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Free Amino Acids in Gut Contents

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Intravenous feeding is less effective than feeding by mouth. An amino acid mixture substituted for a dietary protein is often nutritionally inferior to the protein it simulates(1). These facts suggest that the adequately stimulated digestive system contributes something advantageous to alimentation besides the simple hydrolysis and absorption of ingested food. With this in mind, we decided to examine the free amino acid pattern in gastrointestinal contents of normal healthy animals after ingestion of test meals. These were single feedings and all observations in any experiment were made on the same day. Animals were fed an adequate diet except on the day of experiment. The question of classical dietary deficiency, therefore, was never a complication.

In the rat the mass of nitrogen recoverable from the lumen of the gastrointestinal tract may exceed the nitrogen ingested up to 5 hours after feeding(2). Such results suggest that the gastrointestinal tract during digestion may contribute as much endogenous protein to the luminal contents as dietary protein ingested. The investigation was extend to include various parts of the gastrointestinal tract in the dog. Test meals were fed containing 80 percent protein (either egg albumin or zein), 10 percent lard, and 10 percent sucrose. A non-protein meal consisting of 40 gm lard, and 10 gm sucrose was fed in two experiments. The dogs were killed $1\frac{1}{2}$ hours after feeding and the contents of stomach, duodenun, jejunum and ileum collected separately(3). Total nitrogen in the lumen was the same after feeding either eggalbumin or zein but it was reduced to $\frac{1}{3}$ of this amount after feeding the non-protein meal. Zein is virtually devoid of both tryptophan and lysine but, after ingestion of zein, 89 mg of lysine and 15 mg of tryptophan were found in the small intestine. The quantities of 8 essential amino acids reeovered from the stomach and small intestine, after feeding three different test meals, are given in Table 1. Total quantities of the 8 essential amino acids shown in Table 1 were recovered form the gut in ratios of 1:2:4 for non-protein, zein and egg albumin test meals respectively. This may represent in part the secretagogue effectivenss of these three types

of test meals because differnt types of meals affect differently the quantitative responses of digestive glands.

Table 1 : Microbioligically Available Essential Amino Acids in Contents of Dog Stomach and Small Intestine (Milligrams)

Test Meal	Egg Albumin		Zein		Lard-Sucrose	
Contents	Stomach	Gut	Stomach	Gut	Stomach	Gut
Histidine	1.5	61.6	1.0	33.5	1.6	16.4
Leucine	71.3	262.7	55.5	160.5	7.8	57.8
Lysine	3.3	163.0	2.5	88.5	4.5	46.8
Methionine	9.0	48.6	1.2	31.5	1.4	11.2
Phenylalanine	12.0	133.3	4.5	57.5	2.9	30.9
Threonine	24.0	149.2	14.5	83.0	3.4	41.9
Tryptophan	3.4	44.2	0.6	14.7	2.2	10.4
Valine	41.3	184.3	9.5	68.5	5.4	39.4

Table 2 : Molar Ratios of Essential Amino Acids in Test Meal Proteins and in Jejunal Contents Derived from Feeding Them(Threonine=1.000)

	Test Meal Proteins		Jejunal Contents		
	Egg Albumin	Zein	Egg Albumin	Zein	Non-Protein
Histidine	0.44	0.44	0.27	0.31	0.32
Leucine	2.03	7.17	1.45	1.69	0.93
Lysine	1.25	0.00	0.80	0.83	0.90
Methionine	1.04	0.61	0.22	0.28	0.16
Phenylalanine	1.28	1.54	0.56	0.49	0.56
Threonine	1.00	1.00	1.00	1.00	1.00
Tryptophan	0.21	0.02	0.15	0.10	0.16
Valine	1.55	1.02	1.11	0.84	0.97

Amino acid molar ratios in the test meal proteins and in jejunal contents derived from feeding them, are given in Table 2. Qualitatively the amino acid mixture in the jejunum relatively constant regardless of the amino acid composition of protein fed. The last column shows that even in the absence of ingested protein the amino acid mixture remains relatively constant. This phenomenon of amino acid homeostasis in the gut lumen have nutritional significance. It is probably a regulatory mechanism that serves to modulate the unusual amino acid mixtures that could arise in blood plasma from certain dietary proteins if only simple hydrolysis and absorption were involved in the digestive process.

