The Sites of Hydrolysis of Dipeptides containing Leucine and Glycine by Rat Jejunum *in vitro*

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1. The effect of peptides containing leucine and glycine on accumulation of leucine and glycine by everted jejunal rings was studied. 2. It was shown that, on a molar basis, leucyl-leucine is a more effective inhibitor of uptake of [^{14}C]leucine than is either leucylglycine or glycyl-leucine. These latter dipeptides behave alike. 3. The concentration of the dipeptides and their constituent amino acids in both the incubation medium and the tissue has been followed in these experiments by amino acid analysis. No leucine-containing peptides were observed in the tissue. 4. The inhibitory effects of the mixed dipeptides are altered by pH changes in an analogous way to the alterations in peptidase activity. 5. The experimental results indicate that leucine-containing peptides are hydrolysed before the transport step. 6. Glycylglycine, on the other hand, has only a small effect on the accumulation of glycine, although large amounts of the peptide accumulate unchanged in the tissue. This suggests that glycylglycine is taken up by a different mechanism to that for the leucine dipeptides.

It is generally accepted that during digestion proteins are broken down to their constituent amino acids before being absorbed into the portal bloodstream (Denton, Gershoff & Elvehjem, 1953; Hartmann, Lenz, Lopez-Calleja & Münzenberg, 1956; Levenson, Rosen & Upjohn, 1959; Dawson & Holdsworth, 1962). The terminal stage of this process, hydrolysis of di- and tri-peptides, is, however, not completely understood.

There are suggestions that hydrolysis of dipeptides may take place outside the cells (Kushak & Ugolev, 1966), within the intestinal brush border (Rhodes, Eichholz & Crane, 1967) or they may enter the cells unchanged and there be hydrolysed (Newey & Smyth, 1960, 1962). It has also been postulated (Matthews, Craft, Geddes, Wise & Hyde, 1968*b*) that some amino acids and peptides share a common membrane transport step.

If an assumption is made that active transport of nutrients occurs at the brush border (McDougal, Little & Crane, 1960; Kinter, 1961) there are two possible mechanisms for the entry of peptides (XY) into the mucosal cell; either the peptide enters the tissue and is then hydrolysed or hydrolysis precedes the transport of the constituent amino acids (X and Y).

We have studied the problem by determining the effect of non-radioactive peptide (XY) on the accumulation of one of its constituent amino acids X, present as a radioactive tracer X^* . The study

has involved the measurement of the rate of intracellular accumulation of X^* together with the changes in the extracellular and intracellular concentrations of X, Y and XY. If hydrolysis precedes transport, the amino acids X and Y would be liberated into the medium. X would lower the specific radioactivity of X^* and Y might compete with X^* for the transport carrier; this would be seen as a decrease in the intracellular accumulation of X^* . The increase in concentration of X and Y in the medium would be expected to precede any intracellular increase in concentration of X and Y. The other conditions in which the entry of X^* might be decreased are if XY competes with X^* for the carrier site or if X, generated by the intracellular hydrolysis of XY, is effluxed from the tissue so as to decrease the specific radioactivity of X^* in the medium. In these situations, unless the rates of efflux of X and Y are more rapid than the rate of entry of XY, the increase in the concentrations of X and Y in the medium would be expected to follow an increase in concentration of X and Y in the tissue.

METHODS

White rats (95–110 g.) derived from the Sprague-Dawley strain were fed on Spillers laboratory small animal diet (autoclaved), and were killed by decapitation. The small intestine was removed, washed in Krebs-Ringer bicarbonate buffer, everted over a glass rod and cut into segments

2–3 mm. long, beginning 12 cm. from the pylorus. Care was taken to exclude Peyer's patches. Segments, one from each of three rats, were placed in a conical flask containing 2 ml. of Krebs–Ringer bicarbonate buffer, pH 7.4 (Umbreit, Burris & Stauffer, 1946) (which contained no glucose) and the appropriate dipeptide and amino acids. The solution was gassed with $O_2 + CO_2$ (95:5). When other H^+ concentrations were used, Krebs–Ringer phosphate buffer (Umbreit *et al.* 1946), was employed, and the solution gassed with 100% O_2 . Most incubations were carried out for 10 min. After incubation with shaking at 37°, the tissue, which weighed 60–80 mg., was processed as described by Manchester & Young (1960). Distribution ratios between the intracellular fluid compartment and the incubation medium were calculated according to the method of Kipnis & Cori (1957). The inulin space (extracellular fluid) was 13% of the tissue wet weight. Total water comprised 80% of the tissue weight.

