The Catabolism of Plasma Albumin in the Rabbit

Its rate and regulation

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ABSTRACT From I^{131}-albumin studies and previously defined mathematical formulations, rates of breakdown were estimated for native plasma albumin in rabbits. These rates of catabolism per unit weight of animal were remarkably constant and were independent of variations in the steady state values of albumin concentration in the plasma. These results imply that, at least between animals, the breakdown of plasma albumin follows a kinetic process of approximately zero order. It seems plausible that the process operates similarly in individual animals, and hence that albumin is maintained at normal steady state levels in the healthy animal primarily by means of a regulated rate of synthesis.

INTRODUCTION

In the previous paper (1) I^{131}-albumin experiments on rabbits were detailed, the results of several mathematical formulations were compared with the tracer data, and the reality of the formulations was discussed. It was concluded that the estimate of breakdown rate obtained from the plasma data and the equations of mathematical model A (or model D) must be near the true value of the rate of albumin catabolism. These estimates are considered here with various other measurements made on the rabbits. Our work (1) and that of others (2–5) imply that albumin is catabolized at a site or sites outside the plasma. It is here shown that in healthy animals albumin destined for catabolism leaves the plasma according to a kinetic process approximating zero order. This behavior may be due to the zero order nature of the transport mechanisms or may be a reflection of the saturation of the proteolytic enzyme systems responsible for catabolism.

Methods

The rabbits and their treatment, the preparation of I^{131}-labelled albumin, the methods of making the measurements, and the mathematical analysis used have already been

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described (1). The weights given in Table I were the mean weights during the first 10 days of the experiments. The hematocrit values of heparinized whole blood are corrected for trapped plasma (0.03 of the packed cell volume) and are mean values for the first 10 days of the experiments. Plasma volume (P. V.) was determined from the quotient of the activity of the injected I^{131}-albumin divided by the activity per milliliter of the plasma sample, withdrawn approximately 10 minutes after the injection. Blood volume (B. V.) was determined from the equation B. V. = P. V. $\times$ 100/(100 − 0.9 H), in which H is the corrected per cent hematocrit value (6). Three animals, Nos. 5-94, 5-98, and 5-99, were exercised twice daily by 20 minutes of running in a rotating cage. No. 5-94 was exercised for about 1 week before, and during the course of the experiment, Nos. 5-98 and 5-99 for a number of weeks before, and during the course of the experiment. The latter two rabbits in particular were lean, muscular, and vigorous.

RESULTS

A. GENERAL OBSERVATIONS Table I summarizes measurements and calculations of k_3 and $k_3\bar{x}$ on nine rabbits for which complete data are available and Table II presents the same results expressed per kilogram body weight. The calculations of k_3 were made with Equations 7, 8, and 9 of the previous paper (1) with k_4 set to 0 and it has already been shown (1) that these values must be near to the true values. All rabbits appeared healthy and Table I shows little variation in their hematocrit levels, 37 to 44.5 with a mean of 40.7 and their total plasma proteins, 5.8 to 6.4 gm. per cent with a mean of 6.2. There was more variation in the plasma albumin concentration, which ranged between 2.95 and 4.1 gm. per cent, with a mean of 3.6. Rabbits 1-11 and 1-01 showed low levels of plasma albumin compared with the others. Table II shows that the exercised rabbits had higher plasma volumes than the mean of 30.6 ml./kg. and rabbits 1-02 and 1-01 had lower levels; the exercised rabbits also had higher levels of blood volume than the mean of 47.4 ml./kg., and rabbits 1-02 and 1-01 had lower levels. Certain correlations between the measurements of Tables I and II and $k_3\bar{x}$ and k_3 are now examined.

