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ABSORPTION FROM A MIXTURE OF SEVENTEEN FREE AMINO ACIDS BY THE ISOLATED SMALL INTESTINE OF THE RAT

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SUMMARY

1. Absorption and secretion from a mixture of seventeen free amino acids has been measured in isolated perfused rat small intestine.

2. The absorption rate of an amino acid from this mixture is proportional to its concentration in the perfusate and independent of its chemical constitution. The constant of proportionality is the same as that previously observed when the perfusate contained peptides as well as amino acids.

3. Amino acids are concentrated, on average, sixfold during passage across the mucosa, and the free amino acid composition of the secretion into the tissue fluid is very similar to that of the luminal perfusate.

4. Peptides do not appear to be added to the tissue fluid during absorption of free amino acids.

5. It is concluded that the mechanisms for absorption of free amino acids are in general independent of those for absorption of peptides.

INTRODUCTION

A previous paper (Gardner, 1975) described the absorption by an isolated intestine preparation of free and peptide-bound amino acids from a partial hydrolysate of casein in which 50% of the nitrogen was present as free amino acids. Several striking findings appeared to require further investigation: (i) the rate of absorption of each amino acid, whether free or peptide-bound, from the casein digest was directly related to the perfusate concentration of that amino acid; (ii) the rate of absorption of each free amino acid was highly negatively dependent on the rate of absorption of the peptide-bound form of that amino acid; (iii) over a third of the amino nitrogen appearing in the secretion at the serosal surface was in the form of peptide-bound amino acids; (iv) the amino

acid composition of the peptides in the secretion was positively correlated with the total (i.e. free + peptide-bound) amino acid concentration in the luminal perfusate.

The present study of absorption from a synthetic mixture of free amino acids at a low concentration (1·6 mM total) was made to test: (i) whether the rate of amino acid absorption was proportional to amino acid concentration but independent of the nature of the amino acid; (ii) whether the apparent inhibition by peptides of free amino acid absorption had been due to backflux into the lumen of amino acids produced by mucosal hydrolysis of peptides or whether it was a true inhibition of uptake; and (iii) whether peptides appeared in the intestinal secretion on to the serosal surface when the perfusate contained only free amino acids.

The amino acid composition of the mixture was intended to be the same as that of the free amino acid fraction of the partial digest of casein used in the previous investigation.

METHODS

Experimental procedure. The isolated small intestine of adult female rats (from the Ligament of Treitz to the ileocaecal valve) was perfused slowly in a single pass with a bicarbonate-saline medium containing glucose (28 mM) and an amino acid mixture (see below). Full details of the segmented flow perfusion technique are given by Fisher & Gardner (1974).

The effluent from the intestinal lumen and the secretion produced by the intestine at the serosal surface were collected during successive 15 min periods. The composition of this secretion reaches a steady state during the fourth 15 min period (Gardner, 1975): the fluids collected during this period therefore were taken for analysis.

TABLE 1. Control values for apparent rates of absorption and secretion during nitrogen-free perfusion, $\mu g\ N.cm^{-1}.hr^{-1}$. These values relate to the fourth 15 min collection and are means ± S.E. of mean of four experiments

	Absorption	Secretion
Free amino acid-N	−1·76 ± 0·456	9·02 ± 0·649
Peptide-N	−3·16 ± 1·23	5·33 ± 1·17

Controls. Two experiments were made in which no amino acids were present in the perfusate. The results of these experiments were pooled with those already obtained in the two control experiments of Gardner (1975) to give the results shown in Table 1.

Amino acid mixture. A mixture was made up which approximated closely to the free amino acid fraction of the casein hydrolysate used by Gardner (1975). Four amino acid analyses were carried out on this mixture. A comparison of the results of these analyses with the corresponding values for the casein hydrolysate used previously are given in Table 2.

Analytical methods and computation of results. Amino acid analyses on samples

of perfusate, luminal effluent and secretion were performed by ion exchange chromatography before and after total acid hydrolysis. The analytical procedures were identical to those used in the earlier work (Gardner, 1975) except that the samples of intestinal secretion were deproteinized by the addition of solid 5-sulphosalicylic acid (30 mg/ml. secretion) according to Perry & Hansen (1969). Computation of results was also as previously described by Gardner (1975).