Feeding a single amino acicd to a guinea pig will readily induce maked shifts in distribution of various other amino acids between liver cells and extracellular fluid(4). In man the intravenous administration of single amino acids, as well as certain amino acid mixtures, can cause seri ous signs and symptoms, such as hypotension, headache, nausea, vomiting, fever, azotemia and hepatic dysfunction(5). These results indicate the importartance of maintaining a certain normal balance among the 20-odd amino acids found in blood plasma. The first step in amino acid homeostasis is accomplished in the gut lumen even before amino acid absorption occurs.

The rates of absorption of individual amino acids vary considerably, depening upon whether they are introduced singly into the intestine or mixed with other amino acids. Competition occurs among different amino acids for transport sites in the mucosa and hence absorption rates determind for single amino acids are not valid under normal conditions of digestion and absorption. If some amino acids were absorbed much more rapidly than others from the

normal mixture present during digestion, the compisition of the mixture should change with time and translation along the gut. The mixture should become impeverished in the more rapidly absorbed amino acids as the mixture either approached the ileum or the end of the digestive process at any specific point along the small intestine. Conversely, the more slowly absorbed amino acids should tend to accumulate. There is no clear evidence that this occurs(6). If the absorption of the various amino acids were not fairly uniform throughout the digestive period, the effect in the blood stream would be comparable to administering a few amino acids, including some essentials, at one time and the others somewhat later. This is the type of amino acid influx that has been shown to be inefficient for protein synthesis(7. 8).

The relative amounts of endogenous and exogenous protein in the digestive tract were determind by feeding a labeled protein. Two dogs provided with jejunostomies were fed test meals containing c^{14}-labeled casein(6). Jejunal contents were collected for 10 hours in 4 periods of 2.5 hours each. The results are summarized in Table 3. Rats were fed the radioactive casein and killed 1, 2, and 4 hours later. Contents of stomach and small intestine were

Table 3: Engogenus and Exogenous N in Intestinal Contents Collected from Jejunostomies after Feeding Test Meals Containing C^{14}-Labeled Casein[1] to Dogs.

	Sample[2]				Totals
	1	2	3	4	
Dog A:					
Volume recovered, ml	47	40	154	133	174
Total solids recovered, gm	3.7	2.8	8.7	4.0	19.1
Total N, mg	163	117	249	258	787
Exogenous N[3], mg	41.7	23.4	15.1	4.2	82
Endogenous N[4], mg	122	94	234	254	703
Exogenous N, % of total	25.6	19.9	6.1	1.6	10.7
Dog B:					
Voluume recovered, ml	24	71	38	88	221
Total solids recovered, gm	1.2	5.3	3.1	10.1	19.9
Total N, mg	67	260	136	233	696
Exogenous N mg	7.1	41.7	18.6	12.3	80
Endogenous N[4], mg	59	219	117	220	615
Exogenous N, % of total	10.7	16.0	13.7	5.2	11.5

1. Thirty grams of diet were fed containing 10 gm of radioactive casein and 1.350 gm of N
2. Samples were collected in 4 equal time intervals for 10.0 hours after feeding.
3. Attributable to labeled casein
4. By difference

analyzed separately for total nitrogen, amino acid and radioactive carbon. At all three time intervals dietary protein constituted 91–95 percent of the total protein in the stomach. There was a striking reversal in the small intestine, where dietary protein accounted for only 14 percent of the total protein recovered. The results are summarized in Table 4. In the several hours required to accomplish these experiments there may have been some recycling of C^{14}, in which case dilution of ingested protein with endogenous protein was greater than indicated by the data.

The evidence cited above demonstrates that a large mass of protein moves rapidly into the lumen of the digestive tract in response to feeding. The digestive tract therefore must contain a large and mobile protein reserve. The mucous membrane and the digestive glands evidently recover quickly from the demands made upon them during digestion, suggesting that these tissues synthesize protein very rapidly. In an investigation of this problem, rats were either fasted or fed non-protein diets for several days (9). After 1, 2, 4, and 8 days of fasting, animals were killed and total nitrogen determined in whole stomach, pancreas, small gut and liver. In 8 days of fasting the gut and liver total nitrogen content was reduced to 1/2 of the control value. The pencreas lost a little less and the

Table 4: Endogenous and Exogenous N in Stomach and Small Intestine of Rats after Feeding a Test Meal Containing C^{14}-Labeled Casein[1] (Two rats used at each time interval)[2].

