The Role of the Gut in Albumin Catabolism

II. *Studies in enterectomized rabbits*

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ABSTRACT   Using I^{131}-albumin tracer methods, albumin breakdown rates were estimated in 7 rabbits following extensive gastrointestinal resection, including 5 with nearly complete enterectomy, 1 with total gastroenterectomy, and 1 with gastrectomy only, and in 4 sham operated rabbits. Breakdown rates in the resected animals varied from 48 to 187 per cent of the corresponding controls, with an average of 96 per cent. It is concluded that no more than one-half, and probably much less, of albumin breakdown occurs in the gastrointestinal tract.

INTRODUCTION

In experiments described in the previous paper (1), we found that removal of 75 to 90 per cent of the jejunum and ileum of rabbits caused no reduction in the plasma albumin catabolic rate as determined by analysis of I^{131}-albumin tracer data. In this paper we report experiments in rabbits with more extensive bowel resections. Measurement of albumin breakdown is difficult in such animals because they survive surgery only a few hours. Relatively crude estimates can be made by relating the release of I^{131}-albumin breakdown products into the blood to changes in the plasma specific activity. When these estimates are compared with similar estimates in sham operated animals, some assessment of the residual capacity of resected animals to break down albumin can be made.

METHODS

Preparation and Screening of I^{131}-Albumin

I^{131}-albumin was prepared according to a method described previously (2) except that labeling was carried out using McFarlane's iodine monochloride technique (3).

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Non-protein-bound activity was removed by passage through an Amberlite IR4B ion exchange column (4). Labeled protein with a high specific activity and with an iodide to albumin molar ratio of less than 3 was obtained and was observed to be 97 to 99 per cent albumin by paper electrophoresis. Within 30 minutes after preparation the labeled albumin was injected into a small (1 to 2 kg) rabbit for "biologic screening" (4, 5). Forty to 48 hours later, 30 to 50 ml of blood was removed and the whole plasma used within 2 hours in experiments described below. Ninety-seven to 99 per cent of the radioactivity in this plasma was bound to albumin as determined by paper electrophoresis, and less than 2 per cent of the activity remained in solution after precipitation with 12 per cent trichloroacetic acid.

Injection, Sampling, and Measurement

Four to 25 μc of I^{131}-albumin in 5 to 20 ml of plasma was injected into the marginal ear veins of male New Zealand White rabbits immediately following the surgery described below. This injection was followed by 2 to 10 μc of NaI^{130} in a separate syringe. Three-milliliter blood samples were collected at intervals of 2 to 6 hours; urine and gastric juice excreted over the same intervals were also collected, the latter in flasks containing a few grams of $NaHCO_3$. Plasma volume, albumin and total protein concentrations, and hematocrits were measured as previously described (1). Non-protein-bound radioactive breakdown products of I^{131}-albumin, which have been shown to consist mainly of iodide^{131} (6), and iodide^{130} were separated from I^{131}-albumin by treating plasma or gastric juice with 5 volumes of acetone. After 30 minutes in the cold, the mixture was centrifuged in a Hemmings filter and the protein-free filtrate analyzed for radioactivity. Experiment showed that recovery of iodide was close to 100 per cent with no detectable protein-bound activity in the filtrate. I^{131} and I^{130} were counted with a Technical Measurement Corporation S-2 Dual Peak Analyzer at 0.36 mev (5 volt window) and above a base line of 0.50 mev, respectively. Appropriate corrections were made for energy peak overlap using simultaneous algebraic equations.

Preparation of Animals

For 3 days prior to the experiment, the animal was given drinking water containing 200 mg NaI and 1.8 gm $NaCl$ per liter. Surgery was performed using aseptic technique under pentobarbital anesthesia; anesthesia was maintained throughout the experiment. Rectal temperature was monitored continuously and maintained close to 101°F with a heat lamp and/or heating pad and a YSI Constant Temperature Controller, model 71. Fluid output was measured and replaced by equal volumes of physiologic saline; an additional 75 ml of saline per 24 hours was given in divided amounts during an experiment. Two to 6 hours after surgery, 100 mg of streptomycin and 60,000 units of crystalline and procaine penicillin were given intramuscularly. Surgical mortality was high. Sufficient data for analysis were obtained from 11 rabbits surviving 6 to 24 hours, but nearly 3 times that many were operated, the remainder dying during or shortly after surgery. Rabbits E-1, E-2, E-3, E-6, and E-7 underwent nearly complete enterectomy. That portion of the gut extending from a few centimeters below the ampulla of Vater to below the rectosigmoid junction was removed

