The Nature and Rates of Excretion of Radioactive Breakdown Products of I^{131}-Albumin in the Rabbit

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ABSTRACT When I^{131}-albumin is given intravenously to rabbits, the radioactive breakdown products that are released into the plasma and urine can be extracted into acetone. Paper chromatography and paper electrophoresis show that about 80 per cent of these are I^{131}-iodide and the remainder are organic I^{131}-iodine compounds. When I^{131}-iodide is given to rabbits taking iodide in their drinking water, the radioactivity is quantitatively excreted, without being accumulated in the tissues and without becoming attached to the plasma proteins. The rate of excretion can be defined by a first order rate process with a rate constant, a, ranging between 1 and 3day^{-1}. The organic I^{131}-iodine compounds liberated during the metabolism of I^{131}-albumin can be closely matched by a mixture of the organic I^{131}-iodine compounds liberated during the metabolism of I^{131}-monoiodotyrosine, I^{131}-diiodotyrosine, and the amino acids released by digestion from I^{131}-albumin. These organic I^{131}-iodine compounds are not accumulated in the body and their radioactivity does not become attached to the plasma proteins. Their radioactivity is excreted as fast or faster than that of I^{131}-iodide, and, to a satisfactory approximation, the same equations describing the excretion of I^{131}-iodide with the same constants may be used for describing the excretion of the organic I^{131}-iodine. These results permit improved estimates of the distribution and catabolism of I^{131}-albumin.

INTRODUCTION

For the analysis reported subsequently (1) knowledge was required of the nature, and of the metabolic and excretory behavior, of the radioactive metabolic end products of I^{131}-albumin in the rabbit. Preparations of I^{131}-albumin were therefore made essentially by McFarlane's method (2), and injected intravenously, and it was found that about 80 per cent of the radioactive end products appearing in the plasma and urine was I^{131}-iodide, and the remainder compounds containing I^{131} in organic combination. In theory at least, the radioactive end products might be completely excreted, or partly excreted and partly retained, and the retained radioactivity might be

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either recycled through the plasma proteins or accumulated at other sites in the body, or both. The rate and completeness of excretion of I^{131}-iodide, administered intravenously, were therefore studied, and observations were made to determine whether there was recycling or accumulation of I^{131}-iodide. Since the organic iodine end products have not been completely characterized, a similar direct approach was not possible with them. Instead an indirect approach was adopted. The iodination of albumin leads to the formation of mono- or diiodotyrosine in the protein (3, 4). On the likely assumption that catabolism of I^{131}-albumin leads first to liberation of the contained amino acids and that these are then subsequently metabolized, the behavior of I^{131}-mono- and I^{131}-diiodotyrosine when given intravenously was studied. In case other labelled amino acids were also present in the protein, the behavior of the radioactive metabolites released when I^{131}-albumin was given by mouth, so that its contained amino acids were liberated by digestion, was also studied. In all the experiments, no evidence was obtained for recycling of I^{131} through plasma proteins or, under the experimental conditions used, for accumulation of I^{131} in the thyroid or elsewhere, and, within the experimental errors, the radioactive breakdown products were excreted completely, chiefly in the urine. To a sufficiently good approximation the rate of excretion of inorganic and organic I^{131} could be described as a first order process with a rate constant ranging from 1.5 to 3.5 day^{-1}.

Methods

Experiments were made on white New Zealand male rabbits weighing 2 to 3.5 kg. They were fed Purina Chow with weekly supplements of fresh greens and were housed in separate metabolism cages. Daily collections of the separated urine and feces were made, and at this time the cages were rinsed out with 100 ml. of 0.2 per cent KI solution and the rinsings were added to the urine. The urine was collected in a jar containing 10 ml. KI solution and 2 ml. of toluene. The feces were homogenized in a Waring blendor with about five times their volume of water and a few crystals of KI to give a thick even suspension that could be transferred by pipette. In all the experiments, to prevent accumulation of radioactivity in the thyroid and other tissues and to promote its excretion, the rabbits were given drinking water containing 200 mg. KI and 1.8 gm. NaCl per liter. They were handled with gentleness, since undue disturbance tended to cause irregular urinary excretion.

The I^{131}-iodide was obtained as carrier-free NaI from Oak Ridge, Tennessee, through the Abbott Laboratories, Chicago. The I^{131}-albumin was prepared essentially according to McFarlane's method (2) as described in (1) and was used within 3 days of preparation. I^{131}-*l*-monoiodotyrosine and I^{131}-*l*-diiodotyrosine were obtained from the Abbott Laboratories, Chicago. These amino acids on receipt had specific activities of 1 to 5 mc. per mg. and were used within 1 week of receipt. Chromatography showed negligible deterioration in this period. All measurements of radio-

activity were made in a well scintillation counter with a sensitivity of 10^6 counts per μc. I^{131} per minute.

