotometer at 540 mμ.

Urine collection. Samples (24 hr.) were collected at 9 a.m. throughout the period of observation. In two rabbits the bladder was emptied at the end of each 24 hr. period by catheterization and irrigation.

Urinary creatinine estimations. Urinary creatinine was estimated by the colorimetric method of Bosnes & Taussky (1945).

Measurement of radioactivity

^{131}I-*Samples.* Solutions were measured by scintillation counting, using one of two cylindrical NaI crystals in conjunction with an 11-stage electron multiplier. The larger crystal (1·5 in. diameter) had a central well to hold 3 ml. in a glass tube and measured the γ-radiation with an efficiency of 33%. The smaller one (0·75 in. diameter) had an annular jacket holding up to 10 ml. of solution and measured the γ-rays with an efficiency of 7·4%.

^{14}C-*Samples.* These were converted into CO_2 by combustion and the radiation from the purified gas was measured in a cylindrical counting tube with central tungsten anode and graphite cathode as described elsewhere (Bradley, Holloway & McFarlane, 1954); the efficiency was 75–80%.

Corrections for sampling. Repeated blood sampling results in a cumulative removal of substances which may be assumed to be promptly replaced since there was no reduction in the plasma protein concentration, haematocrit or plasma volume during the course of these experiments. The labelled protein removed in the blood sample must therefore be replaced by unlabelled molecules, and this dilution must effect the decay curve and lead to a false estimate of the half-life.

The correction for this error is based on the fact that injected protein equilibrates in a pool which is taken to be equivalent to 2·4 times the plasma volume (approximately 11% of the body weight). In a rabbit weighing 2·72 kg., the plasma protein pool will be equivalent to 300 ml. of plasma. Repeated removal of 2 ml. plasma samples after the first necessitates correction of sequential specific activities by factors of 300/298, 300/296, 300/294, etc. Also since the urinary excretion of ^{131}I is proportional to the prevailing specific activity of plasma protein (see later) a similar correction is required for the daily ^{131}I urinary values.

RESULTS

Iodine-labelled proteins

Elimination of intravenously injected protein from the plasma

Total plasma protein. The plasma elimination of intravenously injected ^{131}I-labelled protein has been followed for periods of 18–32 days in 13 male rabbits. Either homologous or autogenous plasma obtained from 9 separate iodinations was administered. The results of these experiments are summarized in Table 1.

The form of the plasma protein elimination curve was similar in all animals and is illustrated by the experiment shown in Fig. 1. The dilution of administered label at 2 min. after injection is assumed to be due only to intravascular mixing. Plasma protein specific activity (counts/min./mg. of protein) shows an initial rapid fall which in every instance is followed by a more gradual exponential decline lasting throughout the period of observation (up to 32 days).

The initial fall in specific activity is attributed to the distribution of labelled protein within the total exchangeable intra- and extra-vascular plasma protein pool. Equilibration is assumed to be complete when specific activity values fall on the later linear portion of the decay curve. On this basis the time taken for injected protein molecules to equilibrate with the plasma protein pool varied from 40 to 100 hr. (mean 66 hr.). The size of the total plasma protein pool was estimated from the zero time extrapolation value of the exponential decay curve, using the principle of isotope dilution. In conjunction with plasma protein specific activities measured at 2 min. the values obtained by extrapolation provide measures of the relative masses of intra- and extra-vascular proteins in the total exchangeable plasma protein pools. From Table 1 it will be seen that the ratio of the total plasma protein pool to the intravascular pool (or distribution ratio) varied between 1·92 and 2·86 (mean 2·36). The size of the intravascular pool was relatively constant (8·0–11·4 g.), while the extravascular pool showed greater variation (8·7–18·9 g.).

Table 1. *Distribution and elimination of ^{131}I-labelled plasma protein in rabbits*

In Expts. 126–129 a single batch of iodinated plasma was used; in Expts. 138 and 142 a different iodinated plasma was injected. A = autogenous; H = homologous plasma protein. For methods of calculating distribution ratio, equilibration time and turnover rate see text.

Expt. no.	Material injected	Dose ^{131}I (μc)	Duration of expt. (days)	Fibrinogen		Plasma protein					Urine	
				Distribution ratio	$t_{\frac{1}{2}}$ (hr.)	Equilibration time (hr.)	Distribution ratio	Intravascular pool (g.)	Extravascular pool (g.)	$t_{\frac{1}{2}}$ (hr.)	Turnover rate (g./day)	$t_{\frac{1}{2}}$* (hr.)	Percentage of dose excreted at $t_{\frac{1}{2}}$
106	A	138	32	1·37	52	70	2·49	8·2	12·1	185	1·87	190	48·2
107	A	165	32	—	66	60	2·42	8·4	12·0	220	1·55	215	50·4
112	H		18	—	—	60	2·40	—	—	185	—	195	—
113	A	93	28	1·31	75	60	2·18	8·7	10·2	205	1·53	200	43·1
114	A	155	30	2·09	70	70	2·00	11·4	11·4	202	1·87	200	49·0
115	A	127	28	—	—	60	2·45	8·0	11·6	215	1·51	205	43·7
116	A	101	29	—	—	40	1·92	10·6	8·7	170	1·99	—	43·5
126	H	86	25	1·35	68	100	2·86	10·1	18·9	212	2·27	205	46·5
127	H	83	25	1·21	68	70	2·36	8·4	11·4	225	1·47	230	49·2
128	H	79	25	1·00	62	60	2·34	9·1	12·2	190	1·87	180	47·1
129	H	86	25	1·03	68	90	2·60	10·8	17·2	250	1·87	240	45·9
138	H	204	28	1·86	65	70	2·40	7·3	10·1	220	1·33	220	50·0
142	H	204	28	1·50	65	65	2·13	8·3	9·3	175	1·60	175	47·9
Range		79–204	18–32	1·00–2·09	52–75	40–100	1·92–2·86	7·3–11·4	8·7–18·9	170–250	1·33–2·27	175–240	43·1–50·4
Mean		127	27	1·42	66	67	2·34	9·2	12·1	203	1·73	205	47·0

