Relationships between mucosal hydrolysis and transport of two phenylalanine dipeptides

D. B. A. SILK¹, J. ALEX NICHOLSON, AND Y. S. KIM

From the Gastrointestinal Research Laboratory, Veterans Administration Hospital, San Francisco, and the Department of Medicine, University of California, School of Medicine, San Francisco, California, USA

SUMMARY In order to investigate the source of free amino acids found in the gut lumen during absorption of dipeptides, as well as evaluating the role of brush border peptidases in the mucosal hydrolysis of dipeptides during absorption, rates of dipeptide disappearance and appearance of hydrolytic products were measured during perfusion of rat jejunum and ileum *in vivo* with buffered and unbuffered 10 mM solutions of glycyl-L-phenylalanine (Gly-Phe) and L-phenylalanyl-glycine (Phe-Gly). Mucosal brush border peptidase activity was then measured in the perfused segments *in vitro* at luminal pH and at two substrate concentrations. In addition cytosol peptidase activity in the perfused segments was measured at pH 7.4 and at 10 mM substrate concentrations. In the jejunum, there was a relationship between rates of free phenylalanine appearance *in vivo* (Phe-Gly > Gly-Phe) and rates of brush border (Phe-Gly > Gly-Phe) rather than cytosol (Gly-Phe > Phe-Gly) peptidase activities. No constant relationship between free phenylalanine appearance and hydrolysis of the dipeptides by either brush border or cytosol peptidases was observed in the ileal studies. These findings suggest that, in the jejunum, hydrolytic products originate from the surface of the cell whereas, in the ileum, hydrolytic products originate from both the intracellular compartment as well as from the surface of the mucosal cell. In the jejunum, *in vitro* rates of brush border hydrolysis of Gly-Phe were always less than *in vivo* disappearance rates, whereas rates of Phe-Gly brush border hydrolysis always exceeded luminal disappearance rates. These data imply that Gly-Phe is predominantly transported intact and hydrolysed by cytosol peptidases. In contrast, brush border peptidases play an important role in the mucosal hydrolysis of Phe-Gly.

Although a few dipeptides with a low affinity for intestinal mucosal peptidases have been detected in peripheral and portal plasma during absorption experiments (Peters and MacMahon, 1970; Adibi, 1971; Boullin *et al.*, 1973), most dipeptides removed from the lumen of the small intestine during the processes of protein digestion and absorption (Silk, 1974; Matthews, 1975; Matthews and Adibi, 1976) are hydrolysed by intestinal mucosal peptidases.

The subcellular mucosal site or sites of dipeptide hydrolysis are unclear at present. On the one hand, results of many experiments have been interpreted as indicating that dipeptides are hydrolysed by intracellular enzymes (Adibi, 1971; Matthews, 1971; Silk, 1974; Matthews, 1975). These conclusions are

supported by (1) subcellular fractionation studies, which show that mucosal dipeptidase activity is predominantly located to the cytoplasmic compartment of the cell (Robinson, 1963; Peters, 1970; Donlon and Fottrell, 1972), and (2) the recent characterisation of a carrier mediated transport system for di- and tripeptides (Addison *et al.*, 1972; Matthews *et al.*, 1974; Addison *et al.*, 1975a; Addison *et al.*, 1975b; Sigrist-Nelson, 1975).

On the other hand a common finding in almost all *in vivo* and *in vitro* dipeptide absorption experiments has been the detection of dipeptide hydrolytic products (amino acids) in the medium bathing the intestinal mucosa (Silk, 1974; Matthews, 1975; Matthews and Adibi, 1976). The source of free amino acids produced during dipeptide absorption has not been determined, but their presence has been taken to indicate that some hydrolysis of dipeptides occurs at the surface of the mucosal cell (Adibi, 1971;

¹Address for correspondence: Dr D. B. A. Silk, Liver Unit, King's College Hospital, London S.E.5.

Cheng *et al.*, 1971; Silk *et al.*, 1973). Supporting a superficial site of hydrolysis, recent studies indicate that the brush border fraction as well as the cytosol fraction of intestinal mucosa contains a number of enzymes capable of hydrolysing a wide variety of dipeptides (Rhodes *et al.*, 1967; Kim *et al.*, 1972; Fujita *et al.*, 1972; Heizer *et al.*, 1972; Wojnarowska and Gray, 1975; Kim and Brophy, 1976; Kim *et al.*, 1976). The activity of these enzymes is high, even in relationship to sucrase (Auricchio *et al.*, 1972) and it has therefore been suggested that they play a role in the terminal stages of protein digestion at the peptide level (Rhodes *et al.*, 1967; Kim *et al.*, 1972; Fujita *et al.*, 1972; Heizer *et al.*, 1972; Wojnarowska and Gray, 1975).