[$U-^{14}C$]Glycine (5.0 mc/m-mole) and [$U-^{14}C$]leucine (10 mc/m-mole) (The Radiochemical Centre, Amersham, Bucks.) were used as tracers at a concentration of 25 nc/ml. Unless otherwise stated, L-leucine and glycine (Sigma (London) Chemical Co., London S.W.6) were present at 0.25 mM. These substances, and also L-leucyl-L-leucine (Schwartz BioResearch Inc., Orangeburg, N.Y., U.S.A.), glycyl-L-leucine, L-leucylglycine and glycylglycine (Sigma), yielded single spots when subjected to electrophoresis at 1000 v for 10 hr., with acetic acid–pyridine–water (10:1:200, by vol.; pH 3.6) as an electrolyte, and also on paper chromatography in butan-1-ol–acetic acid–water (12:5:3, by vol.). After incubation of tissue with leucine, at least 95% of the radioactivity in the tissue and medium was recovered as unchanged amino acid, as shown by chromatography in butan-1-ol–acetic acid–water. With glycine the corresponding figure was 90%.

Efflux experiments were performed by preloading the tissue for 10 min. with radioactive amino acid (1 mM). The preparation was then removed from the medium, washed twice in saline, blotted and transferred to 3 ml. of medium containing non-radioactive amino acid (0.25 mM). The incubation was continued at 37°, during which time 0.2 ml. samples of medium were taken at 4 min. intervals. At the end of the incubation the tissue was processed to measure the remaining radioactivity (see above).

Radioactivity in the various tissue extracts and in the incubation media was determined by pipetting 0.2 ml. of sample into a mixture containing 2.8 ml. of ethanol and 7 ml. of scintillator [0.01% 1,4-bis-(5-phenyloxazol-2-yl)benzene and 0.457% 2,5-diphenyloxazole in toluene]. Each sample was counted at least four times for a period of 5 min. The samples were counted at 42% efficiency, in a Packard Tri-Carb liquid-scintillation counter.

Each point shown graphically represents the mean of three experiments. The coefficient of variation for each point was less than 16%.

Preparation of samples for amino acid analysis. After incubation, the tissue in each flask was homogenized in a Potter–Elvehjem ground glass homogenizer with picric acid (12.5 g./l.). The picric acid homogenates from each of three flasks that had been incubated under identical conditions were combined and centrifuged. The resulting supernatant was stored at –20° before being processed. Extracts were thawed and picric acid was removed from the combined extract by passage through a Dowex 2 column (Stein & Moore, 1954). The eluates were concentrated under

Table 1. *Elution times of amino acids and dipeptides on automatic analysis*

Amino acid	Elution time (ratio to elution time of norleucine)
Glycine	0.57
Alanine	0.61
Methionine	0.82
Glycylglycine	0.86
Isoleucine	0.93
Leucine	0.96
Norleucine	1.00
(internal standard)	
Tyrosine	1.06
Leucylglycine	1.08
Phenylalanine	1.09
Glycyl-leucine	1.19
Ammonia	1.21
Leucyl-leucine	1.36
Lysine	1.48

reduced pressure at 40° in a rotary evaporator and adjusted to pH 2.0. The samples were analysed for amino acid content with a Technicon amino acid AutoAnalyzer with a simplified buffer system (Kacser, 1966) and a flow rate of 0.6 ml./min. The incubation media were similarly processed. The elution times of the four dipeptides used in this study are given in Table 1. No detectable hydrolysis of dipeptides occurred in the presence of cold picric acid.