TABLE 2. Amino acid composition of the perfusate, μM. For comparison the amino acid composition of the free amino acid fraction of the perfusate used in the previous experiments of Gardner (1975) is shown in parentheses. Values are means ± S.E. of mean of four analyses

Aspartic acid	61 ± 1·4 (51)	Methionine	83 ± 5·9 (73)
Threonine	82 ± 7·3 (81)	Isoleucine	113 ± 2·8 (112)
Serine	121 ± 5·2 (133)	Leucine	235 ± 9·6 (273)
Glutamic acid	125 ± 7·3 (112)	Tyrosine	57 ± 2·3 (61)
Proline	75 ± 5·2 (77)	Phenylalanine	109 ± 1·6 (102)
Glycine	36 ± 2·3 (33)	Histidine	48 ± 1·7 (47)
Alanine	85 ± 2·8 (87)	Lysine	198 ± 13 (193)
Valine	135 ± 9·5 (144)	Arginine	84 ± 1·4 (90)
	Total 1645 ± 41·4 (1669)		

Note: tryptophan (19 μM) was also present but this was not estimated routinely.

TABLE 3. Distribution of amino acid-N in luminal perfusate, effluent and secretion during the fourth 15-min collection, μg N/ml. Mean ± S.E. of mean of four experiments

	Luminal perfusate	Luminal effluent	Secretion
Amino acid + peptide-N*	32·3 ± 0·44	25·5 ± 2·40	214 ± 12·6
Amino acid-N*	30·7 ± 0·80	19·9 ± 0·87	193 ± 10·3
Peptide-N†	1·66 ± 0·87	5·58 ± 1·82	21·2 ± 3·70

* By ion-exchange chromatography.
† By difference.

RESULTS

Composition of perfusate, luminal effluent and secretion

The distribution of nitrogen in the perfusate, luminal effluent and secretion is shown in Table 3. As a check on the analytical procedure the perfusate was routinely analysed before and after acid hydrolysis even though peptides were known to be absent. The fact that no significant amounts of peptide were detected in the perfusate supports the accuracy of the methods and the fact that the difference is positive (although not significantly different from zero) suggests, as previously assumed, that no detectable losses of amino acid occurred during the acid hydrolysis. Significant amounts of peptide-bound amino acids were found in the luminal effluent and in the secretion.

Table 4 shows the concentration of each free amino acid in the secretion

in experiments with the amino acid mixture (column 1) and in the control experiments (column 2). The ratios of concentrations of free amino acids in the secretion to those in the intestinal lumen (column 4) and the ratios after the secretion concentrations have been corrected for the values in control experiments (column 5) are also shown. Each ratio in column 4 is significantly greater than unity, and the over-all ratio is around 6:1. The actual mean concentration ratio is higher than this since the over-all ratio of secretion concentration to luminal effluent concentration is 9·3:1 because absorption reduces the effluent concentrations to about 60% of perfusate concentrations (see below).

TABLE 4. Concentrations of free amino acids in secretion (μM), and ratios of concentration in secretion to concentration in perfusate. All values refer to the fourth 15 min collection. Mean ± S.E. of mean of four experiments

Amino acid	Concentration in secretion (1)	Concentration in secretion in controls (2)	Difference (1 − 2) (3)	Ratio of column 1 to perfusate concentration (4)	Ratio of column 3 to perfusate concentration (5)
Aspartic acid	219	107	112	3·6 ± 1·3	1·84
Glutamic acid	343	287	56	2·8 ± 0·5	0·45
Glycine	416	391	25	11·6 ± 0·9	0·69
Alanine	972	652	325	11·6 ± 1·0	3·82
Leucine	1575	194	1381	6·7 ± 0·3	5·88
Isoleucine	668	107	561	5·9 ± 0·3	4·96
Valine	965	267	698	7·2 ± 0·5	5·17
Serine	514	214	300	4·3 ± 0·8	2·48
Threonine	537	187	350	6·7 ± 0·7	4·27
Phenylalanine	693	120	573	6·4 ± 0·4	5·26
Tyrosine	369	101	268	6·5 ± 0·3	4·70
Methionine	606	132	474	7·3 ± 0·4	5·71
Proline	530	359	171	7·3 ± 1·4	2·28
Lysine	1480	271	1209	7·6 ± 1·0	6·11
Histidine	248	94	154	5·2 ± 0·4	3·21
Arginine	424	58	366	5·0 ± 0·4	4·36
Over-all ratio				6·4 ± 0·4	

Individual ratios in column 4 are not significantly different ($P > 0·02$) from those obtained in the earlier work with the exceptions of those for leucine and proline. The ratios in column 5 are similar for all amino acids except the dicarboxylic amino acids, serine, proline and glycine.