In this paper we report experiments that were undertaken to further investigate the mucosal hydrolysis of dipeptides with the aim of (1) determining the source or sources of free amino acids detected during jejunal and ileal perfusion of dipeptides *in vivo*, and (2) evaluating the role of brush border enzymes in the mucosal hydrolysis of dipeptides during absorption *in vivo*. Using two substrates glycyl-L-phenylalanine (Gly-Phe) and L-phenylalanyl-glycine (Phe-Gly) dipeptide disappearance rates and free amino acid appearance rates were measured during *in vivo* perfusion of rat intestine. Rates of hydrolysis of the peptides by mucosal brush border peptidases, obtained from the mucosa of the perfused segments, were then measured *in vitro* at the pH of intestinal contents and mucosal cytosol peptidase activity measured at pH 7.4. In this way, relationships between enzyme activities, disappearance of luminal substrate, and luminal appearance of the products of dipeptide hydrolysis could be examined. Initially, perfusion experiments were performed using unbuffered solutions. The pH of jejunal and ileal contents was different; as activities of intestinal mucosal peptidases are pH dependent (Kim *et al.*, 1974) the pH of jejunal and ileal contents was reversed by perfusing buffered solutions so that the above relationships could be examined in more detail.

Methods

PERFUSION PROCEDURE AND TISSUE PREPARATION

Unfasted female Wistar rats (220-240 g) were used for all experiments, since it is known that fasting has a deleterious effect on mucosal brush border peptide hydrolase activity (Kim *et al.*, 1973). 20-25 cm segments of rat jejunum or ileum were prepared and perfused as previously described (Lane *et al.*, 1975). Test solutions were perfused at 0.3 ml/min and, after

a 30 minute equilibration period for each solution, a single 10 minute collection of intestinal contents was made and the pH measured immediately. Three additional 10 minute collections were then made into tubes positioned in a mixture of dry ice and ethanol. These samples were then boiled to inactivate possible luminal peptidases.

At the end of the perfusion experiments, the jejunal and ileal loops were removed and transferred to a cold room maintained at 4°C. Mucosae were scraped, the scrapings weighed and immediately homogenised in 14% glycerol (20 ml/g).

CHOICE OF SUBSTRATES AND COMPOSITION OF PERFUSION SOLUTIONS

The dipeptides Gly-Phe and Phe-Gly were chosen as substrates because mucosal brush border peptidase activity in mucosal homogenates can be readily distinguished from mucosal cytosol activity by the different sensitivities of the enzymes in these two fractions to the inhibitor p-hydroxy mercuribenzoate (PHMB, Heizer *et al.*, 1972; Nicholson and Kim, 1975). For the first series of experiments, 10 mM concentrations of Gly-Phe and Phe-Gly were dissolved in 140 mM sodium chloride. 4 g/l polyethylene glycol (PEG) labelled with 2 μ Ci 14 C PEG/l was added and the solutions adjusted to pH 7.0 with 0.1 M NaOH.

In the second series of experiments jejunal solutions were prepared by adding dipeptide (final concentration 10 mM) to Krebs Henseleit Ringer bicarbonate (KHRB) buffer pH 7.4. Ileal solutions were prepared by adding dipeptide to 75 mM acetic acid: sodium acetate buffer pH 5.4 and the resultant mixture made isotonic by adding sodium chloride.

CHEMICAL ANALYSIS OF INTESTINAL CONTENTS

Suitably diluted aliquots of intestinal contents were assayed for free L-phenylalanine (Phe) using the L-amino acid oxidase reagent described by Nicholson and Kim (1975). Previous studies in this laboratory have shown that neither glycine, Gly-Phe or Phe-Gly react with L-amino acid oxidase (Nicholson and Kim, 1975). Concentrations of unhydrolysed dipeptide were estimated from the difference between concentrations of Phe in intestinal contents after and before acid hydrolysis. 0.5 ml intestinal content was mixed with 0.5 ml 6 N HCl, and incubated in sealed glass tubes at 110°C for 20 hr. Hydrolysates were then neutralised and assayed for free Phe. When intestinal contents collected during perfusion of isotonic saline were assayed as described above, absorbance readings corresponded to less than 1.2 nmol Phe.C 14 PEG was estimated as previously described (Silk *et al.*, 1974).