The succus entericus was obtained from male Sprague–Dawley-derived rats weighing 400 ± 50 g. The animals were starved for 12 hr., then fed with Spillers laboratory small animal diet (autoclaved); after a further 4 hr. they were decapitated. A 20 cm. section of the jejunum 20 cm. from the pylorus was squeezed manually and the exuded fluid centrifuged. A known volume of the supernatant was added to norleucine and picric acid as before and stored at –20°. Three regions in the resulting chromatograms were examined, namely those between (i) methionine and isoleucine, which corresponds to the elution time of glycylglycine, (ii) phenylalanine and ammonia, which corresponds to the elution times of glycyl-leucine and leucylglycine and (iii) ammonia and ornithine, which corresponds to the elution time of leucyl-leucine. Ten peaks that were not attributable to known amino acids were detected in these regions and were observed in all rats. None of these compounds was present at a concentration of more than $40 \mu M$.

RESULTS

Fig. 1 shows the effect of leucyl-leucine on accumulation of [^{14}C]leucine by jejunal rings; it can be seen that increasing concentrations of leucyl-leucine produce increasing inhibition of uptake of [^{14}C]leucine. Fig. 2 demonstrates the effects of glycine, a mixture of glycine and leucine, glycyl-leucine and leucylglycine on accumulation of [^{14}C]leucine. All the inhibitors were present at

Fig. 1. Inhibition of uptake of [¹⁴C]leucine with various concentrations of leucine in the medium. The jejunal segments were incubated for 10 min. at 37° in Krebs-Ringer bicarbonate buffer (see the Methods section). The abscissa shows the initial concentration (mm) of [¹⁴C]leucine in the medium. The ordinate shows the ratio of the distribution of ¹⁴C radioactivity between tissue and medium. ○, 2 mM-leucyl-leucine + 0.25 mM-leucine; △, 0.5 mM-leucyl-leucine + 0.25 mM-leucine; ●, 0.25 mM-leucine.

Fig. 2. Inhibition of uptake of [¹⁴C]leucine by leucylglycine (1 mM), glycyl-leucine (1 mM), glycine (1 mM) and a mixture of glycine (1 mM) + leucine (1 mM). The jejunal segments were incubated for 10 min. at 37° in Krebs-Ringer bicarbonate buffer. The abscissa shows the initial concentration (mm) of [¹⁴C]leucine in the medium. The ordinate shows the ratio of distribution of ¹⁴C radioactivity between tissue and medium. □, Leucine + glycine; ▲, leucylglycine; ○, glycyl-leucine; △, glycine; ●, control.

1 mM. Only a marginally greater inhibition was produced with the mixture of amino acids than with glycyl-leucine or leucylglycine; glycine by itself had a negligible effect. The inhibition by leucine + glycine, glycyl-leucine and leucylglycine at 1 mM was similar to that caused by 0.5 mM-leucyl-leucine (cf. Fig. 1). Fig. 3 illustrates the results obtained by performing incubations with [¹⁴C]-leucine in the presence of various concentrations of the compounds used in the previous two experiments. It is apparent that, on a molar basis,

Fig. 3. Change in percentage inhibition of uptake of [¹⁴C]leucine (0.25 mM) produced by various concentrations of inhibitor in the medium. The jejunal segments were incubated for 10 min. at 37° in Krebs-Ringer bicarbonate buffer, plus the following additives: □, leucine + glycine (each at the indicated concentration); ●, leucyl-leucine; ▲, leucylglycine; ■, glycyl-leucine; △, preload for 10 min. with leucyl-leucine at the concentrations indicated on the abscissa, followed by incubation for 10 min. in [¹⁴C]leucine (0.25 mM).

Table 2. *Hydrolysis of leucyl-leucine in the presence of leucine (0.25 mM)*

Hydrolysis is indicated by the decrease in concentration of the substrate and by the increase in concentration of the product in the medium and tissue. Conditions of incubation are as for Fig. 1.