in toto. Tubes were inserted into the duodenal stump and the urinary bladder for collection of gastroduodenal fluids and urine. In most animals one or both femoral veins or arteries were catheterized to facilitate blood sampling and fluid replacement. Rabbit E-5 was gastrectomized only. Rabbit E-4 survived 6 hours following hepatic artery ligation and a complete gastroenterectomy, extending from the cardioesophageal junction to the rectum. Numerous attempts to repeat this procedure in other animals were unsuccessful. Four animals were sham operated. Rabbits C-1, C-3, and C-4 underwent laparotomy only, while rabbit C-2 underwent, in addition, section and reanastomosis of the gut at the ligament of Treitz and the rectosigmoid junction.

RESULTS

Plasma I^{130} Radioactivity

Fig. 1 shows a semilogarithmic plot of the I^{130} activity in the plasma in an enterectomized and in a sham operated animal. After the first few hours, the
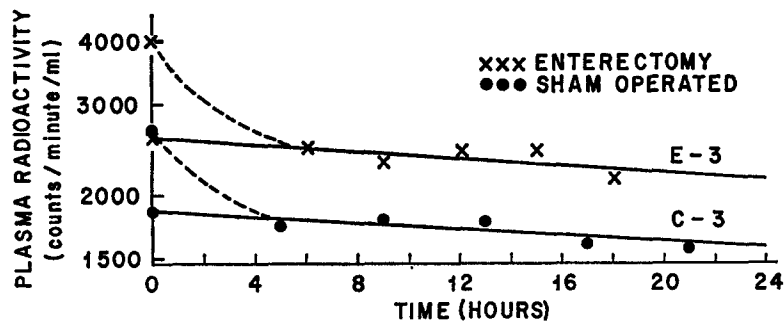

FIGURE 1. Plasma concentration of iodide[130] in postoperative animals.

data can be fairly well fitted, using the method of least squares, to a single component exponential function. The slope of this function gives an estimate of the iodide excretion rate, termed k_5 (7). The extrapolated intercept of this function, at $t = 0$, divided into the total amount of injected I^{130} activity gives an estimate of the volume of the iodide space, V_s. k_5 was greatly reduced in these animals, ranging from 0.071 to 0.739 days^{-1}, as compared with a normal value of 2.5 days^{-1} (6). Small values are not unexpected in view of the greatly reduced output of urine observed in these experiments. V_s averaged 10 times the plasma volume, as compared with an average value of 8 found by Zizza *et al.* (6). I^{130} data were not obtained from rabbits E-1 and E-2. V_s and k_5 were estimated for these animals by using the means from all other experiments.

Plasma I^{131} Radioactivity

Figs. 2 *a* and 2 *b* show plots of I^{131} activity in the plasma for all experiments. The upper function, *s*, in each figure represents the fractional plasma specific

activity, *i.e.*, the protein-bound I^{131} radioactivity per gram of albumin at any time t divided by the protein-bound I^{131} radioactivity per gram of albumin at $t = 0$. The lower function is termed z and represents the amount of radioactive breakdown products of I^{131}-albumin in the animal at any time t expressed as a fraction of the total I^{131} radioactivity injected at $t = 0$. z is obtained by multiplying the concentration of non-protein-bound I^{131} radioac-
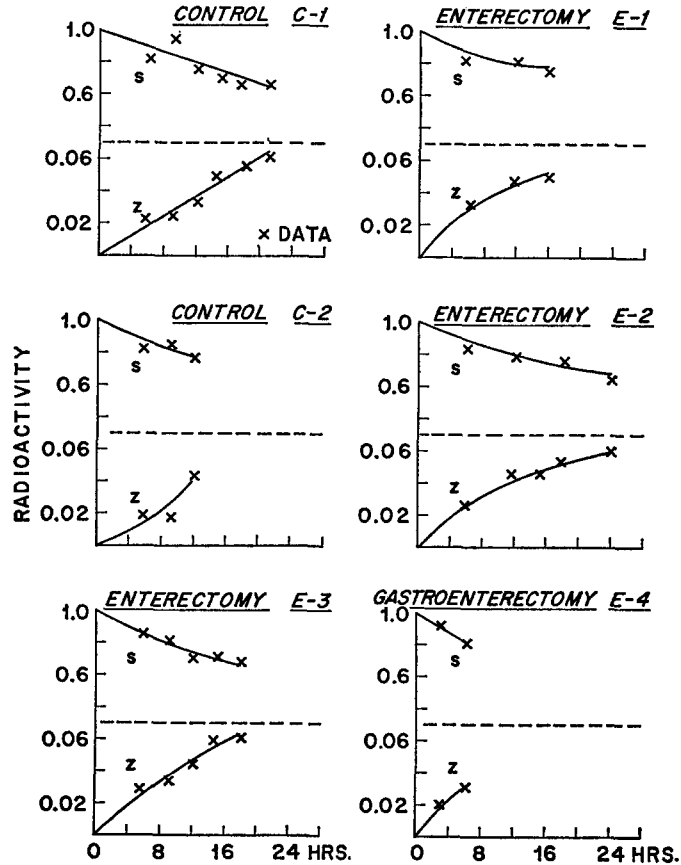

