The Kinetics of the Distribution and Breakdown of I^{131}-Albumin in the Rabbit

Observations on several mathematical descriptions

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ABSTRACT Rabbit plasma albumin was labelled with I^{131}, injected intravenously, and measurements were made of the radioactivity in plasma, urine, and feces over many days. In some experiments plasma radioactivity was fractionated into I^{131}-albumin activity and that of labelled breakdown products. Curves of these radioactivities were compared with corresponding curves predicted by four mathematical models. Each model included a vascular and extravascular albumin compartment in transfer equilibrium, a radioactive breakdown products compartment, and an excretion compartment; but model A supposed I^{131}-albumin catabolism to occur within the vascular system, model B within the extravascular compartment, model C within both, and model D within a separate compartment receiving albumin for catabolism from the plasma. The experimental data were reasonably well predicted by models A and C. However, model D, though data were insufficient for its complete validation, gave the best predictions and agrees with present knowledge of albumin catabolism.

Various methods for calculating the rate of albumin breakdown are discussed. When calculations are based solely on the plasma radioactivity data, identical rates are predicted by models A, C, and D. When, as a valuable independent method, catabolism is calculated from plasma *and* excreted radioactivities, an error (ordinarily small) is incurred unless account is taken of the rate of passage of I^{131}-albumin to the breakdown sites, and of the rate of excretion of the radioactive breakdown products.

For the measurement of the rates of exchange of albumin between the plasma and tissue fluids, and the rate of breakdown of plasma albumin, a first requirement is a satisfactory tracer-labelled albumin. A number of labels such as C^{14} (1), N^{15} (2), S^{35} (3), and I^{131} (4), have been used, but there has been doubt whether certain of these tracer-labelled preparations, and particularly those incorporating I^{131} (5), behave identically as does the animal's unlabelled

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albumin. However, McFarlane and coworkers (6–9) have shown recently that if sufficient precautions are taken, plasma proteins can be labelled with I^{131} without altering their behavior in the experimental animal,—at least as judged by the almost identical behavior of proteins "biologically" labelled with C^{14}. A second requirement is a satisfactory mathematical description of the behavior of the tracer-labelled albumin, for the rates of exchange and breakdown calculated depend on the mathematical formulation used. A number of mathematical formulations are possible, and the only basis of choice between them is (*a*) whether the formulation is physiologically reasonable and, more particularly, (*b*) whether it predicts quantitatively the experimental measurements that can be made. For the test of possible mathematical formulations of the behavior of albumin in the body, I^{131}-albumin has a number of advantages. The behavior of the tracer in the plasma after intravenous injection is easily followed, and is not made more complex by recycling of released radioactivity into the plasma proteins (10). Further, radioactive metabolic end products are released which can be measured in the plasma, and which are excreted quantitatively at a known rate (10). This behavior offers opportunity for careful test of mathematical formulations which define not only the behavior of the I^{131}-labelled albumin in the plasma, but also the behavior of the I^{131} breakdown products in the plasma and excreta.

A very simple mathematical description of the time course of disappearance of plasma proteins labelled with I^{131} was first used by Sterling (4) and Myant (11). This was extended and improved by Berson and his collaborators (12, 13). Campbell *et al.* (8) suggested a much improved mathematical description which has recently been fully detailed by Matthews (14). Reviews of previous mathematical formulations are given by McFarlane (15) and Berson and Yalow (16). Here we have defined a further number of the simpler mathematical models which include that of Campbell *et al.* (8), that might describe the behavior of the injected I^{131}-labelled albumin and its radioactive metabolites and have tested them against our experimental data. All these can reasonably be eliminated except one, which can then be further interpreted in terms of current knowledge of I^{131}-albumin metabolism. Our work extends that of Campbell *et al.* (8) and Matthews (14) and puts it on a firmer basis.

In what follows are described first, the labelling without denaturation of fairly pure unaltered rabbit plasma albumin by McFarlane's method (6); second, its injection and the obtaining of samples of blood, urine, and feces, and the method of making measurements on these samples; third, a description of the results obtained from these measurements; fourth, a description of possible kinetic models with the differential equations describing them; fifth, a comparison of the results with the predictions of the various models, and

the choice of the model that gives the best agreement; sixth, the utilization of appropriate models to calculate the albumin fluxes, that is, the albumin transfer and breakdown rates; seventh, methods for calculating the total quantity of extravascular albumin; eighth, an alternative method for calculating the rate of breakdown of albumin; and finally a discussion of the "meaning" of the mathematical models.

Methods

The animals were 2.2 to 3.7 kg. New Zealand male rabbits, mostly 6 months to 1 year old, housed in individual metabolism cages, and fed Purina chow and fresh greens. Food and water were given *ad libitum*; for 3 days before and during the experiment, the drinking water contained 200 mg. KI and 1.8 gm. NaCl per liter. Collections of urine and feces were made as described previously (10). The animals were handled gently at all times (10).

PREPARATION OF I^{131}-ALBUMIN The method of I^{131} labelling was that of McFarlane (6), with two modifications. The first of these was removal of the Na_2SO_3, which interferes with iodination, from the carrier-free NaI^{131} obtained from Oak Ridge. This was accomplished by mixing 1 or 2 drops of 1 N HCl with the amount of NaI^{131} required for iodination, and heating in a boiling water bath for no longer than 2 to 5 minutes (17). The evolved SO_2 is removed in a stream of air, without significant loss of I^{131}. The second modification was prompted by the lack of flexibility of McFarlane's method of jet iodination. Two other systems were used and found to be satisfactory. In the first, the I^{131} solution was rapidly forced through polyethylene tubing into the buffered protein, while this was briefly mechanically shaken. In the second method reduced pressure in a closed system was used to suck the I^{131} solution in a jet through the albumin solution in the closed system.

The procedure for albumin separation and iodination was as follows: Heparinized plasma from about 12 ml. of blood from a fasting donor rabbit was mixed with an equal volume of ammonium sulfate solution (saturated at 26 to 30°C.), left overnight at 4°C., and then centrifuged in the cold until the supernatant was clear. The supernatant was then dialyzed in a rocking dialyzer in the cold against 0.2 per cent NaCl solution. (By paper electrophoresis the protein in the resulting preparation was 90 to 95 per cent albumin with the remainder α_1-globulin and a trace of β-globulin.) Six to 12 ml. of this dialyzed solution (containing 90 to 180 mg. protein) was reduced to about 3 ml. by blowing air with a fan on the dialyzing bag; this was buffered to pH 9.1 with 1.5 to 2.0 ml. of McFarlane's pH 9.3 glycine buffer (6) and then iodinated by rapid mixing with an iodine solution containing approximately 0.5 mg. free iodine and from 200 to 700 μc. I^{131}. The latter mixture was prepared at ice water temperature by adding to the requisite amount of sulfite-free NaI^{131}, some 0.10 to 0.20 ml. of McFarlane's iodide-iodate solution (6). After the protein and iodine had reacted from 1 to 5 minutes, about 5 mg. of carrier NaI solution was added and the mixture was passed through an amberlite IR4B resin column (6) and then dialyzed for 8 to 16 hours against 0.9 per cent NaCl at 4°C. Usually about 20

per cent of the I^{131} was bound to the protein. The final preparations had albumin concentrations of about 2 per cent and contained 0.3 to 3.0 μc. I^{131} per mg. protein. More than 98 per cent of the iodine was protein-bound, as shown by precipitation with trichloroacetic acid in the cold, and the ratio of iodine atoms to protein molecules ranged between 0.5 and 1.0. Paper electrophoresis revealed that the radioactivity was 90 to 95 per cent in albumin, with the remainder in α_1-globulin and none in β-globulin.

INJECTION, SAMPLING, AND MEASUREMENT Two to 5 ml. of the above preparations was injected, usually within 16 hours and always within 36 hours of iodination, into the marginal ear veins of rabbits. The quantity injected was determined from the weight of the syringes before and after filling, less the residual activity washed out of the syringes by 0.1 N NaOH. Triplicate standards were prepared by diluting portions of the I^{131}-albumin solution with 1 per cent NaCl mixed with non-radioactive plasma (18). Following injection, heparinized blood samples of about 3 ml. were withdrawn at accurately noted times at approximately 10 minutes, 12 hours, 24 to 36 hours, and at 1 or more day intervals. Some or all the following measurements were made: hematocrit values, hemoglobin concentration, total plasma protein and plasma albumin concentrations, and the radioactivities of whole plasma, plasma albumin, and of the protein-free plasma. Protein concentrations were measured by the biuret or micro-Kjeldahl procedures; both methods showed close agreement. The specific activity of plasma albumin was measured in two ways. In the first the albumin activity was taken as that of the whole plasma and the albumin concentration was measured as follows:—0.2 ml. of plasma was mixed with 2 ml. of a sodium sulfate–sodium sulfite solution (19) in a test tube, incubated 30 ± 2 minutes at 37°C., cooled to 31 ± 1°C. in a water bath, 0.4 ml. of ether was added and brought to the same temperature, and the tube was then stoppered and gently inverted 15 times without shaking; finally the tube was spun for 10 minutes in a centrifuge warmed to about 30°C., the clear subnatant fluid was removed with a capillary pipette, and analyzed for protein. This method gave consistent results which closely approximated albumin concentration. In the second method albumin was first separated and then both the radioactivity and albumin content of the separated solution were determined. To do this 1 volume of plasma was treated with 3 volumes of 2.43 M Na_2SO_4 (kept in solution by storing at 37°C.) and the mixture was incubated overnight at 37°C. The next morning the mixture was brought to 31 ± 1°C., treated with ether, and centrifuged as described for the first method. The subnatant was then analyzed for radioactivity and albumin content. Specific activities were expressed as counts per milligram albumin. Both methods showed close agreement but method 1 gave the more consistent results. The radioactivity not firmly bound to protein was determined from either the supernatant of plasma treated with 5 to 10 volumes of 6 per cent trichloroacetic acid at 4°C., or from the acetone extract (10). The activity in urine and feces was measured as described elsewhere (10). Measured activities were corrected for decay by reference to the standards, and the excreted activity by 1/0.95 for loss of counts in collection when both urine and feces were collected (10) and by 1/0.90 when the urine only was collected (see later). No correction was made for increase in body weight, although in a few rabbits this increased

by 5 to 10 per cent. Cohen's correction (7) was made for the removal of radioactivity with each plasma sample, *i.e.* each specific activity was multiplied by $250/250 - 2(n - 1)$ in which n is the sample number, on the reasonable assumption that the protein contained in each 2 ml. of plasma removed at sampling represented one-one hundred twenty-fifth of the animal's total protein and was rapidly replaced with unlabelled protein.

