Nature and Appearance of Protein Digestion Products in Upper Mesenteric Blood. (24873)

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The long standing question of whether a protein meal is hydrolyzed completely to free amino acids before absorption into the portal blood seems to have been answered recently in the affirmative by Parshin and Rubel(1), Whipple and his co-workers(2), and by Denton and Elvehjem(3,4). Whipple, *et al.* reached their conclusion from the fact that C^{14} lysine fed to dogs was metabolized in precisely the same way regardless of whether it was free or bound in protein. The others based their conclusions on failure to find significant rises in peptide concentrations of portal blood plasma after protein meals. Many years ago, some workers(5) purported to find large amounts of peptides in portal blood of animals after protein meals, but this work suffered from the difficulty that there was no method available for specific analysis of individual amino acids or peptides. The approach was usually to measure amino nitrogen of a protein free filtrate before and after hydrolysis, or to measure the difference in amino nitrogen of plasma filtrates after precipitation with different reagents, *e.g.*, trichloroacetic acid and tungstic acid. Conclusions based on such methods are still being put forward(6). We made a direct search for peptides by newer methods, *i.e.*, ultrafiltration and ion exchange fractionation of all low molecular plasma amino compounds. There is no recent work using these methods which, to our knowledge, unequivocally demonstrates that chemically measurable peptides appear in portal blood plasma as a result of protein meal. We are not dealing here with minute amounts of peptides and whole proteins which must be absorbed to result in the well known immunological phenomena that occur in all animals. It has seemed reasonable, therefore, to conclude that proteins are digested with complete degradation to amino acids. Dent and Schilling(7) however, found no rises of free amino acids in portal blood of partially gastrectomized dogs fed dog plasma protein, from which they concluded that this protein not only escaped complete hydrolysis, but was absorbed whole, escaping even the peptide stage. They used paper chromatography and the hydrolysis procedure mentioned previously. Besides the fact that their dogs were partially gastrectomized, their experiments were marked with difficulties in getting their animals to accept the meal without immediate vomiting and diarrhea; when this occurred, these excreta were refed or eaten voluntarily.

Materials and methods. We attempted to recreate this apparently unique protein absorption process with normal, ungastrectomized dogs. They were prepared by cannulation of a branch of superior mesenteric vein by surgical technic of Denton, *et al*(8). We chose this vein, rather than the portal vein, to avoid admixture of gastric, splenic and large bowel venous blood. We believe that our study is unique in this respect. The catheters were kept open by daily irrigations with saline and heparin. In addition, we obtained lymph from one dog by cannulation of the thoracic duct. The dogs were allowed to recover for about a week after operation, and fed orally, or by stomach tube, about 70 g of cooked ground beef liver, or 30 g dry lyophilized homologous plasma protein. Plasma amino acids were fractionated and analyzed by the method of Moore and Stein(9,10). This method is uniquely suited for detection of peptides, since the smaller peptides are chromatographable on Dowex 50, and can be recognized among the familiar landmarks of known amino acids. In addition, they can be isolated and analyzed by hydrolysis and rechromatographing. We(11) isolated an acidic amino acid conjugate from plasma of nor-

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TABLE I. Micromols Amino Acid per 100 ml Superior Mesenteric Plasma Ultrafiltrate.

	Dog A (liver meal)			Dog B (liver meal)		Dog C (plasma meal)		
	Hr after feeding							
	0	3	6	0	6½	0	3	6
Taurine	4	10	8	5	30	3	5	5
Aspartic acid	0	1	1	4	14	1	3	14
Threonine	15	47	47	13.6	60	14	34	86
SGA*	42	50	44	78	91	48	87	130
Glutamic acid	8	10	9	10	9	7	6	31
Proline	13	20	60	13	69	16	26	48
Glycine	26	33	36	22	69	22	29	52
Valine	17	24	38	21	83	20	34	80
Methionine	3	13	11	6	28	4	3	11
Isoleucine	5	27	19	7	60	6	6	18
Leucine	9	47	37	15	91	9	47	101
Tyrosine	2	6	7	4	14	3	12	30
Phenylalanine	2	12	8	9	32	2	13	40
	Composition of conjugate							
Serine	0	0	0	0	0	0	2	0
Glutamic acid	7	0	0	0	6	2	8	0
Glycine	17	49	59	24	23	25	31	24
Alanine	0	3	6	0	0	0	0	0

* SGA = Serine + glutamine + asparagine.

mal human subjects in this way.

Results. Table I shows levels of mesenteric plasma amino acids in 2 dogs which received liver. Increases in amino acid concentrations are by no means uniform, and cannot be correlated with the composition of the fed protein.

We analyzed the lymph of one dog after a liver meal (Table II). The rises are similar to those of the mesenteric plasma.