B. RATE OF BREAKDOWN OF ALBUMIN $k_3\bar{x}$ This rate has the units of grams of albumin/day, and Table I shows a mean rate of breakdown of 0.84 gm./day with a range of 0.63 to 0.94. Table II shows that if the values of the exercised rabbits, 5-98 and 5-99, are excluded, the breakdown rate per kilogram rabbit is remarkably constant, ranging between 0.23 and 0.26 gm./day. Rabbits 5-98 and 5-99 had much less subcutaneous fat than the others and the probable reason for the higher values in them is the higher proportion of "lean body mass" to total body weight in them. Fig. 1 *a* shows that $k_3\bar{x}$/kg. is quite independent of the plasma albumin concentration, par-

TABLE I

VARIOUS MEASUREMENTS ON RABBITS

Rabbit No.	Wt.	Ht.	P.V.	B.V.	T. Pr.	Alb.	$\bar{x}$	k_3	$k_3\bar{x}$
	kg.	*per cent*	*ml.*	*ml.*	*gm./100 ml.*		*gm.*	*day*$^{-1}$	*gm./day*
21	2.55	37	74	111	6.3	3.8	2.81	0.223	0.627
E 5-94	3.50	36	122	180	6.4	3.3	4.03	0.225	0.907
9-8	3.70	40	115	180	6.4	3.95	4.54	0.189	0.858
1-02	3.60	43	90	147	6.1	4.1	3.69	0.254	0.937
E 5-98	2.85	38	98	149	6.1	3.8	3.72	0.226	0.841
E 5-99	2.80	38	99	150	6.3	3.7	3.66	0.243	0.889
1-09	3.10	40.5	93	147	5.8	3.65	3.39	0.221	0.749
1-11	3.50	41.5	108	172	6.3	3.0	3.24	0.278	0.901
1-01	3.65	41.5	91	145	6.1	2.95	2.68	0.324	0.868
Mean		39.5			6.2	3.6		0.243	

Ht. = hematocrit value. Alb. = plasma albumin.
P.V. = plasma volume. $\bar{x}$ = total albumin in plasma.
B.V. = blood volume. k_3 = tracer rate constant.
T. Pr. = plasma total protein. $k_3\bar{x}$ = catabolic rate for albumin.
E indicates exercised animals.

ticularly among the animals that were not exercised. There are no strong correlations as judged from the plots (not shown) of $k_3\bar{x}$ with the volume of plasma, the volume of blood or the total plasma albumin, $\bar{x}$.

C. FRACTIONAL RATE OF BREAKDOWN OF TOTAL PLASMA ALBUMIN, k_3

The tracer rate constant, k_3, has the units of the fraction of tracer, x, removed by catabolism per day. In the Appendix it is established that this fractional

TABLE II

VARIOUS MEASUREMENTS PER KILOGRAM BODY WEIGHT

Rabbit No.	P. V.	C. V.	B. V.	$\bar{x}$	$k_3\bar{x}$
	ml./kg.	*ml./kg.*	*ml./kg.*	*gm./kg.*	*gm./kg. day*
21	29.0	13.0	43.5	1.10	0.246
E 5-94	34.9	14.6	51.5	1.15	0.259
9-8	31.2	15.5	48.7	1.23	0.232
1-02	25.0	14.5	40.8	1.03	0.26
E 5-98	34.4	15.8	52.3	1.31	0.295
E 5-99	35.4	16.4	53.6	1.31	0.318
1-09	30.0	15.5	47.5	1.09	0.242
1-11	30.9	16.5	49.1	0.927	0.257
1-01	24.9	13.5	39.8	0.735	0.238
Mean	30.6	15.3	47.4	1.10	0.261
				0.735–1.31	0.232–0.318

C. V. = red cell volume; other symbols as in Table I.

rate in the single experiment is identical with the fractional rate of breakdown of $\bar{x}$, the total plasma albumin. Table I shows that the values of k_3 vary from 0.19 to 0.32. Plots of the data of Tables I and II (not shown)