Rates of absorption from the lumen and rates of secretion

Table 5 shows the corrected rates of absorption of nitrogen from the intestinal lumen and of secretion on to the serosal surface in the form of free and peptide-bound amino acids. Results for absorption from the partial hydrolysate of casein are also shown in parentheses. All rates in this Table have been corrected by subtraction of values found in the absence of nitrogen in the perfusate.

TABLE 5. Rates of absorption of nitrogen from the intestinal lumen and rates of secretion on to the serosal surface corrected by subtraction of values found in the absence of added amino acids, μg N.cm^{-1}.hr^{-1}. Mean of four experiments and four controls ± S.E. of mean. Results from Gardner's (1975) experiments with mixture of free amino acids plus peptides are shown in parentheses (five experiments and four controls)

	Absorption		Secretion	
Free amino acid-N	28·6 ± 2·7 (25·2 ± 1·7)	N.S.	20·9 ± 3·0 (30·1 ± 2·7)	$P < 0{\cdot}05$
Peptide-N	−4·5 ± 4·3 (24·7 ± 3·4)	$P < 0{\cdot}001$	−2·2 ± 1·2 (25·5 ± 12·0)	$P < 0{\cdot}001$

N.S., difference is not significant.

The rate of absorption from the lumen is marginally greater than that in the corresponding experiments with the partial digest. However, the rate of appearance of free amino acids in the secretion is significantly less than that observed in the previous experiments. The rate of secretion of peptide-bound amino acids on to the serosal surface was not significantly different from zero: this is in contrast to the results from the experiments with the partial hydrolysate.

The absorption rate from the lumen of each free amino acid is given in Table 6, together with the corresponding results of the earlier experiments in which the luminal perfusate also contained peptides. The rates of secretion on to the serosal surface are also shown in Table 6.

The relationship between the rate of absorption of each free amino acid and its concentration in the luminal perfusate is shown in Fig. 1. The linear relation observed is very similar to that found in the earlier experiments with the casein hydrolysate (Gardner, 1975).

Relationship between composition of the secretion and that of the perfusate

The relationship between the rate of free amino acid secretion (after correction for control secretion) and the free amino acid concentration in the perfusate is shown in Fig. 2. There is a closely linear relationship,

and since the rate of secretion of any solute is directly related to its concentration in the intestinal secretion it follows that the composition of the secretion reflects closely that of the luminal perfusate. The exceptions from this relationship appear to be glutamic acid and serine.

TABLE 6. Corrected rates of absorption and rates of secretion of each free amino acid. Mean values ± S.E. of mean for four experiments with synthetic free amino acid mixture and four control experiments. (Mean values for five experiments with partial digest of casein and four control experiments are shown in parentheses)

