The Role of the Gut in Albumin Catabolism

I. *Studies in the jejunoileectomized rabbit*

J. J. FRANKS, E. L. MOSSER, and GEORGE L. ANSTADT

From the Biokinetics Branch and the Veterinary Services Branch of the School of Aerospace Medicine, Brooks Air Force Base, Texas. Dr. Franks' present address is Department of Medicine, United States Air Force Hospital, Lackland Air Force Base, Texas

ABSTRACT I^{131}-albumin metabolic studies were carried out in 5 rabbits before, 3 weeks after, and several months after removal of 70 to 90 per cent of the jejunum and ileum. A sixth animal was studied before and 11 weeks after a sham operation. During postoperative experiments, the animals were found to be in a highly unsteady state with large losses of albumin from the vascular compartment. Despite these losses, the plasma albumin concentration was maintained at a relatively constant level. No decrease in the albumin efflux occurred following nearly complete jejuno-ileectomy. The data suggest that albumin catabolism is a first order process.

INTRODUCTION

Several reports published during the past few years point to the gastrointestinal tract as a major site of albumin catabolism. Ullberg *et al.* (1), using commercially prepared human I^{131}-albumin in cats and in mice, reported the accumulation and excretion of the labeled protein by the small intestine. Wetterfors *et al.* (2), using a similar labeled preparation in patients at surgery, found accumulation of protein-bound activity in the walls and secretions of the stomach, duodenum, and jejunum. Wetterfors' estimates of albumin breakdown by the upper gastrointestinal tract were large enough to account for the bulk of albumin degradation in the body. Similarly, experiments by Armstrong *et al.* (3), who used isolated sections of intestine in the rabbit, indicated passage of labeled protein into the lumen in quantities sufficient to account for most of the albumin breakdown in this animal. Recently, Campbell *et al.* (4) showed the presence of significant quantities of albumin in the jejunal contents of sheep. Organs other than the gastrointestinal tract have been implicated in albumin catabolism. Gitlin *et al.* (5) found reduced albumin catabolism in nephrectomized and in partially hepatectomized mice; Gordon and Humphrey (6), Cohen and Gordon (7), and Katz *et al.* (8) reported low

rates of breakdown in perfused liver and kidney. Recently Katz and coworkers noted continued albumin breakdown in totally eviscerated rats (9).

In this paper we report measurements of albumin breakdown rates in rabbits before, 3 weeks after, and several months after removal of 70 to 90 per cent of the jejunum and ileum. In a subsequent paper we report acute experiments comparing albumin breakdown in sham operated and partially to totally enterectomized rabbits (10). Many of the methods devised by Reeve and coworkers (11–13) were used in these experiments.

MATERIALS AND METHODS

The animals were male New Zealand White rabbits fed Purina Rabbit Chow. During an experiment they were housed in individual metabolism cages, and daily collections of the separated urine and feces were made. At this time the cages were rinsed with 100 ml of 0.2 per cent NaI and the rinsings added to the urine. The urine was collected in a bottle containing 10 ml of 0.2 per cent KI and 2 ml of toluene. The feces were homogenized in a Waring Blendor with about 5 times their volume of 0.2 per cent KI and 15 gm of starch agar. This resulted in a thick, even suspension that could be transferred by pipette and did not settle out during counting. For 3 days before and during an experiment the rabbits were given drinking water containing 200 mg KI and 1.8 gm NaCl per liter to prevent accumulation of radioactivity in the thyroid. Rabbit I^{131}-albumin was prepared using a modification (12) of McFarlane's method (14) except that dialysis for 20 to 24 hours was used in place of the Amberlite IR4B column to remove residual radioactive iodide. No less than 97 per cent of the radioactivity in the final preparation was protein bound as indicated by precipitation with trichloroacetic acid in the cold.