RESULTS

1 A. BEHAVIOR OF RABBIT I^{131}-ALBUMIN IN THE PLASMA AFTER INTRAVENOUS INJECTION Fig. 1, curve X, represents specific activity (s.a.) of the plasma albumin, plotted semilogarithmically against time, for a typical animal, B1X. The experimental values, denoted by solid triangles, were

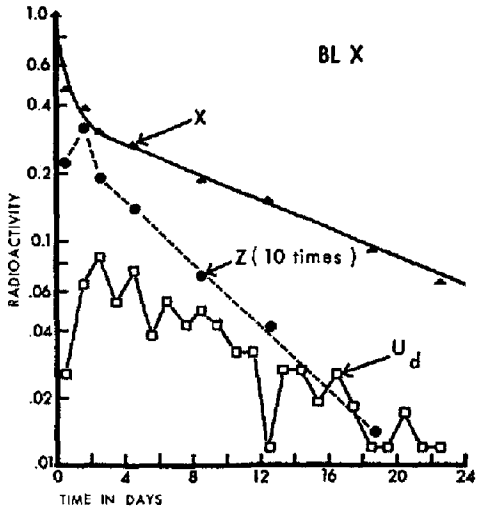

FIGURE 1. Plasma albumin radioactivity is represented by curve X (see section 1 *a*). Curve Z represents values of the total radioactivity of the breakdown products within the animal (times 10) (see section 1 *b*). Plot U_d gives radioactivity excreted per day (see section 1 *c*).

plotted at the times of withdrawal of the blood samples. Graphical analysis of the best smooth curve that could be drawn through these points showed that it could be described by an equation of the form $X = C_1 e^{-at} + C_2 e^{-bt}$. The smooth curve, X, of Fig. 1 was drawn by means of this equation with constants determined from the actual graphical analysis (for the methods of graphical analysis, see for instance (20)) and it can be seen that the equation describes the data reasonably well over an interval of 3 weeks. Fig. 2, curve X, shows a similar graphical analysis compared with the experimental points for

another rabbit, No. 21. To allow comparison of the data of one animal with those of another the following conventions have been used. The initial samples, withdrawn about 10 minutes after the injection of I^{131}-albumin, were assigned unit s.a. and plotted at zero time, giving $X = 1$ when $t = 0$, and thus $C_1 + C_2 = 1$; and the specific activities of subsequent samples were plotted as decimal fractions of the specific activity of the initial samples. The resulting graph is considered equivalent to a graph of the fraction of the total activity in the plasma at any time. The linear tail of the curve X, or the slow component, had slope a, and was extrapolated to zero time, t_0, to yield C_1. The fast component with slope b was extrapolated to yield C_2, which by

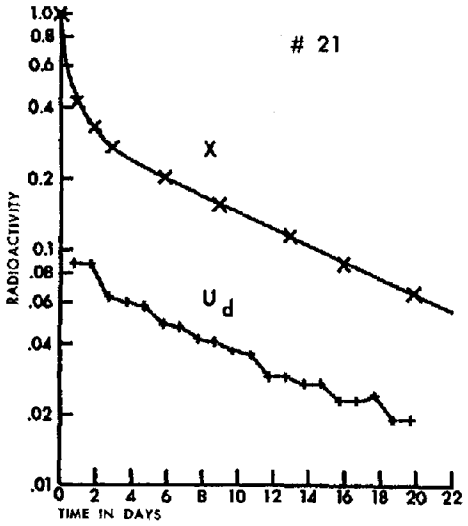

FIGURE 2. Curve X is the radioactivity of plasma albumin and curve U_d the radioactivity excreted daily. Compare with Fig. 1 and see sections 1 *a* and 1 *c*.

definition was $1 - C_1$. What has previously been described as the "biological half-life" of the I^{131}-albumin and used to define the slope, a, is obtained from $t_{1/2} = 0.693/a$. In rabbit B1X it was 9.7 days, and in rabbit 21, 8.3 days.

Strictly speaking the curve X does not exactly represent the specific activity of the plasma albumin, since a fraction of the total activity present is due to radioactive breakdown products of I^{131}-albumin. It is shown shortly, however, that this did not exceed 1 to 2 per cent of the total radioactivity present, and it can therefore safely be neglected.

Since in our experience the behavior of the plasma I^{131}-albumin s.a. over time can be reasonably accurately represented by the relation $X = C_1 e^{-at} + C_2 e^{-bt}$, the plasma data for eleven rabbits have been summarized by recording the four constants of this equation in Table I. Seven different preparations of I^{131}-albumin were used and in several experiments the same prepa-

ration was given simultaneously to two rabbits. The total radioactivity injected is given in column 7 of Table I, and the animals were observed for from 17 to 25 days (column 8). Values of C_1 ranging from 0.30 to 0.40, and a, ranging from 0.0715 to 0.1118 day^{-1}, or expressed as half-times from 6.2 to 9.7 days, are seen to be in reasonably good agreement with values taken from the data of Cohen *et al.* (7) given at the bottom of the table.

TABLE I

PLASMA AND OTHER DATA ON THE RABBITS

	1	2	3	4	5	6	7	8
Rabbit No.	I^{131}-albumin Preparation No.	C_1	a [day^{-1}]	$t_{\frac{1}{2}}$ for a [days]	C_2	b [day^{-1}]	I^{131}	Days followed
							$\mu c.$	
B1X	27	0.354	0.0715	9.7	0.646	1.81	76.5	24
21	10	0.337	0.0835	8.3	0.663	1.497	14.3	20
5-94	10	0.33	0.0803	8.65	0.67	1.955	19.6	20
9-8	22	0.40	0.0806	8.6	0.60	2.005	38.2	21
1-02	22	0.31	0.0866	8.0	0.69	1.885	38.3	21
5-98	12	0.37	0.0924	7.5	0.63	1.497	33.4	24
5-99	12	0.354	0.0943	7.35	0.646	1.786	33.6	24
1-09	20	0.37	0.0873	7.95	0.63	2.10	29.3	25
1-10	18	0.375	0.0950	7.3	0.625	2.04	15.1	17
1-11	24	0.30	0.0921	7.5	0.70	2.02	14.3	21
1-01	24	0.31	0.1118	6.2	0.69	2.165	15.1	21
Mean		0.346		7.9				
range		0.30–0.40		6.2–9.7				
Cohen *et al.* (7)	A	0.33		7.5				
		(0.31–0.40)		(6.7–8.7)				
	B	0.40		8.2				
		(0.36–0.485)		(7.9–8.6)				
	C	0.41		8.5				
		(0.35–0.47)		(7.7–9.6)				

A = 6 younger rabbits given C^{14}-labelled protein.
B = 3 rabbits given C^{14}-labelled protein.
C = 5 rabbits given I^{131}-labelled protein.

1 B. THE RADIOACTIVE BREAKDOWN PRODUCTS IN THE PLASMA AND THE TOTAL QUANTITY OF RADIOACTIVE BREAKDOWN PRODUCTS IN THE ANIMALS It has elsewhere been shown (10) that after I^{131}-albumin is given intravenously radioactive breakdown products appear in the plasma, 80 per cent or more of which are I^{131}-iodide and the remainder probably the metabolites of amino acids labelled with I^{131}. These breakdown products behave as if they were distributed in a volume 7 to 10 times the plasma volume and are rapidly

excreted. Curve Z in Fig. 1 shows calculations of the changes with time in the *total* quantity of radioactive breakdown products in rabbit B1X, expressed as a decimal fraction of the total radioactivity injected into the animal. The calculations were made from measurements of the radioactivity of the breakdown products extracted from the plasma by acetone and the assumption that this radioactivity was distributed in 8 times the plasma volume. Curve Z shows that the maximum retention of radioactive breakdown products occurred at about 1.5 days after the injection. At this time analysis showed the

TABLE II

CUMULATIVE EXCRETION OF RADIOACTIVITY, AFTER I.V. I^{131}-ALBUMIN, EXPRESSED AS A DECIMAL FRACTION

Rabbit No.	Fraction Day 1*	Day 1	2	3	5	7	9	11	13	15	Fraction of radioactivity in feces
B1X	0.4	0.025	0.089	0.173	0.299	0.390	0.480	0.554	0.598	0.652	N.C.‡
21	0.8	0.088	0.175	0.238	0.356	0.452	0.535	0.608	0.666	0.720	0.07
5-94	0.8	0.081	0.133	0.227	0.378	0.468	0.552	0.640	0.690	0.732	0.24
9-8	0.8	0.063	0.133	0.206	0.318	0.395	0.502	0.589	0.656	0.715	0.03
1-02	0.8	0.000	0.000	0.003	0.093	0.161	0.244	0.305	0.356	0.400	0.11
5-98	0.6	0.064	0.143	0.203	0.330	0.438	0.522	0.590	0.642	0.693	0.038
5-99	0.6	0.035	0.139	0.220	0.331	0.442	0.525	0.602	0.659	0.710	0.034
1-09	0.8	0.088	0.172	0.241	0.361	0.446	0.545	0.612	0.670	0.742	N.C.
1-10	0.8	0.120	0.178	0.250	0.385	0.455	0.559	0.633	0.687	0.736	N.C.
1-11	0.4	0.031	0.116	0.215	0.340	0.457	0.546	0.616	0.682	0.736	0.058
1-01	0.4	0.000	0.105	0.232	0.405	0.517	0.624	0.700	0.769	0.830	0.03

* Day 1 was not a complete day but the fraction of a day indicated.