Amino acid	Absorption rate (μM.100 cm^{-1}.hr^{-1})	Secretion rate (μM.100 cm^{-1}.hr^{-1})
Aspartic acid	3·7 ± 0·4 (0·2 ± 3·2)	2·0 ± 1·9 (0·9 ± 0·5)
Glutamic acid	4·9 ± 2·3 (−0·5 ± 1·3)	0·8 ± 0·9 (1·2 ± 0·9)
Glycine	0·0 ± 1·0 (−1·9 ± 0·8)	0·4 ± 1·0 (3·1 ± 0·6‡)*
Alanine	3·9 ± 2·4 (4·0 ± 1·2)	5·2 ± 2·2 (12·2 ± 1·5‡)*
Leucine	21·7 ± 1·6 (30·6 ± 1·4)*	21·4 ± 2·6 (23·9 ± 1·8‡)
Isoleucine	12·3 ± 0·9 (10·4 ± 0·9)	8·7 ± 1·0‡ (12·8 ± 1·1)*
Valine	12·3 ± 4·0 (11·6 ± 1·3)	10·8 ± 1·5 (18·8 ± 2·5‡)*
Serine	7·1 ± 2·9 (7·4 ± 1·5)	4·4 ± 1·2 (15·2 ± 1·4‡)*
Threonine	5·9 ± 1·0 (4·0 ± 1·3)	5·7 ± 1·7 (7·1 ± 0·8)
Phenylalanine	12·0 ± 2·7 (10·0 ± 1·3)	8·9 ± 1·0 (9·8 ± 0·9)
Tyrosine	4·7 ± 1·1 (5·0 ± 0·9)	4·2 ± 0·7 (5·8 ± 0·5)
Methionine	8·5 ± 1·3 (7·6 ± 0·4)	7·4 ± 1·2 (6·8 ± 0·8)
Proline	5·5 ± 3·2 (−6·3 ± 3·1)*	3·0 ± 2·0 (21·5 ± 3·7‡)*
Lysine	23·8 ± 4·3 (20·1 ± 2·9)	18·4 ± 1·9 (20·0 ± 2·1)
Histidine	3·3 ± 0·4 (2·8 ± 1·6)	2·5 ± 0·6 (4·2 ± 0·4)*
Arginine	11·1 ± 0·7 (12·2 ± 1·2)	5·6 ± 0·6‡ (5·8 ± 0·7‡)

* Rate for free amino acid mixture significantly different from rate in parentheses for amino acid plus peptide mixture.

‡ Secretion rate significantly different from corresponding absorption rate.

Multiple regression analyses

Table 7 summarizes the significant first-order regressions obtained by automatic elimination with 'MULTREG' (see Gardner, 1975). Again, as in the previous paper the data for glutamic acid and alanine have not been included. None of these regression coefficients differ significantly from the corresponding coefficients reported earlier (Gardner, 1975) for absorption from the mixture containing both amino acids and peptides. The coefficients from the earlier work are included in Table 7 in parentheses. However, no significant regression was found in the present results when the secretion concentration (uncorrected) of peptide-bound amino acids was the dependent variable.

TABLE 7. Significant regressions computed with progressive automatic elimination of insignificant variables by MULTREG for absorption from the synthetic mixture of free amino acids. The corresponding coefficients for absorption from the partial hydrolysate of casein are shown in parentheses for comparison; they are similar to those reported by Gardner (1975) but data for two additional control experiments have been incorporated. Concentrations are expressed in μmole/ml. and rates in μmole.cm^{-1}.hr^{-1}

	Independent variable (coefficient ± S.D.)			
Dependent variable	Free amino acid concentration in perfusate	Rate of absorption of free amino acid	Intercept (± S.D.)	Significance
1. Rate of absorption of free amino acid	1·13 ± 0·119 (1·20 ± 0·084)	—	− 0·022 ± 0·014	$P < 0.001$
2. Rate of absorption of total amino acid	1·25 ± 0·13 (1·20 ± 0·92)	—	− 0·042 ± 0·016	$P < 0.001$
3. Free amino acid concentration in secretion	6·80 ± 0·55 (4·94 ± 0·971)	—	− 0·22 ± 0·064	$P < 0.001$
3*a*. Free amino acid concentration in secretion	—	5·67 ± 0·433	− 0·060 ± 0·050	$P < 0.001$
4. Peptide-bound amino acid concentration in secretion	(No significant regressions found for absorption from free amino acid mixture)			
5. Total amino acid concentration in secretion	1·09 ± 0·097 (1·42 ± 0·142)	—	− 0·046 ± 0·011	$P < 0.001$
5*a*. Total amino acid concentration in secretion	—	0·907 ± 0·074 (1·16 ± 0·103)	− 0·020 ± 0·008	$P < 0.001$