IN VITRO ASSAY OF MUCOSAL PEPTIDASE ACTIVITIES

It is necessary during the process of absorption for molecules to pass through the unstirred water layer (Dietschy *et al.*, 1971) as well as the lipid membrane of the microvillus surface. The unstirred water layer has been shown to distort the kinetic parameters of carrier mediated transport processes for hydrophilic solutes *in vitro* (Wilson and Dietschy, 1974). In the present study, concentrations of dipeptide in the bulk water phase of the small intestinal lumen may be greater than at the surface of the mucosal cells. The magnitude of this difference is unknown, but, in order to obviate possible effects of the unstirred water layer on juxta mucosal concentrations of dipeptide, all assays of brush border peptidase activity were carried out at infusion substrate concentration (10 mM) and also at 2 mM. Initial rates of dipeptide hydrolysis by cytosol and brush border peptidases in mucosal homogenates were measured over a 20 minute period in the absence and presence of 0.5 mM PHMB using a two step L-amino acid oxidase method (Nicholson and Kim, 1975). Substrates were dissolved in the following buffers: (1) 50 mM Tris-maleate buffer for estimation of brush border peptide hydrolase activity after the saline-dipeptide perfusions. The pH was adjusted to the pH of intestinal contents. (2) KHRB buffer pH 7.4 for estimation of brush border peptide hydrolase activity after jejunal perfusion of dipeptides dissolved in this buffer. (3) 50 mM Tris-maleate buffer pH 5.7 for estimation of ileal brush border enzyme activity after ileal perfusion of dipeptide dissolved in 75 mM acetic acid: sodium acetate buffer. (4) 50 mM Tris-maleate buffer pH 7.4 for estimation of jejunal and ileal cytosol peptide hydrolase activity.

CALCULATION OF DATA

Disappearance rates of unhydrolysed dipeptide, and appearance rates of free Phe during the *in vivo* dipeptide perfusions were calculated from standard formulae (Adibi, 1971) and results expressed as $\mu\text{mol/h/g}$ mucosal scrapings. For the *in vitro* enzyme assays, mucosal brush border peptidase activity is the activity observed in the presence of PHMB, and cytosol peptidase activity is the activity in the ab-

sence of PHMB minus the activity observed in its presence (Silk and Kim, 1975). Results are expressed as μmol dipeptide hydrolysed/h/g mucosal scrapings. The statistical significance of differences in results was evaluated by the paired *t* test and by Student's *t* test where appropriate.

Results

pH OF INTESTINAL CONTENTS

The pH of intestinal contents is shown in Table 1. During the dipeptide-saline perfusions jejunal contents had a lower pH than ileal contents. Jejunal perfusion of dipeptides dissolved in KHRB buffer resulted in luminal pH values very similar to those found during ileal perfusion of dipeptide-saline mixtures. Similarly, ileal perfusion of dipeptides dissolved in acetic acid: sodium acetate buffer resulted in luminal pH values similar to those found during jejunal perfusion of dipeptide-saline mixtures.

FREE PHENYLALANINE APPEARANCE AND MUCOSAL DIPEPTIDE HYDROLYSIS

Dipeptide-saline perfusions (Table 2)

When the unbuffered dipeptide-saline solutions were perfused in the jejunum, Phe appeared faster during Phe-Gly perfusion than Gly-Phe perfusion ($P < 0.05$). Table 2 shows that at 10 mM substrate concentration brush border peptidase activity was greater for Phe-Gly than Gly-Phe ($P < 0.005$). This was also the case when 2 mM substrate concentrations were used in the assays ($P < 0.005$). In contrast, cytosol peptidase activity was 10 times greater for Gly-Phe than Phe-Gly ($P < 0.001$). When the same solutions were perfused in the ileum, Phe appeared faster during Gly-Phe perfusion than Phe-Gly perfusion ($P < 0.02$). As in the jejunum, brush border peptidase activity was greater for Phe-Gly than Gly-Phe at both substrate concentrations ($P < 0.02$ or less) and cytosol peptidase activity was greater for Gly-Phe than Phe-Gly ($P < 0.001$).

Buffered dipeptide perfusions (Table 3)

Phe appearance during jejunal perfusion of Gly-Phe and Phe-Gly increased when the jejunal luminal pH

Table 1 pH of intestinal contents*

Composition of perfusion solutions	Jejunum		Ileum	
	Gly-Phe	Phe-Gly	Gly-Phe	Phe-Gly
Dipeptide-saline (pH 7.0)	5.3-6.2	5.6-6.1	6.9-7.5	6.7-7.9
Dipeptide-buffer†	7.0-7.5	7.0-7.4	5.4-6.0	5.4-5.9

*Values are range, $n = 6$.

†Dipeptides (final concentration 10 mM) dissolved in pH 7.4 Krebs Henseleit Ringer bicarbonate buffer for the jejunal perfusions and pH 5.4 acetic acid: sodium acetate buffer for the ileal perfusions.

