Degradation of Albumin by Isolated Segments of the Gastrointestinal Tract in Rats.* (29487)

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The specific sites of catabolism of serum albumin are not known, but there is evidence that the gastrointestinal tract participates in this process. Endogenous serum albumin has been identified in both gastric juice(1-5) and intestinal fluid(6,7) of normal man and experimental animals, indicating that the protein permeates into the gut lumen. Degradation of albumin within the gut lumen has been observed in normal man(8), rabbits(7), and rats(9). Furthermore, enterectomy prolongs significantly the biologic half-life of serum albumin in the rat(10). These studies contribute considerably to the understanding of catabolism of serum albumin by the gastrointestinal tract, but the capability of specific segments of the gut to catabolize albumin and the role of pancreatic enzymes in this process have not been fully investigated.

This communication reports 3 groups of experiments designed to explore these aspects. The first series consisted of studies of absorption of radioactive sodium iodide (NaI^{131}) from the lumen of the isolated rat stomach and selected segments of proximal, middle, and distal small intestine, and was conducted to aid in the design of the second and third groups. The second group of studies was performed to determine the potential of these same sites to catabolize rat albumin injected into the isolated segments. The third group was similarly conducted 24 hours after diversion of biliary and pancreatic secretions to study the effect of absence of these secretions upon albumin degradation.

Materials and methods. Animals used were male rats (250-375 g) of the Sprague-Dawley strain fasted for 24-48 hours before each study. Sodium pentobarbital administered intraperitoneally or open drop ether was used for anesthesia.

Albumin was isolated from pooled rat serum by diethylaminoethyl cellulose column chromatography after ammonium sulfate precipitation of the major globulin fraction as previously described(11). The albumin was radioiodinated by the iodine monochloride method(12), and was demonstrated to be electrophoretically pure and homogeneous by ultracentrifugation. After precipitation with equal volumes of chilled 10% trichloroacetic acid (TCA) less than 5% of the radioactivity was recoverable in the supernatant.

Isolation of segments was done through abdominal incisions by ligating the stomach at the cardia and pylorus with #50 polyethylene tubing, and similarly separating 5-10 cm portions of proximal, middle and distal small intestine. The proximal ligature of the first small bowel segment was carefully made below the pancreaticobiliary tree, thus excluding it, and occlusion of visible vessels was avoided in all segments. The abdominal contents were bathed with Krebs-Ringer phosphate buffer (pH 7.4), and bleeding was minimized. In the third group of rats, diversion of pancreatic juice and bile was accomplished 24 hours prior to study by severing the duodenum 10 cm from the pylorus and creating a duodenal-cutaneous fistula with each cut end. One-half to one ml of NaI^{131} or I^{131}-albumin (1.7-2.6 mg) was injected through a #25 needle into each isolated lumen, and the incisions closed.

Extraction of radioactivity. One hour after intraluminal injection the animals were sacrificed, the segments excised, and the serosal surfaces washed with isotonic saline. No distention was noted and gross blood was present in only 3 of the 62 segments studied. Serosal surface areas were measured, the segments homogenized in chilled isotonic saline in a Potter-Elvehjem homogenizer, and the

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TABLE I. Recovery and Loss of Radioactivity 1 Hour After Injection of NaI^{131} into Isolated Segments of Gastrointestinal Tract. Net gain of radioactivity in stomach segments reflects gastric secretion of circulating isotope absorbed from all 4 segments.

Segment	No. rats	% recovered Mean	% recovered S.D.	% lost/cm² serosal surface Mean	% lost/cm² serosal surface S.D.
Stomach	4	118.5	.6	—	—
Proximal	6	6.4	4.9	15.3	3.6
Middle	6	26.0	8.3	13.3	3.5
Distal	6	13.3	4.6	14.7	1.0

homogenates diluted to known volumes. Aliquots of radioactivity standards, homogenized segments, and samples of peritoneal fluid were monitored for radioactivity. In the studies utilizing I^{131}-albumin, additional aliquots of homogenates were precipitated with equal volumes of chilled 10% TCA. After standing overnight at 4°C, these TCA solutions were agitated, the mixtures centrifuged in a capillary tube at 12,000 × *g* for 2½ minutes, and the volume of the precipitates and supernatants estimated by a microhematocrit reader. Following centrifugation at 3,000 rpm for 15 minutes, aliquots of the supernatants were monitored for radioactivity.