Initial concn. of leucyl-leucine in the medium (mm)	Time of incubation (min.)	Concn. in medium at end of incubation (mm)		Concn. of leucine in tissue at end of incubation (mm)
		Leucyl-leucine	Leucine	
1.9	0	1.9	0.25	2.2
	3	0.5	2.1	6.4
	7	0.3	2.6	8.8
	10	0.1	2.9	10.1
0.5	0	0.5	0.25	2.3
	3	0.1	0.7	2.5
	7	0.0	0.8	4.7
	10	0.0	0.7	6.2

leucyl-leucine has a greater inhibitory effect on uptake of [¹⁴C]leucine than either of the mixed dipeptides and has an effect similar to that of twice the molar concentration of leucine (presented as a leucine + glycine mixture). Maximal inhibition was produced at a concentration of approximately 3 mM of all the peptides tested.

Table 3. *Hydrolysis of leucylglycine (0.85 mm) and glycyl-leucine (1.05 mm) in the presence of leucine (0.25 mm)*

Hydrolysis is indicated by the decrease in concentration of the peptide and by the increase in concentration of the hydrolytic products, glycine and leucine, in the medium and tissue. Conditions of incubation are as for Fig. 2.

Peptide	Time of incubation (min.)	Concn. in medium at end of incubation (mm)			Concn. in tissue at end of incubation (mm)	
		Peptide	Leucine	Glycine	Leucine	Glycine
Glycyl-leucine	0	1.05	0.25	0.0	2.2	4.3
	3	0.7	0.5	0.4	2.3	4.3
	7	0.4	0.8	0.7	4.0	4.4
	10	0.3	1.0	1.2	5.2	4.6
Leucylglycine	0	0.85	0.25	0.0	2.3	4.4
	3	0.6	0.5	0.3	2.4	4.2
	7	0.4	0.6	0.7	4.5	4.5
	10	0.3	0.7	0.8	5.5	5.0

The variation of concentrations of leucine and leucyl-leucine in both the medium and the tissue (Table 2) was followed by amino acid analyses, under conditions similar to those used in the experiment described in Fig. 1 but with a single leucine concentration of 0.25 mm. No leucyl-leucine was detected in the tissue. The hydrolysis of this peptide was extremely rapid at both concentrations; when leucyl-leucine was initially present at 0.5 mm none was detected in the medium after 3 min. (Table 2). The hydrolytic product, leucine, accumulated in both the medium and the tissue at a rate that reflected the hydrolysis of the peptide. The tissue maintained a leucine concentration of 2.2 mm throughout a 10 min. incubation in the presence of leucine alone (0.25 mm). In an analogous manner the contents of glycyl-leucine, leucylglycine and their hydrolytic products in the medium and the tissue were measured in experiments similar to those reported in Fig. 2 with a single leucine concentration of 0.25 mm (Table 3). No glycyl-leucine or leucylglycine was detected in the tissue at any time throughout the experiments. The hydrolysis of both peptides occurred at approximately the same rate. The disappearance of the peptides from the medium was reflected in the increased concentrations of glycine and leucine in the medium. The intracellular leucine concentration increased throughout the incubations but the intracellular concentration of glycine remained at 4.5 ± 0.5 mm, irrespective of incubation conditions (Table 3). Under the conditions of these studies, the uptake of radioactive leucine (1 mm) from the medium is far more rapid than that of glycine (1 mm); the distribution ratio of [^{14}C]leucine was 4.5 after 10 min., whereas for [^{14}C]glycine the value was unity.

Table 4 illustrates the effect of concentration on the hydrolysis of the two mixed dipeptides, under

Table 4. *Hydrolysis of leucylglycine and glycyl-leucine at various concentrations*

Hydrolysis is indicated by the decrease in concentration of the peptide in the medium and by the simultaneous increase in concentration of the hydrolytic products, glycine and leucine. Conditions of incubation are as for Fig. 2.