‡ N. C., feces not collected.

radioactive breakdown products only accounted for about 1 per cent of the total radioactivity in the plasma, and thus, as already noted, it can be neglected in measuring the specific activity of albumin when using whole plasma. Calculations of Z from other isolated measurements of the quantities of breakdown products in other animals generally agree with the results in Fig. 1.

1 C. THE EXCRETION OF RADIOACTIVE BREAKDOWN PRODUCTS Fig. 1, curve U_d, shows the fraction of the total radioactivity excreted each day by rabbit B1X, and Fig. 2, curve U_d, the same data for rabbit 21. Similar experiments were made on eleven animals. Table II summarizes the cumulative excretions of radioactivity during the first 15 days of the experiments and shows that in the eight animals in which the feces and urine were collected from 0.03 to 0.24 of the radioactivity was in the feces. Excluding No. 5-94, in which the feces were obviously contaminated by urine, the average excre-

tion in the feces was about 0.08 of that given, with a minimum of 0.03. In the three animals in which the feces were not collected a conservative correction was therefore made by assuming that the feces contained 0.05 of the total activity excreted, as described under Methods. Figs. 1 and 2 show that after about the 3rd day the excretion rates, U_d, parallel the a slopes of the plasma curves. Cumulative excretions for four representative animals are plotted in Fig. 3. The nature of these curves and the U_d curves in Figs. 1 and 2 suggests that cumulative excretion of radioactivity, u, can be described after

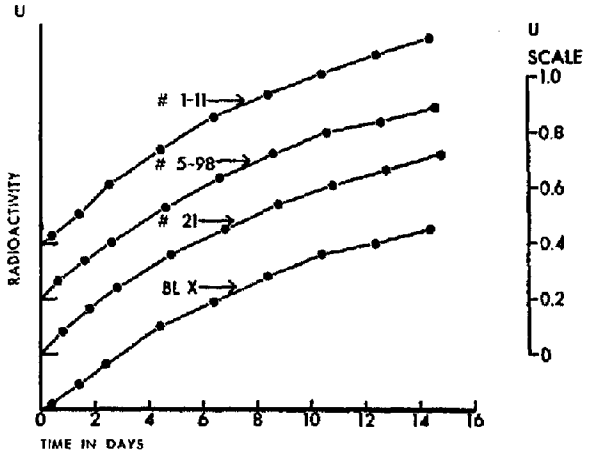

FIGURE 3. The cumulative radioactivity excreted, U, as a fraction of that given. To avoid the superimposition of the four curves, the zeros of the ordinate have been successively elevated, and the scale intervals indicated on the right. (See section 1 *c*.)

the first few days by an equation of the form $u = 1 - Ke^{-at}$ in which K is a constant. Fig. 8, presented with section 3 *b*, shows this to be true, and its significance will appear shortly.

1 D. THE TOTAL QUANTITY OF RADIOACTIVITY AS EXTRAVASCULAR I^{131}-ALBUMIN This quantity unlike the others cannot be measured directly but only by difference. It is defined by $y = 1 - x - z - u$, in which 1 is the total radioactivity injected, x is the total activity in the plasma, and z is the total activity of the radioactive breakdown products in the body, both at the time of measurement, and u is the total activity of the breakdown products that have been excreted up to this time. The values of y obtained in this manner for B1X are shown as "y (observed)" in Fig. 5 presented with section 3 *a*. Since a precise estimate of y requires measurements of z, y can only be approximately calculated for the other animals. The quantity y, the total activity associated with extravascular albumin, must be clearly differentiated from the specific activity of the extravascular albumin, which in later terminology is $y/\bar{y}$.

2. THE KINETIC MODELS FOR ALBUMIN AND I^{131}-ALBUMIN

(*a*) *Unlabelled Albumin* Albumin in animals is assumed to be normally present in constant amount, part of which is in plasma, say $\bar{x}$ gm. albumin, and the remainder in the extravascular fluids (lymph and tissue fluids) namely $\bar{y}$ gm. Fig. 4 A shows the plasma and extravascular albumin as two compartments which are in transfer equilibrium, albumin passing out of the minute vessels at a rate $\bar{k}_1$ gm./day to the extravascular fluids, and returning

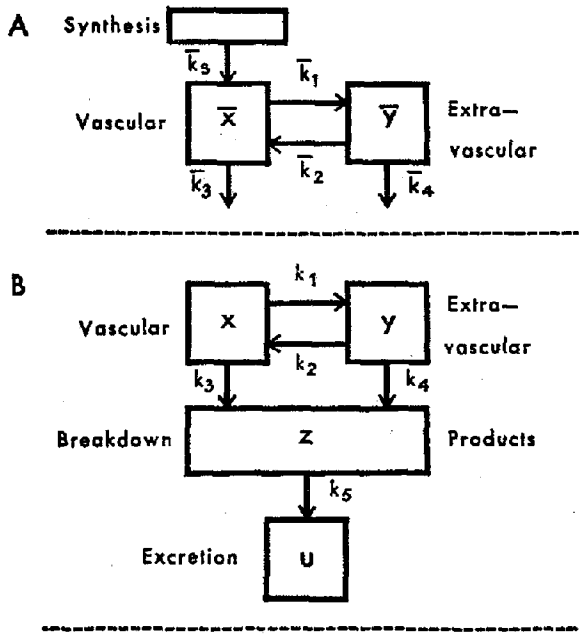

FIGURE 4. A, schema of distribution and transfer of unlabelled albumin in an animal in the steady state. (See section 2 *a*.) B, schema of distribution, transfer, and excretion of radioactivity following an injection of I^{131}-albumin. (See section 2 *b*.)

primarily *via* the lymphatics to the plasma at a rate $\bar{k}_2$ gm./day. Albumin is also continually broken down, and the catabolized protein is continually replaced by newly synthesized protein. The site or sites of breakdown are uncertain and therefore may, in a general way, be pictured as occurring in the plasma compartment in which $\bar{k}_3$ gm./day protein are broken down, and in the extravascular compartment in which $\bar{k}_4$ gm./day are broken down (Fig. 4 A). (The physiological significance of these two rates is discussed later.) Then the rate of synthesis $\bar{k}_s = \bar{k}_3 + \bar{k}_4$ in the steady state. Note, however, that newly synthesized protein may, in the theory at least, either enter the plasma compartment or the extravascular compartment. In Fig.

4 A it is represented as entering the plasma compartment, since albumin is known to be synthesized by the liver (21, 22) and must therefore reach the blood stream either directly or by rapid lymphatic pathways. The four constants k_1, k_2, k_3, and k_4 may be termed the "flux" constants, and define the changes (in grams of protein per day) undergone by unlabelled albumin. They must be distinguished from the "tracer" k's which are now discussed.

2 b. I^{131}-Albumin A kinetic model for I^{131}-albumin is portrayed in Fig. 4 B. Initially I^{131}-albumin is injected into the vascular compartment in which it becomes mixed within a few minutes and *thereafter its behavior is assumed to follow that of unlabelled albumin.* At any time, t, thereafter, the fraction of the total administered radioactivity attached to albumin in the *vascular compartment* is x and in the *extravascular compartment* is y. Since the I^{131}-albumin is continually broken down with liberation of radioactive breakdown products there are two additional compartments in Fig. 4 B, a *breakdown products compartment* which at t contains a fraction, z, of the total activity, as radioactive breakdown products, and an *excretion compartment* in which radioactivity accumulates and which at t contains the fractional activity u. The rate of transfer of tracer from one compartment to another at t, is taken to be proportional to the amount present in the former compartment at t. For example, the rate of breakdown of I^{131}-albumin in the plasma compartment and its rate of passage to the extravascular compartment are both assumed proportional to the total I^{131}-albumin in the plasma at the specific time. Since x, y, z, and u are all continuous functions of time, the rates must be considered as "instantaneous rates" which implies first derivatives with respect to time.

We thus obtain the following system of differential equations.

$$dx/dt = k_2y - (k_1 + k_3)x \tag{1}$$

$$dy/dt = k_1x - (k_2 + k_4)y \tag{2}$$

$$dz/dt = k_3x + k_4y - k_5z \tag{3}$$

$$du/dt = k_5z \tag{4}$$

It is required to find solutions for the "tracer" k's (*i.e.* rate constants), k_1, k_2, k_3, and k_4, that will permit their determination from the tracer data. A value of k_5 is also required but, from (10), this may be taken as between 1.5 and 3.5 day^{-1}.

Since the tracer is initially placed in the plasma compartment and all the activity is ultimately excreted, we have as initial conditions,

$$\text{for} \quad t = 0, \qquad x = 1, \qquad y = z = u = 0$$

and as final conditions:

$$\text{for} \quad t \to \infty, \qquad x = y = z = 0, \qquad u = 1$$

Since Equations 1 and 2 are independent of z and u, these may be solved simultaneously for x. Letting $\alpha = k_1 + k_3$ and $\beta = k_2 + k_4$ we obtain in operator notation:

$$[D^2 + (\alpha + \beta)D + \alpha\beta - k_1k_2]x = 0 \tag{5}$$

With the roots of the auxiliary equation of 5 real and distinct,

$$x = C_1e^{r_1t} + C_2e^{r_2t}$$

Since $x \to 0$ as $t \to \infty$, let $r_1 = -a$ and $r_2 = -b$ with $a > 0$ and $b > 0$, thus insuring k's > 0; then

$$x = C_1e^{-at} + C_2e^{-bt} \tag{6}$$

From Equations 1 and 6

$$dx/dt = k_2y - \alpha x = -C_1ae^{-at} - C_2be^{-bt}$$

and by initial conditions,

$$\alpha = k_1 + k_3 = C_1a + C_2b \tag{7}$$

Next from the roots of the auxiliary equation of 5 we obtain,

$$r_1 + r_2 = -(a + b) = -(\alpha + \beta) \quad \text{and} \quad r_1r_2 = ab = \alpha\beta - k_1k_2,$$

thus:

$$\beta = k_2 + k_4 = a + b - \alpha \tag{8}$$

$$\gamma = k_1k_2 = \alpha\beta - ab \tag{9}$$

Since, as noted earlier, C_1, C_2, a, and b are defined by graphical analysis of the plasma tracer data from the equation $x = C_1e^{-at} + C_2e^{-bt}$, Equations 7, 8, and 9 provide three relations for defining the four rate constants k_1, k_2, k_3, and k_4 of the complete system. If in a simplified system either k_3 or $k_4 = 0$, then the three equations are sufficient to define the three remaining k's, or if from a fourth equation one of the rate constants can be determined, then all four can be determined.