One to 3.3 ml containing 8 to 14 μc of labeled albumin was injected, within 24 hours of preparation, into the marginal ear vein of the rabbits. The amount injected was determined from the net weight of the syringe contents less the residual activity washed out of the syringe with 0.1 N NaOH. Quadruplicate standards were prepared by diluting portions of the I^{131}-albumin solution in 1 per cent saline containing 2 per cent by volume non-radioactive rabbit plasma (15). Blood samples of about 3 ml were withdrawn 15 minutes after injection and at intervals of 1 or more days thereafter for 14 days. Total protein and albumin concentrations were measured by a biuret method (12). For the albumin measurement, 0.2 ml of plasma was mixed with 4 ml of a sodium sulfate–sodium sulfite solution (16) in a test tube, incubated 30 minutes at 37°C, and cooled to 31°C in a water bath. One-half milliliter of 1 per cent Tween 80 in ether (17) was added and brought to the same temperature; the tube was then stoppered and gently inverted 10 times. Finally the tube was centrifuged for 10 minutes, and the clear subnatant fluid was removed with a capillary pipette and analyzed for protein. Hematocrits were obtained by centrifuging heparinized blood in Wintrobe tubes 30 minutes at 1450 *g*.

The radioactivity in plasma, urine, and feces was measured in a Nuclear-Chicago DS-5 or in a Technical Measurement Corporation DS 1A well scintillation counter.

Measured activities were corrected for decay by reference to the standards. Excreted radioactivity was corrected for collection loss by multiplying by 1/0.96 or 1/0.93, depending upon the cage used.

Three experiments were performed on each of 5 animals. Six to 17 days after the first experiment, the animal was anesthetized with sodium pentobarbital and 120 to 150 cm of small bowel removed proximal to about 15 cm above the cecum. More extensive resection than this invariably resulted in death of the animal. The remaining bowel was rejoined by a side to side anastomosis. At the time of surgery it was estimated that over half of the small intestine was removed; results of postmortem examinations detailed below indicate that 60 to 75 per cent of the small intestine and 70 to 90 per cent of the jejunum and ileum had been removed. Twenty-one to 22 days after surgery a second experiment was carried out, and 92 to 148 days after surgery a third.

V_p, the plasma volume in milliliters, was calculated from the injected radioactivity divided by the counts per minute per ml in the 15 minute plasma sample. Correction was made for the small fraction of radioactivity not precipitable by trichloroacetic acid (18). In all postenterectomy experiments but one, and in both experiments on sham operated rabbit 49, a second measurement of V_p was obtained at the end of the experiment by injecting additional I^{131}-albumin.

RESULTS

W, c, V_p, and $\bar{x}$

Table I shows W, the body weight in kilograms, c, the albumin concentration in grams per milliliter, V_p, the plasma volume in milliliters, and $\bar{x}$, the total intravascular albumin in grams, at the beginning and end of each experiment. A significant decrease in $\bar{x}$ ($p < 0.01$) occurred during the course of all postoperative experiments except Experiment III in rabbit 43. Despite this decrease the albumin concentration remained relatively stable throughout most experiments. As Table I shows, this was at the expense of a considerable drop in plasma volume. In rabbit 40, Experiments II and III, the final plasma volume and the associated albumin concentration were measured the day after the animal had been removed from the metabolism cage and taken off iodide drinking water. During these intervals, this rabbit apparently increased V_p considerably as indicated by a fall in the albumin concentration from the previous day's level. The latter is given in parentheses and is close to the concentrations measured at the beginning and throughout both experiments. Only in rabbit 39, therefore, was the fall in total intravascular albumin during an experiment accompanied by a fall in the albumin concentration.