The radioactivity was extracted from the urine and plasma by the following methods. Fresh urine, taken before the cages were washed out with KI solution, was adjusted to pH 5.0 with 6.0 N HCl, and was thoroughly shaken to remove dissolved CO_2. To 0.5 to 2 ml. of this in a 200 ml. Erlenmeyer flask were added traces (less than 1 mg.) of carrier NaI and ascorbic acid or sodium sulfite, and the water was immediately evaporated under reduced pressure at a temperature below 42°C. When the urinary solids were distributed as a thin even dry cake, these were shaken with 5 ml. of acetone-water (88 ml. acetone, 12 ml. of water), which was decanted. Two further extractions were made and the combined extracts were placed in a flask and evaporated to dryness at reduced pressure below 42°C. The residue from the extracts was then dissolved in a measured small volume of water, and this solution was used for paper electrophoresis, chromatography, and assay of radioactivity. Plasma was extracted as follows: to 0.2 ml. were added a trace of carrier NaI and ascorbic acid and about 1 mg. of diiodotyrosine. Then 0.5 to 1 ml. of acetone-water was added and the mixture was thoroughly shaken to give a fine precipitate of protein. After centrifuging at 3000 R.P.M. for 10 minutes the supernatant was separated and a second and sometimes a third extraction of the precipitate were made. The acetone extracts were then evaporated and the residues treated in the same way as those from the urine extracts.

PAPER ELECTROPHORESIS AND PAPER CHROMATOGRAPHY The filter paper, Schleicher and Schuell No. 507, was washed in 0.1 M citric acid and then rinsed repeatedly with distilled water.

(*a*) *Electrophoresis* This was performed in filter papers supported horizontally at 4°C., with two buffers of pH 5.0 and 10.0. The test solution was first applied to a small strip of filter paper 0.5 × 6 cm. This was then placed transversely between two long strips of filter paper, 7 × 45 cm., moistened with buffer, which were pressed firmly together in exact apposition. These two strips, with their enclosed application strip, were wrapped in a sheet of polyethylene lightly smeared with vaseline, and the resulting sandwich was placed between two glass plates 30 × 12.5 × 0.5 cm. that were clamped together with light pressure. The protruding ends of the filter papers were dipped into buffer contained in two pyrex baking dishes. The electrophoretic separation was made at 450 volts for 1 to 2 hours, and the free ends of the filter paper were then rapidly torn off flush with the glass plates and the filter paper strips were separated and hung horizontally in a current of air. When nearly dry they were lightly sprayed with the developing reagents and were then cut in transverse strips which were counted in a well scintillation counter. The acid buffer (pH 5.0) consisted of 14.8 ml. of 0.2 M acetic acid and 35.2 ml. of 0.2 M sodium acetate made to 100 ml. with water. The alkaline buffer (pH 10) consisted of 25 ml. of 0.2 M glycine and 16 ml. of 0.2 M NaOH made to 100 ml. with water. The developing reagents are described below.

(*b*) *Chromatography* 10 to 50 μl. of the test solution was placed in a narrow streak 2 cm. from the base of 15 × 10 cm. rectangles of filter paper. The long edges

of the rectangles were stapled together to form cylinders which were stood in pyrex cylinders, kept at room temperature in the dark. Ascending chromatography was used. The solvents were (1) *n*-butanol (78 volumes), glacial acetic acid (5 volumes), and water (17 volumes) (5); (2) *n*-butanol saturated with 2 N NH_4OH (5). The chromatograms were run for 6 to 10 hours till the solvent had almost reached the top of the paper, and they were then dried in a stream of air. They were developed with (1) 0.25 per cent palladous chloride solution in water, or (2) 0.4 per cent ninhydrin solution.

Electrophoresis generally separated the radioactivity into a fraction traveling 6 to 12 cm. from the origin associated with, or just ahead of, the inorganic iodide, and a fraction of "organic" iodine remaining close to the origin. Chromatography separated the radioactivity into well defined fractions, but there was some variation of the R_f of the fractions, probably because relatively crude extracts were examined and at times to get sufficient counts the papers were rather heavily loaded. In butanol-acetic acid there was (1) a fraction remaining close to the origin; (2) the iodide staining fraction; (3) a fraction, extending for a variable distance immediately ahead of the iodide; (4) a fraction, sometimes composite, traveling far ahead with an R_f ranging between 0.5 and 0.9, mean about 0.8. In butanol-ammonia there was (1) a fraction, sometimes composite, behind the iodide, (2) the iodide staining fraction, (3) a fraction immediately ahead of this, and (4) sometimes a fraction far ahead with a mean R_f of about 0.75. The results to be described will be given in terms of these fractions.

RESULTS

1. The Radioactive Breakdown Products of I^{131}-Albumin in the Plasma and Urine

Intravenous injections of I^{131}-albumin were given to a number of rabbits. Table I, section A, summarizes data on acetone extracts of the urine and plasma from one animal which is representative of the others; column 5 shows that 81 to 93 per cent of the radioactivity was extracted from the urine

FOOTNOTES TO TABLE I *facing page*

* El. Mean per cent obtained from electrophoresis at pH 5.0 and pH 10.0, expressed as per cent of counts on paper at end of electrophoresis.

‡ Ch. Mean per cent obtained from chromatography in butanol-acetic acid and butanol-ammonia, expressed as per cent on paper at end of chromatography.