To derive the fourth relation for determining the rate constants of the general system we proceed first to obtain solutions for y, z, and then u as

follows: From Equations 1 and 6

$$y = (1/k_2)\,[C_1(\alpha - a)e^{-at} + C_2(\alpha - b)e^{-bt}]$$

From initial conditions:

$$y = D(e^{-at} - e^{-bt}), \tag{10}$$

in which the constant D is given by

$$D = C_1(\alpha - a)/k_2 \tag{11}$$

By means of Equations 6 and 10, Equation 3 may be solved for z and the solution written in the form:

$$z = Ae^{-at} + Be^{-bt} + Ee^{-k_5 t}, \tag{12}$$

in which

$$A = \frac{k_3C_1 + k_4D}{k_5 - a} \qquad B = \frac{k_3C_2 + k_4D}{k_5 - b} \tag{13}$$

and initial conditions give $E = -(\mathrm{A} + \mathrm{B})$. Finally, solution for u by indefinite integration of Equation 12 in Equation 4 gives

$$u = k_5\left[\frac{A}{a}(1 - e^{-at}) + \frac{B}{b}(1 - e^{-bt}) + \frac{E}{k_5}(1 - e^{-k_5 t})\right] \tag{14}$$

Now it may be shown that by graphical analysis of the experimental data defining either y, or z, or u, a solution for k_2 can be obtained. Since our data for u are good but for z and hence for y are less than adequate, graphical analysis of u will be used.

Since $k_5 \geq b > a$, after some time t_1, *i.e.* for $t > t_1$, Equation 14 is well represented by

$$u_1 = \frac{k_5A}{a} + \frac{k_5B}{b} + E + \frac{k_5A}{a}e^{-at}$$

Moreover, using final conditions, inspection of Equation 14 reveals that as $t \to \infty$ the first three terms approach unity. We thus write for an approximation:

$$u_1 = 1 - (k_5A/a)e^{-at}, \qquad (t > t_1)$$

or the equivalent expression

$$\ln(1 - u_1) = \ln(k_5A/a) - at \tag{15}$$

It is clear from Equation 15 that the semilog plot of $(1 - u_1)$ against t is linear, and that the intercept, C_3, at $t = 0$ defines k_5A/a. By means of Equations 13, 11, 7, 8, and 9, the relation $C_3 = k_5A/a$ yields

$$k_2 = \frac{[(\alpha - a)\beta - \gamma]C_1}{C_3 j - aC_1} \tag{16}$$

in which

$$j = a(k_5 - a)/k_5 = a - (a^2/k_5)$$

It should be noted that since k_5 is about twenty times greater than a, the j term and hence k_2, are relatively insensitive to small changes in k_5. *Thus with k_5 independently determined from previous observations (10), the graphical analysis of x and u together with Equations 16, 7, 8, and 9 allows the calculation of the tracer k's—k_1, k_2, k_3, and k_4.*

3. THE PREDICTIONS OF THE PROPOSED KINETIC MODELS COMPARED WITH THE TRACER MEASUREMENTS; THE ELIMINATION OF SEVERAL MODELS AND THE SELECTION OF MODEL C

(*a*) *Simplified Models—Models A and B* Graphical analysis of the plasma data provides values for C_1, C_2, a, and b (Figs. 1 and 2). Moreover, if either k_4 or k_3 is set to zero, then values for the three remaining "tracer" k's (excluding k_5) can be obtained solely from the plasma data and Equations 7, 8, and 9. Thus, referring to Fig. 4 B, if k_4 is set to zero and the breakdown of I^{131}-albumin is allowed to occur only in close relationship with the vascular compartment, values can be obtained for k_1, k_2, and k_3. This simplification of the complete model is termed model A, and when restricted to the plasma data and the solutions of Equations 1 and 2, is essentially the model proposed by Campbell *et al.* (8).

Similarly, if breakdown of I^{131}-albumin is allowed to occur exclusively in the extravascular compartment and $k_3 = 0$, then values for k_1, k_2, and k_4 can be obtained. This we term model B. Examination of Equations 5, 7, 8, and 9, shows that models A and B, yield the same type of equation for x, and therefore the plasma data yield no evidence as to which model is to be preferred. However, examination of Equations 10 and 11 shows that models A and B yield different values for y, Equations 12 and 13 reveal that models A and B give distinct values for z, and finally Equation 14 shows that u is different for the two models.

Fig. 5 shows y curves obtained from the relevant equations of models A and B and values of C_1, C_2, a, and b obtained from graphical analysis of the plasma data. The "observed" y in Fig. 5, determined from $y = 1 - x -$

$z - u$, is close to the predictions of model A, and is within the region bounded by the curves defined according to the two models.

The theoretical curves for z and u require in addition values for k_5. Fig. 6 shows z curves calculated for models A and B with $k_5 = 1.0$ day^{-1} and 3.0 day^{-1} (10). The measured values indicate a curve which is closely related to that predicted by model A with $k_5 = 3.0$. Fig. 7 compares measured values of u for two rabbits with those calculated for models A and B with $k_5 = 1.0$

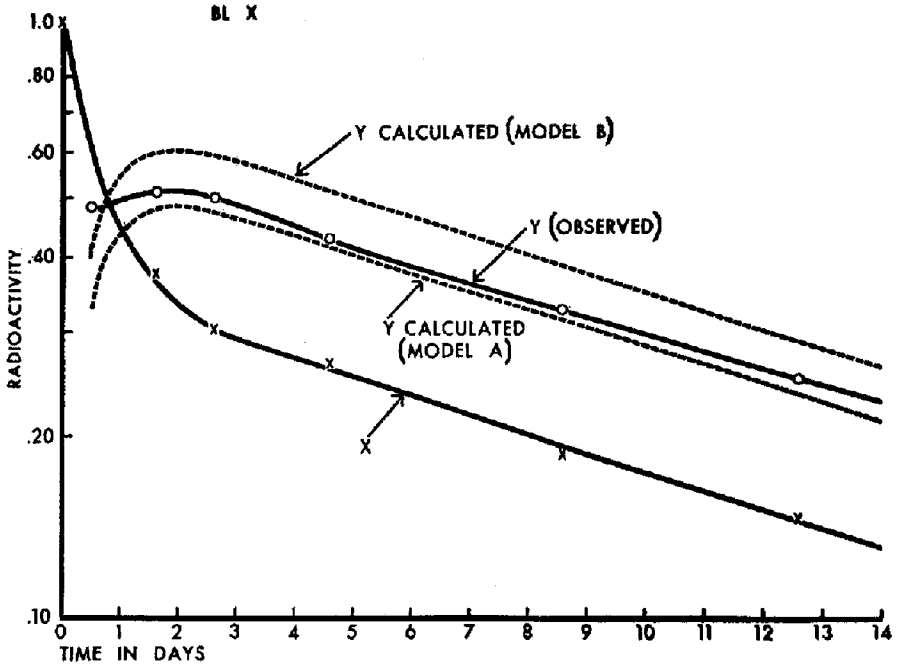

FIGURE 5. Comparison of observed values of y, the radioactivity in the extravascular compartment, with curves predicted by models A and B. (See sections 1 *d* and 3 *a*.)

and $k_5 = 3.0$. In neither animal is model B capable of describing the experimental data, which, however, lie fairly close to the predictions of model A.

When neither k_3 nor k_4 is allowed to vanish, as was done to obtain models A and B, the general system of Equations 1, 2, 3, 4 defines model C, the solutions of which are bounded by curves defined by models A and B. Clearly the observed values of y and u lie close to the boundary defined by model A, and it would thus be expected that the particular curves of model C would be close to the model A boundary, and would give a rather better description.

3 b. The Complete Model—Model C Equation 16 shows that the fourth relation necessary to calculate the four tracer k's can be obtained from the excretory data. Fig. 8 shows typical semilogarithmic plots of $1 - u$ against time, with the intercept, C_3, obtained by extrapolating to zero time. In most

instances, as illustrated, after the first 2 or 3 days the points lay very close to a straight line. Equations 6 and 15 demand that the slope of this line which may be termed a_u, should be the same as that of the slow component, a, of the plasma data. Table III compares values of a, determined from the plasma data, with the slope a_u. In only one animal, No. 1-02, is agreement poor, and in this animal for unknown reasons only about half the expected radioactivity

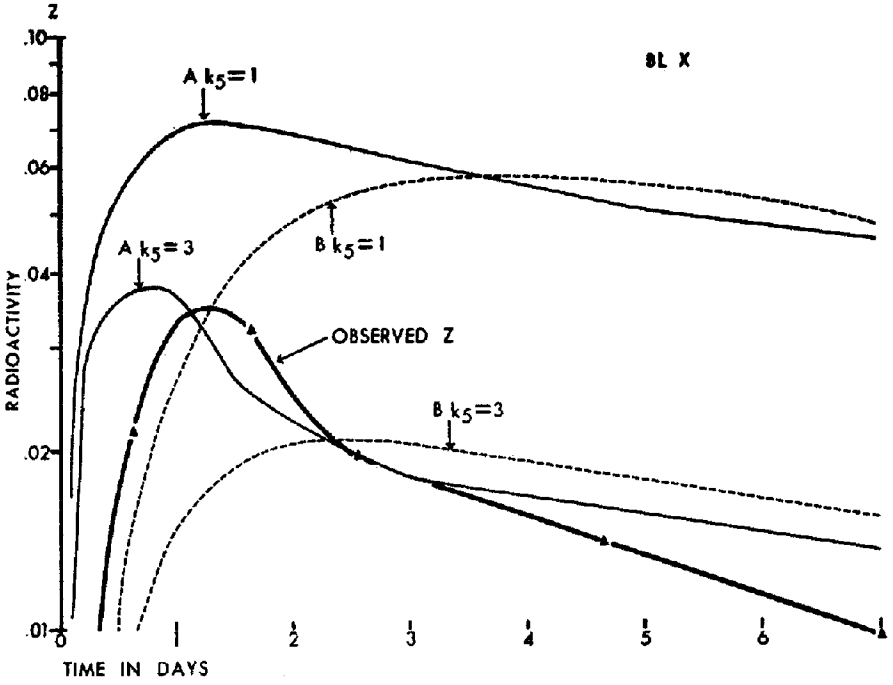

FIGURE 6. Comparison of the observed values of z, the radioactivity in the breakdown products compartment, with the curves predicted by models A and B. To allow for the variation in excretory rate, two z curves are shown for each model, utilizing $k_5 = 1$ and $k_5 = 3$ day^{-1}. (See section 3 *a*.) Latterly the observed z curve declines more rapidly than predicted. Since, at this time, z levels were low, this may be from errors of measurement.