Peptide	Initial concn. of peptide in medium (mm)	Concn. in medium after 10 min. incubation (mm)		
		Peptide	Leucine	Glycine
Leucylglycine	4.0	3.3	0.8	0.8
	2.0	1.3	0.7	0.6
	1.0	0.4	0.6	0.5
Glycyl-leucine	4.0	2.4	1.3	1.3
	2.0	1.6	0.6	0.5
	1.0	0.5	0.5	0.4

conditions similar to those described for Fig. 3. Clearly, as the peptide concentration is increased, proportionally less is hydrolysed over the concentration range studied.

The effect of preincubating intestinal segments with leucyl-leucine for 10 min. on the uptake of [^{14}C]leucine (0.25 mm) during a subsequent incubation for 10 min. is also shown in Fig. 3. The inhibition of uptake of leucine observed under these circumstances is much less than that obtained when leucyl-leucine is concomitantly present in the medium. When the tissue was preloaded with [^{14}C]leucine or [^{14}C]glycine (1 mm) and the incubation was continued in a medium containing leucine or glycine respectively (0.25 mm) it can be seen (Fig. 4) that [^{14}C]glycine effluxes from the tissue at a similar rate to that of [^{14}C]leucine.

Decreasing the pH of the incubation medium was

Fig. 4. Efflux of [^{14}C]leucine and [^{14}C]glycine, into a Krebs-Ringer bicarbonate solution containing the respective amino acids (0.25 mM), from tissue preloaded with either [^{14}C]leucine or [^{14}C]glycine (1 mM). ■, Leucine; ●, glycine.

Fig. 5. Changes in inhibition of uptake of [^{14}C]leucine (0.25 mM) by glycyl-leucine and by leucylglycine, with change in pH. The effect of pH variation on the accumulation of leucine (i.e. the ratio of distribution of ^{14}C between tissue and medium over the same period in the absence of inhibitors) is illustrated in the lower portion of the graph. The jejunal segments were incubated for 10 min. at 37° in Krebs-Ringer phosphate buffer. ▲, Glycyl-leucine (4 mM); ■, leucylglycine (4 mM); ●, leucine (0.25 mM).

associated with a halving of the inhibitory effect of glycyl-leucine on the uptake of leucine (Fig. 5). Neither the inhibitory effect of leucylglycine nor the accumulation of leucine in the absence of inhibitors was significantly changed when the pH was altered.

The effect of glycylglycine on the accumulation of glycine is illustrated in Fig. 6. The inhibition resulting from the presence of this dipeptide was

Fig. 6. Inhibition of uptake of [^{14}C]glycine with various concentrations of glycine in the medium. The jejunal segments were incubated for 10 min. at 37° in Krebs-Ringer bicarbonate buffer. The abscissa shows the initial concentration (mM) of [^{14}C]glycine in the medium. The ordinate shows the ratio of the distribution of ^{14}C radioactivity between tissue and medium. ○, Glycylglycine (10 mM) + glycine; □, glycyl-leucine (10 mM) + glycine; ●, glycine only.

Table 5. *Hydrolysis of glycylglycine (2 mM) in the presence of glycine (0.25 mM)*

Hydrolysis is indicated by the decrease in concentration of the peptide in the incubation medium and tissue. The increase in the glycine concentration in the medium is also illustrated. Conditions of incubation are as for Fig. 1.

Incubation time (min.)	Concn. in medium at end of incubation (mM)		Concn. in tissue at end of incubation (mM)	
	Glycyl-glycine	Glycine	Glycyl-glycine	Glycine
0	2.0	0.25	0.0	3.1
3	1.9	0.4	0.5	5.8
7	1.7	0.5	0.5	6.9
10	1.6	0.5	0.4	7.5

slight when compared with that obtained in the presence of glycyl-leucine. The concentrations of glycine and glycylglycine in the medium and tissue were followed under similar conditions to those used to study the effect of leucyl-leucine on uptake of leucine (Fig. 1); glycine was initially present at 0.25 mM and glycylglycine at 2.0 mM. The disappearance of glycylglycine from the incubation medium progressed at a much slower rate (Table 5) than that for leucyl-leucine (cf. Table 2). The glycine concentration in the medium was slightly higher in the presence of glycylglycine than in its absence. In contrast to the incubation mixtures involving mixed glycine peptides (Fig. 3) there was a large increase in the intracellular glycine concentration (Table 5) and appreciable quantities of

the unchanged peptide were detected in the tissue. The tissue maintained a glycine concentration of 3.0 mM throughout a 10 min. incubation in the presence of glycine alone (0.25 mM).