§, ‖, ¶ Per cent of counts recovered on paper running behind iodide (Beh), immediately ahead of iodide (Im.Ah.), and far ahead of iodine (Far Ah.) during chromatography in the two solvent systems.

** Per cent of the total radioactivity in the urine or plasma extracted by acetone.

‡‡ Per cent of the total counts applied to the paper recovered *after* electrophoresis (El.) or chromatography (Ch.).

§§ This animal, BS, broke down I^{131}- albumin given by mouth more slowly than the others. Its values are indicated in parentheses.

‖ ‖ This animal, No. 1-20, showed a marked delay in excretion.

TABLE I

THE RADIOACTIVE BREAKDOWN PRODUCTS EXTRACTED FROM URINE AND PLASMA

	Time	(1) Per cent as iodide		(2) Per cent near origin	Per cent organic iodine						(5) Acetone** extract, per cent	(6) Paper‡‡ recovery, per cent	
					(3) Butanol-acetic acid			(4) Butanol-ammonia					
	Day	El.*	Ch.‡	El.*	Beh.§	Im. Ah.‖	Far Ah.¶	Beh.§	Im. Ah.‖	Far Ah.¶		El.*	Ch.‡
					A. After giving I^{131}-albumin by vein (1 rabbit)								
Urine	1	75	75	18	5	—	6	6	—	8	85	90	96
	2	85	78	8	4	6	7	6	5	6	86	86	91
	3	86	80	11	4	—	6	7	7	4	90	93	90
	5	88	88	10	3	—	6	5	5	—	93	84	90
	9	89	96	7	—	—	—	—	—	—	81	88	93
	12	88	90	10							89	76	78
Plasma	1	80	78	8	7	—	—						
	2	80	92	12	5	—	—						
	3	88	85	9	—	—	5						
	5	—	86	—	5	—	8						
					B. After giving I^{131}-albumin by mouth (4 rabbits)								
	Day												
Urine	1	88–95	90–92	0–10	—	—	5	4	—	—	96–98	93–100	90–100
		(30)§§	(35)	(60)	—	—	(30)	(5)	(10)	(45)			
	2	84–95	80–97	5–10	—	—	5–10	10	—	—	88–92	88	94–98
	Hrs.												
Plasma	2	90	95	5				—	4	—	73–96		94
		(70)§§	(60)	(30)	—	—	(25)						
	6	95	90	3	4	—	—	—	8	—	86–97		82
		—	(36)	—	—	(12)	(30)						
					C. After giving I^{131}-monoiodotyrosine by vein (4 rabbits)								
	Day												
Urine	1	70–80	75–83	10–20	5	—	12	—	6–9	—	108	75–85	90
	2	90	92	3–5	—	—	3	—	—	—	94	83–90	96
	Hrs.												
Plasma	2	88	80	5	15	5	—	—	12	—	90	80	98
	7	96	95	0	—	—	—	—	—	—	77–90	78	95
					D. After giving I^{131}-diiodotyrosine by vein (6 rabbits)								
	Day												
Urine	1	10–20	10–25	70–85	—	5–10	65–70	75–80	—	—	90–95	90–95	80–95
	2	30–80	37–84	18–65	—	0–10	14–45	3–60	—	—	85–100	80–100	90–100
	3‖‖	8	15	91	5	5	75				80	93	90
	4‖‖	89	90	8	—	—	—	10	—	—		95	91
	Hrs.												
Plasma	2.5	35	36–60	63	—	0–4	20–55	12–58	0–6	—	88		
	7.5	52–82	62–83	18–45	0–5	—	11–38	13–37	0–5	—	88		
	18.5‖‖	84	93	5	—	5	—	—	6	—	96		
	49‖‖	95	100	—	—	—	—				92		

and column 6, that 76 to 96 per cent of that placed on the paper for analysis was recovered.

Column 1 shows the percentages of the counts, recovered from the paper, running with inorganic iodide as demonstrated with palladous chloride. On the assumption that any counts lost during electrophoresis or chromatography were lost equally from all the I^{131}-containing fractions, *75 to over 90 per cent of the radioactivity in the urine extract ran with iodide.* Seventy-eight to 92 per cent of the radioactivity of the plasma extracts recovered from the paper ran with iodide. Table 1 A, columns 2, 3, and 4, summarize data (also shown as percentages of the counts recovered on the papers), on the "organic" iodine fractions. By electrophoresis 7 to 18 per cent of the radioactivity remained at or near the origin. Chromatography in butanol-acetic acid broke up this fraction into a portion remaining near the origin and a portion running far ahead with a mean R_f of about 0.8. In butanol-ammonia three fractions could be distinguished, one behind the iodide, one immediately ahead of the iodide, and one running far ahead with a mean R_f of about 0.75. Thus, on the assumption that counts lost during electrophoresis or chromatography are lost equally from all fractions, *7 to 18 per cent of the total radioactivity of the acetone extracts of plasma and urine consisted of I^{131} in organic combination.*

TABLE II

PERCENTAGES OF TOTAL DOSE OF RADIOACTIVITY GIVEN BY VEIN AS I^{131}-IODIDE FOUND AT POSTMORTEM EXAMINATION IN CERTAIN TISSUES IN EACH OF THREE RABBITS