Fig. 1a

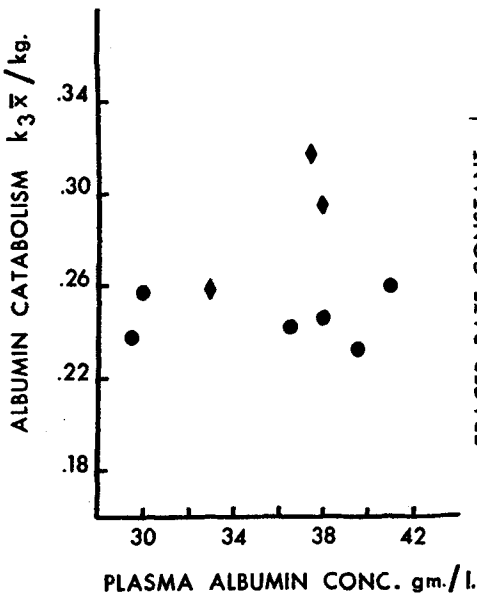

FIGURE 1 *a*. The rate of breakdown of albumin per unit body weight, $k_3\bar{x}/W$ (gm. albumin/kg. day), plotted against the concentration of albumin in the plasma (gm./liter). The diamonds, ♦, represent the exercised animals. Excluding the exercised animals, because of their greater proportion of "lean body mass," the catabolic flux is seen to be independent of the concentration. This relationship indicates that the catabolic process is of zero order, as noted in the Appendix, Equation 6.

Fig. 1b

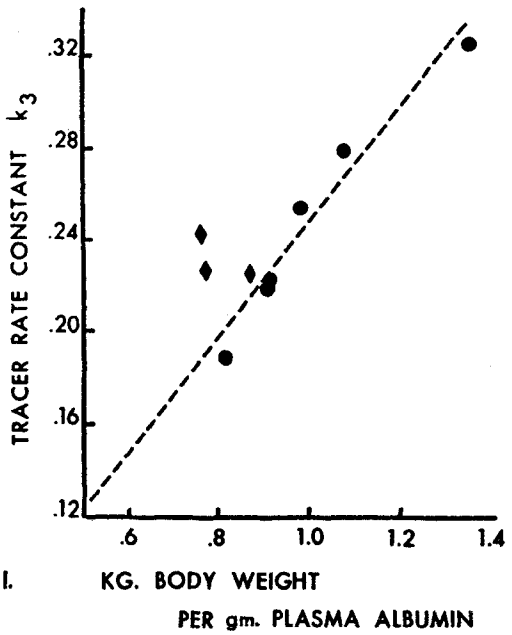

FIGURE 1 *b*. The tracer rate constant, k_3, is plotted against the body weight per unit weight of albumin in the plasma, $W/\bar{x}$ (kg./gm.). For a first order process, as noted in Equation 6 *a* of the Appendix, k_3 should vary linearly with concentration times the ratio $W/\bar{x}$, namely $\bar{c}(W/\bar{x})$, but if zero order k_3 should be a linear function simply of $W/\bar{x}$, as is seen in the figure. With a zero order process, a plot of k_3 against $W/\bar{x}$ should yield a straight line passing through the origin; the dashed line shows the upper segment of such a line. The diamonds, ♦, represent the exercised animals.

show no clear correlation of k_3 with body weight, the volume of plasma or of whole blood. However, there is some correlation between k_3 and the reciprocal of the albumin concentration, and Fig. 1 *b* shows clear correlation between k_3 and the reciprocal of $\bar{x}$/kg. The two exercised animals, 5-98 and 5-99, differ a little from the others, but in a way to be expected from their greater lean body mass.

