Nitrogen Content of Gastrointestinal Tracts of Rats During Absorptive Period.* (23699)

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In a series of earlier papers(1a,b,2,3) we reported the effects of stomach emptying, intestinal propulsion, and enzymatic breakdown on the absorption and utilization of dietary proteins. In these experiments adult male rats were trained to eat definite quantities of different proteins or other test meals and the nitrogen present in the gastrointestinal contents was determined at intervals following ingestion. It was found that, 2 hours after feeding, in most cases 50-70% of the ingested nitrogen disappeared from the gastrointestinal tract and the speed of disappearance and the distribution of the residual nitrogen between stomach and intestine depended upon the nature of the protein.

Conflicting results were obtained by Dreisbach and Nasset(4) who, using a similar method, consistently recovered more nitrogen from the digestive tract in rats during the absorptive period than had been fed. From these results and from experiments with dogs(5), they postulated existence of a "homeostatic mechanism" whereby the digestive tract could supply to a deficient meal the missing amino acids or those present in insufficient quantities. This is presumably accomplished by secretion of digestive juices and by desquamation of mucosal epithelium. Due to the importance of this concept, if substantiated, and due to the contradiction existing between the results of Nasset and Dreisbach and our own, we decided to investigate the problem by: (1) determining the amount of nitrogen present in gastrointestinal contents of rats after a protein-free meal; (2) studying changes in these values under conditions of progressive protein depletion; and (3) investigating the nitrogen content of the gastrointestinal tract after feeding different skim milk powders. Skim milk powders were chosen as test meals because we had found (1) that milk protein is eliminated from the gastrointestinal tracts of rats slower than other proteins, and consequently any accumulation of endogenously supplied nitrogen would be made more conspicuous.

Methods. Male, albino rats of the Wistar strain, weighing between 140 and 220 g were kept in individual cages, water being provided at all times. Body weights were recorded daily. The animals were starved 24 hours (previous work had shown that the residual nitrogen in the gut after 24 hours was negligible) and then offered a one-half gram test meal consisting of 80% starch and 20% butterfat. Only those rats who ate the meal immediately without spilling were used. An hour and a half or 3 hours *post cibum*, rats were killed by a blow to the head. The abdominal cavity was opened and ligatures placed at the cardia and at the pyloric and ileocaecal sphincters. The intact stomach and small intestine were removed, and contents collected separately by washing repeatedly into a flask. The washing has to be performed carefully immediately after killing in order to prevent post mortem desquamation which may alter results. The washings were acidified and analyzed for nitrogen by the micro-Kjeldahl method. In several cases the accuracy of this method was controlled by Nesslerization of parallel samples and determination of nitrogen with a Lumetron colorimeter. Rats on stock diet received Rockland rat cubes. For protein depletion the rats received the following diet *ad libitum*: Cornstarch, 81.0%; butterfat, 2.5%; wheat germ oil, 2.5%; lard, 3.2%; choline HCl, 0.4%; methyllinoleate, 0.4%; U.S.P. Salt Mixture #5, 4.0%; Ruffex (methyl cellulose), 6.0%; vitamins were supplemented to previously established levels(6). Four control animals,

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TABLE I. mg of Nitrogen Present in Gastric and Intestinal Contents 90 Min. after Ingestion of a Protein-Free Meal. (Avg values obtained with 4 rats each.)

Days on protein-free diet	mg N present 90 min. *post cibum*		
	In stomach	In intestine	Total
0 (controls)	1.18 ± .41	4.02 ± .57	5.20 ± .80
2	1.75 ± .59	5.09 ±1.00	6.85 ± .42
4	.54 ± .17	5.34 ± .96	5.88 ± .91
6	1.31 ± .39	4.01 ± .52	5.34 ± .95
8	1.05 ± .38	2.91 ± .27	3.96 ± .60
18	2.01 ± .39	3.08 ± .43	5.09 ± .59

previously fed stock diet, were starved for 24 hours and killed. Groups of 4 animals each were sacrificed after 2, 4, 6, and 8 days of protein depletion. In a second group, 2 g of various skim milk preparations, supplying 96-107 mg of nitrogen, were fed as a test meal to rats kept on stock diet. The milk powders were wetted immediately before feeding by addition of approximately 0.5 ml of water and one or two drops of tuna juice for flavor.

Results. The results of the first group of experiments are summarized in Table I. The nitrogen content of the stomachs and intestines of the control animals kept on a stock diet, fasted for 24 hours and then given the protein-free test meal, was found to be 5.20 mg (4.59-5.99 mg). Subjecting the animals to continued protein inanition did not cause any significant diminution of these nitrogen values. After 18 days on the protein-free diet, the nitrogen content was found to be 5.09 mg (3.83-6.26 mg) and on no single day was there any significant deviation from these mean values.