The chromatograms showed no ninhydrin reactive components which could be peptides. We did, however, isolate an amino acid conjugate (supra) from each sample. These components gave no color with ninhydrin, and descended the columns rapidly, emerging ahead of aspartic acid. Subsequent hydrolysis in sealed tubes at 110°C for 12 hr, followed by rechromatography, yielded results shown in Table I. Glycine is the major component; we do not know the origin of these conjugates.

The effect of feeding homologous plasma protein is also shown in Table I. The rises of amino acids are, if anything, more dramatic than in liver fed dogs. No peptide fractions were found, nor was the conjugate remarkably elevated.

Discussion. Our results indicate that the normal dog digests homologous plasma proteins in the same way as other proteins, *i.e.*, with complete degradation to amino acids; the rises of free amino acids in the mesenteric plasma are at least as great in the dog fed plasma as in those fed liver. The increase in amino acid concentrations after meals followed no predictable pattern, nor did this process bear any relation to composition of the meal. The explanation for this may be that the amino acids have different absorption rates, that they compete with each other in the absorption process, or that their absorption is inhibited by nonprotein components of the diet. This last possibility was discussed by Denton and Elvehjem(3).

The amino acid conjugate, consisting mainly of glycine and glutamic acid was found in all plasma samples, and in lymph. We do not know the origin of this substance, nor the reason for its varying composition.

Fisher(12), theorizes that peptides are the basic units of protein synthesis, rather than free amino acids. If this is true, these peptides could not have a direct dietary origin, at least if liver or plasma are protein sources, nor does it seem from our experiments that amino acid conjugate(s) could arise directly from the diet.

Since our work was done, Newey and Smyth (13) have shown with *in vivo* experiments

TABLE II. Micromols Amino Acid/100 ml Thoracic Duct Lymph Ultrafiltrate.

	Dog C (liver meal)		
	Hr after feeding		
	0	2.5	4.5
Taurine	3	5.8	8.5
Aspartic acid	2	8	7
Threonine	13	27	27
SGA*	45	74	124
Glutamic acid	10	46	15
Proline	17	23	27
Glycine	23	32	35
Valine	24	28	23
Methionine	3	11	13
Isoleucine	6	14	15
Leucine	11	25	19
Tyrosine	5	11	12
Phenylalanine	5	20	18
	Composition of conjugate		
Serine	0		12
Glutamic acid	3		15
Glycine	7		8.5
Alanine	0		0

* SGA = Serine + glutamine + asparagine.

that a number of dipeptides introduced into the intestine of the dog appear in mesenteric plasma almost completely as free amino acids. Their results, then, are in general agreement with our own. Similar experiments done *in vitro* by Newey and Smyth confirm earlier work of Agar, *et al.*(14) which showed that dipeptides introduced on the mucosal side appeared on the serosal side almost entirely as free amino acids. The small and variable though definite passage of some peptide leaves open the possibility that such absorption might occur *in vivo* in cases of surgically altered or diseased gastrointestinal tract.

Summary. Dogs prepared with catheters in the superior mesenteric vein were fed meals of liver and homologous plasma protein. Analysis of the superior mesenteric blood plasma showed marked rises in amino acids up to 6 hours later. Digestion of the homologous plasma protein took essentially the same course as that of liver protein, contrary to results reported by others(7). No peptides were found and both proteins were apparently completely degraded to amino acids. Rises in lymph amino acids were qualitatively similar to those of the mesenteric plasma, though not as marked. An amino acid conjugate was isolated from all plasma samples, and its amino acid composition was determined. Its changing structure, and failure to respond in an obvious way to the meals leads us to believe that it is not of direct dietary origin.

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ECHO-9 Virus Antibody Titrations in a HeLa Cell System. (24874)

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The need to perform ECHO-9 virus antibody titrations during an outbreak of this infection in summer of 1957(1,2) prompted us to attempt the use of HeLa cells for these titrations because HeLa cells were more readily available to us than monkey kidney cells. HeLa cell adapted(3) ECHO-9 virus (Hill strain) was used as the antigen.* The results reported here clearly demonstrate that ECHO-9 antibody titrations can be readily executed in HeLa cell tissue culture.

* Seed virus was kindly supplied by Dr. Herbert A. Wenner, Univ. of Kansas School of Med., Kansas City.

Materials and methods. HeLa cells were grown in milk dilution bottles according to methods originally outlined by Syverton and Scherer(4). Lactalbumin hydrolysate-yeast extract medium (LHYE) containing 20% human serum was used as growth medium. After 7 days incubation at 37°C, bottles were scraped, cells dispersed in growth medium, a cell count made, and suspension prepared to dispense 50,000 to 60,000 cells/tube in 0.75 ml of growth medium. Tubes were incubated at 37°C for 3 or 4 days. On day tubes were used, the growth medium was removed, the cell sheet rinsed 3 times with Hanks' balanced salt solution (BSS) following which