* Calculated from ^{131}I excretion during successive 72 hr. periods.

It is assumed that the slow exponential decay results from progressive dilution of labelled by unlabelled protein molecules. Since plasma protein concentration did not alter significantly during the course of these experiments, the exponential decay also provides a measure of the rate of plasma protein degradation. In each experiment the biological half-life of plasma protein was obtained graphically from a linear semi-logarithmic plot of the slow exponential decay curve. Values varied between 170 and 250 hr., the average half-life being 204 hr. (8·5 days). The fractional turnover rate* (% of plasma protein pool/day) is calculated from the expression $0{\cdot}693/t_{\frac{1}{2}}$ (days). The turnover rate of plasma protein (g./day) is equal to total plasma protein pool (g.) × fractional turnover rate/100. In the animals investigated 1·51–2·27 g. of plasma protein were replaced daily (mean, 1·78 g./day).

Six rabbits received autogenous plasma protein and seven were given homologous plasma protein (Table 1). In both groups the form of protein elimination curves, time taken for equilibration, distribution ratios of labelled material, biological half-lives and turnover rates of plasma protein were closely similar. It appears therefore that homologous and autogenous plasma proteins are identical with regard to distribution and metabolism in the healthy rabbit.

Albumin and globulin. The specific activity of albumin and globulin has been followed in five rabbits after intravenous injection of homologous protein obtained from three separate iodinations (Table 2). In these animals equilibration of albumin occurred within 40–65 hr. and the distribution ratio was 2·10–2·84. The biological half-life of albumin varied from 185 to 230 hr. (mean 203), and the turnover rate was 0·82–1·19 g./day (mean 0·99 g./day). The albumin specific activity at 100 hr. expressed as a percentage of the specific activity at 2 min. after injection (S_{100} value) was 23·9–33·4 (mean 28·6).

In two animals equilibration of globulin occurred after 65–70 hr., but in the remaining three rabbits complete equilibration was not achieved until 115–140 hr. after administration of labelled protein. In all experiments the biological half-life of globulin was shorter than that of albumin and varied from 140 to 155 hr. (mean 149). The turnover rate of globulin in four rabbits was 0·68–1·26 g./day (mean 1·00 g./day) and the specific activity of globulin at 100 hr. was 20·0–28·0 % (mean 24·3 %) of the value at 2 min. after injection. In the remaining rabbit (Table 2, Expt. 126) the S_{100} value was 13·5 %, the extravascular globulin pool was unusually large and the replacement rate 1·70 g./day.

* The nomenclature adopted here is that suggested by Zilversmit (1955).

Table 2. *Distribution and elimination of* ^{131}I*-labelled albumin and globulin in rabbits*

Labelled homologous plasma was administered intravenously in each experiment. Dose of ^{131}I in Expt. 98 was not determined. For dose administered in remaining experiments and for details of total plasma protein turnover and urinary ^{131}I excretion see Table 1. In Expts. 127, 138 and 142 ^{14}C-labelled serum was simultaneously injected (see Table 6). For methods of calculating equilibration time, S_{100} value, distribution ratio and turnover rate see text.

Expt. no.	Equilibration time (hr.)	S_{100}	Distribution ratio	Intravascular pool (g.)	Extravascular pool (g.)	$t_{\frac{1}{2}}$ (hr.)	Turnover rate (g./day)
				^{131}I-Albumin			
98	65	23·9	2·84	4·7	8·5	185	1·19
126	40	26·4	2·55	5·6	8·6	200	1·18
127	60	28·9	2·31	4·9	6·5	230	0·82
138	60	30·5	2·39	4·5	6·3	212	0·84
142	55	33·4	2·10	5·2	5·7	190	0·95
Range	40–65	23·9–33·4	2·10–2·84	4·5–5·6	5·7–8·6	185–230	0·82–1·19
Mean	56	28·6	2·44	5·0	7·1	203	0·99
Ammoniacal iodination	75	16·6	4·20	3·3	10·6	180	1·21
Over-iodination	90	9·0	7·7	5·5	30·0	168	4·54
				^{131}I-Globulin			
98	115	23·4	3·06	3·5	7·1	140	1·26
126	140	(13·3)	(5·35)	2·9	(12·4)	150	(1·70)
127	115	20·0	3·64	3·2	5·7	150	0·99
138	65	26·0	2·43	3·3	4·7	155	0·86
142	70	28·0	2·28	3·5	4·6	150	0·90
Range	65–140	20·0–28·0	2·28–3·64	2·9–3·5	4·6–7·1	140–155	0·86–1·26
Mean	101	24·3	2·85	3·3	5·5	149	1·00
Ammoniacal iodination	200	6·9	9·4	3·2	26·7	146	3·32
Over-iodination	90	9·0	5·3	5·4	25·4	98	4·64