FIGURE 2 *a*.   *s* and *z* in postoperative animals. *s*, plasma albumin specific activity; *z*, total non-protein-bound activity (see text).

tivity in the plasma (in counts per minute per milliliter) by V_s/R where R is the total I^{131} activity in counts per minute injected at $t = 0$. This estimate of z assumes that the breakdown products of I^{131}-albumin are evenly distributed throughout the iodide space, an assumption that is probably roughly true. Correction was made for the small amount of non-protein-bound radioactivity in the injected material. Both *s* and *z* can be empirically fitted to polynomial functions. Using the method of least squares,

$$s = \alpha_0 + \alpha_1 t + \alpha_2 t^2 \qquad\qquad (1)$$

and

$$z = a_0 + a_1 t + a_2 t^2 \qquad\qquad (2)$$

In Fig. 2, the curves through the data points represent these quadratic functions.

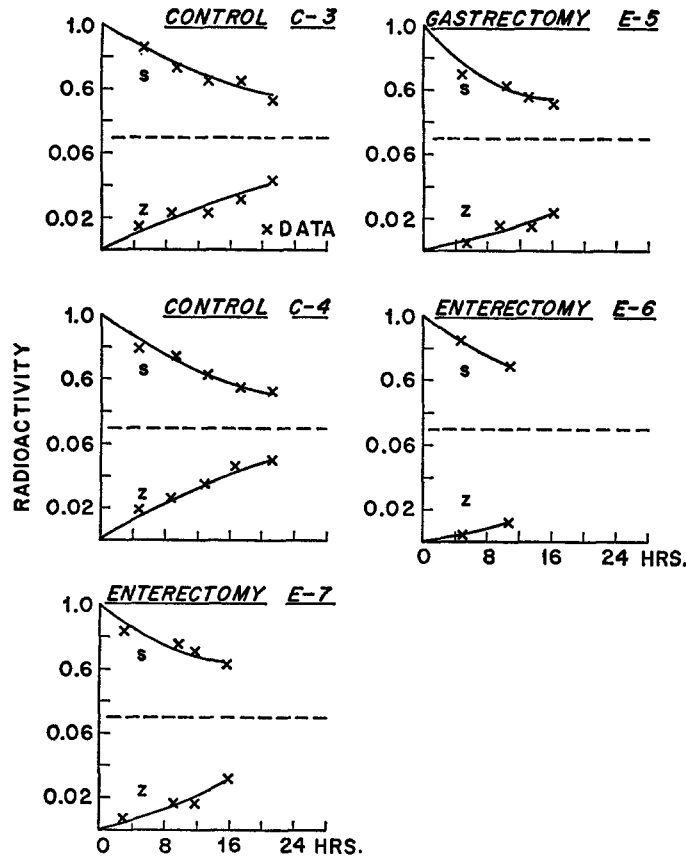
FIGURE 2 *b*.   For legend see facing page.

Urine and Gastroduodenal Secretions

Urinary output was greatly reduced in all animals; the total volume collected during the course of an experiment was never more than 20 ml. The volume of gastroduodenal secretions collected varied from 25 to 150 ml. In no experiment did the total I^{131} radioactivity collected in this fluid exceed 0.5 per cent of the injected I^{131}-albumin, and of this, 75 to 85 per cent was not protein bound. About 4 per cent of the injected iodide[130] ultimately appeared in the gastroduodenal secretions.