apparently was excreted, though the plasma activity behaved in the usual manner. In the remaining animals, in half of which agreement between a_u and a is excellent, the mean value for a_u is 0.0878 and for a 0.0889, which is satisfactory. Rabbits 5-98 and 5-99 in which agreement is not too good were growing during the course of the experiment and it is believed that increase in the circulating albumin with growth resulted in some dilution of the activity in the plasma with resulting apparent increase in the value of a. It must also be remembered that small collection errors and small errors made in correcting for losses in collection of excreted radioactivity by using an average correction factor of 1/0.95 will appreciably alter the slope a_u.

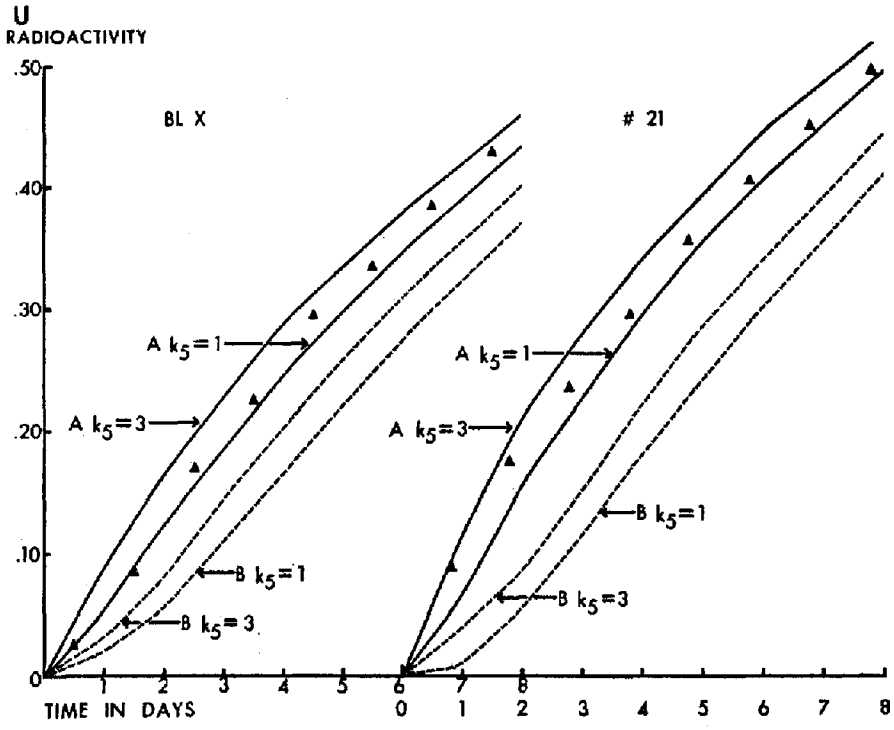

FIGURE 7. Comparison of the observed values of u, the cumulative excretion of radioactivity shown by the isolated ▲, with curves predicted by models A and B. To allow for variations in excretory rate, two u curves are shown for each model, utilizing $k_5 = 1$ and $k_5 = 3$ day^{-1}. (See section 3 *a*.)

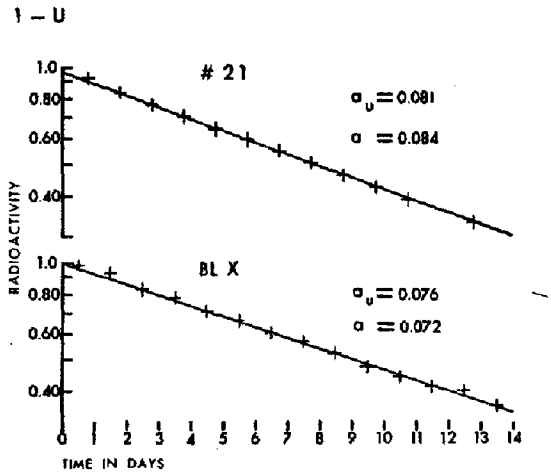

FIGURE 8. Semilogarithmic plot of $1 - u$, in which u = observed cumulative values of excreted radioactivity. After the 3rd day a good linear relation is evident. (See section 1 *c*, Equation 15, and section 6.) Slopes defined by these excretory lines are denoted a_u while values denoted as a are the corresponding plasma slopes.

Table III also summarizes the values for C_3 determined by graphical analysis, and with these values k_2 may be calculated by Equation 16 provided k_5 is designated. Since k_5 lies between 1.5 and 3.5 (10) a value of 2.5 may be taken which gives a value for k_2 that is little altered by the range in k_5. This holds because of the nature of the j term as noted above with Equation 16. The remaining rate constants k_1, k_3, and k_4 are then readily obtained from Equations 7, 8, and 9. Table IV shows the calculated values of all four k's of model C and the three k's of model A. Since model A, in which $k_4 = 0$,

TABLE III

OBSERVED AND CALCULATED VALUES OF a, C_3, AND u

Rabbit No.	a	a_u	C_3*	C ‡ Calculated	u§ Observed	u‖ Model A	u¶ Model C	Period of observation
								days
B1X	0.0715	0.0787	1.00	0.965	0.785	0.81	0.80	22.5
21	0.0835	0.0831	0.962	0.933	0.830	0.825	0.82	20
5-94	0.0803	0.0907	0.975	0.955	0.825	0.81	0.805	20
9-8	0.0806	0.0841	1.01	0.972	0.835	0.820	0.815	21
1-02	0.0866	0.0432	—	0.940	0.54	0.85	—	21
5-98	0.0924	0.0812	0.965	0.936	0.85	0.90	0.895	24
5-99	0.0943	0.0835	0.972	0.946	0.89	0.90	0.895	24
1-09	0.0873	0.0862	0.972	0.971	0.91	0.89	0.89	25
1-10	0.0950	0.0862	0.942	0.965	0.78	0.805	0.81	17
1-11	0.0921	0.0907	0.975	0.940	0.875	0.86	0.855	20.5
1-01	0.1118	0.1135	0.975	0.935	0.96	0.91	0.905	20.5
Means excluding 1-02	0.0889	0.0878	0.975	0.952	0.854	0.853	0.849	

* Determined by graphical analysis.

‡ C_3 calculated $= \frac{k_2 k_5 C_1}{a(k_5 - a)}$ in which $k_5 = 2.5$.

§ u observed = fraction of total radioactivity excreted during the period of the observations.

‖ u(model A) = fraction of total radioactivity excreted calculated from $u = 1 - C_3 e^{-at}$, in which C_3 is $C_3^{\ddagger}$.

¶ u (model C) = fraction of total radioactivity excreted calculated from $u = 1 - C_3 e^{-at}$ in which C_3 is C_3^*.

was found to predict measured values reasonably well, the value of k_4 in model C would be expected to be small, and Table IV shows the mean value is 0.027. One animal, 1-10, shows a small negative value for k_4 which is impossible according to the model, but may be regarded as due to experimental errors, since the calculated values of k_4 are sensitive to the extrapolated value of C_3 which depends on a number of measurements. Though, because of the complexity of the measurements, it is thought that there may be some error in individual values of k_4, their consistency in the group of animals, in which errors may be expected to cancel out, indicates that k_4 is small. Comparison of the values of k_1 and k_2 of model C with those of model A shows minor changes of 5 per cent or less caused by the small extravascular leak in model

C. However, the differences in k_3 are seen to be considerable. As a check on the predictions of model C, Table III shows the measured excretions of radioactivity and the excretion of radioactivity calculated from the plasma data, the excretory intercept C_3, and Equation 15. Excluding 1-10, the mean observed excretion is 0.854, and that calculated with model C is 0.849. If model A is used, as shown in Table III the mean calculated excretion is 0.853, very slightly though not significantly better than model C.

TABLE IV

COMPARISON OF THE TRACER k's GIVEN BY MODEL C AND MODEL A

Rabbit	Model C from plasma and excretory data k_1	k_2	k_3	k_4	Model A from plasma data only k_1	k_2	k_3
B1X	1.062	0.651	0.133	0.036	1.006	0.686	0.189
21	0.863	0.517	0.158	0.043	0.798	0.560	0.223
5-94	1.143	0.680	0.193	0.019	1.112	0.699	0.225
9-8	1.104	0.805	0.131	0.045	1.046	0.850	0.189
1-02	—	—	—	—	1.074	0.644	0.254
5-98*	0.790	0.583	0.185	0.031	0.751	0.612	0.226
5-99*	0.987	0.663	0.200	0.030	0.944	0.693	0.243
1-09	1.141	0.827	0.214	0.005	1.134	0.832	0.221
1-10	1.037	0.855	0.274	−0.030	1.076	0.824	0.235
1-11	1.235	0.632	0.207	0.039	1.164	0.671	0.278
1-01	1.293	0.697	0.235	0.051	1.205	0.748	0.324
Means omitting 1-02	1.065	0.691	0.193	0.027	1.024	0.717	0.235

* Making correction for growth by substituting a_u for a and making resulting slight alterations in C_1 and C_2 reduced k_4 in both animals by approximately 0.015.