Tissue	No. 1-02 4th day after injection, *per cent*	No. 1-09 4th day after injection, *per cent*	No. 1-08 6th day after injection, *per cent*
Thyroid	0.05	0.03	0.03
Liver	0.03	0.03	0.02
Kidneys	0.01	0.01	0.01
Spleen	0.01	0.01	0.01
Stomach	Neg.*	Neg.	Neg.
Small intestine	Neg.	Neg.	Neg.
Bladder	Neg.	Neg.	Neg.
Parotid	—	Neg.	Neg.
Sublingual	Neg.	Neg.	—
Submaxillary	Neg.	—	Neg

* Neg. = negligible.

2. *Experiments on Rabbits Given Intravenous I^{131}-Iodide*

A number of rabbits were given 30 to 50 μc. of I^{131}-iodide with 40 μg. carrier sodium iodide intravenously, serial blood samples were withdrawn, and the urine and feces were collected and analyzed daily. The acetone method extracted 85 to 100 per cent of the radioactivity from the plasma and urine,

and paper electrophoresis and chromatography showed that at least 90 per cent of this ran with inorganic iodide. When allowance was made for losses from the paper, essentially all the radioactivity ran with iodide. The observations on the plasma strongly suggested that the I^{131} did not become fixed to the plasma proteins and other observations confirmed this. Thus, on many occasions at various intervals after giving I^{131}-iodide by mouth or vein, the plasma proteins were sampled, were precipitated by trichloracetic acid, and the quantity of I^{131} bound to them was determined. On no occasion was a significant fraction found attached to them. Table II summarizes observations made to determine whether under our experimental conditions I^{131} was accumulated by any rabbit tissue. Three rabbits were autopsied 4 to 6 days after receiving I^{131}-iodide, and even in the thyroid, which of the tissues examined contained the most radioactivity, the proportion of the total dose retained was negligible.

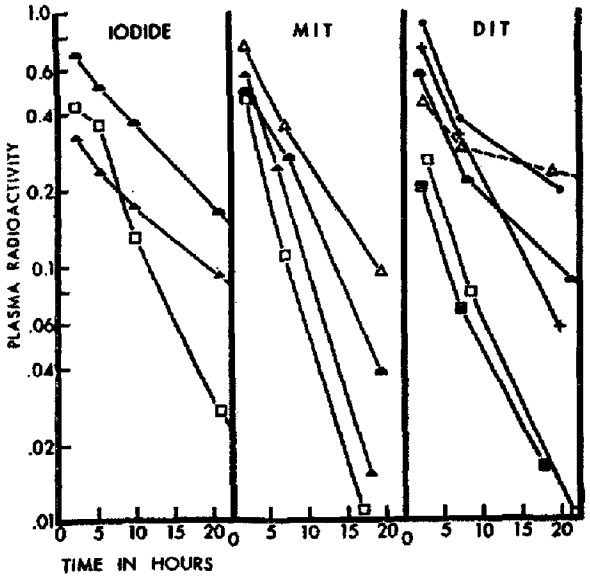

FIGURE 1. The disappearance of radioactivity from the plasma of rabbits after intravenous injection of I^{131}-iodide, I^{131}-monoiodotyrosine (MIT), and I^{131}-diiodotyrosine (DIT). The symbols △---△ show the data from rabbit 1-20, which excreted no urine for the first 2 days of the experiment.

Fig. 1 displays the results of three experiments in which the rate of disappearance of the I^{131}-iodide from the plasma was followed, and shows that when the logarithms of the counts of plasma radioactivity are plotted against time, most of the points for each animal fall close to a straight line. The points may therefore be described fairly well by the equation, $y = y_0 e^{-at}$, (compare

(6)), in which y is the plasma concentration of I^{131} at any time t, y_0 is the concentration obtained by extrapolation, at t_0, the time of injection, and a is the plasma iodide "removal" constant. When t is expressed in days a ranges from 1.5 to 3.5. When the total counts injected are divided by y_0, the counts in 1 ml. of plasma, a volume of distribution, termed the "iodide space," is obtained (6). In the three animals shown this ranged from 670 to 930 ml. and was seven to nine times the plasma volume.

Table III records the radioactivity excreted daily in the urine and feces, expressed as a percentage of that given, for seven rabbits. In all seven animals the major part was excreted by the end of the 2nd day, and excretion was

TABLE III

THE DAILY EXCRETION OF I^{131} IN THE URINE AND FECES IN SEVEN RABBITS GIVEN INTRAVENOUS I^{131}-IODIDE

The quantities excreted are expressed as percentages of the dose given

Day	No. 1-09		No. 1-02 No. 1-08		No. 28		No. 1-00		No. 8*		No. 22*	
	U	F	U	F	U	F	U	F	U	F	U	F
1	70	2	72	1.8	0.4	0.1	30.5	3.8	64.2	13.8	35.3	21
2	24	0.7	13.8	0.5	87.5	1.5	50.7	2.5	11.5	0.5	20.1	1.5
3	1.2	0.2	3.5	0.1	1.0	0.1	4.2	1.0	0.9	0.1	6.8	0.7
4			1.0	0.2	1.0	0.1	0.4	0.2	0.4	0.1	2.8	0.6
5					0.3	0.1	0.3	0.1	0.3	—	0.8	0.2
6									0.2	—	0.1	0.1
7									0.2	—	0.4	0.1
Totals	95.2	2.9	90.3	2.6	90.2	1.9	86.1	7.6	77.7	14.5	66.3	24.2
Combined	98.1		92.9		92.1		93.7		92.2		90.5	