Time after feeding	Stomach	Intestine
1 Hour		
Total N, mg	129.8	25.9
Exogenous N[3], mg	118.5	3.5
Endogenous N[4] mg	11.3	22.4
Exogenous N, % of total	91.3	13.4
2 Hours		
Total N, mg	93.4	19.6
Exogenous N[3], mg	88.5	2.8
Endogenous N[4], mg	4.9	16.8
Exogenous N, % of total	94.7	14.3
4 Hours		
Total N, mg	51.2	19.5
Exogenous N[3], mg	48.7	2.9
Endogenous N[4], mg	2.5	16.6
Exogenous N, % of total	95.2	14.9

1. Four grams of diet containing 1 gm of radioactive casein and 138.2 mg of N was fed by stomach tube.
2. Only one gut sample was obtained at 1 hour.
3. Attributable to labeled casein.
4. By difference.

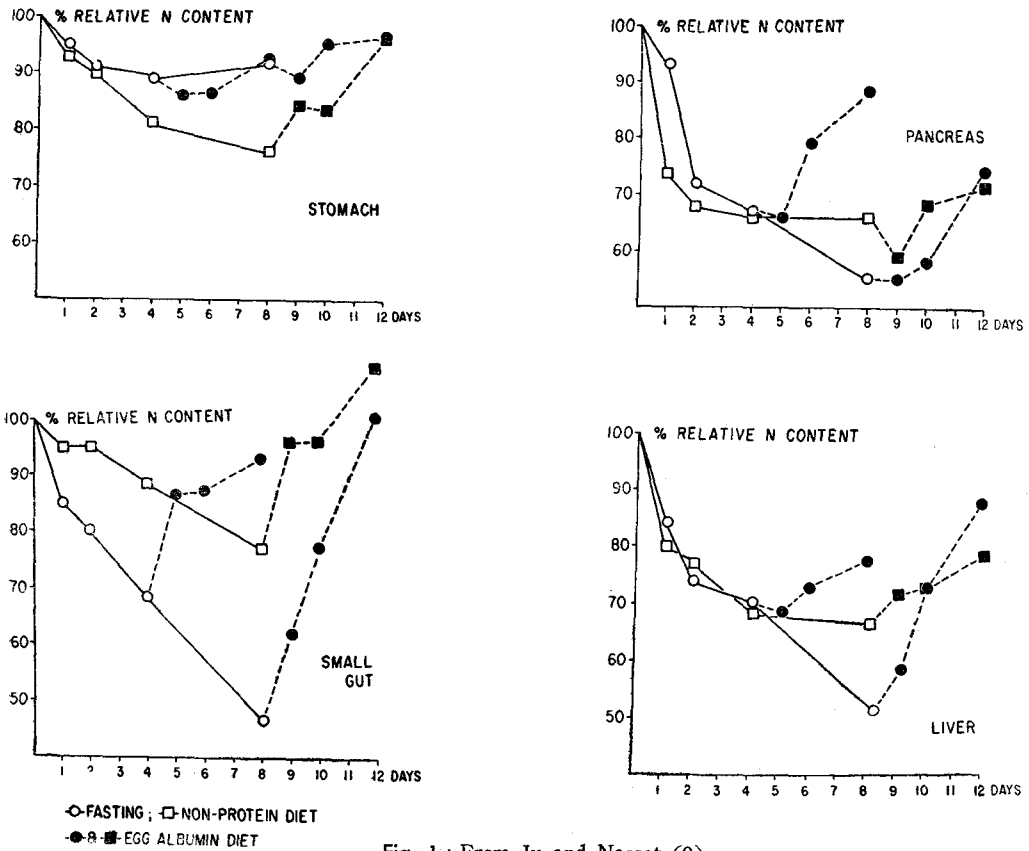

Fig. 1 : From Ju and Nasset (9)

stomach lost only 8 percents. On realimentation with a diet containing egg albumin, the small gut recovered most completely in 4 days and the liver was a close second. In the first day or two of realimentation, the stomach and pancreas either lost still more nitrogen or made very small gains. Figure 1 represents the changes observed.