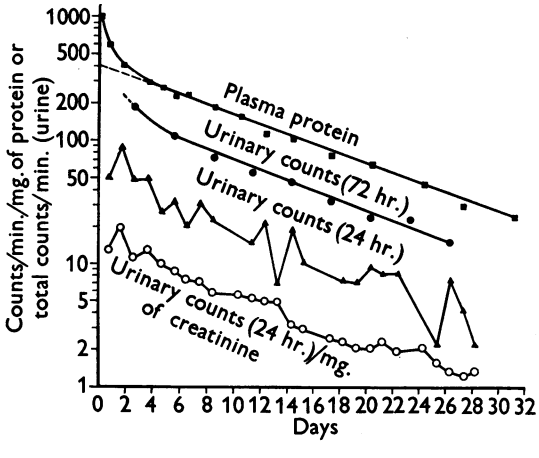

Fig. 1. Plasma and urinary elimination of ^{131}I after injection of iodinated plasma protein (Expt. 106).

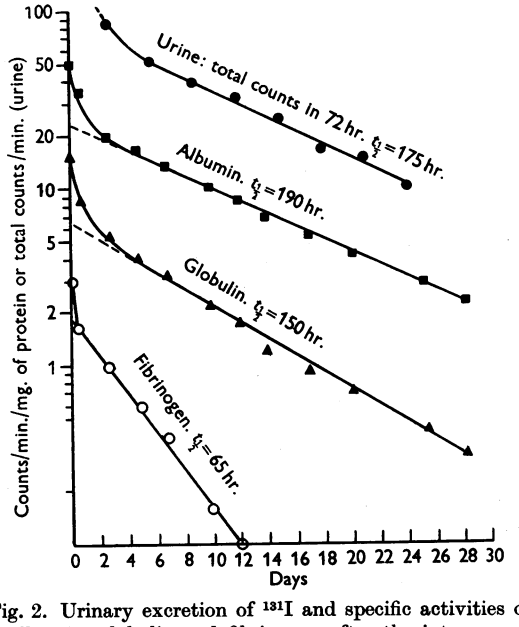

Fig. 2. Urinary excretion of ^{131}I and specific activities of albumin, globulin and fibrinogen after the intravenous administration of iodinated plasma protein (Expt. 142).

The distribution ratio, pool size, replacement rate and S_{100} value obtained in two experiments using other methods of iodination differed very considerably from values observed with jet-iodinated plasma (Table 2).

Fibrinogen. The specific activity of fibrinogen has been followed in ten rabbits given ^{131}I-labelled plasma protein by intravenous injection. The initial rapid drop in specific activity was always less marked than that observed for albumin and globulin (Fig. 2). This difference is illustrated by the fact that the distribution ratio for fibrinogen was 1·00–2·09 (mean 1·42), which is considerably less than that observed for total plasma protein, albumin or globulin (Tables 1 and 2). The initial fall in fibrinogen specific activity was followed by an exponential decline which continued throughout the period of observation (8–11 days). The biological half-lives, which varied from 52 to 75 hr. (mean 66), were considerably shorter than those of albumin or globulin. In these experiments the dose of fibrinogen-bound ^{131}I was not accurately determined and the fibrinogen pool size could not be estimated.

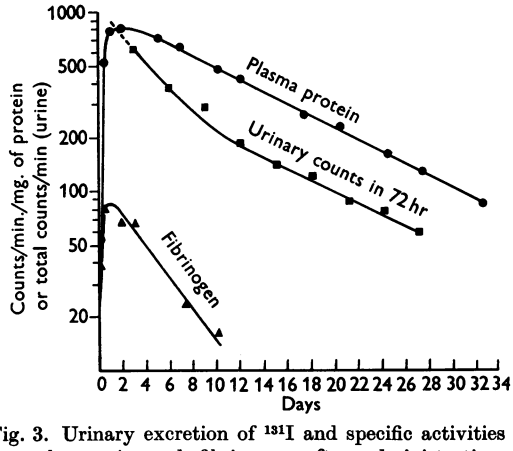

Fig. 3. Urinary excretion of ^{131}I and specific activities of total protein and fibrinogen after administration of iodinated plasma into multiple subcutaneous sites.

Elimination of subcutaneously injected protein from the plasma

^{131}I-Labelled plasma protein was administered to a rabbit by subcutaneous injection into five sites over the ventral surface of the thorax and abdomen. From the dose of labelled protein given and assuming the rabbit to have an exchangeable plasma protein pool of 22 g., the hypothetical specific activity following complete and instantaneous equilibration is estimated to be 920 counts/min./mg. protein. From Fig. 3 it is seen that the radioactivity of the recipient's plasma protein rose sharply during the initial 24 hr. after injection, at which time the specific activity was 782 counts/min./mg. protein. The specific activity remained stable for the ensuing 100 hr., and thereafter showed an exponential rate of decline throughout a further 600 hr. of observation. The biological half-life estimated from this portion of the decay curve was 205 hr.