U, urine. F = feces. * These animals were housed in unsatisfactory cages.

negligible after the 4th day. Recovery of the radioactivity given ranged from 90.5 to 98.1 and averaged close to 93 per cent. In control experiments, in which a measured amount of I^{131}-iodide solution was placed in metabolism cages containing uninjected rabbits, 94 to 98 per cent was recovered with an average of 95 per cent. Thus, when I^{131}-iodide is injected into rabbits under our experimental conditions, essentially all the radioactive iodide is eliminated. In five rabbits 1.9 to 7.6 per cent of the radioactivity was in the feces, but in the other two, 14.5 and 24.2 per cent. For these two animals an unsatisfactory form of cage was used, and the feces were heavily contaminated with urine.

The rate of removal of I^{131}-iodide from the plasma can be related to its rate of excretion on the assumption that under our experimental conditions the I^{131}-iodide leaving the plasma is excreted instantaneously and 95 per cent

of that excreted can be recovered. Then defining the total dose of radioactivity administered as 1, the radioactivity, u, appearing in the urine between t_0 and t is given by $u = 0.95\ (1 - e^{-at})$. Fig. 2 shows two curves plotted for this equation, one for $a = 1$ day^{-1}, the second for $a = 3$ day^{-1}. (These values rather than the values of 1.5 and 3.5 determined from the plasma data were chosen for ease in computation in the subsequent paper; the changes made no material difference.) Also plotted are the data of Table III, most of which lie within or close to the shaded area between the two curves. Thus it appears that the excretion of I^{131}-iodide can be described

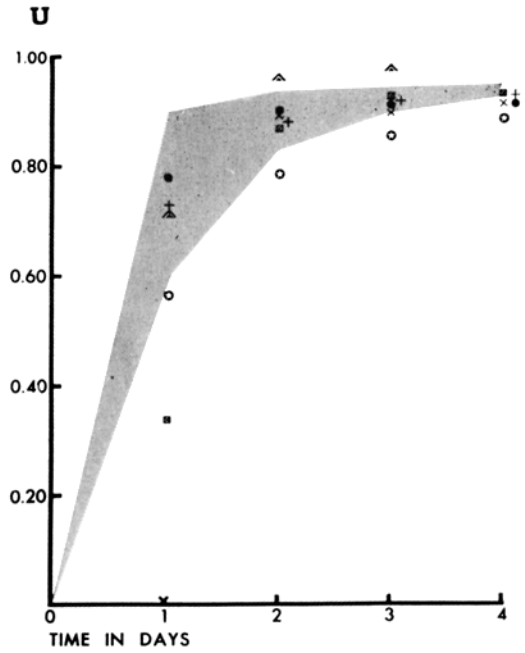

FIGURE 2. The measured cumulative excretions of I^{131}-iodide, following a single intravenous injection, are compared with the predicted excretions. The cumulative excretion of radioactivity, u, is plotted against time. The predicted excretions were calculated from the equation $u = 0.95(1 - e^{-at})$, in which 1.00 was defined as the total dose of radioactivity given (see text). Two values were used for a, namely $a = 3$ day^{-1}; the curve given with this value is the top edge of the shaded area, and $a = 1$ day^{-1}; the curve given with this value is the bottom edge of the shaded area. It is seen that the area between the two curves (the shaded area) described fairly well the observed values.

reasonably well by a first order rate constant, a, ranging in value from 1 to 3 day^{-1}, and acting on a quantity of iodide roughly defined at any time, t, by the product of the iodide space and the plasma iodide concentration.

3. Observations on Rabbits Given I^{131}-Iodotyrosines by Vein

(A) MONOIODOTYROSINE (MIT) Four rabbits were given 20 to 30 μg. of I^{131}-labelled l-monoiodotyrosine. Table I, section C, shows that in the plasma by 2 hours after injection over 80 per cent and by 7 hours after injection, over 90 per cent of the radioactivity ran with inorganic iodide. In the 1st day's urine 70 to 80 per cent of the radioactivity ran with the inorganic iodide,