DISCUSSION

Because of the stability of albumin, and of I^{131}-albumin when kept sterile, I^{131}-albumin in the body must be catabolized by proteolytic enzymes. Since we find no evidence of breakdown in rabbit blood (1), these enzymes must be situated either on the internal surfaces of vessels, or at extravascular sites such as on the surfaces of, or inside, cells. Fig. 2, taken from model D (1), represents the various breakdown sites as a single compartment outside the plasma containing $\bar{v}$, grams of albumin reacting with proteolytic enzymes. In theory, the rate of breakdown might be controlled either by the rate at which albumin enters the breakdown compartment, when the proteolytic enzymes are in excess, or by the quantity of the proteolytic enzymes, if these are maintained saturated or nearly saturated with substrate. In the former case, if the rate depends, for instance, on diffusion or on an unsaturated transport mechanism (7), it should have the characteristics of a first order

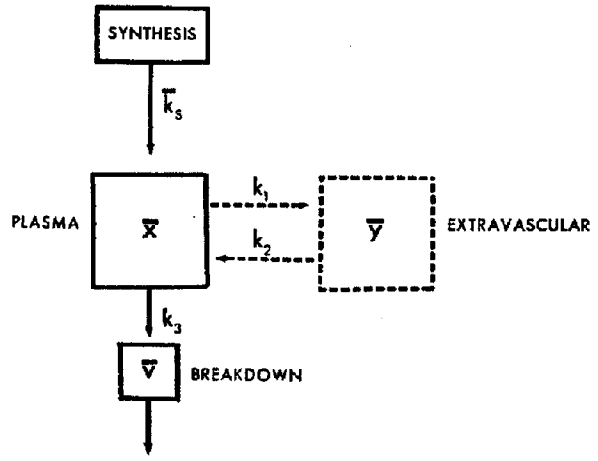

Figure 2. Diagrammatic representation of the model (D) of albumin synthesis and catabolism (1).

$\bar{x}$ = total intravascular albumin (gm.).
k_3 = fractional rate constant acting on $\bar{x}$.
$k_3\bar{x}$ = breakdown of albumin (gm./day).
$\bar{v}$ = quantity of albumin reacting with proteolytic enzyme in the breakdown site.

rate process. If, however, the transport system to the breakdown site or the proteolytic enzymes in the site were saturated with albumin, the behavior should be zero order. Assuming that each of the animals of Tables I and II was healthy, the data in these tables may be used to distinguish between these two possibilities. Two findings are then clear: (1) Excluding for reasons already given, the exercised animals, 5-98 and 5-99, the rate of catabolism

per unit body weight, $k_3\bar{x}/W$, is remarkably constant irrespective of the plasma albumin concentration (Fig. 1 *a*); (2) the tracer rate constant, k_3, is not independent of the plasma albumin concentration, but rather varies approximately as its reciprocal (Fig. 1 *b*). In the Appendix it is shown that (1) implies a zero order process. It is also shown that if the breakdown of native albumin follows a first order rate process, then k_3 should be independent of plasma albumin concentration which (2) contradicts. Finally, the Appendix shows that higher order catabolic processes can be ruled out. These findings strongly suggest that *within this range of albumin concentration*, the rate of albumin breakdown is controlled by a saturated proteolytic enzyme system or transport system, the activity of which closely parallels body weight. This conclusion assumes, of course, that the behavior of the process between animals is indicative of the nature of the process within individual animals, and it does not preclude an unsaturated system at lower albumin concentrations.

These ideas are at variance with other current ideas. Thus Gitlin (8) on the basis of the exponential decline of gamma-globulin infused into the blood stream of children suffering from agammaglobulinemia, and of fibrinogen infused into children with afibrinogenemia, concludes that breakdown of the plasma proteins follows a first order rate process, and McFarlane (9) on the basis of the exponential decline of infused pneumococcus antibody globulin in rabbits, and other less direct evidence, comes to the same conclusion. Such evidence is suggestive but may only be relevant for plasma globulins. McFarlane (9) proposes as a possible mechanism of regulation of plasma protein level a constant rate of synthesis by the liver and an exponential rate of breakdown. Our results suggest for albumin in healthy animals the direct opposite, namely a variable (regulated) rate of synthesis and a relatively constant rate of breakdown. At present little is known about the factors affecting albumin breakdown in man or animals. Rothschild and collaborators (10, 11) have shown by measurements of the breakdown flux with I^{131}-albumin that desiccated thyroid, prednisone, and hydrocortisone may increase the rate of breakdown in patients by 30 per cent. It has been claimed (12) that a protein-free diet in rats may reduce, and a high protein diet may increase, the rate of albumin breakdown, though objections may be raised to the methods used for calculating breakdown flux, and to some of the animals not being in a steady state. Presumably these hormone and dietary effects act on the enzyme or transport system. Something is known of the breakdown sites. The experiments of Miller and coworkers (2) with isolated perfused rat livers showed that $C^{14}O_2$ was released when C^{14}-labelled plasma proteins were perfused. Gordon (3) and Cohen and Gordon (5) showed that the rate of release of I^{131} from "screened" preparations of rat I^{131}-albumin by the isolated perfused rat liver could only account for from one-tenth to one-