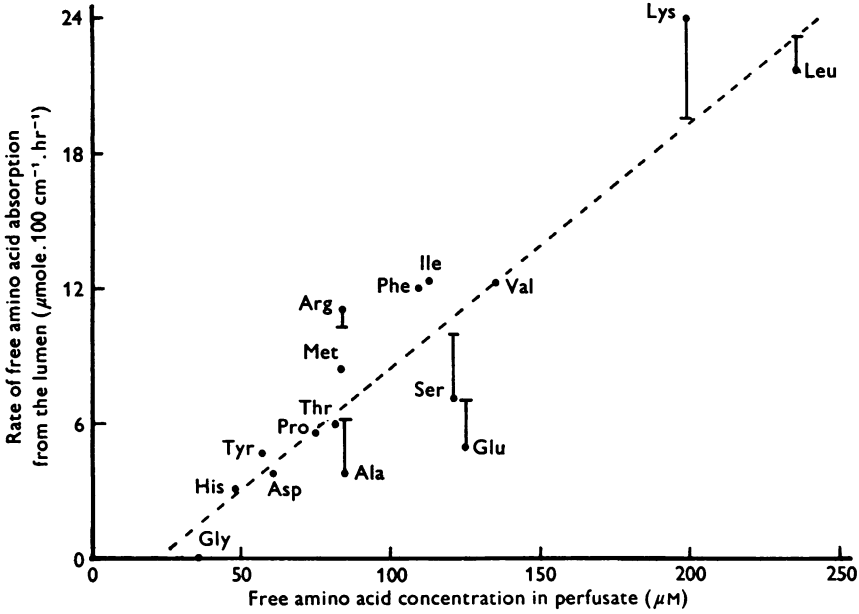

Fig. 1. Relationship between the rate of absorption of each free amino acid from the intestinal lumen and the concentration of that amino acid in the luminal perfusate. Results are means of four experiments (corrected by four control experiments). The S.E. of the mean is shown in some instances. The dashed line is the first-order regression line.

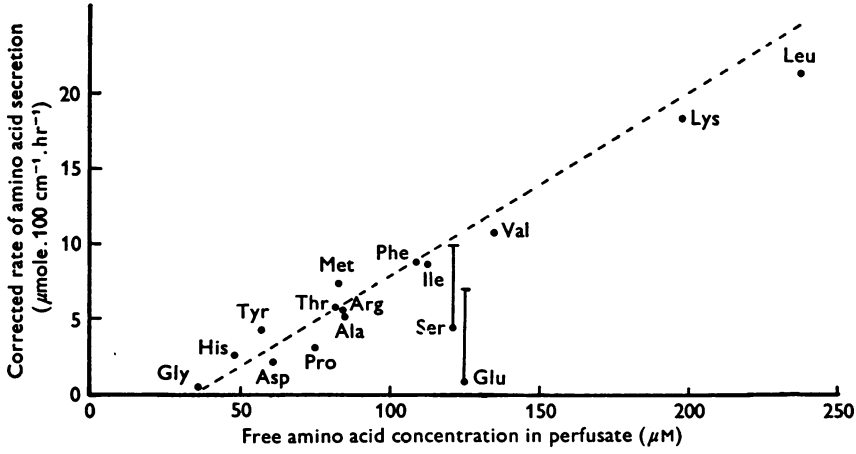

Fig. 2. Relationship between the rate of secretion of each free amino acid on to the serosal surface and the concentration of that amino acid in the luminal perfusate. Results are means of four experiments (corrected for secretion of endogenous amino acids in four control experiments with nitrogen-free perfusion). The S.E. of the mean is shown for serine and glutamic acid. The dashed line is the first-order regression line through all data except for glutamic acid and serine.

DISCUSSION

Composition of the perfusate and rates of absorption

The amino acid composition of the perfusate (Table 2) was on the whole closely similar to that of the free amino acid fraction in the partial digest of casein which was used in the earlier investigation by Gardner (1975). The mean leucine content was however only 86 % of the intended value.

The over-all absorption rate from the lumen is marginally (but not significantly) greater than the free amino acid absorption rate observed from the partial digest (Table 5). The instances in which absorption of free amino acids was substantially different from the rates observed with the partial hydrolysate are proline and leucine (Table 6). In the case of proline the net rate of absorption in the earlier investigation had been lowered to a negative value apparently by substantial backflux of free proline liberated during intracellular hydrolysis of proline-containing peptides (Gardner, 1975). The present experiments in which peptides were absent from the luminal perfusate therefore more truly represent the absorption of free proline. About half the discrepancy observed for leucine absorption can be accounted for by the fact that the leucine concentration in the synthetic mixture of free amino acids was only 86 % of that in the hydrolysate.