The Plasma Specific Activity and Excretory Curves

Fig. 1 shows a semilogarithmic plot of plasma and excretory data from a tracer experiment. The upper function, s, represents the fractional plasma specific activity, *i.e.*, the radioactivity per gram of albumin at any time t divided by

the radioactivity per gram of albumin at $t = 0$ (15 minute sample). The lower curve is termed $1 - u$, in which u represents the cumulative values of urinary and fecal radioactivity. The derivation and relationships of s and $1 - u$ are

TABLE I

VARIOUS MEASUREMENTS IN RABBITS AT THE BEGINNING AND END OF EACH EXPERIMENT

Rabbit		Experiment I Preoperative		Experiment II Postoperative		Experiment III Postoperative	
5	Time (days)	−19*	−5	+21*	+35	+162	+177
	W‡	3.12	3.12	2.92	3.03	3.28	3.25
	c§	0.0413	0.0428	0.0405	0.0448	0.0384	0.0409
	V_p‖	131	—	103	—	114	94
	$\bar{x}$¶	5.43	—	4.17	—	4.38	3.83
40	Time	−20	−6	+22	+36	+107	+122
	W	2.90	2.99	3.18	3.16	3.70	3.54
	c	0.0387	0.0367	0.0427	0.0342 (0.0417)	0.0394	0.0368 (0.0403)
	V_p	101	—	103	95	112	102
	$\bar{x}$	3.93	—	4.40	3.26	4.41	3.75
41	Time	−20	−6	+22	+36	+107	+122
	W	2.79	2.94	2.87	2.86	3.48	3.27
	c	0.0410	0.0402	0.0443	0.0439	0.0444	0.0422
	V_p	110	—	115	91	109	97
	$\bar{x}$	4.51	—	5.09	4.01	4.84	4.15
39	Time	−20	−7	+21	+35	+148	+163
	W	3.22	2.94	2.80	3.18	3.70	3.51
	c	0.0414	0.0430	0.0450	0.0356	0.0350	0.0313
	V_p	111	—	107	110	114	97
	$\bar{x}$	4.60	—	4.81	3.92	3.99	3.02
43	Time	−21	−7	+21	+35	+92	+106
	W	3.30	3.21	3.50	3.65	3.88	3.88
	c	0.0399	0.0387	0.0447	0.0422	0.0397	0.0390
	V_p	134	—	162	155	134	154
	$\bar{x}$	5.35	—	7.24	6.54	5.32	6.00
49	Time	−28	−14	+78	+93		
	W	3.55	3.52	3.73	3.31		
	c	0.0328	0.0350	0.0362	0.0375		
	V_p	129	117	154	117		
	$\bar{x}$	4.23	4.10	5.57	3.76		

* −, days before surgery; +, days after surgery.
‡ Weight in kilograms.
§ Plasma albumin concentration in grams per milliliter.
‖ Plasma volume in milliliters.
¶ Intravascular albumin (in grams) = $V_p \cdot c$.

detailed more fully elsewhere (19). As illustrated by Fig. 1, the plasma data can be described fairly well by

$$s = E_1 e^{-\alpha t} + E_2 e^{-\beta t} \tag{1}$$

and since $\beta \gg \alpha$, by

$$s = E_1 e^{-\alpha t},\ t \geq t_a . \tag{2}$$

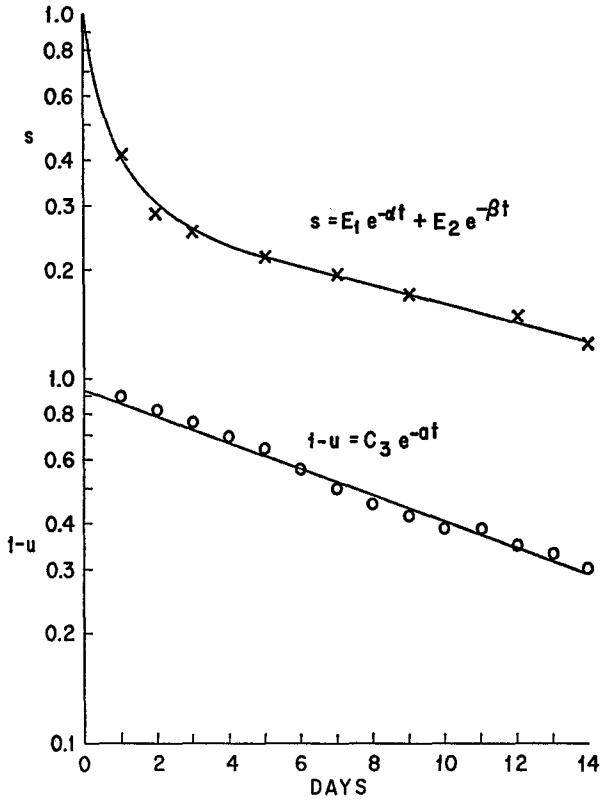

FIGURE 1. Plasma specific activity and excretory data in the rabbit (rabbit 41, Experiment III).