seventh of the rate of breakdown in the living rat. Gitlin and coworkers (4) claim that the kidney and reticulo-endothelial system of mice are also sites of breakdown of I^{131}-albumin, but the experiments of Freeman and coworkers (13) using the isolated perfused rat liver seem to exclude the Kupffer cells as sites of breakdown of I^{131}-albumin. In the living rat Freeman and coworkers found that injections of carbon particles increased the fractional rates of breakdown of I^{131}-albumin but greatly depressed the plasma albumin concentrations. Their data are insufficient to determine whether the carbon injections altered the absolute breakdown flux in the whole animal, but they might perhaps be explained by reduced synthesis with not greatly altered breakdown of albumin.

APPENDIX

A. THE FIRST ORDER NATURE OF THE TRACER RATE CONSTANT, k_3, IN A SINGLE EXPERIMENT IN A SINGLE ANIMAL IN A GIVEN STEADY STATE Consider an I^{131}-albumin experiment in which the volume of plasma remains constant. Let the total amount of albumin in the plasma, $\bar{x}$ (grams), be maintained at a constant value by a constant influx, $\bar{k}_s$ (grams/day), from synthesis and a constant efflux, B (grams/day), of albumin destined for breakdown (Fig. 2). The steady state equation is thus

$$\frac{d\bar{x}}{dt} = \bar{k}_s - B = 0 \tag{1}$$

Now if a small amount of tracer (I^{131}-albumin), $x \ll \bar{x}$, is added to the plasma, so that $x + \bar{x} \simeq \bar{x}$, we accept the approximation of replacing $\bar{x}$ by $\bar{x} + x$ in Equation 1. Next because the tracer is also participating in lymphatic exchange we write for emphasis $x = x(t)$, so that the arguments hold for the general plasma activity function. Equation 1 can then be written in the form

$$\left\{\frac{d}{dt}\left[\bar{x} + x(t)\right]\right\} \simeq \bar{k}_s - B\left[\frac{\bar{x}}{\bar{x} + x(t)} + \frac{x(t)}{\bar{x} + x(t)}\right] = 0 \tag{2}$$

in which the bracketed derivative is understood to be that rate of change of $x(t)$ related to the breakdown process. In the last term, consider again, $\bar{x} + x \simeq \bar{x}$, so that

$$\left\{\frac{d\bar{x}}{dt}\right\} + \left\{\frac{dx(t)}{dt}\right\} \simeq \bar{k}_s - B - B\frac{x(t)}{\bar{x}}$$

by applying Equation 1,

$$\begin{aligned}\left\{\frac{dx(t)}{dt}\right\} &= -\frac{B}{\bar{x}}x(t) \\ &= -k_3 x(t)\end{aligned} \tag{3}$$

in which $k_3 = B/\bar{x}$ represents a first order rate constant of I^{131}-albumin breakdown under these conditions, irrespective of the order of the reaction governing the catabolism of native albumin, as was assumed in the previous paper (1). Thus k_3, the fractional rate of breakdown of I^{131}-albumin, is also under these conditions identical with the fractional rate of breakdown of native albumin, and $B = k_3\bar{x}$.