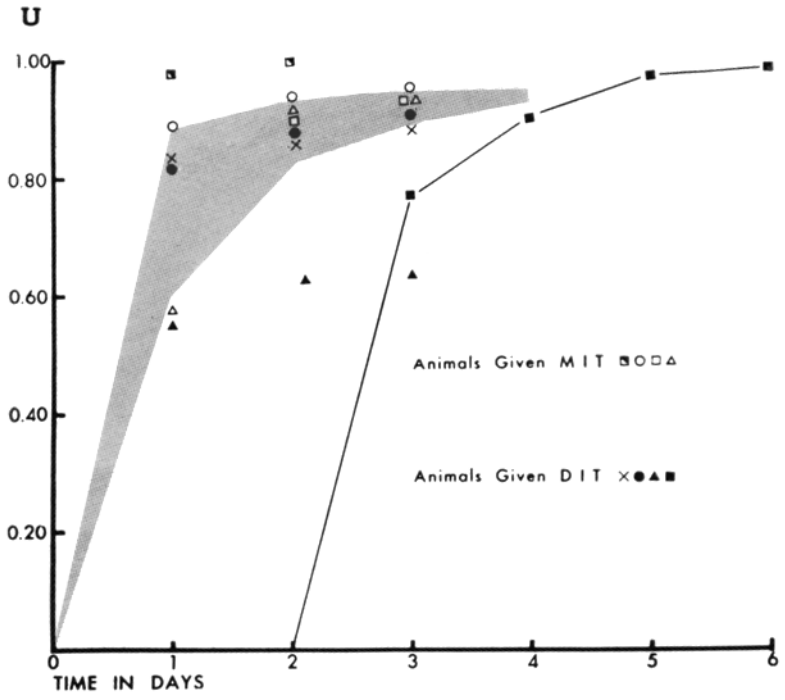

FIGURE 3. The measured cumulative excretions of radioactivity after single intravenous injections of I^{131}-mono- and I^{131}-diiodotyrosine, compared with those predicted for intravenous I^{131}-iodide. The shaded area is calculated in the same way as for Fig. 2. The symbols ■——■ are the data from 1-20, the rabbit that failed to excrete urine for the first 2 days.

but 10 to 20 per cent remained at the origin during electrophoresis, and chromatography showed at least two organic iodine compounds to be present. By the 2nd day almost all the radioactivity behaved as inorganic iodide, but by this time most had been excreted (Fig. 3). Examination of the plasma during the course of these experiments showed no binding of radioactivity to the proteins and 4 days after the injections showed no radioactivity remaining, and thus no labelling of the plasma proteins by MIT or its metabolites.

Measurement of the plasma removal constant, a, by the same methods used for iodide gave values of 3.0 to 5.6 day^{-1}. The MIT space, calculated in the same way as was the iodide space, was approximately ten times the plasma volume. The disappearance rates from the plasma and the excretory rates are shown in Figs. 1 and 3, where it is seen that these are comparable to or faster than those shown by I^{131}-iodide. Excretion of radioactivity was just as complete as with I^{131}-iodide. From 1 to 7 per cent (average about 3 per cent) of the total radioactivity excreted was in the feces.

(B) DIIODOTYROSINE (DIT) Four rabbits were given I^{131}-DIT with carrier DIT totalling 2 mg. per rabbit and two rabbits were given a total of 20 μg. each. Each rabbit received 50 to 150 μc. of I^{131}. The rabbits given the small amounts of DIT showed no essential difference from those given the larger amounts. Table I, section D, shows that initially only 30 to 40 per cent of the radioactivity in the plasma behaved as inorganic iodide, though this percentage progressively increased with time till almost all the radioactivity behaved as inorganic iodide. In the 1st day's urine only 10 to 25 per cent of the extracted radioactivity behaved as iodide but in subsequent days the proportion increased to 80 to 90 per cent. In the extracts from both plasma and urine, initially a large fraction remained close to the origin during electrophoresis, and this was separated by chromatography into a *major* portion running far ahead in butanol-acetic acid and remaining behind the iodide in butanol-ammonia, and a *minor* portion running just ahead of the iodide in butanol-acetic acid. Examination of the plasma proteins during the experiment showed no significant binding of radioactivity to them. Fig. 1 shows the disappearance rates of DIT from the plasma. In three instances these are clearly not linear. Excluding rabbit 1-20, shown by the triangles and dashes, which is dealt with below, the results suggest a more rapid disappearance rate early when there is a considerable proportion of organic iodine present and a slower loss rate later as the DIT is broken down to inorganic iodide, and the observations on the urine suggest that the organic iodine fractions are more rapidly excreted than the inorganic iodide. If relatively straight sections of the graphs of the disappearance of radioactivity from the plasma are taken, a ranges between 1.7 and 5.2 day^{-1}, with a mean of about 3. Calculation of the diiodotyrosine space is uncertain because of the uncertainty of extrapolation but it ranges between fifteen and twenty times the plasma volume. Fig. 3 shows that excluding one rabbit, in which it is believed the 2nd day's collection was incomplete, excretion was as rapid and complete as in the intravenous iodide experiments, so that it appears that in spite of the lack of linearity in the plasma data the excretion of the radioactive breakdown products of DIT can be satisfactorily described by the same equation and constants describing iodide excretion. In five of the six animals 88 to 98 per cent of the

radioactivity given was collected in the excreta of which 0.5 to 3 per cent was in the feces.