Table 2 Free phenylalanine appearance during in vivo perfusion of unbuffered 10 mM dipeptide-saline solutions, mucosal brush border peptidase activity in perfused segments measured in vitro at luminal pH and at 10 mM substrate concentration and mucosal cytosol peptidase activity in perfused segments measured in vitro at pH 7.4 and at 10 mM substrate concentration

	Jejunum (pH 5.3-6.2)		Ileum (pH 6.7-7.9)	
	Gly-Phe	Phe-Gly	Gly-Phe	Phe-Gly
Free Phe appearance (μ mol/h/g mucosa)	0.01 $\pm$ 0.01	0.76 $\pm$ 0.38	7.53 $\pm$ 1.06	4.41 $\pm$ 0.62
Brush border peptidase activity (μ mol/h/g mucosa)	38.4 $\pm$ 10.0	295.4 $\pm$ 37.1	165.5 $\pm$ 39.8	312.2 $\pm$ 47.3
Cytosol peptidase activity mmol/h/g	7.5 $\pm$ 0.6	0.7 $\pm$ 0.1	9.3 $\pm$ 0.7	0.4 $\pm$ 0.0

Values are the mean $\pm$ SE, n = 6.

Table 3 Free phenylalanine appearance during in vivo perfusion of buffered 10 mM dipeptide solutions, mucosal brush border peptidase activity in perfused segments measured in vitro at luminal pH and at 10 mM substrate concentration, and mucosal cytosol peptidase activity in perfused segments measured in vitro at pH 7.4 and at 10 mM substrate concentration

	Jejunum (pH 7.0-7.5)		Ileum (pH 5.4-6.0)	
	Gly-Phe	Phe-Gly	Gly-Phe	Phe-Gly
Free Phe appearance (μ mol/h/g mucosa)	1.82 $\pm$ 0.96	48.2 $\pm$ 1.42	3.53 $\pm$ 0.64	3.36 $\pm$ 0.26
Brush border peptidase activity (μ mol/h/g mucosa)	329.8 $\pm$ 31.2	1725.0 $\pm$ 91.6	4.2 $\pm$ 2.8	72.8 $\pm$ 12.6
Cytosol peptidase activity mmol/h/g	7.5 $\pm$ 0.6	0.7 $\pm$ 0.1	9.3 $\pm$ 0.7	0.4 $\pm$ 0.0

Values are the mean $\pm$ SE, n = 6.

Table 4 Rates of dipeptide disappearance during in vivo perfusion of 10 mM unbuffered dipeptide-saline solutions and mucosal brush border peptidase activity in perfused segments measured in vitro at luminal pH and at 2 mM and 10 mM substrate concentrations

(μ mol/h/g mucosa)	Jejunum (pH 5.3-6.2)		Ileum (pH 6.7-7.9)	
	Gly-Phe	Phe-Gly	Gly-Phe	Phe-Gly
Dipeptide disappearance	77.7 $\pm$ 8.5	75.5 $\pm$ 9.7	67.5 $\pm$ 8.8	74.3 $\pm$ 4.1
Brush border peptidase activity (2 mM)	26.4 $\pm$ 11.0	101.4 $\pm$ 11.0	69.5 $\pm$ 13.6	246.6 $\pm$ 26.1
(10 mM)	38.4 $\pm$ 10.0	295.4 $\pm$ 37.1	165.5 $\pm$ 39.8	312.2 $\pm$ 47.3

Values are the mean $\pm$ SE, n = 6.

was raised (pH 7.0-7.5) compared with appearance rates seen during the jejunal dipeptide-saline perfusions ($p < 0.05$ for both dipeptides). Similarly, jejunal mucosal brush border peptidase activity for both dipeptides at the two substrate concentrations was also greater when measured at the pH of buffered than unbuffered intestinal contents ($p < 0.05$ or less). As during the jejunal dipeptide saline perfusions (Table 2), Phe appearance during the buffered perfusions was greater for Phe-Gly than Gly-Phe ($p < 0.02$) and brush border peptidase activity was also greater for Phe-Gly than Gly-Phe ($p < 0.001$).

Ileal mucosal brush border peptidase activity for both dipeptides at the two substrate concentrations was reduced when measured at the lower pH of the buffered (pH 5.4-6.0) than unbuffered (pH 6.7-7.9) ileal contents ($p < 0.05$ or less). As during the ileal

dipeptide-saline perfusions (Table 2) brush border peptidase activity was greater for Phe-Gly than Gly-Phe ($p < 0.05$ or less). Phe appearance rates during the buffered ileal perfusions was the same for Gly-Phe and Phe-Gly.