At 4 hr. after injection the specific activity of fibrinogen was 70% of the total plasma protein value. The maximum specific activity of fibrinogen observed at 24 hr. was only 10% of the total plasma protein specific activity at the same time. The subsequent elimination of plasma fibrinogen followed an exponential curve with a half-life of 88 hr.

Urinary excretion of ^{131}I after the injection of labelled protein

The daily urinary excretion of ^{131}I has been estimated for periods of up to 4 weeks in thirteen rabbits injected with labelled plasma protein. The pattern of excretion was similar in all animals, and is illustrated by the experiment shown in Fig. 1.

The elimination of ^{131}I was somewhat irregular during successive 24 hr. periods, but in general showed a progressive decline during the course of the experiment. In two rabbits a tenfold increase in the sodium iodide content of the drinking water 10 days after administration of the labelled protein did not alter the pattern of ^{131}I excretion. The irregular daily elimination of ^{131}I, which was observed in all rabbits, was also present in two animals subjected to daily catheterization and does not, therefore, result from incomplete emptying of the bladder. Nevertheless, the expression of counts eliminated per 24 hr. period in terms of the creatinine content of the urine (counts/24 hr./mg. creatinine) produces an appreciable smoothing of the urinary elimination curve. Moreover, in all animals studied excretion of ^{131}I per 72 hr. period showed an exponential decline after about the first 100 hr. The slope of this elimination curve was closely similar to that of the corresponding plasma protein decay curve.

It is evident that after equilibration the renal excretion of ^{131}I considered over 3-day periods is proportional to the prevailing plasma protein specific activity; this is true whether labelled protein is given by intravenous or subcutaneous injection (Figs. 1, 3). It was thus possible to obtain a measure of the rate of plasma protein degradation from the 72 hr. urinary excretion curve, and in every instance this corresponded closely with the value obtained from plasma figures. Thus, as shown in Table 1, the biological half-life of plasma protein estimated from plasma specific activity and from excretion of ^{131}I during successive 72 hr. periods,

did not differ by more than 5 % in any of the twelve rabbits studied.

The urinary excretion of ^{131}I during the initial 72 hr. was always within 2 % of the value calculated on the basis of the dose injected and the exponential decay of plasma protein specific activity. On the other hand, after injection of jet-iodinated pathological human plasma or homologous plasma iodinated by other methods, the urinary excretion of ^{131}I exceeded the anticipated value during the initial 72 hr. by an amount equivalent to 9–18 % of the dose (Table 3).

Re-utilization of ^{131}I for plasma protein synthesis

From the findings presented above it is apparent that a small proportion of ^{131}I removed from the exchangeable protein pool is not immediately excreted and can be identified in diffusible form. It is therefore necessary to ascertain whether iodinated compounds derived from plasma protein breakdown are re-utilized in plasma protein synthesis, since such a process would affect the plasma protein decay curve.

The nature of all iodinated compounds derived from the breakdown of ^{131}I-labelled plasma protein

Table 3. *Urinary excretion of ^{131}I during the initial 72 hr. following the injection of iodinated plasma protein*

The predicted ^{131}I excretion was calculated from the dose injected and the slow exponential decay rate of plasma protein specific activity.

Iodinated plasma injected	Expt. no.	Predicted ^{131}I excretion (72 hr. × 10^6) (counts/min.)	Observed ^{131}I excretion (72 hr. × 10^6) (counts/min.)	Difference as percentage of dose
Autogenous: rabbit	107	5·60	5·95	+1·3
	113	3·37	3·41	<0·1
	114	5·59	5·90	+1·2
	115	4·40	4·21	−0·2
Homologous: rabbit	126	3·01	3·20	+1·4
	127	2·76	2·85	+0·1
	128	3·10	3·26	+1·3
	129	2·63	2·46	−1·2
	138	6·98	7·50	+1·7
	142	8·33	8·40	<0·1
Homologous: rabbit (over-iodination)	139	10·62	16·43	+18·4
Homologous: rabbit (ammoniacal iodination)	130	5·99	7·84	+9·1
Human myeloma plasma	131	3·92	6·17	+13·7

The results in Table 1 show that 43–50 % (mean 47 %) of the injected ^{131}I was recovered in the urine at the biological half-life estimated from plasma protein specific activities. In two rabbits the amount of ^{131}I in the body at different times and not bound to protein was estimated by multiplying the diffusible counts in 1 ml. of whole blood by 37 % of the body weight in g. (cf. Dixon & Talmage, 1951). This investigation carried out in Expts. 114 and 116 showed a retention of 1·1 and 3·9 % of the respective doses as unbound ^{131}I at the half-life of plasma protein. In another rabbit 46·4 % of injected ^{131}I had been recovered in the urine 8 days after injection of labelled protein. At this time 48·4 % of the dose was present in the exchangeable protein pool (11 % of the body weight), 1·2 % in the non-protein iodide pool (37 % of the body weight), while the thyroid gland contained less than 0·1 % of the ^{131}I administered dose.

is not known. However, it appeared feasible to investigate the problem of re-utilization by studying whether or not plasma protein became labelled by iodinated compounds absorbed after digestion of orally administered ^{131}I-labelled plasma protein. The specific activity of plasma protein following intravenous injection of ^{131}I-labelled mono- and di-iodotyrosine was also investigated. In these experiments labelled plasma was administered to unanaesthetized rabbits by stomach tube. Plasma proteins were separated from non-protein components of plasma by precipitation with trichloroacetic acid at a concentration of 10 %. Results are summarized in Table 4, from which it is seen that neither iodinated products of plasma protein digestion nor labelled iodotyrosines were incorporated to any detectable extent into plasma protein synthesized within 40–64 hr. of isotope administration.