The behavior of rabbit 1-20 noted in Table I and Figs. 1 and 3 was of interest. This rabbit after receiving DIT excreted no urine for 2 days during which time the disappearance rate of radioactivity from the plasma (Fig. 1) was much reduced. At the end of this time (Fig. 3) excretion of radioactivity started and was 98 per cent complete 2 days later. During the first 2 days of the experiment the plasma radioactivity was maintained at high levels but there was no demonstrable combination with plasma protein.

4. Observations on Rabbits Given I^{131}-Albumin by Stomach Tube

Seven rabbits were given 50 to 100 mg. of rabbit albumin labelled with 20 to 50 μc. I^{131}. Table I, section B, shows that in three of four animals so studied

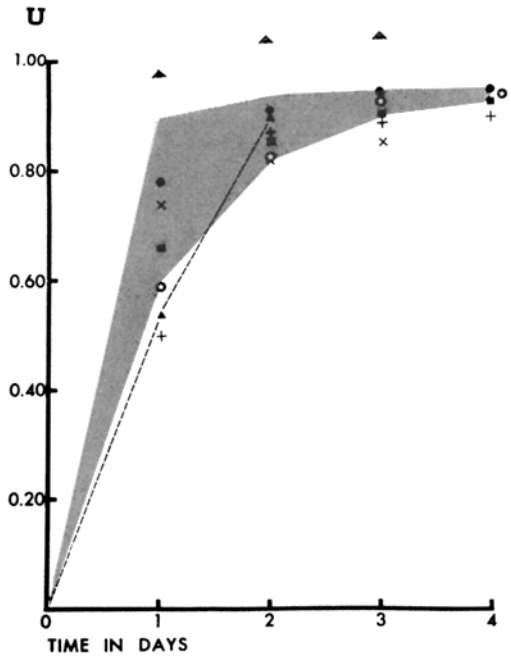

FIGURE 4. The measured cumulative excretions of radioactivity after I^{131}-albumin was given as a single dose by stomach tube, compared with the rates predicted for intravenous I^{131}-iodide. The shaded area is calculated in the same way as for Fig. 2. The symbols ▲---▲ are the data from rabbit BS (see Results, section 4).

the radioactivity in the plasma and urine was predominantly iodide. With these animals 10 per cent or less of the counts remained at the origin during electrophoresis, and small amounts of a compound running far ahead of the

iodide in butanol-acetic acid and behind or just ahead in butanol-ammonia could be distinguished. Rabbit BS, whose results are given in parentheses, showed a slower breakdown to inorganic iodide. Two samples of plasma withdrawn during the 1st day showed only 36 to 70 per cent of the radioactivity running with iodide, and the 1st day's urine contained only 30 to 35 per cent in the form of iodide. Chromatography of these samples showed a fraction running far ahead in butanol-acetic acid and three organic iodine fractions in butanol-ammonia. By the 2nd day the radioactivity excreted in the urine of this animal was 85 per cent or more iodide and the organic iodine fractions were similar to those in the urine of the other animals. Examination of the plasma of all the rabbits gave no evidence of labelling of the plasma proteins by I^{131}. The excretions of radioactivity, including that of rabbit BS (the triangles linked by the dashed line) are shown in Fig. 4 and compared with those calculated for iodide. As would be expected from the high proportion of iodide excreted there is a striking similarity, but even with rabbit BS which excreted much radioactivity as organic iodine, there is no evidence of reduced or delayed excretion. The proportion of the total counts excreted in the feces ranged from 5 to 8 per cent.

DISCUSSION

1. Technical Methods

For studying the I^{131}-metabolites of iodinated amino acids most workers (*e.g.* 5, 7, 8) have analyzed the body fluids directly or extracted them with butanol at pH 1.0. Rabbits excrete an alkaline urine of high salt content so that we preferred to extract the radioactivity, but substituted acetone for butanol. If the urine is brought to pH 5.0, evaporated under reduced pressure at temperatures below 42°C. to give a thinly spread residue, and is extracted with 88 per cent acetone, about 90 per cent of the radioactivity can be extracted leaving much insoluble material behind. Plasma can be extracted, without drying, with 4 to 5 volumes of acetone. The acetone extracts iodide, MIT, DIT, a number of unidentified iodinated compounds, and certain thyronines. Since there is always the risk that the more fragile organic iodine compounds may be altered while standing before extraction or during the extraction process, all extracts of plasma were made shortly after withdrawing the blood samples. Control observations in which MIT and DIT were placed in urine containers and the urine from a control rabbit was collected as usual overnight showed no change in the chromatographic behavior of these compounds. Iodine readily exchanges with iodotyrosines (7, 9), and to prevent this traces of Na_2SO_3 or ascorbic acid were added prior to extraction (9).

Comparison of measurements made with and without the reducing agents yielded no evidence that these caused the appearance of compounds not initially present, or materially altered the relative concentrations of the radioactive compounds present.

2. *The Catabolism of Plasma Proteins and the Nature of the Radioactive Breakdown Products of I^{131}-Albumin*

Little is at present known of the routes by which plasma proteins are broken down, though it has been claimed that two sites of isotope-labelled albumin catabolism are the liver (10, 11) and the kidneys (11). It has not yet been demonstrated whether the final breakdown products are polypeptides or amino acids, but there is much evidence according to Goldsworthy and Volwiler (12) in favor of the latter view. Assuming that during I^{131}-albumin catabolism the constituent amino acids are liberated, then the contained iodinated amino acids will also be liberated and metabolized; and if MIT and DIT are the main radioactive amino acids, then the metabolic end products of these should make up the major part of the radioactive end products formed during I^{131}-albumin catabolism.