DIPEPTIDE DISAPPEARANCE RATES AND BRUSH BORDER PEPTIDASE ACTIVITIES

Dipeptide-saline perfusions (Table 4)

Rates of brush border hydrolysis of Phe-Gly (measured at the same pH of intestinal contents using 2 mM and 10 mM substrate concentrations) were the same (2 mM) or greater (10 mM) than the rates of disappearance of unhydrolysed Phe-Gly from the gut lumen during jejunal and ileal perfusion of the unbuffered (10 mM) dipeptide-saline solutions. The same was the case during the ileal experiments with

Table 5 Rates of dipeptide disappearance during *in vivo* perfusion of 10 mM buffered dipeptide solutions and mucosal brush border peptidase activity in perfused segments measured *in vitro* at luminal pH and at 2 mM and 10 mM substrate concentrations

(μ mol/h/g mucosa)	Jejunum (pH 7.0-7.5)		Ileum (pH 5.4-6.0)	
	Gly-Phe	Phe-Gly	Gly-Phe	Phe-Gly
Dipeptide disappearance	81.3 $\pm$ 7.0	80.8 $\pm$ 4.4	87.2 $\pm$ 4.8	80.1 $\pm$ 7.2
Brush border peptidase activity				
(2 mM)	39.1 $\pm$ 10.0	1131.0 $\pm$ 48.1	1.9 $\pm$ 1.9	24.6 $\pm$ 7.6
(10 mM)	329.8 $\pm$ 31.2	1725.0 $\pm$ 91.6	4.2 $\pm$ 2.8	72.8 $\pm$ 12.6

Values are the mean $\pm$ SE, n = 6.

Gly-Phe, but hydrolysis of Gly-Phe by brush border peptidases in jejunal mucosa was less than jejunal disappearance rates of the dipeptide.

BUFFERED DIPEPTIDE PERFUSIONS (Table 5)
Altering the luminal pH did not significantly affect jejunal or ileal disappearance rates of either dipeptide. Thus, values of jejunal Phe-Gly brush border peptidase activity, measured at two substrate concentrations and at the pH of buffered jejunal contents, were several times greater than luminal disappearance rates. In the case of Gly-Phe, jejunal brush border peptidase activity measured at 10 mM substrate concentration was also greater than the rates of luminal disappearance, but enzyme activity measured at 2 mM was less than the rate of luminal disappearance. In the ileal experiments, when luminal pH was lowered, brush border activities for both the dipeptides, under all assay conditions, were less than luminal disappearance rates.

Discussion

A common finding in almost all *in vivo* and *in vitro* dipeptide absorption experiments has been the detection of dipeptide hydrolytic products (amino acids) in media bathing intestinal mucosal preparations (Silk, 1974; Matthews, 1975; Matthews and Adibi, 1976). These amino acids could arise as a result of (1) hydrolysis of dipeptides by peptidases in the medium, (2) hydrolysis of dipeptides by mucosal brush border peptidases, with subsequent diffusion of liberated amino acids into the medium, or (3) hydrolysis of dipeptides by mucosal cytoplasmic peptidases with subsequent movement of liberated amino acids out of the intracellular compartment back into the medium. The first alternative may be of major importance in *in vitro* experiments since mucosal peptidases are rapidly secreted into the medium (Josefsson and Sjöström, 1966; Silk and Kim, 1976). However, in *in vivo* perfusion experiments luminal peptidase activity is low (Adibi, 1971; Silk *et al.*, 1973; Lane *et al.*, 1975), and does not significantly contribute to the production of free

amino acids detected during dipeptide perfusion. The first aim of the present study was therefore to determine if appearance of free amino acids during *in vivo* dipeptide transport is related to brush border or cytoplasmic peptidase activity.

When unbuffered solutions of Gly-Phe and Phe-Gly were perfused in the jejunum, free Phe appearance was greater during perfusion of Phe-Gly than Gly-Phe. Brush border peptidase activity measured at luminal pH (5.3-6.2) was greater for Phe-Gly than Gly-Phe. In marked contrast cytosol peptidase activity (measured at pH 7.4) was 10 times greater for Gly-Phe than for Phe-Gly. These data suggest that appearance rates of free Phe during jejunal perfusion of the two dipeptides is a function of the relative rates of hydrolysis of the two dipeptides by brush border rather than cytosol peptidases. This conclusion is supported by the results of buffered perfusions. When luminal pH was raised from pH range 5.3-6.2 to 7.0-7.5 *in vivo* rates of free Phe appearance during perfusion of both dipeptides increased as did *in vitro* rates of brush border hydrolysis. Furthermore, the differential between rates of appearance of free Phe and rates of hydrolysis of the dipeptides was maintained (Table 3).