Similarly, the excretory data can be described by

$$1 - u = C_3 e^{-at},\ t \geq t_b . \tag{3}$$

Table II gives numerical values for the constants of equations 1 and 3 for all experiments as determined by the method of least squares. Log s and log $(1 - u)$ generally became linear when $t \geq 5$ and $t \geq 3$ respectively, *i.e.*, $t_a = 5$ days and $t_b = 3$ days. Consequently, E_1 and α were determined from data obtained when $5 \leq t \leq 14$, and C_3 and a from data obtained when $3 \leq t \leq 14$.

The Albumin Breakdown Rate

In the first paper of this series (19), it was shown that in both the steady and non-steady states, $t_1 \geq t_a > t_b$,

$$\bar{\bar{k}}_3 = \bar{x}_0 \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5} \frac{\int_{t_1}^{t_2} \frac{u'}{s} dt}{t_2 - t_1} \tag{4}$$

where $\bar{\bar{k}}_3$ = the mean albumin breakdown rate in grams per day between t_1 and t_2 and $\bar{x}_0$ = intravascular albumin in grams at $t = 0$. k_4 and k_5 are tracer rate constants associated with the breakdown and excretory compartments

TABLE II

CONSTANTS FOR THE EXCRETORY AND PLASMA SPECIFIC ACTIVITY FUNCTIONS (EQUATIONS 1 AND 3)

Rabbit	Experiment	C_3	a	$\frac{\hat{\sigma}_{(1-u)\cdot t}*}{1-u}$	E_1	α	$\frac{\hat{\sigma}_{s\cdot t}‡}{s}$	E_2	β
5	I (pre-op.)	0.971	0.0595	0.0257	0.400	0.0668	0.0464	0.600	2.14
	II (post-op.)	1.116	0.0774	0.0239	0.347	0.0834	0.0497	0.653	1.39
	III (post-op.)	0.992	0.0779	0.0241	0.357	0.0789	0.0315	0.643	1.26
40	I	0.975	0.0719	0.0373	0.336	0.0664	0.0294	0.664	1.40
	II	1.005	0.0706	0.0384	0.391	0.0890	0.0756	0.609	2.14
	III	1.080	0.0866	0.0535	0.304	0.0716	0.0829	0.696	1.49
41	I	0.932	0.0624	0.0171	0.347	0.0709	0.0226	0.653	1.64
	II	0.896	0.0709	0.0300	0.377	0.0811	0.0315	0.623	1.47
	III	0.924	0.0827	0.0279	0.290	0.0595	0.0182	0.710	1.54
39	I	1.057	0.0801	0.0415	0.274	0.0557	0.0230	0.726	1.87
	II	1.090	0.0627	0.0400	0.364	0.0818	0.0257	0.636	1.60
	III	1.074	0.0718	0.0472	0.282	0.0466	0.0503	0.718	1.29
43	I	0.969	0.0666	0.0286	0.363	0.0545	0.0406	0.637	1.22
	II	0.940	0.0765	0.0396	0.422	0.0677	0.0402	0.578	2.01
	III	1.250	0.0945	0.0506	0.349	0.0672	0.0637	0.651	1.04
49	I	1.080	0.0682	0.0333	0.359	0.0798	0.0429	0.641	2.02
	II	1.020	0.0528	0.0489	0.419	0.0597	0.0277	0.581	2.81

* Relative standard error of estimate for excretory data (28).