These experiments suggest that a large proportion of the protein in gut, pancreas and liver is available to sustain the more essential protein metabolism of the fasting animal. The rapidity with which the intestine recovers during realimention suggests that this organ, having first access to absorbed amino acids, makes good use of its advantageous position and proceeds promptly to the synthesis of protein. As its rate of recovery indicates, the liver occupies the next most favorable position with respect to access to amino acids being absorbed. All other organs must compete for the amino acids present in peripheral blood. Ingestion of food stimulates prompt secretion and hence loss of more N in both stomach and pancreas but, since they must compete with other organs and tissues for amino acids, their recovery of nitrogen is slow.

The changes in nitrogen content of the abdominal viscera were different when, instead of fasting, a non-protein diet was fed. The small gut lost only half as much as in fasting and up to 4 days the changes in liver were the same as in fasting. The stomach lost over twice as much as in fasting and the pancreas lost still more nitrogen at a very rapid rate for at least 2 days. On realimentation, the small gut again made the fastest and most complete recovery, the liver and stomach were slow and the pancreas again failed to gain in the first day or two.

Even a non-protein meal is an adequate stimulus for the digestive glands, and the result is that stomach and pancreas lose nitrogen in their secretions more rapidly than when the animal is fasting. Apparently these organs are not able quickly to recoup their losses from the presumably limited supply of amino acids in the arterial blood. From the beginning of a non-protein diet the nitrogen losses from the small gut are minimized. This is an obvious protein-sparing effect, but not in the classical sense. The explanation may be that the proteins in salivary, gastirc, pancreatic, and hepatic secretions, as well as shed mucosal cells, are digested and recovered immediately in the intestinal mucosa for the synthesis of protein. Since the liver apparently gains no advantage for several days as a result of feeding a non-protein diet, it appears as if the intestinal mucosa retains a large proportion of the amino acids recovered from the proteins lost by the stomach, pancreas, and other glands in the process of secretion. Changes in amylase activity in pancreas, gut, and liver under similar conditions are approximately parallel with the changes in total nitrogen discussed above and suggest that enzyme protein loss is proportional to loss of total nitrogen (10).

If the mucosa removes and sequesters unknown quantities of amino acids from the mixture being absorbed, as the work described above indicates, the changes in the amino acid composition of mesenteric and portal vein blood could not be accurately predicted from a knowledge of the amino acid composition of either ingested protein or gut contents. This was demonstrated in dogs fed lean beef muscle, zein, casein, gelatin, and wheat gluten (11, 12, 13, 14).

The proteins in the digestive secretions represent only a portion of the endogenous protein available for digestion in the lumen of the alimentary tract. The well-known rapid turnover of the mucosa results in the constant shedding of epithelial cells which promptly disintegrate and release their contents in the gut lumen. The composition of feces does not change appreciably with changes in diet and digestive enzyme activity of feces is low or absent. It is evident from numerous classical works in nutrition that most of this cellular material must be hydrolyzed and recovered. It has been estimated that in man the mass of mucosal cells shed daily into the gut lumen may exceed 200 gm (15). From these and other considerations it seems likely that in man endogenous protein from shed mucosal cells may approach 70–90 gm/day, and from digestive secretions as much as 60–260 gm/day, making a total of 130–350 gm/day (16). It is not surprising, therefore, that the gut amino acid homeostasis described above for dogs and rats is also found in man. Even in the duodenum the free amino acid mixture is quite different from that derivable from hydrolysis of the ingested protein alone (17).

What is the nutritional significance of amino acid homeostasis in the gut lumen? It is evident that either feeding a single amino acid or giving it intravenously can cause serious disturbances of normal metabolism (4, 5). Such results suggest that amino acids are not necessarily innocuous compounds. The regulatory mechanism found in the

digestive tract obviously prevents wide fluctuations in composition of the amino acid mixture available for absorption after a meal. Nothing is known about how long this mechanism operates in the presence of dietary deficiency of essential amino acids or other essential nutrients. It may be unwise, for example, to add one or two essential amino acids as a supplement to the diets of a malnourished man or child. The added amino acids, being water soluble, might be absorbed long before the bulk of the amino acids derived by digestion of dietary and endogenous protein. More information is needed and I hope that other workers will become interested enough to join in advancing our knowledge of this subject.

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