The relationship between absorption rate and amino acid concentration is a closely linear one; Fig. 1 (see below).

That there was no fundamental difference in the absorption of amino acids in the two series of experiments is amply borne out by the identity of the regression coefficients for absorption seen in Table 7. Therefore it is confirmed that the interactions between peptide absorption and amino acid absorption (net rate) which were detected in the earlier work do reflect backflux of hydrolysis products, and not an inhibition of amino acid absorption by peptides.

Composition of the secretion

The peptides found in the steady-state secretion (Table 3) appear to be derived wholly from loss of endogenous peptides, since the corrected rate of peptide secretion shown in Table 5 is not significantly different from zero. In the previous experiments with the partial hydrolysate a substantial fraction of the nitrogen entering the tissue fluid was peptide-bound, but the origin of these peptides was not clear. Since peptides were not added to the tissue fluid in the present experiments with only free amino acids in the lumen it appears that the origin of the secretion peptides in the previous investigation must have been luminal peptides.

The poor correlation ($r = 0{\cdot}223$) between the concentration of each individual amino acid in the perfusate peptides and in the secretion peptides in the previous investigation makes it unlikely that unchanged peptides in general crossed the mucosa to the tissue fluid to an important extent. One possibility is that some peptides crossed the mucosa intact, while others were partially hydrolysed within the cells. This does not explain why the rate of secretion of some peptide-bound amino acids was greater than their rate of absorption from the lumen in the previous work. Elucidation of this point and of the fate of particular peptides in partial digests will require more detailed knowledge of the compositions of individual peptides in the perfusates and the secretion.

The rate of free amino acid secretion during perfusion with the synthetic mixture of free amino acids was significantly less than during the experiments with the partial digest (Table 5). This apparently only reflects the fact that peptides were present in the perfusate of the earlier experiments and that some of these were being hydrolysed intracellularly. Indeed the regression coefficients relating the secretion composition to the free amino acid concentration in the perfusate or to the rate of absorption of free amino acid from the lumen do not differ significantly between the two series of experiments with the different perfusates (Table 7). Therefore the presence of peptides in the perfusate of the earlier work probably did not modify the secretion of free amino acids into the tissue fluid, but probably simply augmented this with the products of intracellular peptide hydrolysis.

Interactions between peptide and amino acid absorption

The strong identity between the regression coefficients obtained in the two series of experiments as discussed above suggests that the presence of peptides in the partial hydrolysate which was first investigated did not alter the characteristics of absorption of the free amino acids in the mixture (Table 7).

The implication is that there are independent mechanisms for the absorption of free amino acids and of peptides. This is in agreement with the current views stemming particularly from the work of Matthews and of Milne (e.g. Craft, Geddes, Hyde, Wise & Matthews, 1968; Asatoor, Cheng, Edwards, Lant, Matthews, Milne, Navab & Richards, 1970; Milne, 1972; Matthews, 1972).

Whether or not free amino acids interact with the absorption of peptides cannot be established at this time, although such an interaction would not be expected if the mechanisms for free amino and peptide absorption are indeed independent.

Interactions between amino acids in absorption

The linear relationship seen in Fig. 1 is in close agreement with the relationship which was observed when the perfusate also contained peptides (Gardner, 1975). A similar relationship was reported by Bronk & Leese (1974) and it was pointed out by Gardner (1975) that such relationships imply a constant V_{max}/K_m ratio for all amino acids. Calculations from Adibi & Gray's (1967) data for absorption from amino acid mixtures in man supported this conclusion.

Thus the rate of absorption of each amino acid from a mixture at a low concentration appears to be determined by its concentration in the intestinal lumen and not by the nature of the amino acid. Nevertheless the absorption process is clearly an active one since concentration gradients of 6·4:1 on average and of up to about 12:1 exist across the intestinal mucosa at the steady state (Table 4).

It has been noted that the kinetics of transport exhibit the same ratio of V_{max}/K_m for all amino acids when they are presented as a mixture, although this is apparently not seen when the absorption of single amino acids is studied. This appears to be evidence of interactions between amino acids in the mixture, and contrasts with the finding that peptides do not interact with the absorption of free amino acids.

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