‡ Relative standard error of estimate for plasma data $\equiv \hat{\sigma}_{\log s \cdot t}$ = standard error of estimate for the regression of log s on t.

(19), with numerical values of 2.0 and 2.5 days^{-1}, respectively (11, 12). Substitution from equations 2 and 3 gives the computing form,

$$\bar{\bar{k}}_3 = \bar{x}_0 \frac{k_4 - a}{k_4} \cdot \frac{k_5 - a}{k_5} \cdot \frac{aC_3}{E_1} \cdot \frac{e^{(\alpha-a)t_2} - e^{(\alpha-a)t_1}}{(\alpha - a)(t_2 - t_1)}. \quad (5)$$

Table III shows $\bar{\bar{k}}_3$ as calculated from equation 5 for each of seventeen

TABLE III

THE MEAN ALBUMIN EFFLUX WITH ITS RANGE, AND THE MEAN ALBUMIN EFFLUX PER KILOGRAM BODY WEIGHT, BEFORE AND AFTER JEJUNO-ILEECTOMY

Rabbit	Experiment	$\bar{\bar{k}}_3$ (gm/day)	Range* (gm/day)	$\bar{\bar{k}}_3/\overline{W}$ (gm/day/kg)
5	I (pre-op.)	0.800	0.767–0.819	0.257
	II (post-op.)	1.032	0.997–1.052	0.346
	III (post-op.)	0.880	0.887–0.895	0.274
40	I	0.730	0.745–0.709	0.248
	II	0.895	0.820–0.969	0.282
	III	1.085	1.164–1.016	0.297
41	I	0.777	0.744–0.804	0.272
	II	0.888	0.847–0.928	0.310
	III	0.952	1.053–0.855	0.283
39	I	1.048	1.169–0.939	0.340
	II	1.021	0.940–1.137	0.341
	III	0.807	0.898–0.716	0.222
43	I	0.795	0.838–0.756	0.244
	II	1.032	1.095–1.012	0.288
	III	1.284	1.439–1.121	0.328
49‡	I	0.909	0.861–0.956	0.259
	II	0.732	0.707–0.752	0.205

* Range about $\bar{\bar{k}}_3$ is $\bar{k}_3$ (5) to $\bar{k}_3$ (14) calculated from equation 20 of the preceding paper (19).
‡ Sham operated animal.

experiments with $t_1 = 5$ days and $t_2 = 14$ days. The range, *i.e.* the breakdown rate at t_1 and t_2, is also given. The weight-corrected rate, $\bar{\bar{k}}_3/\overline{W}$, in grams per day per kilogram ($\overline{W}$ = mean weight during the experiment) permits comparison of albumin catabolism in animals of different size. As indicated in Table III, $\bar{\bar{k}}_3/\overline{W}$, did not fall after enterectomy but actually increased in all but one postenterectomy experiment. This slight rise probably reflects the smaller proportion of body fat in the postoperative animals rather than any significant increase in their capacity to break down albumin (13).

Postmortem Findings

Four surviving rabbits, nos. 5, 39, 40, and 41, were available for tissue examination 8 to 14 months after small bowel resection. At necropsy, the remaining small bowel was measured, and sections of liver, duodenum, jejunum, ileum, and the anastomosis were taken for histologic examination. There was relatively little intraperitoneal fat. No hypertrophy or other gross abnormalities were noted except for slight dilatation of the bowel proximal to the anastomosis in rabbit 5. The anastomoses were patent. Microscopically, all tissues were within normal limits except for slight to moderate jejunal muscle hypertrophy above the anastomoses. The residual small bowel measured 90 to 145 cm in the 4 rabbits. The length of small bowel in 8 normal animals was 350 ± 60 cm with 60 to 75 cm of duodenum. These measurements indicate that 60 to 75 per cent of the entire small bowel and 70 to 90 per cent of the jejunum and ileum had been excised.