B. THE RELATIONSHIP BETWEEN THE TRACER RATE CONSTANT k_3, THE FLUX, $k_3\bar{x}$, AND THE PLASMA ALBUMIN CONCENTRATION, $\bar{c}$, WITH DIFFERENT ORDERS OF THE BREAKDOWN PROCESS IN A SERIES OF EXPERIMENTS Suppose that an animal can change from one steady state, in which $d\bar{x}/dt = \bar{k}_s - B = 0$ and $\bar{x}$ is fixed in value, to another, in which $\bar{x}$ has changed and $\bar{k}_s = B$ may or may not have changed. The breakdown process acting on native albumin might obey zero order, first order, or n order kinetics. Let k_b represent the rate constant of any order of catabolic reaction. In conformity with classical kinetic theory k_b "acts" on the concentration of albumin raised to some power. Let $\bar{c}$ (gm./liter) be the albumin concentration; then $k_b\bar{c}^n$, in which $n = 0, 1, 2 \ldots$ or some fractional power, is the order of the reaction, and is related in some way (requiring definition) to B, and hence to $k_3\bar{x}$.

CASE 1 Suppose the breakdown occurs through all the plasma and is mediated by proteolytic enzymes contained in the plasma. Note that $\bar{x}$ is defined by $\bar{c}V$, in which V(liters) is the volume of plasma and hence also the volume through which the catabolic enzyme is distributed. It thus follows that

$$k_3\bar{x} = k_b\bar{c}^nV \tag{4}$$

and hence

$$k_3 = k_b\bar{c}^{n-1} \tag{4 a}$$

Reasons have already been given for concluding that breakdown does not occur in the plasma and therefore case 2 is considered.

CASE 2 Suppose, as in Fig. 2, that breakdown takes place in a breakdown compartment containing $\bar{v}$ grams of albumin. This albumin may be pictured quite generally as that reacting with an enzyme system or distributed through a certain volume of catabolic cells, or may further be considered as that reacting with some transport system. Let $\bar{v} = v(\delta\bar{c})$, in which it is assumed that the concentration associated with $\bar{v}$ is a constant fraction, δ, of the albumin concentration in the plasma, $\bar{c}$, and v may be considered the volume of distribution for $\bar{v}$. Since v, unlike V, cannot be measured, it is presumed to be directly proportional to body weight, W (kg.). Thus $v = jW$, in which j is a constant.

Equation 4 now becomes

$$k_3\bar{x} = k_b(\delta\bar{c})^njW \tag{5}$$

$$= K_b\bar{c}^nW$$

in which $K_b = k_bj\delta^n$, and Equation 4a becomes

$$k_3 = K_b\bar{c}^{n-1}W/V \tag{5 a}$$

When comparing results in animals of different sizes it is convenient to make comparisons on the basis of unit weight. Equation 5 now becomes

$$k_3\bar{x}/W = K_b\bar{c}^n \tag{6}$$

and the solution for k_3 becomes

$$k_3 = K_b\bar{c}^n W/\bar{x} \tag{6 a}$$

which may be rewritten

$$k_3 = K_b\bar{c}^{n-1}W/V$$

Equation 6 defines the flux behavior, and Fig. 1 *a* shows that when $k_3\bar{x}$/kg. is plotted against albumin concentration, $\bar{c}$, the results imply that $K_b\bar{c}^n$ is a constant. It follows that $n = 0$, *i.e.*, the process is of zero order, since for $n > 0$ the function would not behave independently of $\bar{c}$.
From Equation 6 *a* it is noted that for:

$n = 0$, $k_3 = K_bW/V\bar{c}$, and when W/V = *constant*, k_3 is related to $1/\bar{c}$;

$n = 1$, $k_3 = K_bW/V$, and when W/V = *constant*, k_3 is independent of concentration;

$n = 2$, $k_3 = K_b\bar{c}W/V$, and when W/V = *constant*, k_3 is a linear function of concentration.

Note that $W/V = W\bar{c}/\bar{x}$.

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