Intestinal Absorption of Histidine as Influenced by Tryptophan in the Rat.* (19775)

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It has been shown that amino acids have to be available simultaneously and in proper relative concentrations for optimal utilization in the tissues(1). The first step in the utilization of dietary amino acids is the intestinal transfer to the body proper and therefore the speed and rate of this transfer may significantly influence the simultaneous availability of the amino acids(2). There is only little information available on the mechanism of amino acid absorption. Some data have been published in the literature which suggest that the absorption of amino acids is not a process of simple diffusion, but that some other mechanisms probably some "accelerating factors" may be also involved(3). Further it has been shown by several authors that amino acids are absorbed selectively at varying rates from the gastrointestinal tract. It was the purpose of the present study to determine how the absorption of an amino acid, such as histidine or tryptophan, is influenced by the presence of other amino acids in the intestinal tract.

Method. The Cori technic(4) was used to investigate the absorption of amino acids from the gastrointestinal tract of adult male Sprague-Dawley rats. The rats were fed the amino acid solutions via stomach tube. L-tryptophan and dl-phenylalanine were fed as such, and L-histidine and L-arginine were fed as the monohydrochlorides. All of the amino acid solutions were adjusted to a pH of 7.0-8.0 before feeding. After a predetermined absorption period, the residual amounts of histidine and tryptophan which remained in the gastrointestinal tract were colorimetrically determined(5,6). The absorption coefficients were computed on a basis of body surface area rather than of body weight in an attempt to obviate any variability due to differences in the weights of the animals used.

For the determination of histidine Lang's modification of the Pauly-Weiss method was used(5), by which a red color is developed when histidine is treated in alkaline solution with freshly prepared diazotized sulfanilic acid. The only other amino acid giving this color under these conditions is tyrosine, which was completely removed by precipitation with $HgCl_2$. The test for tryptophan is based on the Voisenet-Rhode reaction(6), by which tryptophan specifically produces a blue color in an acidic medium of p-dimethylaminobenzaldehyde. In preliminary control experiments it was shown that the presence of tryptophan in the concentrations used did not interfere with the determination of histidine. Similarly, the color test for tryptophan was not modified by the presence of histidine.

Results. Table I lists the mean values of the absorption coefficients for histidine and tryptophan $\pm$ the standard deviations of the mean. Their absorption rates appear to depend to a certain limit on the concentration in which they were fed. The quantity absorbed increased with increasing concentration of the solution fed from 24 till 50 mg/7.5 ml. Greater concentrations, *i.e.*, 60 to 75 mg/7.5 ml, did not increase further the absorbed quantity of histidine or tryptophan. The data of the table show that the absorption rates of histidine and tryptophan did not differ statistically when each was fed by itself. The results of the experiments wherein histidine and tryptophan were fed simultaneously show that *the presence of l-tryptophan significantly decreases the rate of absorption of histidine from the gut.* This is seen clearly following the feeding of a solution containing 40 to 75 mg each of histidine and tryptophan in 7.5 ml. When histidine was present alone in the gut in this concentration, the absorption coefficient of histidine ranged from 35.7 $\pm$ 8.0 to 43.4 $\pm$ 7.1. When, however, both histidine

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TABLE I. Absorption Rates of Histidine and L-Tryptophan (15-minute absorption period).

Amino acids fed	No. of animals	Amount fed (mg/7.5ml) l-trypto-phan	L-histidine	dl-phenyl-alanine	L-arginine	Absorption coef. (mg absorbed/100 cm^2/hr) l-tryptophan	histidine
l-t†	1	30				*12.7	
	1	50				*43.2	
	1	75				*41	
h†	5		24.3				15.5 ± 4.6
	6		40.5				35.7 ± 8
	4		60.8				43.4 ± 7.1
l-t + h	5	30	24.3			24.8 ± 6.3	9.6 ± 5.1
	5	50	40.5			37.3 ± 8.2	12.9 ± 6.5
	4	75	60.8			47.5 ± 4.2	6 ± 3.1
l-t, h, and dl-phenyl-alanine	2	30	24.3	30		26.2 ± 1.7	4.3 ± 1.3
	2	50	40.5	50		41.1 ± 1.3	10 ± .5
	2	75	60.8	75		44.2 ± 3.8	3.5 ± 3.5
l-t, h, dl-phenylala-nine, and arginine	2	30	24.3	30	24.8	26 ± 3	5.3 ± 5.3
	2	50	40.5	50	41.4	37.6 ± 2.5	1.1 ± .5
	2	75	60.8	75	62	35 ± 7.2	0 ± 0
h, dl-phenylalanine, and arginine	2		24.3	30	24.8		21.1 ± 3.1
	2		40.5	50	41.4		30.2 ± 8.6
	1		60.8	75	62		13.9

* Feeding of identical concentrations of l-tryptophan followed by 10, 20, and 30 min. absorption periods resulted in similar absorption data.