Radioactivity monitoring was conducted in a Nuclear Chicago scintillation well detector (Model C-120-1) for periods sufficient to insure counting errors of less than 5%.

Calculations of 1) percent of radioactivity recovered, 2) percent radioactivity lost/cm² serosa (NaI^{131} studies only), 3) percent albumin catabolized and 4) percent albumin catabolized/cm² serosa were made according to the following equations:

1) % Recovered = [(CPM recovered)/(CPM administered)] × 100

2) % Lost/cm² = (100 − % recovered)/(cm² serosa)

3) % Catabolized = [(CPM administered − CPM TCA precipitable)/(CPM administered)]

CPM TCA precipitable = CPM recovered − CPM TCA soluble

Statistical significance of the difference of means of percent catabolized was calculated utilizing the two sample t test(13).

Results and discussion. The results of NaI^{131} absorption in the first group are presented in Table I. After injection of isotope into all 4 isolated lumens, the gastric segments consistently displayed a net gain of radioactivity. This is explained by the fact that I^{131} absorbed from each segment circulates through the gastric mucosa and is secreted into the gastric lumen and confirms the findings of Small *et al*(14). Although net recovery from intestinal segments varied, absorption expressed as a function of surface area was quite uniform, though not complete, under the conditions of this study. Less than 1% of the injected radioactivity was recoverable in the peritoneal fluid, suggesting that no significant leaking of radioactivity occurred at injection sites.

The results of studies of I^{131}-albumin degradation in animals without (Group II) and with (Group III) pancreatic diversion 24 hours prior to incubation are given in Table II. In animals without pancreatic diversion the gastric segments again demonstrated a net mean gain in radioactivity. Thus it is apparent that recovery of radioactivity from the stomach did not reflect

TABLE II. Degradation of I^{131}-Albumin After Injection of 1.7-2.7 mg into Isolated Segments of Gastrointestinal Tract in Rats Without and With Pancreatic Diversion 24 Hours Prior to Study.

Segment	No pancreatic diversion (5 rats) Mean	No pancreatic diversion (5 rats) S.D.	Pancreatic diversion (5 rats) Mean	Pancreatic diversion (5 rats) S.D.
Stomach				
% Recovered	106.4	9.9	87.6	11.5
% Catabolized	48.3	30.5	35.9	18.9
% Catabolized/cm² serosa	6.4	4.3	4.0	1.1
Proximal small intestine				
% Recovered	60.4	15.6	68.3	14.3
% Catabolized	48.6	20.6	33.5	13.4
% Catabolized/cm² serosa	10.4	5.6	5.1	2.4
Middle small intestine				
% Recovered	45.8	26.1	83.3	11.3
% Catabolized	71.1	32.2	18.6	10.9
% Catabolized/cm² serosa	13.3	8.2	2.5	1.5
Distal small intestine				
% Recovered	63.7	22.8	75.7	10.7
% Catabolized	75.0	35.1	26.1	10.1
% Catabolized/cm² serosa	13.0	5.9	4.9	1.2

albumin degradation in this organ because I^{131} liberated from albumin in other segments was absorbed and secreted into the gastric lumen. Furthermore, it is likely that variability in rates of flux of I^{131} in segments of small intestine rendered total recovery of radioactivity inaccurate as an index of I^{131}-albumin degradation in these sites. Thus calculation of the TCA precipitable I^{131} after incubation seemed necessary to estimate I^{131}-albumin catabolism more accurately.

A gross reflection of diminished albumin degradation in intestinal sites deprived of pancreatic enzymes is apparent in the higher mean percents of administered radioactivity recovered in this group. The mean percent of injected albumin degraded in isolated stomachs of animals with and without pancreatic diversion did not differ significantly, nor was there a significant difference in the mean percent catabolized in proximal segments at 95% confidence limits. The mean percent catabolized per cm^2 in the proximal segment of small intestine in rats with pancreatic diversion was significantly lower than the mean in animals without pancreatic diversion at 90% confidence limits. In middle and distal segments the means of both the percent catabolized and the percent catabolized per cm^2 were significantly lower at 95% confidence levels in animals with prior pancreatic diversion than in those without.