In the ileum, Phe appearance rates were greater for both dipeptides than in the jejunum, when the dipeptide-saline mixtures were perfused (Table 2). At first sight this seemed to be because brush border peptidase activity against the two dipeptides was greater in ileal than jejunal mucosa when assays were carried out at luminal pH. However, in contrast with the jejunal studies, there was no relationship between differential rates of ileal Phe appearance and brush border peptidase activity for the two dipeptides. This was also the case when the ileal perfusions were repeated using buffered dipeptide solutions. Indeed, during the ileal dipeptide-saline perfusions there was a relationship between the differential rates of Phe appearance and cytosol peptidase activity against the two dipeptides. The present study has not therefore been helpful in determining why free amino acid appearance is greater during ileal rather than jejunal perfusions (Adibi, 1971; Silk *et al.*, 1974b; Lane *et al.*,

1975). The results do suggest however that the origin of these free amino acids is quite different in the ileum compared with the jejunum. One possibility is that in the ileum free amino acids move from the intracellular compartment to the mucosal medium after dipeptide hydrolysis by cytoplasmic peptidases (Boyd *et al.*, 1975). Further studies are needed, however, before firm conclusions can be drawn about the source of luminal free amino acids during ileal dipeptide perfusion.

The jejunum is currently thought to be the major site of absorption of protein digestion products (Borgström *et al.*, 1957; Nixon and Mawer, 1970). Here, although rates of disappearance of the two dipeptides were the same, rates of appearance of free Phe and brush border peptidase activity were greater for Phe-Gly than Gly-Phe. This suggests that greater proportions of luminal Phe-Gly than Gly-Phe are hydrolysed at the surface of the cell during absorption. Determination of the precise amounts of either dipeptide hydrolysed at the surface of the cell is difficult. Free amino acids in the lumen represent only those proportions of released amino acids that have not been absorbed, and have diffused across the unstirred layer into the bulk phase. The simultaneous estimation of *in vivo* transport rates and *in vitro* brush border hydrolysis rates allows some conclusions to be drawn about the overall contribution of brush border peptidases in the mucosal hydrolysis of luminal dipeptides. In the case of Gly-Phe, rates of hydrolysis were less than half rates of luminal disappearance. Thus, it is probable that substantial quantities of this dipeptide are absorbed intact.

In the case of Phe-Gly, rates of brush border hydrolysis were greater than luminal disappearance rates. The conditions of the *in vitro* enzyme assays are not necessarily the same as the conditions of the *in vivo* perfusion experiments. Comparisons of *in vivo* disappearance rates and *in vitro* hydrolysis rates are thus subject to a number of limitations. Firstly, concentrations of substrate at the aqueous-lipid interface may be lower than in the bulk perfusion solution (Wilson and Dietschy, 1974). To circumvent this problem brush border peptidase activities were measured at one-fifth infusion concentration as well as at infusion concentration. Even at the lower substrate concentration jejunal Phe-Gly brush border peptidase activity was still greater than jejunal Phe-Gly disappearance rates during perfusion of the dipeptide saline mixture (Table 4). Secondly, although enzyme assays were performed at luminal pH, the pH in the bulk phase of the gut lumen and at the surface of the cell may not necessarily be the same. Indeed surface pH of rat everted sacs *in vitro*, measured with a pH microelectrode was less than the

pH of the incubation medium (Lucas *et al.*, 1975). However, in the present experiments, test solutions were perfused for 30 minutes before samples were collected for analysis. Surface pH values *in vitro* moved towards luminal pH values with time (Lucas *et al.*, 1975), but to date there is no evidence that such changes in surface pH occur *in vivo*. Finally, while the catalytic sites of brush border peptidases are located on the luminal surface of the microvillus membrane (Louvard *et al.*, 1975), luminal substrate is not necessarily exposed to the entire surface area of the villi *in vivo*. It is of interest, though, that brush border peptidase activity is concentrated at the tips of the villi, the area maximally exposed to luminal substrate (Y. S. Kim, unpublished data).

Bearing these limitations in mind, the fact remains that rates of jejunal hydrolysis of Phe-Gly by brush border peptidases were 1-3 to four times greater than rates of dipeptide disappearance. This suggests that, unlike Gly-Phe, the major site of mucosal hydrolysis of Phe-Gly is not necessarily within the cytoplasmic compartment of the cell. Recently, two rat intestinal brush border peptidases have purified to homogeneity (Kim and Brophy, 1976). Studies of the substrate specificity of the two enzymes indicated that, in addition to Phe-Gly, other dipeptides composed of neutral amino acid residues were also rapidly hydrolysed (Kim *et al.*, 1976). The possibility exists, therefore, that while certain peptides such as Gly-Phe are predominantly absorbed intact and hydrolysed by intracellular peptidases, brush border peptidases may play an important role in the mucosal hydrolysis of other dipeptides such as Phe-Gly.