DISCUSSION

As indicated in Table I, many animals were found to be in a highly unsteady state during the 14 day experimental periods, with continual loss of protein and fluid from the vascular compartment. Whether this loss was the result of decreased albumin synthesis or of a net shift of water and protein to the extravascular compartment is not clear, but it probably reflects the inability of animals with little nutritional reserve to withstand the frequent handling, sampling, and other manipulations of experimentation. The problem of calculating the breakdown rate in such animals was resolved by using equation 5 to obtain a mean value for the rate over a given period of time.

The hypothesis that plasma albumin is catabolized by hydrolysis after passage into the gastrointestinal tract from the vascular compartment is an attractive one. It is supported to some extent by experiments showing that the clinical entities of idiopathic hypoalbuminemia (20, 21), giant hypertrophic gastritis (22), sprue (23), regional ileitis, and ulcerative colitis (24, 25) are associated with loss of albumin and other serum proteins into the gastrointestinal tract. It locates the breakdown compartment close to the vascular compartment as required by the model (19) and allows for salvage of the albumin hydrolysate by reabsorption from the gut. Walter, however, has recently reviewed the evidence against simple hydrolysis as the principal means of protein breakdown (26). This evidence suggests that protein catabolism is an energy-sparing process involving an intermediate, non-polypeptide, amino acid derivative which is probably identical with the activated amino acid intermediate of protein anabolism. If serum proteins are catabolized by this mechanism, as is suggested by the work of Whipple and co-

workers (27), the gastrointestinal breakdown hypothesis becomes untenable. The experiments described in this paper do not provide a final answer to this problem. They do demonstrate fairly conclusively that removal of most of the jejunum and ileum does not retard the albumin catabolic rate in the rabbit and, therefore, that these organs play at most only a minor role in normal albumin catabolism.

Another important aspect of albumin catabolism is the relationship of its rate to the plasma albumin concentration, *i.e.*, the reaction order of the

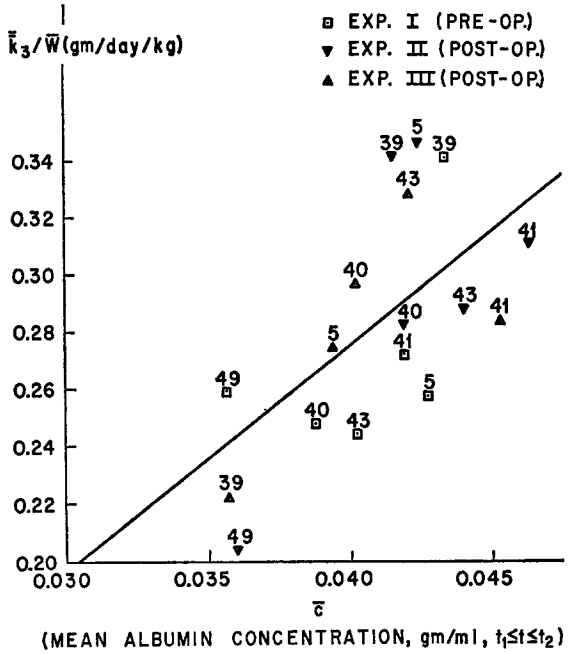

FIGURE 2. Plot of the efflux per kilogram body weight against the mean albumin concentration.

process. Fig. 2 shows a regression curve for a plot of $\bar{k}_3/\bar{W}$, the breakdown rate per unit body weight, against albumin concentration for each experiment. Each result is treated independently since analysis of covariance indicates that changes in $\bar{k}/\bar{W}$ in succeeding experiments in the same animal can be accounted for by changes in the concentration. The slope of the regression curve is significantly greater than zero ($p < 0.05$), indicating that the reaction order is greater than zero. The standard error of estimate is 0.033. Since this latter figure is probably smaller than the variation in $\bar{k}_3/\bar{W}$ (which depends not only on experimental error but also on the range about the mean value $\bar{k}_3$), the analysis suggests that albumin catabolism is a first order process, directly proportional to the albumin concentration within the range of concen-

tration observed. This is contrary to the findings of Reeve and Roberts, who concluded from their data that the reaction order is zero (13).

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