Table 4. *Absence of plasma protein labelling after oral administration of iodinated plasma protein or intravenous injection of labelled mono- and di-iodotyrosine*

Expt. no.	Material	Dose (μc)	Time after injection (hr.)	Plasma protein (counts/ml.)	Non-protein (counts/ml.)	Percentage of dose excreted in urine
120	Autogenous plasma protein (by mouth)	106	2	Nil	10 290	—
			4	Nil	6 000	—
			16	Nil	Nil	88
			40	Nil	Nil	92
121	Homologous plasma protein (by mouth)	106	2	Nil	12 688	—
			4	Nil	6 160	—
			16	Nil	1 568	85
			64	Nil	Nil	100
124	^{131}I Mono-iodotyrosine (intravenous)	55	4	Nil	Nil	—
			22	Nil	Nil	85
			46	Nil	Nil	91
125	^{131}I Di-iodotyrosine (intravenous)	132	4	Nil	321	—
			22	Nil	Nil	86
			46	Nil	Nil	92

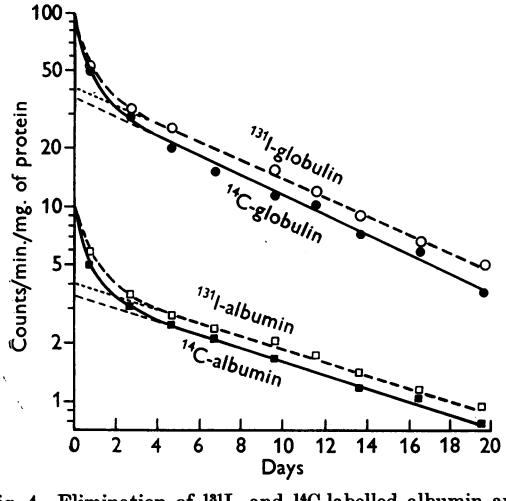

Fig. 4. Elimination of ^{131}I- and ^{14}C-labelled albumin and -globulin administered simultaneously by intravenous injection (Expt. 138).

Iodine- and carbon-labelled proteins

The elimination of ^{131}I- and ^{14}C-labelled plasma proteins has been compared in two series of experiments. In the first, ^{14}C-labelled plasma protein was administered to six rabbits and five of these animals received ^{131}I-labelled plasma 9–12 months later. The results obtained in the six rabbits which received ^{14}C-labelled protein are summarized in Table 5. Four of these experiments have already been published (Dovey *et al.* 1954), but are now re-analysed with corrections for blood sampling. These rabbits were bled with greater frequency at the beginning than at the end of the experiments and this produced the previously reported break in albumin and globulin decay curves. When correction for blood sampling is made, the form of the ^{14}C decay curves is closely similar to that described above for ^{131}I-labelled plasma and no break is apparent (Fig. 4). The distribution ratio of ^{14}C-labelled globulin was consistently slightly greater than that of albumin (Table 5). The biological half-life of ^{14}C-albumin was 160–208 hr. (mean 181 hr.) and of globulin 140–170 hr. (mean 159 hr.). The half-life of ^{14}C-labelled fibrinogen was 55–85 hr. (mean 69 hr.).

In the subsequent ^{131}I experiments on the same rabbits, fractionation of plasma protein was not performed (Table 5). Since albumin contains about 80% of the total counts present in ^{131}I-labelled plasma, it is to be anticipated that the decay rate of total plasma protein will be similar to that of albumin alone. It is apparent from Table 5 that the biological half-life of iodinated plasma protein (170–215 hr.) was, in general, slightly longer than that of ^{14}C-labelled albumin administered about a year earlier (160–208 hr.). The distribution ratio of iodinated plasma protein (1·92–2·45) was always lower than that of ^{14}C-labelled albumin (2·74–3·25). The biological half-lives of ^{14}C- and ^{131}I-labelled fibrinogen were similar in two separate groups of rabbits (Tables 1 and 5).

In a second set of experiments ^{14}C- and ^{131}I-labelled homologous proteins were administered simultaneously by intravenous injection to three rabbits. The results obtained with ^{14}C are shown in Table 6 and the corresponding data for ^{131}I appear in Table 2 (Expts. 127, 138 and 142). In each of these animals the equilibration times and biological half-lives showed satisfactory agreement for both albumin and globulin when estimated with ^{131}I- and ^{14}C-labelled protein (Fig. 4). In two rabbits (Expts. 127 and 138) albumin and globulin pools were slightly larger when measured with ^{14}C-

labelled protein, the difference being confined mainly to the extravascular pool. As a result of this difference in pool size the daily replacement rate of albumin and globulin in Expts. 127 and 138 was slightly greater when measured with ^{14}C than with ^{131}I. In the remaining rabbit (Expt. 142) similar values for pool size and daily replacement rate of albumin and globulin were obtained with ^{131}I- and ^{14}C-labelled proteins.