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References

- Addison, J. M., Burston, D., Dalrymple, J. A., Matthews, D. M., Payne, J. W., Sleisenger, M. H., and Wilkinson, S. (1975a). A common mechanism for transport of di- and tri-peptides by hamster jejunum *in vitro*. *Clinical Science and Molecular Medicine*, **49**, 313-322.
- Addison, J. M., Burston, D., and Matthews, D. M. (1972). Evidence for active transport of the dipeptide glycylsarcosine by hamster jejunum *in vitro*. *Clinical Science*, **43**, 907-911.
- Addison, J. M., Burston, D., Payne, J. W., Wilkinson, S., and Matthews, D. M. (1975b). Evidence for active transport of tripeptides by hamster jejunum *in vitro*. *Clinical Science and Molecular Medicine*, **49**, 305-312.
- Adibi, S. A. (1971). Intestinal transport of dipeptides in man: relative importance of hydrolysis and intact absorption. *Journal of Clinical Investigation*, **50**, 2266-2275.
- Auricchio, S., Pierro, M., Andria, G., and DeRitis, G. (1972). Enzymic activities of the brush border membrane of rat intestine hydrolysing naphthylamides of amino acids,

- leucinamide and dipeptides. *Biochimica et Biophysica Acta*, **274**, 420-425.
- Borgström, B. A., Dahlqvist, A., Lundh, G., and Sjövall, J. (1975). Studies of intestinal digestion and absorption in the human. *Journal of Clinical Investigation*, **36**, 1521-1536.
- Boullin, D. J., Crampton, R. F., Heading, C. E., and Pelling, D. (1973). Intestinal absorption of dipeptides containing glycine, phenylalanine, proline, β -alanine or histidine in the rat. *Clinical Science and Molecular Medicine*, **45**, 849-858.
- Boyd, C. A. R., Cheeseman, C. I., and Parsons, D. S. (1975). Amino acid movements across the wall of anuran small intestine perfused through the vascular bed. *Journal of Physiology*, **250**, 409-429.
- Cheng, B., Navab, F., Lis, M. T., Miller, T. N., and Matthews, D. M. (1971). Mechanisms of dipeptide uptake by rat small intestine *in vitro*. *Clinical Science*, **40**, 247-257.
- Dietschy, J. M., Sallee, V. L., and Wilson, F. A. (1971). Unstirred water layers and absorption across the intestinal mucosa. *Gastroenterology*, **61**, 932-934.
- Donlon, J., and Fottrell, P. F. (1972). Studies on substrate specificities and subcellular location of multiple forms of peptide hydrolases in guinea pig intestinal mucosa. *Comparative Biochemistry and Physiology*, **41B**, 181-193.
- Fujita, M., Parsons, D. S., and Wojnarowska, F. (1972). Oligopeptidases of brush border membranes of rat small intestinal mucosal cells. *Journal of Physiology*, **227**, 377-394.
- Heizer, W. D., Kerley, R. L., and Isselbacher, K. J. (1972). Intestinal peptide hydrolases: differences between brush border and cytoplasmic enzymes. *Biochimica et Biophysica Acta*, **264**, 450-461.
- Josefsson, L., and Sjöström, H. (1966). Intestinal dipeptidases. 4. Studies on the release and subcellular distribution of intestinal dipeptidases of the mucosal cells of the pig. *Acta Physiologica Scandinavica*, **67**, 27-33.
- Kim, Y. S., Birtwhistle, W., and Kim, Y. W. (1972). Peptide hydrolases in the brush border and soluble fractions of small intestinal mucosa of rat and man. *Journal of Clinical Investigation*, **51**, 1419-1430.
- Kim, Y. S., and Brophy, E. J. (1976). Rat intestinal brush border membrane peptidases. 1. Solubilization, purification and physico-chemical properties of two different forms of the enzyme. *Journal of Biological Chemistry*, **251**, 3199-3205.
- Kim, Y. S., Brophy, E. J., and Nicholson, J. A. (1976). Rat intestinal brush border membrane peptidases. 2. Enzymatic properties, immunochemistry and interactions with lectins of two different forms of the enzyme. *Journal of Biological Chemistry*, **251**, 3206-3211.
- Kim, Y. S., Kim, Y. W., and Sleisenger, M. H. (1974). Studies on the properties of peptide hydrolases in the brush-border and soluble fractions of small intestinal mucosa of rat and man. *Biochimica et Biophysica Acta*, **370**, 283-296.