That albumin degradation occurs to any degree in the presence of pancreatic diversion is of interest and suggests the presence of proteolytic bacteria. Cultures of isolated segments after incubation consistently yielded proteus organisms, known to possess proteolytic properties. *Clostridium preferengens,* alpha streptococci and coagulase-negative staphylococci were also encountered, and may have contributed to proteolysis.

The results of this study do not suggest a significant quantitative difference of pancreatic enzymes at various levels of small intestine, as was found by Pelot and Grossman(14) who studied trypsin and chymotrypsin activity of intestinal washings. Differences in both techniques and substrates may explain the discrepancy. Care should be taken not to extrapolate the findings of this report to quantities of albumin normally degraded in various portions of the gastrointestinal tract as fasting, anesthesia, pancreatic exclusion, segment isolation and surgical manipulation provide conditions importantly different from those found in the intact animal. The results do indicate, however, that the lumenal contents of the stomach and entire small bowel of the rat possess significant potential ability to catabolize albumin molecules that reach these sites.

Summary. 1. Studies of NaI^{131} absorption from and I^{131}-albumin catabolism in isolated segments of gastrointestinal tract of rats are described. 2. One hour following the simultaneous injection of NaI^{131} into stomach, proximal, middle, and distal small intestinal segments, there was net gain of radioactivity in the gastric lumen, and a mean loss of 13.3 to 15.3% of the injected dose per cm^2 of serosal surface in the small bowel segments. 3. After injection of 1.7-2.6 mg of I^{131}-albumin into stomach, proximal middle, and distal small bowel segments mean percentages catabolized per cm^2 in one hour in respective segments were 6.4, 10.2, 13.3, and 13.0. 4. Diversion of pancreatic and biliary secretions for 24 hours prior to study lowered these means to 4.0, 5.1, 2.9, and 4.9 respectively. 5. These studies indicate that the stomach and all levels of the small bowel possess significant potential for degrading serum albumin entering the lumen, and that pancreaticobiliary secretions make a significant, but not the only, contribution to this process.

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A Histochemical Study of Succinic and Lactic Dehydrogenases in the Regenerating Forelimb of the Adult Newt, *Triturus*.*† (29488)

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There are few biochemical or histochemical studies on the metabolism of regenerating tissues in amphibians. Okuneff(1) and Vladimirova(2) demonstrated that lactic acid increases in the early regenerate and then declines to normal during differentiation. Associated with the increase in lactic acid is a decrease in pH(3) and respiratory quotient (4).

In the absence of oxygen, pyruvic acid formed during glycolysis is reduced to lactic acid by lactic dehydrogenase instead of being converted to acetyl CoA, one of the first compounds in the Krebs Cycle. The accumulation of lactic acid in the regenerate bud suggests that glycolysis is the principal source of energy and would explain the lowered RQ and pH of these tissues. However, Gezsik and Wolsky(5) found that after an initial decline in the levels of succinic, malic and lactic dehydrogenases in early tail regenerates, the activities rose during "progressive" stages of tail regeneration, particularly for succinic dehydrogenase, indicating that respiratory metabolism via the Krebs cycle might be important. Furthermore, Niwelinski (6) found that the blastemal cells of the regenerating forelimb of the newt reacted intensely when tested histochemically for succinic dehydrogenase and DPNH diaphorase and reached adult levels by the fourth week.

On the other hand, in a more recent study published after the present work was completed, Wolfe and Cohen(7) reported that little or no succinic dehydrogenase and isocitric dehydrogenase were present in the undifferentiated blastema but appeared during later differentiation. However, the blastema reacted strongly for DPNH diaphorase, lactic dehydrogenase and malic dehydrogenase. Furthermore, they reported increased levels of glucose-6-phosphate dehydrogenase and TPNH diaphorase during the undifferentiated blastemal stages.

In the present study several stages during the regeneration of the forelimb were tested histochemically for succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH), the former because of its significance in the respiratory Krebs cycle, and the latter because of its role in glycolysis.

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