In the second group, skim milk powders served as test meals. The results of these experiments are condensed in Table II. The figures indicate that the amount of nitrogen recovered was in no instance higher, but always lower, than the amount of nitrogen consumed with the test meal.

The figures show also that the freeze-dried sample was digested and absorbed approximately at the same rate as the "instant" sample which was spray dried at a considerably higher temperature.

Discussion. Following a protein-free meal, the amount of nitrogen supplied by gastrointestinal secretion and desquamation was about 5 mg, *i.e.* much lower than indicated by Dreisbach and Nasset who reported an average value of 0.29 g of protein, or the equivalent of 45 mg of nitrogen. It is of interest to note here that Junqueira, Rothschild, and Fajer(7) have shown that the total *protein* production of the stimulated rat pancreas is 2.14 mg per hour. The quantities of amino acids supplied endogenously during the absorptive period seem to be far too low to make up for a dietary amino acid deficiency, at least in the rat. Protein depletion induced by feeding a protein-free diet up to 18 days does not influence the quantity of endogenous nitrogen supplied to the gastrointestinal tract. Confirming the results of our earlier experiments, we found consistently less nitrogen present during the absorptive period than had been fed. Differences in animal material and in specific experimental conditions could possibly be responsible for the differences between our results and those of Dreisbach and Nasset.

Summary. 1) The basal level of endogenously supplied nitrogen in adult rats is about 5 mg after 1½ hours, and this level is unaffected by protein depletion. On feeding skim milk powder, 22-27% of the ingested protein disappeared from the gastrointestinal tract within 3 hours. Under the conditions of these experiments the alleged homeostatic action of the gut in supplementing amino acids to deficient protein meals may be only negligible. 2) No significant differences have

TABLE II. mg of Nitrogen Present in Gastric and Intestinal Contents 3 Hr after Consumption of a Skim Milk Powder.* (Avg values for 4 animals.)

Type of skim milk powder	mg N present 3 hr *post cibum*			% less than consumed
	In stomach	In intestine	Total	
"Instant" (98.4 mg N)	67.5 ± .96	8.9 ± .85	76.4 ± 1.47	22
"Low temp." (98.4 mg N)	59.8 ± 3.64	14.7 ± 6.53	74.5 ± 3.18	23
Lyophilized (107 mg N)	63 ± 10.03	7.1 ± .75	70.1 ± 6.62	33

* Samples were supplied by Research Department of Carnation Co. of Los Angeles.

been observed in gastric emptying time, rate of absorption, or digestion between skim milk powders dried at low or high temperature.

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Different Fractions of Serum Proteins in Normal and Scorbutic Guinea Pigs. (23700)

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Electrophoretic patterns of plasma and serum proteins of a few animals have been studied(1,2) by the moving boundary electrophoresis. Greenberg *et al.*(3) observed no significant alteration in the serum proteins patterns of monkeys during early stages of scurvy. An increase in the plasma fibrinogen content has been reported in scorbutic monkeys(4) and guinea pigs(6). The present communication deals with studies on the different fractions of serum proteins in normal and scorbutic guinea pigs by paper electrophoresis as similar studies have not been previously reported.

Methods. The selection of guinea pigs and the feeding of the scorbutic diet by the paired feeding technic were the same as described previously(6). On the 30th day of the experiment when the experimental animals developed severe scurvy, food was withdrawn from the cages in the evening and on the next morning both the scorbutic animal and its normal mate were anesthetized with pentobarbital sodium and blood samples withdrawn by cardiac puncture. A small quantity of blood was taken in oxalated tube and the rest allowed to clot for the separation of serum. Total nitrogen and non-protein nitrogen of serum and plasma fibrinogen were determined by methods described previously(7). Serum proteins were separated by paper electrophoresis. 0.02 cc undiluted serum was used and electrophoresis continued for 16 hours with a current of 1.5 mA and 100 volts. The staining of the paper strips, determination of the optimum density curves of the coloured zones by densitometry and calculation of the amounts of different fractions of serum proteins were the same as described previously (7).

Results. The results are given in Tables I and II. Six clear components of serum proteins were present in guinea pig serum. Serum albumin, α_2 and β_2-globulin values decreased considerably and significantly, α_1-globulins increased significantly and there was no change in γ-globulin and β_1-globulin fractions when the guinea pigs developed scurvy. Total protein value of serum decreased, non-protein nitrogen value of serum and fibrinogen value of plasma increased in scorbutic animals.

Discussion. The lowered albumin globulin ratio in scorbutic guinea pigs agrees with similar observations reported by others(8). The decreased total plasma protein contents has also been reported in scorbutic monkeys (4). Moore(2) could not observe β and γ globulins in guinea pig sera by moving boundary electrophoresis. The demonstration of these components by paper electrophoresis indicates the superiority of the method over moving boundary electrophoresis. Fukuda and Shibatani(9) and Fukuda(10) observed