DISCUSSION

The elimination rates of ^{131}I-labelled plasma proteins and the distribution between intra- and extra-vascular pools varied by about ± 20 % of the mean values in the rabbits studied in this investigation. A similar degree of variation occurred in four animals injected with the same iodinated plasma, suggesting that the wide range of values is attributable to differences among recipient animals rather than to differences between batches of labelled protein. Previous observations in healthy human subjects (Sterling, 1951) and in animals maintained under physiological conditions (Dixon, Talmage, Maurer & Deichmiller, 1952) have revealed similar individual differences in elimination rates of transfused proteins. However, this variation is small in comparison with the wide differences observed between species (Dixon *et al.* 1952).

Comparison between ^{131}I- and ^{14}C-labelled proteins reveals that closely similar plasma elimination

Table 5. *Distribution and turnover of* ^{131}I- *and* ^{14}C-*labelled plasma proteins in rabbits*

The ^{131}I experiments were performed 9–12 months after the ^{14}C experiments. For details concerning donor rabbits see text.

Rabbit no.	^{14}C-Plasma* protein	^{14}C-Albumin Distribution ratio	^{14}C-Albumin $t_{\frac{1}{2}}$ (hr.)	^{14}C-Globulin Distribution ratio	^{14}C-Globulin $t_{\frac{1}{2}}$ (hr.)	^{14}C-Fibrinogen $t_{\frac{1}{2}}$ (hr.)	^{131}I-Plasma protein Distribution ratio	^{131}I-Plasma protein $t_{\frac{1}{2}}$ (hr.)
61	1	2·97	160	—	170	55	—	—
60	1	3·25	160	—	165	75	1·92	170
50	2	2·74	190	3·14	165	68	2·18	205
47	3	3·25	170	3·64	140	65	2·40	185
55	3	2·96	208	3·70	157	85	2·45	215
51	4	—	200	—	—	—	2·00	202
Range		2·74–3·25	160–208	3·14–3·70	140–170	55–85	1·92–2·45	170–215
Mean		3·05	181	3·49	159	69	2·19	195

* 1, Fresh plasma from donor rabbit *B* injected immediately after dialysis; 2, fresh plasma from donor rabbit *A* injected immediately after dialysis; 3, plasma from donor rabbit *A* dialysed, filtered and stored at 3° for 5 weeks prior to injection; 4, plasma from donor rabbit *B*. Supernatant at 18 % Na_2SO_4 frozen-dried. Dried protein dissolved in saline for injection.

Table 6. *Distribution and turnover of* ^{14}C-*labelled serum proteins in rabbits*

Expt. 127: rabbit received serum from donor rabbit *A* dialysed, frozen-dried, and stored at room temp. for 15 months prior to injection. Expts. 138 and 142: rabbits received fresh serum from donor rabbit *C* injected immediately after dialysis. For details concerning donor rabbits and for methods of calculating equilibration time, S_{100} value, distribution ratio and turnover rate see text. These rabbits were simultaneously injected with ^{131}I-labelled plasma (see Table 2).

Expt. no.	Equilibration time (hr.)	S_{100}	Distribution ratio	Intravascular pool (g.)	Extravascular pool (g.)	$t_{\frac{1}{2}}$ (hr.)	Turnover rate (g./day)
				^{14}C-Albumin			
127	60	25·2	2·78	4·7	8·3	200	1·08
138	65	26·5	2·70	4·5	7·7	205	0·99
142	60	34·4	2·06	5·0	5·3	190	0·91
Mean	62	28·7	2·51	4·7	7·1	198	0·99
Mean ^{131}I for these experiments	58	30·9	2·27	4·9	6·2	211	0·87
				^{14}C-Globulin			
127	100	18·5	3·48	3·2	7·9	162	1·14
138	85	23·5	2·64	3·1	5·1	150	0·91
142	65	22·8	2·76	3·0	5·2	150	0·91
Mean	83	21·6	2·96	3·1	6·1	154	0·99
Mean ^{131}I for these experiments	83	24·7	2·78	3·3	5·0	152	0·92

curves are obtained with the two labels. In both instances, equilibration between intra- and extravascular pools was followed by an exponential rate of decay which remained constant during 4 weeks of observation. In rabbits which were injected with ^{14}C-labelled protein and received iodinated plasma after an interval of 9–12 months, half-lives were always slightly longer when measured with ^{131}I. This difference may have been associated with the age of the animals, since a similar discrepancy between labels was not consistently observed in three rabbits which received ^{131}I- and ^{14}C-plasma proteins simultaneously. In these animals the average biological half-lives obtained with the two labels differed by less than 2% with both albumin and globulin. The average half-life of fibrinogen estimated with ^{14}C and ^{131}I in two separate groups of rabbits agreed within 5%. In general albumin was eliminated more slowly than globulin and both much more slowly than fibrinogen.