DISCUSSION

The way in which leucine-containing peptides cause inhibition of accumulation of labelled amino acids, as shown in Figs. 1, 2 and 3, requires consideration, as it is important to the argument presented in favour of hydrolysis before transport. The experiments show that leucyl-leucine is a more potent inhibitor of uptake of radioactive leucine than is either of the mixed dipeptides (Fig. 3); this may be due to the fact that leucyl-leucine yields two leucine molecules on hydrolysis, whereas the mixed dipeptides yield only one; the other moiety of the mixed dipeptides, glycine, is a relatively ineffective inhibitor of uptake of labelled leucine (Fig. 2). At concentrations less than 1 mM the glycine + leucine mixture has an inhibitory effect similar to that of the mixed dipeptides (Figs. 2 and 3), but as the inhibitor concentration is increased, the amino acid mixture becomes the more potent inhibitor (Fig. 3). An explanation for this observation could be that the hydrolytic enzyme systems for the dipeptides become saturated, so the amount of free leucine and glycine liberated does not increase markedly at the higher peptide concentrations.

During these experiments the concentration of leucine-containing peptides in the incubation medium rapidly decreased with time, whereas the concentration of the hydrolytic products increased (Tables 2 and 3). The equal inhibitory activity of glycyl-leucine and leucylglycine (Figs. 2 and 3) is consistent with the observation that they are hydrolysed at similar rates (Table 3). It is also significant that with increasing peptide concentrations there is proportionally less inhibition of uptake of [^{14}C]leucine (Fig. 3), which is paralleled by proportionally less hydrolysis of the peptide (Table 2). In all incubation mixtures involving these leucine dipeptides no dipeptide was detected in the tissue.

Two explanations are possible for these findings: (a) hydrolysis precedes transport and therefore no peptide is found intracellularly; (b) transport precedes hydrolysis, but the former is the rate-limiting step, therefore no peptide will be detected intracellularly. In the latter case the increase in concentrations of amino acids in the medium would arise as a result of efflux from the intracellular pool.

The possibility of inhibition by efflux or 'back diffusion' of the hydrolytic products (the second possibility was rendered unlikely by the observation that there is a change in the ratio of the intracellular concentrations of leucine and glycine when in-

cubated in the presence of the mixed dipeptides; Table 3). Only leucine showed an appreciable rise in concentration. This would be expected if the hydrolysis of peptides occurred extracellularly and the hydrolytic products were then accumulated into the tissue, because the relative rates of accumulation of leucine and glycine are quite different; that of leucine is five times that of glycine over short time periods (see above). Had the peptides undergone intracellular hydrolysis, an equal amount of leucine and glycine would have been generated in the tissue, and as the rates of efflux and metabolism of the two amino acids are similar (Fig. 4, and the Methods section), intracellular equimolarity would have been maintained throughout the experiment. This interpretation of the results was further supported by preloading experiments. When uptake of leucine was studied with tissue that had been preincubated in the presence of leucyl-leucine there was minimum inhibition compared with that observed when the dipeptide was present in the leucine-containing incubation medium (Fig. 3). Thus, under conditions deliberately designed to test the effect of a preloaded intracellular pool on the subsequent uptake of [^{14}C]leucine, no support could be obtained for the hypothesis that effluxing leucine might significantly decrease the uptake of the radioactive marker.

The rate of hydrolysis of leucylglycine is only minimally susceptible to pH change over the range studied in these experiments (Robinson & Shaw, 1960) as is the effect of this dipeptide on inhibition of uptake of leucine (Fig. 5). However, glycyl-leucine, the hydrolysis of which increases rapidly with increasing pH (Robinson & Shaw, 1960), shows a commensurate increase in its inhibitory properties. These results again suggest that the products of hydrolysis, rather than the peptides themselves, are responsible for the observed decrease in accumulation of the radioactive leucine.