Our chromatographic observations suggest that this is true, and that 90 to 95 per cent of the radioactive end products appearing when I^{131}-albumin is given by vein are those that would be released from a mixture of MIT and DIT. The remainder, consisting of organic iodine not detected during the metabolism of MIT and DIT, can be roughly matched by the radioactive end products released when I^{131}-albumin is given by mouth, provided deiodination is not too rapid.

Since about four-fifths of the radioactive end products of I^{131}-albumin given by vein are I^{131}-iodide, deiodination of a large proportion of the organic iodine compounds released during catabolism occurs rapidly. This is true for MIT, for after this is given only 5 to 15 per cent of the total radioactivity is excreted in organic combination. In this respect the rabbit behaves like man (13). It is not true for DIT, for after administration as much as 50 per cent of the radioactivity may be excreted in organic combination. Both man (13) and the rat (14, 15) appear to deiodinate DIT and its breakdown products more rapidly than our rabbits. For the most part the organic iodine compounds excreted are not defined by our observations. However, we do find that very little if any unchanged MIT or DIT is excreted when these are given by vein or I^{131}-albumin is given by vein or mouth. One organic iodine compound that is liberated in considerable amount after feeding DIT is 4-hydroxy-3,5-diiodophenyl lactic acid (DIPL) (16), and this runs far ahead in butanol-acetic acid (15, 17). Thus it is probable that DIPL accounts for

the major part of the organic iodine running far ahead in butanol-acetic acid in the urine of the rabbits given DIT. It may well also be responsible for the radioactivity behaving in the same way after I^{131}-albumin was given intravenously, which made up 5 to 10 per cent of the total excreted radioactivity.

3. Retention and Recycling of Labelled Breakdown Products

Our observations show clearly that there is no retention of inorganic iodide in these animals, and the measurements of excretion indicate that this is also true for organic iodine. We find no evidence for recycling of inorganic or organic iodine through unlabelled protein. These findings agree with those of others (18).

4. The Rate of Excretion of Breakdown Products

Definition of this requires the determination of the nature of the rate process and the rate constants. It has been shown (6, 19) that the disappearance of injected iodide from the plasma can reasonably well be described by an equation of the type $y = y_0 e^{-at}$, and, provided that this removal is mainly due to removal by a single organ (the kidneys), the excretion, u, will be defined by $u = F(1 - e^{-at})$, in which F is a fraction expressing the efficiency of recovery of radioactivity, and here averages 0.95. The removal of iodide from the plasma in our animals behaves as a first order reaction, but the rate constant, a, shows considerable variation ranging from 1.5 to 3.5 day^{-1}. Rabbits are well known to show variable excretory behavior (20) and this probably in part explains the variation in a. At all events the excretion of radioactive iodide in the urine and feces is reasonably well described by the space enclosed between the two curves $u = 0.95(1 - e^{-a_1 t})$ and $u = 0.95\ (1 - e^{-a_2 t})$ when a_1 is 1.0 and a_2 is 3.0. When MIT and DIT are given intravenously a mixture of iodide and organic iodine compounds appears in the plasma and it is difficult to define the rate processes governing the removal of organic iodine from the plasma and its excretion. However, the pattern of removal of organic iodine from the plasma and its excretion into the urine suggests that the organic iodine compounds are excreted even more rapidly than iodide. Further the observation that the excretion of radioactivity after giving I^{131}–albumin by mouth, or MIT or DIT by vein can be described reasonably well by the same equation and rate constants used for iodide even when considerable quantities of organic iodine are present, shows that the excretion of organic iodine can be sufficiently well described by assuming that it be-

haves as inorganic iodide. This was true even when 2 mg. doses of DIT were given, which is 50 to 100 times the maximum quantity of DIT liberated daily from an injected dose of 100 mg. of I^{131}-albumin.

5. Space Occupied by I^{131}-Albumin Breakdown Products

In the following paper (1) it is necessary to have an approximate value of the I^{131}-albumin breakdown products "space." Our observations show that, as might be expected, the iodide and MIT spaces do not differ greatly. However the DIT space may be twice the size of the iodide space suggesting considerable penetration of DIT into cells. Fortunately, as will subsequently appear, no great precision is required for the estimate of the I^{131}-albumin breakdown products space, and since our analyses suggest that less than 10 per cent of the total breakdown products are DIT or metabolites of DIT, the use of the iodide space for the total breakdown products space seems justified.

It will be seen (1) that the observations recorded here aid considerably in the mathematical analysis of the behavior of I^{131}-albumin in the animal body. This analysis is essential for calculation of the quantities of the animal's unlabelled albumin broken down daily, and exchanged daily between the plasma and extravascular fluids. The work recorded here gives such calculations greater reliability and precision than was previously possible.

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