Equilibration of labelled protein with the total exchangeable protein pool was regarded as being complete when plasma specific activity values attained the constant slope of the slow exponential decay curve. Estimated in this way the equilibration time of albumin labelled with ^{14}C or ^{131}I was 40–65 hr. Individual rabbits showed considerable variation in the equilibration times of their globulins (65–140 hr.), but results obtained with the two labels agreed closely. Similar results have been reported with ^{131}I-labelled proteins in man (Sterling, 1951; Myant, 1952; Berson *et al.* 1953). It appears that the overall equilibration time is considerably longer than that deduced by Wasserman & Mayerson (1951) from measurements of protein specific activity in plasma and thoracic duct lymph of dogs. This discrepancy suggests that plasma protein molecules do not penetrate all parts of the extravascular compartment at a uniform rate. Exchange with lymph which eventually drains into the thoracic duct appears to be relatively rapid, but expansion into other parts of the extravascular space is presumably slower. It is of interest in this connexion that in a rabbit which received ^{131}I-labelled plasma by injection into multiple subcutaneous sites, equilibration with plasma was only complete after about 130 hr. Since equilibration may take as long as 6 days, an accurate estimate of plasma protein elimination rate requires observation for at least 3–4 weeks. Where the experimental period is less than 2 weeks the estimated elimination rate as pointed out by Berson *et al.* (1953) is likely to be significantly faster than that obtained in experiments of longer duration.

When equilibration is complete, the mass of plasma protein in the extravascular pool is about 1·5 times that present in the circulation. In general, the proportion of injected globulin which passes into the extravascular pool is slightly greater than that of albumin and the distribution ratio of ^{14}C-labelled albumin and globulin is slightly greater than that of the corresponding iodinated fractions. In the case of fibrinogen no comparison between labels was made, but it is apparent (Tables 1 and 2) that the distribution ratio of iodinated fibrinogen was significantly lower than that of albumin or globulin.

In accordance with generally accepted practice the plasma protein pool size has been calculated in this study by extrapolation of the slow exponential decay curve to zero time. This procedure is based upon the assumption that, throughout the experiment, a constant proportion of the labelled protein molecules retained in the body is degraded per unit time. However, the proportion of labelled molecules broken down during the period of equilibration will be largely dependent upon the relationship of sites of plasma protein degradation to the intra- and extra-vascular pools. Thus, for example, if plasma protein catabolism is confined to molecules within the extravascular pool, a relatively low proportion of labelled molecules will be broken down during the initial hours after intravenous injection and extrapolation of the slow exponential decay curve to zero time will underestimate the actual pool size. Calculation of pool size from the ratio of plasma specific activity at a given time to the dose retained at the same time avoids this error, but cannot be employed for estimating the pool size of individual fractions after injecting whole plasma. It is apparent that the pool size and distribution ratio, as calculated here, may not have the same significance for plasma proteins which differ in regard to equilibration time and site and speed of catabolism; further investigation of this is in progress.

Measurements of pool sizes based upon the dilution of ^{14}C and ^{131}I did not differ by more than 8% in any of the three rabbits which were simultaneously injected with both kinds of labelled protein. The evidence suggests that ^{14}C tends to give slightly higher estimates with the difference confined to the extravascular compartment. Injection of pathological human serum or plasma iodinated by methods other than the jet procedure gave estimates of albumin and globulin pool size up to four times as great as those observed with jet-iodinated or ^{14}C-labelled protein. It has been suggested that this error which is recognized to be due to the presence of rapidly degraded molecules may be avoided by calculating pool sizes from the ratio of plasma specific activity at a given time to the dose retained at the same time (Myant, 1952; Berson *et al.* 1953; Schwartz, 1955). The validity of this procedure is doubtful, however, since it is by

no means certain that all denatured molecules removed from the exchangeable plasma protein pool are promptly catabolized and their attached ^{131}I is quantitatively excreted. Dixon, Bukantz & Dammin (1951) have shown, for example, that iodinated γ-globulin coupled to diazotized *p*-aminobenzoic acid is rapidly removed from plasma, but the attached ^{131}I is retained in liver, spleen and lymph nodes for at least 10 days after administration.

An attempt has been made to detect the presence of rapidly catabolized components in the iodinated plasma used in this investigation by calculating: (*a*) the specific activity of plasma protein at 100 hr. expressed as a percentage of the specific activity at 2 min. after injection (S_{100} value); (*b*) the difference between the observed urinary excretion of ^{131}I during the initial 72 hr. and the predicted excretion (calculated from the dose injected and the slow exponential decay rate of plasma protein specific activity) expressed as a percentage of the dose administered.

In the case of jet-iodinated material the S_{100} values for albumin and globulin were within 5 % of those observed with ^{14}C-labelled serum in the same animals. The difference between the observed and predicted levels of ^{131}I urinary excretion during the initial 72 hr. was never more than 2 % of the dose. Pathological human serum and plasma iodinated by other methods gave S_{100} values for albumin and globulin considerably below the range observed with ^{14}C-labelled or jet-iodinated protein, while the urinary excretion at 72 hr. exceeded the predicted value by an amount equivalent to 9–18 % of the dose.