4. THE PROTEIN FLUXES, $\bar{k}_1$, $\bar{k}_2$, $\bar{k}_3$, AND $\bar{k}_4$

(*a*) *Calculation with Models A and C* It has been seen that model A gives a good prediction of the tracer data but that model C appears preferable. It is now necessary to calculate by means of the tracer k's the protein fluxes, either in grams of protein per day or as fractions of the total plasma albumin $\bar{x}$, or extravascular albumin $\bar{y}$, per day.

Referring to Fig. 4 A, and assuming that newly synthesized albumin enters directly into the plasma, at equilibrium

$$d\bar{x}/dt = \bar{k}_s + \bar{k}_2 - (\bar{k}_1 + \bar{k}_3) = 0 \tag{17}$$

$$d\bar{y}/dt = \bar{k}_1 - (\bar{k}_2 + \bar{k}_4) = 0 \tag{18}$$

in which $\bar{x}$ gm. is the total albumin in the plasma, $\bar{y}$ gm. is the total albumin in the extravascular fluids, $\bar{k}_s$ is grams of albumin synthesized per day, and

the other flux $\bar{k}$'s are the grams of albumin per day being exchanged or broken down as indicated in Fig. 4 A.
At equilibrium

$$\bar{k}_s = \bar{k}_3 + \bar{k}_4, \quad \bar{k}_1 = k_1\bar{x}, \quad \bar{k}_2 = k_2\bar{y}, \quad \bar{k}_3 = k_3\bar{x}, \quad \text{and} \quad \bar{k}_4 = k_4\bar{y},$$

and therefore, provided $\bar{x}$ and $\bar{y}$ are known, all the above albumin fluxes can be determined. The total plasma albumin, $\bar{x}$, can be determined from a measurement of the plasma volume (*e. g.* from the radioactivity of the initial plasma sample after the I^{131}-albumin injection) and the plasma albumin concentration. However, $\bar{y}$, the total extravascular albumin cannot be determined directly, but can only be estimated in terms of $\bar{x}$ by using the assumptions contained in the mathematical description. Equation 18 shows that at equilibrium

$$k_1\bar{x} = (k_2 + k_4)\bar{y}$$

or that

$$\bar{y} = [k_1/(k_2 + k_4)]\bar{x} \tag{19}$$

Thus to determine the fluxes $\bar{k}_2$ and $\bar{k}_4$ in terms of $\bar{x}$ the following equations are obtained:

$$\bar{k}_2 = k_2\bar{y} = [k_1k_2/(k_2 + k_4)]\bar{x} \tag{20}$$

$$\bar{k}_4 = k_4\bar{y} = [k_1k_4/(k_2 + k_4)]\bar{x} \tag{21}$$

So far the fluxes have been given in terms of the general model, model C. The fluxes for model A are easily obtained from the above equations by setting $k_4 = 0$. Then $\bar{k}_s = k_3\bar{x}$, $\bar{k}_1 = k_1\bar{x}$, $\bar{k}_3 = k_3\bar{x}$, and

$$\bar{k}_2 = k_2\bar{y} = (k_1k_2/k_2)\bar{x} = k_1\bar{x} \tag{22}$$

4 b. The Values of the Protein Fluxes Given by Models A and C, and Certain Mathematical Identities between the Models Table V compares the fluxes calculated for model C, with those calculated for model A. The fluxes are calculated as fractions of the total plasma albumin, $\bar{x}$, per day. Whereas it is seen that $k_1\bar{x}$, model C, differs only a little from $k_1\bar{x}$, model A, it is apparent that $k_2\bar{y}$, model C, is identical with $k_2\bar{y}$, model A; the breakdown fluxes $k_3\bar{x} + k_4\bar{y}$, model C, and $k_3\bar{x}$, model A, are also identical. Further it can be shown that these identities are true not just for these particular solutions of model C and model A, but for any solutions defined by Equations 7, 8, and 9. Thus, in terms of Equations 7, 8, and 9

$$k_2\bar{y} \text{ (model C)} = [k_1k_2/(k_2 + k_4)]\bar{x} = (\gamma/\beta)\bar{x}$$

and

$$k_2\bar{y} \text{ (model A)} = k_1\bar{x} = (\gamma/\beta)\,\bar{x} \quad \text{since} \quad k_4 = 0.$$

Similarly with the breakdown fluxes, in terms of Equations 7, 8, and 9

$$k_3\bar{x} + k_4\bar{y} \text{ (model C)} = [k_3 + k_1k_4/(k_2 + k_4)]\bar{x} = (\alpha - \gamma/\beta)\bar{x},$$

and

$$k_3\bar{x} \text{ (model A)} = (\alpha - \gamma/\beta)\bar{x}, \quad \text{since} \quad k_4 = 0.$$

Table V shows that $\bar{k}_2$ in either model averages 1.024 of the albumin in the plasma per day with a range of from 0.75 to 1.2, and that the breakdown,

TABLE V

THE FLUXES* AS FRACTIONS OF THE TOTAL ALBUMIN IN THE PLASMA CALCULATED FROM MODELS A AND C, WITH VALUES FOR THE EXTRAVASCULAR ALBUMIN, $\bar{y}$ (MODELS A AND C) AND $\bar{k}_3$ CALCULATED FROM THE PLASMA AND EXCRETORY DATA

Rabbit	Model C			Model A			Model C	Model A	$\bar{k}_3$‡
	$\bar{k}_1$	$\bar{k}_2$	$\bar{k}_3 + \bar{k}_4$	$\bar{k}_1$	$\bar{k}_2$	$\bar{k}_3$	$\bar{y}/\bar{x}$	$\bar{y}/\bar{x}$	u_{12}/ξ‡
BIX	1.062	1.006	0.189	1.006	1.006	0.189	1.55	1.47	0.197
21	0.863	0.798	0.223	0.798	0.798	0.223	1.54	1.43	0.245
5-94	1.143	1.112	0.225	1.112	1.112	0.225	1.64	1.60	0.241
9-8	1.104	1.046	0.189	1.046	1.046	0.189	1.30	1.23	0.205
1-02	—	—	—	1.074	1.074	0.254	—	1.67	—
5-98	0.790	0.751	0.226	0.751	0.751	0.226	1.29	1.23	0.244
5-99	0.987	0.944	0.243	0.944	0.944	0.243	1.42	1.36	0.265
1-09	1.141	1.134	0.221	1.134	1.134	0.221	1.38	1.36	0.247
1-10	1.037	1.076	0.235	1.076	1.076	0.235	1.21	1.31	0.239
1-11	1.235	1.164	0.278	1.164	1.164	0.278	1.84	1.74	0.310
1-01	1.293	1.205	0.324	1.205	1.205	0.324	1.73	1.61	0.395
Means omitting 1-02	1.065	1.024	0.235	1.024	1.024	0.235	1.49	1.43	0.259

* To turn the $\bar{k}$ values shown into units of grams of albumin/day they are multiplied by $\bar{x}$, the total plasma albumin. Values for $\bar{x}$ for most animals are given in Table I of the subsequent paper (25).

‡ $\bar{k}_3 = u_{12}/\xi = k_3 \cdot k_5/(k_5 - a)$.

$\bar{k}_3$ $(+\bar{k}_4)$, averages 0.235 of the albumin in the plasma per day with a range of 0.19 to 0.32. In Table V, the values of $\bar{k}_1$ for both models are close together and range from about 0.75 to 1.3 of the albumin in the plasma per day. In fact the range of $\bar{k}_1$ in the complete system is limited between α when $\bar{k}_3 = 0$ (model B) and $\alpha - a$ when $\bar{k}_4 = 0$ (model A), and since a is 0.1 or less of α, the maximum possible variation in $\bar{k}_1$ is about 10 per cent. These findings

concerning the fluxes are important since they show that the true breakdown flux can be derived directly by using the simpler model A.

5. THE TOTAL EXTRAVASCULAR ALBUMIN, $\bar{y}$ It has been noted that $\bar{y}$ cannot be measured directly but can only be estimated in terms of $\bar{x}$, the total albumin in the plasma. Equation 19 shows that $\bar{y}$ is affected by the values of k_1, k_2, and k_4. Table V compares values of $\bar{y}$, expressed as a decimal fraction of $\bar{x}$, calculated for model A ($k_4 = 0$) and model C. The differences are small, $\bar{y}/\bar{x}$ of model A averaging 1.43 and of model C, 1.49, but it should be noted that the greater is k_4, the greater is $\bar{y}/\bar{x}$. Campbell *et al.* (8) proposed an alternative method of determining $\bar{y}/\bar{x}$ which avoids measuring the rate constants and can be shown to apply either with model A or with model C. The specific activities, *i. e.* the radioactivities per unit weight protein, of plasma and extravascular albumin are defined by $x/\bar{x}$ and $y/\bar{y}$. Dealing first with the denominators, Equation 18 shows that in a steady state $k_1\bar{x} = (k_2 + k_4)\bar{y}$. Dealing now with the extravascular radioactivity, Fig. 5 shows that y rises to a maximum, y_{max} at t_{max}, and then declines. At its maximum $dy/dt = 0$ and from Equation 2, $k_1x_{max} = (k_2 + k_4)y_{max}$, in which x_{max} and y_{max} are the values of x and y at t_{max}. If now the numerator $k_1x_{max} = (k_2 + k_4)y_{max}$ is divided by the denominator $k_1\bar{x} = (k_2 + k_4)\bar{y}$, then at t_{max} $x/\bar{x} = y/\bar{y}$. To determine $\bar{y}/\bar{x}$, plots of y and x are required so that y_{max} and x_{max} can be obtained. Since $y = 1 - x - z - u$, z must be determined, and Figs. 5 and 6 show that at t_{max} z approached its maximum value of about 0.035. Fig. 5 also shows that t_{max} is not sharply defined by y, and therefore $\bar{y}/\bar{x}$ is not sharply defined. Despite these limitations, this method, which holds whether k_4 is or is not zero, offers an alternative approximate value for $\bar{y}/\bar{x}$. In rabbit B1X, the only animal for which there are sufficient data, by this method $\bar{y}/\bar{x}$ lies between 1.49 and 1.74 compared with 1.55, the value in Table V.