- Kim, Y. S., McCarthy, D. M., Lane, W., and Fong, W. (1973). Alterations in the levels of peptide hydrolases and other enzymes in the brush border and soluble fractions of rat small intestinal mucosa during starvation and refeeding. *Biochimica et Biophysica Acta*, **321**, 262-273.
- Lane, A. E., Silk, D. B. A., and Clark, M. L. (1975). Absorption of two proline containing peptides by rat small intestine *in vivo*. *Journal of Physiology*, **248**, 143-149.
- Louvard, D., Maroux, S., Vannier, C., and Desnuelle, P. (1975). Topological studies on the hydrolases bound to the intestinal brush border membrane. 1. Solubilization by papain and Triton X-100. *Biochimica et Biophysica Acta*, **375** (2), 236-248.
- Lucas, M. L., Blair, J. A., Cooper, B., and Matty, A. J. (1975). Further investigations with pH microelectrodes into the jejunal microclimate in rat and man. *Gut*, **16**, 844.
- Matthews, D. M. (1971). Protein absorption. *Journal of Clinical Pathology*, **24**, suppl. 5, 29-40.
- Matthews, D. M. (1975). Intestinal absorption of peptides. *Physiological Reviews*, **55**, 537-608.
- Matthews, D. M., Addison, J. M., and Burston, D. (1974). Evidence for active transport of the dipeptide carnosine (β -alanyl-L-histidine) by hamster jejunum *in vitro*. *Clinical Science and Molecular Medicine*, **46**, 693-705.
- Matthews, D. M., and Adibi, S. A. (1976). *Gastroenterology*, **71**, 151-161.
- Nicholson, J. A., and Kim, Y. S. (1975). A one-step L-amino acid oxidase assay for intestinal peptide hydrolase activity. *Analytical Biochemistry*, **63**, 110-117.
- Nixon, S. E., and Mawer, G. E. (1970). The digestion and absorption of protein in man. 1. The site of absorption. *British Journal of Nutrition*, **24**, 227-240.
- Peters, T. J. (1970). The subcellular localization of di- and tri-peptide hydrolase activity and guinea-pig small intestine. *Biochemical Journal*, **120**, 195-203.
- Peters, T. J., and MacMahon, M. T. (1970). The absorption of glycine and glycine oligopeptides by the rat. *Clinical Science*, **39**, 811-821.
- Rhodes, J. B., Eichholz, A., and Crane, R. K. (1967). Studies on the organisation of the brush border intestinal epithelial cells. 4. Aminopeptidase activity in microvillus membranes of hamster intestinal brush borders. *Biochimica et Biophysica Acta*, **135**, 959-965.
- Robinson, G. B. (1963). The distribution of peptidases in subcellular fractions from the mucosa of the small intestine of the rat. *Biochemical Journal*, **88**, 162-168.
- Sigrist-Nelson, K. (1975). Dipeptide transport in isolated intestinal brush border membrane. *Biochimica et Biophysica Acta*, **394**, 220-226.
- Silk, D. B. A. (1974). Peptide absorption in man. *Gut*, **15**, 494-501.
- Silk, D. B. A., and Kim, Y. S. (1975). A study of intraluminal peptide hydrolase activity in the rat. *Clinical Science and Molecular Medicine*, **49**, 523-526.
- Silk, D. B. A., and Kim, Y. S. (1976). Release of peptide hydrolases during incubation of intact intestinal segments *in vitro*. *Journal of Physiology*, **258**, 489-497.
- Silk, D. B. A., Perrett, D., and Clark, M. L. (1973). Intestinal transport of two dipeptides containing the same two neutral amino acids in man. *Clinical Science and Molecular Medicine*, **45**, 291-299.
- Silk, D. B. A., Perrett, D., Webb, J. P. W., and Clark, M. L. (1974a). Absorption of two tripeptides by the human small intestine: a study using a perfusion technique. *Clinical Science and Molecular Medicine*, **46**, 393-402.
- Silk, D. B. A., Webb, J. P. W., Lane, A. E., Clark, M. L., and Dawson, A. M. (1974b). Functional differentiation of human jejunum and ileum: a comparison of the handling of glucose, peptides, and amino acids. *Gut*, **15**, 444-449.
- Wilson, F. A., and Dietschy, J. M. (1974). The intestinal unstirred layer: its surface area and effect on active transport kinetics. *Biochimica et Biophysica Acta*, **363**, 112-126.
- Wojnarowska, F., and Gray, G. M. (1975). Intestinal surface peptide hydrolases. Identification and characterization of three enzymes from rat brush border. *Biochimica et Biophysica Acta*, **403**, 147-160.