Only about 16 % of ^{131}I in jet-iodinated plasma is bound to globulin. For this reason the detection of rapidly degraded globulins is relatively inefficient when whole plasma or serum has been injected. Thus, for example, if 10 % of injected globulin molecules are denatured the globulin S_{100} value will be about 10 % below the expected figure; however, the maximum increment in urinary ^{131}I will be only 1·6 % of the initial dose so that the 72 hr. excretion value will remain within the range normally observed (Table 3) and differentiation between rapidly degraded globulin molecules and a true enlargement of the extravascular globulin pool will be impossible. The difficulty is illustrated by experiment 126 (Tables 2 and 3), in which it remains uncertain whether the unusually large globulin pool indicates the presence of denatured globulin molecules in the injected plasma. It appears that this difficulty can be overcome only by separate iodination of the globulin fraction.

Dixon & Talmage (1951) have provided evidence which suggests that ^{131}I detached during catabolism of homologous globulin is rapidly excreted by the rabbit and provides a direct measure of the rate of protein degradation. In rabbits used in this investigation 47 % of the injected ^{131}I had appeared in the urine when 50 % of the iodinated protein had been eliminated from the exchangeable pool. At this time, non-protein bound ^{131}I retained in the body accounted for almost all the remaining ^{131}I which had been detached from labelled protein, and the thyroid gland contained less than 0·1 % of the injected dose. The irregular daily excretion of ^{131}I may have resulted either from variations in renal function or from fluctuations in the rate of plasma protein catabolism; the former mechanism would account for the fact that urinary creatinine and ^{131}I values were frequently displaced in the same direction. As shown above, the urinary excretion of ^{131}I considered over 72 hr. periods showed an exponential decline with a half-life corresponding closely to that of plasma protein specific activity. Such agreement is to be expected in animals under physiological conditions. It is apparent, however, that the ability to make independent measurements of protein replacement and degradation rates by means of ^{131}I may be of great value in the investigation of factors concerned in the regulation of plasma protein metabolism.

The results of the present investigation differ from those of Berson *et al.* (1953), who observed that iodinated albumin administered to human subjects contained several components having different rates of decay. In addition, the present comparison between ^{131}I- and ^{14}C-labelled proteins contradicts the view put forward by Volwiler *et al.* (1954) and supported by Armstrong *et al.* (1954, 1955) that iodination results in sufficient denaturation of plasma protein to produce a biological half-life shorter than that of protein labelled by biosynthesis. The lack of agreement between results reported here and those of other recent investigations is probably attributable mainly to differences in the technique of iodination; in addition, it is possible that the use of pooled protein samples for iodination is undesirable in metabolic studies.

SUMMARY

1. Jet-iodinated plasma protein has been administered to fourteen adult male rabbits by intravenous injection. The elimination rate of ^{131}I-labelled protein and its distribution between intra- and extra-vascular pools showed considerable individual variation ($\pm$ 20 %). This is due more to animal variation than to differences between batches of labelled protein. Homologous and autogenous plasma proteins were indistinguishable in regard to distribution and rate of elimination.

2. Comparison of the fate of ^{131}I- and ^{14}C-labelled plasma proteins administered to the same

animals revealed that (*a*) equilibration times were closely similar, the equilibration of globulin (65–140 hr.) being generally slower than that of albumin (40–65 hr.). (*b*) Equilibration was followed by a decline in plasma specific activity which maintained a constant exponential rate during 3–4 weeks of observation. Biological half-lives of plasma proteins were closely similar when measured with ^{14}C and ^{131}I. In general, albumin (160–230 hr.) was eliminated more slowly than globulin (140–170 hr.) and both much more slowly than fibrinogen (52–85 hr.). (*c*) When equilibration was complete, the mass of protein in the extravascular compartment was about one and a half times that in the blood stream. Albumin had a lower extra-/intravascular distribution ratio than globulin, while that of fibrinogen was considerably less. The complex significance of these ratios is discussed.

3. The presence of denatured, rapidly eliminated components in the injected protein was associated with a high urinary output of ^{131}I in the first 72 hr. and with unusually low plasma specific activity values subsequently, the latter giving rise to incorrectly high estimates of pool size.

4. ^{131}I detached from plasma protein was rapidly and almost completely excreted in the urine and provided an accurate measure of the protein elimination rate. There was no detectable re-incorporation of ^{131}I into newly synthesized plasma protein. Retention of ^{131}I by the thyroid gland was negligible in animals receiving iodide in the drinking water.

5. It is concluded that rabbit plasma can be labelled with radioactive iodine in such a way as to provide data concerning the *in vivo* distribution and elimination of albumin and globulin closely similar to that obtained with the corresponding ^{14}C-labelled proteins.

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The Kinetics of Acid Hydrolysis of Dipeptides

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Hydrolysis by means of acid is the method most generally used for the degradation of proteins. Although all the peptide bonds in a protein molecule are susceptible to hydrolysis by acid, the rate of hydrolysis of a particular bond will depend in large measure upon its accessibility to the approach of hydrogen ions. Thus it is to be expected that the rate of hydrolysis of the different bonds along a peptide chain will be greatly influenced by the electrostatic and steric properties of the chain, as well as by the nature of the amino acids forming each particular bond. The cumulative effects of these and other factors in a long polypeptide chain will vary as hydrolysis proceeds, but it seems reasonable to assume, at least as a first approximation, that the stability to hydrolysis of each bond will depend mainly on the nature of its constituent amino acids, in such a way that the

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