6. AN ALTERNATIVE METHOD FOR CALCULATING THE BREAKDOWN FLUX The breakdown flux measurements in Table V were derived from plasma measurements, with or without measurements of excreted radioactivity, made over many days, and only single values for the fluxes could be obtained. A method is, however, available for obtaining repeated measurements of breakdown flux from analyses of the plasma albumin specific activity and the excreted radioactivity. Both McFarlane and Berson and their respective collaborators (9, 13) proposed this method which essentially depends on relating the total quantity of radioactivity excreted daily to the quantity of albumin from which it has been split. The analysis necessary for its use is now given. Since the breakdown flux is identically predicted by models A and C, the analysis is based on the simpler model A. From Equations 6 and 15 it is seen that after some time t (about 3 days in the rabbit) the equations simplify to $x = C_1e^{-\alpha t}$ and $u = 1 - C_3e^{-\alpha t}$. We now wish to relate the excreted radio-

activity to the plasma activity. Consider first the mean value of the plasma activity x, during some interval t_1 to t_2, and term this value ξ. Then

$$\xi = \frac{\int_{t_1}^{t_2} x dt}{t_2 - t_1} = \frac{C_1(e^{-at_1} - e^{-at_2})}{a(t_2 - t_1)} \tag{23}$$

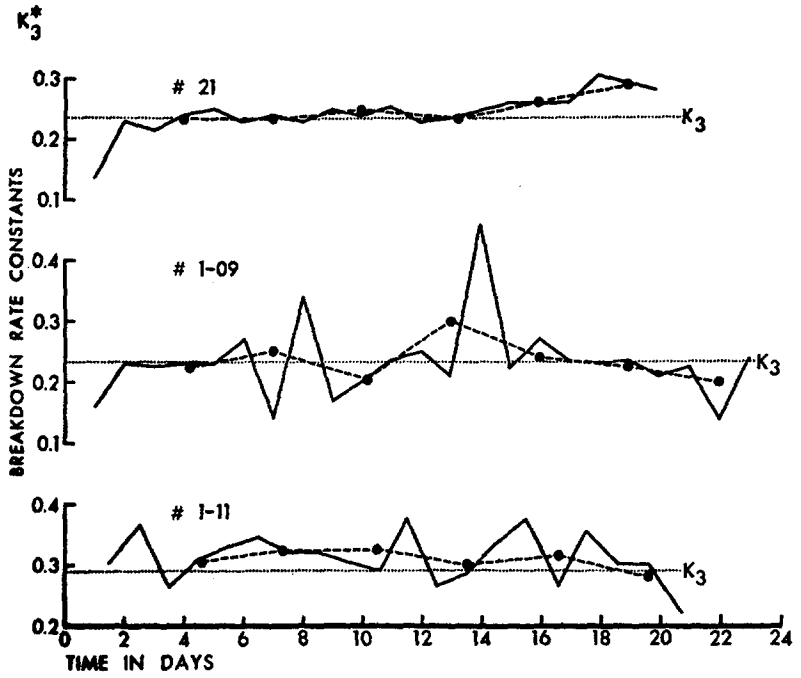

FIGURE 9. Breakdown rate constants, k_3, calculated from model A, fine horizontal lines, compared with values k_3^* estimated from measurements of excreted and plasma activities (see section 6), over intervals of 1 day, ———, and 3 days, ---. The ordinate represents fractions of the total albumin in the plasma which are being catabolized per day.

Define the activity excreted between t_1 and t_2 as u_{12}. Then

$$u_{12} = u_2 - u_1 = C_3(e^{-at_1} - e^{-at_2}) \tag{24}$$

By forming the ratio u_{12}/ξ and using $C_3 = k_3k_5C_1/a(k_5 - a)$

$$k_3 = \frac{u_{12}}{\xi(t_2 - t_1)} \cdot \frac{k_5 - a}{k_5} \tag{25}$$

and when $\bar{x}$ is known the flux $\bar{k}_3$ is obtained from $k_3\bar{x}$.

The value of u_{12} can be obtained by measuring all radioactivity excreted between t_1 and t_2 and correcting for collection losses. The mean plasma ac-

tivity, ξ, can be closely approximated by measuring x at the midtime interval between t_1 and t_2, or by measuring x_1 at t_1 and x_2 at t_2, and taking $\xi = (x_1 + x_2)/2$. With our data the latter method gave values within 1 per cent of the value from the integral, (Equation 23), when t_1 to t_2 was 3 days. Equation 25 may be rewritten $k_3k_5/(k_5 - a) = u_{12}/\xi(t_2 - t_1)$ in which the term on the right side becomes what McFarlane (9) has termed the "metabolic rate" of albumin. Because of the time lag in excretion of breakdown products this "metabolic rate" differs from k_3 by the factor $k_5/(k_5 - a)$. When k_5, the excretory rate constant, is 2.5 it is about thirty times a, and $k_5/(k_5 - a)$ is about 1.03. Hence, if the factor $k_5/(k_5 - a)$ is omitted values 2 to 3 per cent too high will be obtained for k_3. If k_5 is slower (*e.g.* in other animals) greater errors will result. Fig. 9 shows typical values of $k_3k_5/(k_5 - a)$ determined from u_{12}, x_1, and x_2 for intervals of 1 and 3 days compared with k_3 as calculated solely from the plasma data and the model A equation, $k_3 = \alpha - \gamma/\beta$. As would be expected, the determinations made at daily intervals show the effects of irregular excretion which are less evident in the 3 day intervals between $t = 4$ and $t = 15$ days. With the exception of animal 1-01 agreement between these values and k_3 determined from the plasma data and model A is good, the average $k_3k_5/(k_5 - a)$ being 0.259, about 10 per cent higher than the average k_3 of 0.235. If in Equation 25 correction of 0.97 is made for $(k_5 - a)/k_5$, the mean value for k_3 is 0.251, about 7 per cent higher than the mean value given by k_3, model A. Plausible reasons for this residual overestimate will be found in sections 7 *c* and 7 *d*.

DISCUSSION

7 A. THE I^{131}-ALBUMIN Except that it was also dialyzed after preparation the labelled albumin used in these experiments was made essentially by McFarlane's method (6). Table I (given earlier) provides a comparison of our data with those of Cohen *et al.* (7) but the experiments are not quite comparable, since Cohen *et al.* injected a whole plasma preparation containing a number of labelled plasma proteins and then separated an "albumin" fraction from the recipient's plasma which contained 15 to 20 per cent alpha- and beta-globulins, and we separated an "albumin" fraction prior to labelling which, after labelling, had 90 to 95 per cent of the radioactivity attached to albumin and the remainder to α_1-globulin. Taking into account the experimental differences, which include breed and age of the rabbits, agreement between our results and those of Cohen *et al.* seems satisfactory.

7 B. THE PLASMA ACTIVITY EQUATION Though the graphical analysis of plasma activity is often reported to yield two exponential terms, in some instances a third component is described, giving $x = C_1e^{-at} + C_2e^{-bt} + C_3e^{-ct}$. Thus Berson and Yalow (13) report both types of equation from human

experiments, and of two rabbits studied by Matthews (14) one required a two term and the other a three term exponential equation. The third exponential is only required for the description of the plasma activity during the first few hours after the injection. In our experiments the plasma activity has been well described by a two term exponential equation during its whole course, though our observations have been insufficient to rule out the need for a third exponential to define the very early course. An accurate examination of this early period requires the withdrawal of a number of samples over a few hours, and since this may lead to undue excitation of the rabbits with temporary suppression of urine, and perhaps circulatory alterations, this has not been attempted.

7 c. THE MATHEMATICAL MODELS AND THEIR RELATION TO REALITY It has been shown that models A and C rather closely describe the tracer data except for the Z function. It is now necessary to inquire how physiologically reasonable are the descriptions implied by these models. The models consist of two parts, one describing the intravascular-extravascular transfer, and the other the catabolism of I^{131}-albumin. The transfer model pictures the intravascular albumin as a single compartment from which tracer passes at a rate, k_1x, to a single extravascular compartment, from which it is returned at a rate, k_2y. While the intravascular albumin may reasonably be considered a single compartment in which rapid mixing occurs, this is not so for the extravascular albumin, which is better considered as a group of different compartments between which there is little communication. The models thus imply a much oversimplified description of the extravascular behavior of albumin. Matthews (14) on the basis of the presence at times of a third exponential, has proposed equations for a two compartment extravascular system, but it is doubtful if this is more realistic than the single compartment models. In spite of these defects the simple transfer models described here satisfactorily predict the extravascular radioactivity during the major part of an experiment.

The part of the models describing I^{131}-albumin catabolism is also oversimplified. In essence it states that I^{131}-albumin is catabolized in the plasma or extravascular spaces, that the radioactive end products are excreted at a known average exponential rate, and that the quantities of I^{131}-albumin broken down in the plasma and extravascular fluids can be approximately determined from the time relations of the excreted radioactivity. If model A precisely defined the system it would indicate that catabolism occurred either in the blood stream or, perhaps, in the entire vascular endothelium. We have found no evidence of breakdown when I^{131}-albumin is incubated for 24 hours under sterile conditions with whole blood, plasma, or red cells, and the small positive values for k_4 suggest that catabolism does not occur solely in the vascular endothelium. Model C could mean that about three-quarters of the

catabolism occurred in the vascular system, and the remainder in an extravascular site separated by a significant time interval. But it could equally well mean that breakdown occurred entirely in other extravascular sites separated by a short passage time from the plasma. Miller (23) and Gitlin (24) with their collaborators have claimed that plasma proteins are broken down in the liver and kidneys, which could thus be such sites. As now shown, a single breakdown compartment to represent extravascular breakdown sites can readily be incorporated into model A, and thus renders the breakdown model more realistic in terms of our present knowledge.