Calculation of the Albumin Breakdown Rate

In the first paper of this series (7), a method was given for the calculation of the albumin breakdown rate, using s and z data only based on a 5 compartment model. Such a model includes a breakdown compartment containing a fraction, v, of the total activity. v cannot be measured directly but can be estimated from z. Since such an estimate is subject to very large error in short experiments, we chose to eliminate v from the calculation of these data

TABLE I

ALBUMIN EFFLUXES* IN CONTROL AND RESECTED
ANIMALS, FROM EQUATION 3

I *a*

Control rabbits	$T = 6\ddagger$	$T = 11$	$T = 15.5$	$T = 16$	$T = 18$	$T = 21$
C-1	0.264	0.278	0.292	0.294	0.302	0.314
C-2	0.137	0.248	—	—	—	—
C-3	0.302	0.324	0.339	0.341	0.347	—
C-4	0.322	0.325	0.333	0.335	0.337	—
Average	0.256	0.294	0.321	0.323	0.329	0.314

I *b*

Resected rabbits	Procedure	$T\ddagger$	Efflux	Average§ control efflux
E-1	Enterectomy	16	0.337	0.294
E-2	Enterectomy	21	0.314	0.314
E-3	Enterectomy	18	0.325	0.329
E-4	Gastroenterectomy	6	0.478	0.256
E-5	Gastrectomy	16	0.179	0.323
E-6	Enterectomy	11	0.142	0.323
E-7	Enterectomy	15.5	0.210	0.321

* $\bar{k}_3$ in grams per day.
‡ T in days.
§ Each resected animal's efflux is compared with the average of control animals' effluxes for the same T.

entirely. This leads to an underestimate of the breakdown, since it ignores a significant amount of catabolized I^{131}-albumin retained in the animal (8); however, comparison of control and experimental animals is probably more valid, and the calculated breakdown rates can be accepted as minimum values. Eliminating v from equation 23 in our earlier paper (7), we obtain, as with equation 25, a solution for the mean efflux,

$$\bar{k}_3 = \frac{x_0 k_5 \int_0^T \frac{z}{s} dt + \int_0^T \frac{z'}{s} dt}{T}, \quad T > 0, \tag{3}$$

where $\bar{k}_3$ = the mean albumin breakdown rate in grams per day between $t = 0$ and $t = T$, and $\bar{x}_0$ = the total intravascular albumin at $t = 0$. Substituting from equations 1 and 2 with numerical values for the constants α and a, $\bar{k}_3$ can be calculated for any period of time 0 to T. Table I gives values for $\bar{k}_3$ in grams per day for all experiments. Table I a gives $\bar{k}_3$ and the averages of $\bar{k}_3$ for different periods of time in control animals. Table I b gives $\bar{k}_3$ for resected animals for the duration of each experiment and compares each value with the average of control effluxes computed for the same period of time. Effluxes in resected animals varied from 48 to 187 per cent of the corresponding control values, with a mean of 96 per cent.

DISCUSSION

The albumin breakdown rates obtained in both control and resected animals are about one-third to one-half the rates found in normal animals (9). This difference is due in part to the method of equation 3, which underestimates the efflux, and in part to the poor condition of the animals during experiments. However, considering the experimental and analytical difficulties involved, the results do not vary unreasonably. Three enterectomized animals and one gastroenterectomized animal with a ligated hepatic artery (rabbit E-4) had calculated effluxes greater than the corresponding controls. Two enterectomized animals and a gastrectomized animal had effluxes less than control values. The gastrectomized animal developed intravascular hemolysis with a rapidly falling hematocrit and deep jaundice shortly after surgery and was in very poor condition throughout the experiment.

From these studies, we conclude that in the rabbit no more than half of albumin breakdown occurs in the gastrointestinal tract and that this upper limit is probably too high. These studies support the conclusions of Katz *et al.* (10), who, using somewhat similar experimental methods in rats, estimated that about one-half of albumin catabolism took place in extravisceral sites. If there is any substantial breakdown of albumin by the digestive tract, it probably takes place in the stomach and upper duodenum, since we demonstrated in the previous paper that the jejunum and ileum are not required for catabolism of albumin at normal rates. This is indirectly suggested by the work of Borgström *et al.* (11), who showed that there is a large influx of fluid into the stomach and duodenum during digestion. We found that no more than 0.5 per cent of the total I^{131} activity injected into these animals appeared in the gastroduodenal secretions and that most of this was unbound. That some of this activity was excreted as iodide seems likely since about 4 per cent of the injected iodide[130] also appeared in these secretions. However, if we assume that all of the I^{131} activity collected from the stomach and the duodenum was the result of albumin catabolism, this still acounts for less than 10 per cent of the total albumin breakdown during that period.

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