Thus all the experiments reported in this paper indicate that decrease in the uptake of [^{14}C]leucine occurs in a way that can be predicted from the known behaviour of leucine peptide hydrolases, and that these hydrolases act extracellularly. This conclusion is an agreement with that of Kushak & Ugolev (1966), who showed that in the presence of sodium fluoride, which inhibits active transport, the products of hydrolysis of glycyl-leucine entered the intestinal mucosa in a manner that was consistent with a model based on extracellular, rather than intracellular, breakdown.

In contrast with these results Newey & Smyth (1960) suggested that dipeptides are able to enter the mucosal cells without previous hydrolysis, and support for this concept has come from Matthews, Craft & Crampton (1968a), Matthews *et al.* (1968b) and Craft, Crampton, Lis & Matthews (1968), who

have suggested that intact dipeptides share a rate-limiting step with amino acids for entry into the intestinal mucosal cell. That some dipeptides are able to enter the mucosal cell is consistent with the findings of Agar, Hird & Sidhu (1953) and Wiggins & Johnston (1958), who demonstrated that glycylglycine could pass unchanged across the intestinal wall. In view of the contradiction between the findings with leucine-containing dipeptides (Kushak & Ugolev, 1966 and the present work) and those with glycylglycine, it was decided to study glycylglycine in the same way as the leucyl peptides. From the results shown in Fig. 6 and Table 5 it became obvious that there was a completely different mode of accumulation of the hydrolytic product, in this case glycine. The rate of hydrolysis of the peptide was much slower than that of the leucine-containing peptides, but more significantly, glycylglycine was observed in the tissue at relatively high concentrations (Table 5). Further, the appearance of glycine in the jejunal tissue was greater in the presence of glycylglycine than in the control despite only a small change in the concentration in the medium (Table 5). This is to be contrasted with the glycine concentrations in the incubation mixtures with the mixed peptides (Table 3), where despite a very large increase in the concentration in the medium there was only a small increase in the concentration of glycine in the tissue. This implies that, in tissue incubated in the presence of glycylglycine, a large proportion of the intracellular glycine originated from intracellular hydrolysis of glycylglycine, in accordance with the scheme proposed by Matthews *et al.* (1968a) and Newey & Smyth (1960, 1962).

An important consideration in the assessment of peptide hydrolase activity is the concentration of dipeptides in the gastrointestinal tract. The succus entericus of rats both in the starving and non-starving states was studied by amino acid chromatography, and it was shown by these studies that the concentration of individual dipeptides in the lumen of the jejunum never exceeded $40\mu\text{M}$. Consequently, studies in which a very much higher concentration of dipeptides is used may well give rise to unphysiological results. At high concentrations, the Lineweaver-Burk plot for the accumulation of glycine by jejunal tissue deviates towards the origin (E. B. Fern, R. C. Hider & D. R. London, unpublished work). This finding is similar to that made by Smith (1967) in brain tissue and interpreted by him as indicating a diffusion process. It may be significant that the work reported by Matthews *et al.* (1968a) and Craft *et al.* (1968) used amino acid and peptide concentrations of about 100mM.

The experiments in this paper indicate that, for dipeptides containing the hydrophobic amino acid

leucine, hydrolysis precedes transport, whereas glycylglycine diffuses into the cell before hydrolysis. That glycylglycine enters the jejunal mucosal cell may simply reflect lack of hydrolysing ability of the brush border for this peptide. The findings with this peptide should be considered in the light that glycylglycine is not representative of naturally occurring dipeptides (Newey & Smyth, 1960; Smyth, 1964); and is hydrolysed by an enzyme of low activity (Wiggins & Johnston, 1958; Rhodes *et al.* 1967). Thus the intracellular breakdown of this substance may not be relevant to the absorption of protein from the lumen of the small intestine.

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