7 D. AN IMPROVED MATHEMATICAL DESCRIPTION OF I^{131}-ALBUMIN CATABOLISM The new system which may be termed model D, is shown in Fig. 10 which now includes a breakdown compartment containing radioactivity, v,

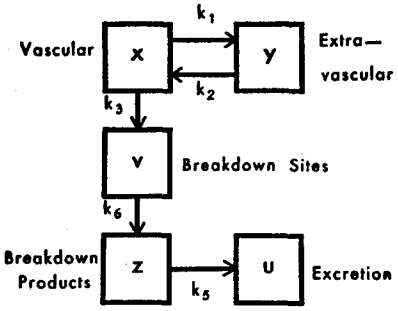

FIGURE 10. A modified and extended version of the model in Fig. 4 B, in which k_4 has been assumed to be zero and an additional compartment has been added. This compartment contains an amount, v, of radioactive albumin being catabolized and the radioactive end products are removed at a rate indicated by k_6. This is termed model D. (See section 7 *d*.)

attached to albumin, (this may be pictured as situated on the surface or in the interior of the catabolic cells in the liver and kidney), a new first order rate constant, k_6, defining the rate of release of breakdown products from v, and k_4 is now zero. The relevant equations are:

$$dx/dt = k_2y - (k_1 + k_3)x \tag{1 a}$$

$$dy/dt = k_1x - k_2y \tag{2 a}$$

for which the solutions are those given in Equations 7 through 11 when $k_4 = 0$

$$dv/dt = k_3x - k_6v \tag{3 a}$$

$$dz/dt = k_6v - k_5z \tag{4 a}$$

$$du/dt = k_5z \tag{5 a}$$

for which the new solutions are

$$v = Fe^{-at} + Ge^{-bt} - Fe^{-k_6 t} - Ge^{-k_6 t} \qquad \text{(compare with Equation 12)} \quad (6\,a)$$

$$z = k_6\left[\frac{F}{k_5 - a}(e^{-at} - e^{-k_5 t}) + \frac{G}{k_5 - b}(e^{-bt} - e^{-k_5 t}) + \frac{F + G}{k_5 - k_6}(e^{-k_5 t} - e^{-k_6 t})\right] \quad (7\,a)$$

in which

$$F = \frac{k_3 C_1}{k_6 - a} \quad \text{and} \quad G = \frac{k_3 C_2}{k_6 - b}$$

In a manner similar to the reduction of Equation 14 to 15, we have

$$u = 1 - \frac{k_6}{k_6 - a} \cdot \frac{k_5}{k_5 - a} \cdot \frac{k_3 C_1}{a} \cdot e^{-at} \qquad \text{(compare with Equation 15)} \quad (8\,a)$$

and C_3, the intercept at t_0 of the plot of log $(1 - u)$ against time is

$$C_3 = \frac{k_6}{k_6 - a} \cdot \frac{k_5}{k_5 - a} \cdot \frac{k_3 C_1}{a} \quad (9\,a)$$

When k_3, C_1, and a are obtained from the plasma data and k_5 is known,

$$k_6 = \frac{aH}{H - 1} \quad \text{in which} \quad H = \frac{a(k_5 - a)C_3}{k_3 k_5 C_1} \quad (10\,a)$$

Finally k_3 can be obtained independently from the plasma and excretory data from

$$k_3 = \frac{k_6 - a}{k_6} \cdot \frac{k_5 - a}{k_5} \cdot \frac{u_{12}}{\xi(t_2 - t_1)} \qquad \text{(compare with Equation 25)} \quad (11\,a)$$

provided k_5 and k_6 are known approximately since, as will be seen, k_6, like k_5, is probably much greater than a.

Rabbit B1X is the only animal providing sufficient data for test of this new system of equations. Taking $k_5 = 2.5$, from Equation 11 a $k_6 = 2.1$. Fig. 11 compares y obtained from $1 - x - v - z - u$ with y, model A, calculated from the plasma data, shows a plot of v calculated from Equation 6 a, and compares the observed z values with z calculated from Equation 7 a. Fig. 12 compares the observed u values with u calculated from Equation 8 a. Agreement between theory and observation is excellent. The new v compartment, as the equations indicate, is now very like the calculated z compartment of model A, $k_5 = 3$, shown in Fig. 6, and reaches an early maximum content of radioactivity of 0.05, which then declines to become quite small. Equation 11 a gives a value for k_3 for B1X of 0.185 compared with 0.189 given by model A

(Table V). The other animals provide insufficient data for a critical examination, but the new system assuming, as the data suggest, an average k_6 of about 2.0, would result in a very close correspondence of the values of k_3, model A, and k_3 determined from Equation 11 *a*. Two features of model D should be noted. First the solutions for the three tracer k's—k_1, k_2, and k_3—and the corresponding flux k's are identical with the solutions for model A. However, second, as shown by Equation 11 *a*, McFarlane's "metabolic rate" (9), which in our terminology is u/ξ, does not give a true measurement of

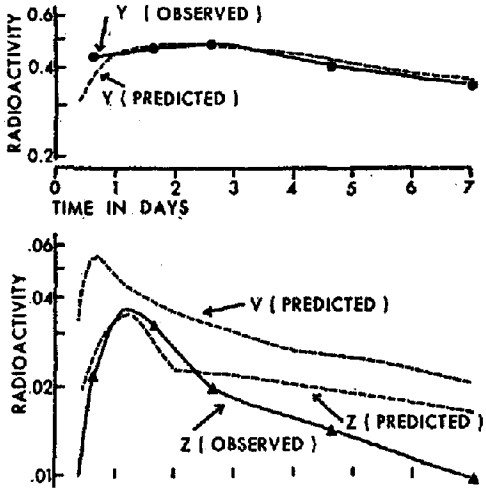

FIGURE 11. Curves predicted by model D compared with observed values of y and z. (See section 7 *d* and Figs. 5 and 6.)

k_3, but rather measures $k_3 \cdot k_6 \cdot k_5/(k_6 - a)(k_5 - a)$, and hence constitutes an overestimate of k_3. Thus, the assumption by McFarlane (9) and Berson (13) and their coworkers that excretion of radioactivity may be considered instantaneous is at best an approximation.

7 E. THE VALIDITY OF THE MEASUREMENTS OF ALBUMIN BREAKDOWN FLUX. AN UPPER BOUND FOR $k_3\bar{x}$ Last, further evidence of the validity of the estimate of $k_3\bar{x}$ is sought. It will first be noted that for each animal two *quite independent* estimates are available, that derived solely from the plasma data over many days, and that derived from the excretory and plasma data over periods of a few days, termed here u/ξ. Summarizing now the findings concerning u/ξ, section 6 shows that if the breakdown occurs solely in the plasma, u/ξ is greater than $k_3\bar{x}$ by the few per cent defined by $k_5/(k_5 - a)$; and section 7 *c* shows that if breakdown occurs at catabolic sites in communication with the plasma, then u/ξ is too great by the slightly larger amount $k_5k_6/(k_5 - a)(k_6 - a)$. A further possibility, that breakdown occurs after passage from the plasma through lymphatic channels to the breakdown site, may be imagined. Suppose that such passage requires an average time, τ. (The analy-

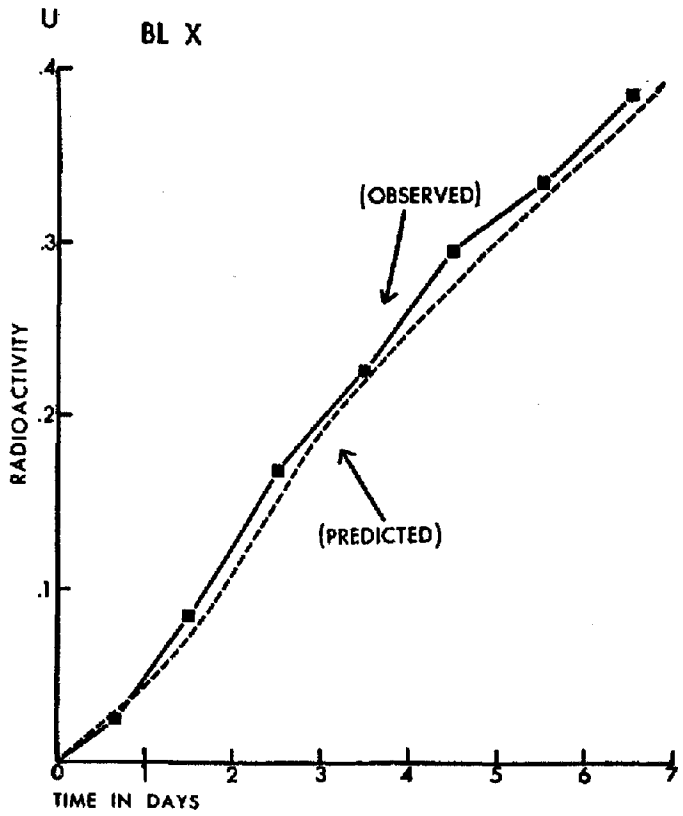

FIGURE 12. Excreted radioactivity predicted by model D compared with observed excretion. (See section 7 *d* and compare with Fig. 8.)

sis of the tracer data in sections 3 *a* and 3 *b* indicates that if there is such passage, then τ is relatively small.) Then if ξ, the mean plasma specific activity between t_1 and t_2 is $C_1e^{-at_m}$, the mean activity at the breakdown site (call this ξ_1) will be $C_1e^{-a(t_m-\tau)}$, and u/ξ is now too great by $(\xi_1/\xi)k_5k_6/(k_5 - a)(k_6 - a)$. Thus u/ξ provides values for $k_3\bar{x}$ which will be too high by an amount depending on the nature and sites of the breakdown process, but in any event will not be much too high. Hence these values provide upper bounds for $k_3\bar{x}$. Since the values of $k_3\bar{x}$ derived solely from the plasma data lie a few per cent below the values given by u/ξ, this is strong evidence that, despite the simplifications of the mathematical model from which they are derived, the values are close to the true values.

In the following paper (25) the values of k_3 and $\bar{x}$ are examined for evidence of the nature of the breakdown process.

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