† t = tryptophan; h = histidine.

and tryptophan were present coincidentally in this concentration. the absorption coefficient of histidine was decreased to 6.0 ± 3.1 to 12.9 ± 6.5. *The absorption rate of l-tryptophan does not appear to be affected by the simultaneous presence of histidine, dl-phenylalanine and arginine in the gut.* The presence of dl-phenylalanine and arginine did not show any significant effect on the absorption of either histidine or tryptophan.

Discussion. Our results support further the assumption of some authors that a competition may exist between amino acids themselves or between amino acids and chemically similar compounds for the selective processes of absorption or concentration. In regard to this, Pitts(7) has shown that a competition exists between alanine and creatine for reabsorption by kidney tubule cells. Creatine may be regarded chemically as a substituted methyl glycine. The *in vitro* studies of Christensen and Streicher(9) indicate also that there may be a competition between alpha-amino acids for specific receptor groups in the tissues. Orten *et al.*(8) found also "alterations in the absorption rate of individual amino acids in the presence of other amino acids."

There is no satisfactory explanation yet for the amino acid imbalance which occurs in animals fed certain amino acid mixtures or proteins. It is possible that an amino acid imbalance may be a result of the differential absorption of amino acids from the gastrointestinal tract. The absorption of proper relative concentrations of the essential amino acids may be hindered by the presence of excessive amounts of some other amino acids. This problem is being presently further investigated.

Summary. The presence of l-tryptophan decreases the rate of intestinal absorption of L-histidine in the rat. The absorption rate of l-tryptophan does not appear to be affected by the simultaneous presence of histidine. Dl-phenylalanine and L-arginine do not seem to affect significantly the absorption of either histidine or tryptophan.

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A Critique of Biological Activity of L-Lyxoflavin.* (19776)

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Pallares *et al.*(1) claimed that lyxose was isolated from human myocardium treated with cobra venom. This group later reported(2) the isolation of lyxoflavin from human myocardium and postulated the release of lyxose by the enzymes of cobra venom. Gardner *et al.*(3,4) and Heyl *et al.*(5) subsequently described syntheses of L-lyxoflavin. Gardner *et al.*(3,4) failed to obtain lyxose from synthetic L-lyxoflavin treated with either cobra venom or Russell's viper venom. It was then reported that L-lyxoflavin has vitamin activity for the rat fed a diet containing 0.5% dessicated thyroid(6,7), and growth promoting activity for the chick(8).

Snell and Strong(9) have shown that other stereoisomers of riboflavin, namely the d- and l-arabityl analogs, enhanced the growth response of *Lactobacillus casei* to limiting amounts of riboflavin although these are devoid of activity when assayed in the absence of riboflavin. Other workers(10,11) have demonstrated that the d-xylityl and l-arabityl analogs of riboflavin have limited effectiveness in replacing riboflavin in rats receiving a riboflavin deficient ration. We wish to report our observations on the similar behavior of L-lyxoflavin in the nutrition of *Lactobacillus casei*, the rat and the chick.

Microbiological experiments. L-Lyxoflavin was tested for riboflavin activity using the assay of Snell and Strong(12). The results are shown in Fig. 1. It is apparent that lyxoflavin shows little activity in the absence of added riboflavin. The failure of the higher levels of lyxoflavin to give increased growth indicates that the slight activity of lyxoflavin is not due to contamination with riboflavin. However, in the presence of 0.10 γ of riboflavin, lyxoflavin approximates the activity of riboflavin at levels of 0.02 to 0.10 γ. At levels of 150 γ or more, lyxoflavin inhibits the response of the microorganisms to 0.10 γ of riboflavin. When these results were completed, Bruins *et al.*(8) stated without detailing the data that lyxoflavin stimulated the growth of *Lactobacillus casei* in the presence of limiting amounts of riboflavin and acts as an antimetabolite at higher concentrations.

Rat experiments. Emerson and Folkers(7) found that lyxoflavin was without activity in a prophylactic rat assay for riboflavin. In our experiments, L-lyxoflavin was tested for riboflavin activity in both prophylactic and curative assays. The ration of Street(13) was modified so that the daily vitamin supplement contained 20 γ B_1, 20 γ B_6, 200 γ calcium pantothenate, 250 γ p-aminobenzoic acid, 500 γ i-inositol, 2 γ biotin, 2 γ folic acid, 1 mg niacin and 4 mg choline chloride. Male, weanling Sprague-Dawley rats were used in these experiments. In the prophylactic assay 3 levels of L-lyxoflavin (7, 35 and 70 γ per day) and 2 levels of riboflavin (3.5 and 7 γ per day) were employed in addition to a negative control. Ten rats were used in each group. The results are plotted in Fig. 2. The greatest growth-promoting effect of lyxoflavin was manifested within the first 2 weeks of the assay, after which time the growth rate declined in contrast to the sustained weight gain of the rats receiving riboflavin. It is also

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