

## Functional Characterization of Dipeptide Transport System in Human Jejunum

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### Research Article

The present studies were performed to determine whether dipeptide absorption in human jejunum exhibits the characteristics of carrier-mediated transport. 15-cm jejunal segments from human volunteers were perfused with test solutions containing varying amounts of either glycylglycine, glycyllucine, glycine, leucine, glycylglycine with leucine or glycine, glycylglycine with glycyllucine, or glycyllucine with an equimolar mixture of free glycine and leucine. Jejunal absorption rates of both glycylglycine and glycyllucine followed the kinetics of a saturable process. The  $K_m$  value in millimoles/liter of glycylglycine was significantly greater than the  $K_m$  value of glycyllucine ( $43.3 \pm 2.6$  vs.  $26.8 \pm 5.9$ ,  $P < 0.05$ ); and the  $K_m$  value of glycine was also significantly greater than the  $K_m$  value of leucine ( $42.7 \pm 7.5$  vs.  $20.4 \pm 5.4$ ,  $P < 0.05$ ). While overlapping occurred among the  $K_m$  values of free amino acids and dipeptides, the transport kinetics of dipeptides were characterized by higher  $V_{max}$  values (in micromoles per minute per 15 centimeters) than those of free amino acids. For example, the  $V_{max}$  values for glycylglycine and glycine were  $837 \pm 62$  and  $590 \pm 56$ , respectively ( $P < 0.02$ ). While jejunal absorption rates of glycylglycine were not significantly affected by free leucine or free glycine, they were competitively inhibited by glycyllucine. The jejunal absorption rate of glycyllucine was not significantly altered by an equimolar mixture of free glycine and leucine. The selective absorption of dipeptides was investigated by [...]

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# Functional Characterization of Dipeptide Transport System in Human Jejunum

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**ABSTRACT** The present studies were performed to determine whether dipeptide absorption in human jejunum exhibits the characteristics of carrier-mediated transport. 15-cm jejunal segments from human volunteers were perfused with test solutions containing varying amounts of either glycylglycine, glycyll-leucine, glycine, leucine, glycylglycine with leucine or glycine, glycyll-leucine with glycyll-leucine, or glycyll-leucine with an equimolar mixture of free glycine and leucine. Jejunal absorption rates of both glycylglycine and glycyll-leucine followed the kinetics of a saturable process. The  $K_m$  value in millimoles/liter of glycylglycine was significantly greater than the  $K_m$  value of glycyll-leucine ( $43.3 \pm 2.6$  vs.  $26.8 \pm 5.9$ ,  $P < 0.05$ ); and the  $K_m$  value of glycine was also significantly greater than the  $K_m$  value of leucine ( $42.7 \pm 7.5$  vs.  $20.4 \pm 5.4$ ,  $P < 0.05$ ). While overlapping occurred among the  $K_m$  values of free amino acids and dipeptides, the transport kinetics of dipeptides were characterized by higher  $V_{max}$  values (in micromoles per minute per 15 centimeters) than those of free amino acids. For example, the  $V_{max}$  values for glycylglycine and glycine were  $837 \pm 62$  and  $590 \pm 56$ , respectively ( $P < 0.02$ ). While jejunal absorption rates of glycylglycine were not significantly affected by free leucine or free glycine, they were competitively inhibited by glycyll-leucine. The jejunal absorption rate of glycyll-leucine was not significantly altered by an equimolar mixture of free glycine and leucine. The selective absorption of dipeptides was investigated by infusing three equimolar mixtures, each containing two different dipeptides. Among the three dipeptides examined, glycylglycine was

the least absorbed. There was no significant difference between the absorption of glycyll-leucine and leucylglycine.

The above studies suggest that absorption of both glycylglycine and glycyll-leucine is mediated by a carrier which is not shared with free neutral amino acids; and that both COOH- and NH<sub>2</sub>-terminal amino acids appear to be influential in imposing the affinity of a dipeptide for the absorption sites.

## INTRODUCTION

Investigations, either by intestinal perfusion studies or by oral tolerance tests, have indicated intact absorption for a wide range of dipeptides in man (1-11). These include: glycylglycine, glycyll-leucine, glycyll-lysine, glycyll-alanine, L-alanyl-glycine, carnosine ( $\beta$ -alanyl-L-histidine), L-phenylalanyl-L-phenylalanine, glycyll-tryptophan, glycyll-tyrosine, and L-arginyl-L-aspartate.

In addition to intact absorption, our previous studies, utilizing glycylglycine and glycyll-leucine as model dipeptides, have suggested that intact absorption is either the exclusive or the major mode of dipeptide disappearance in human intestine (1, 2). The evidence that suggests this includes: (a) either no or minimal peptide hydrolase activity against glycylglycine or glycyll-leucine in the intraluminal fluids; (b) almost unimpaired absorption of amino acid constituents of these dipeptides when the carrier system for free neutral amino acids has been saturated with additional free amino acids; and (c) severe reduction in intraluminal pH, which abolishes the *in vitro* hydrolysis of dipeptides by mucosal enzymes, affecting the luminal disappearance of these dipeptides less than that of free amino acids.

These studies lead to further questions about the mechanism of dipeptide absorption; for example, whether dipeptides are absorbed by a carrier-mediated transport system or by the process of simple diffusion. The evi-

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dence required to establish carrier-mediated transport includes the demonstration of saturation, selective absorption, and competitive inhibition phenomena. The present studies were performed to determine whether the transport of dipeptides in human jejunum exhibits these phenomena.

## METHODS

18 healthy male volunteers were intubated with a double-lumen tube placed in the upper jejunum. The subjects were from 21 to 29 years old. The methods of intubation as well as of positioning the tube in the jejunum were as before (1, 12, 13). In order to minimize the high concentrations of dipeptides in test solutions necessary to achieve near maximal absorption rates in the jejunal segment, a smaller distance between the port of infusion and the port of aspiration was employed. This length was modified from 30 cm in previous studies to 15 cm in this one.

The protocol included perfusion studies of seven separate sets of test solutions. The amino acid or peptide composition of each set of test solutions is detailed in Table I. The volunteers were divided into four groups: the first was studied with solution sets I-V, the second with sets VI-VII, the third with set VIII, and the fourth with set IX. All the test solutions contained 0.4% polyethylene glycol as a nonabsorbable marker and between 110 and 140 mM sodium chloride. The sodium chloride concentration in the test solution was manipulated to minimize net water movements. The pH of test solutions varied from 6.80 to 7.20. Previous studies from this laboratory have shown that the above variations in sodium concentration and in pH of test solutions do not significantly alter the jejunal absorption rates of an amino acid such as leucine (14, 15). Further-

TABLE I  
Protocol for perfusion studies

Sets of solutions	Composition of test solutions
I	20, 50, 75, and 100 mM glycylglycine
II	10, 25, 50, and 75 mM glycine
III	20 mM glycylglycine + 100 mM L-leucine; 50 mM glycylglycine + 100 mM L-leucine; 75 mM glycylglycine + 100 mM L-leucine
IV	10 mM glycylglycine + 50 mM glycyl-L-leucine; 20 mM glycylglycine + 50 mM glycyl-L-leucine; 30 mM glycylglycine + 50 mM glycyl-L-leucine
V	20 mM glycylglycine + 20 mM glycyl-L-leucine; 20 mM glycylglycine + 20 mM L-leucylglycine; 20 mM glycyl-L-leucine + 20 mM L-leucylglycine
VI	20, 30, 40, and 60 mM glycyl-L-leucine
VII	10, 20, 40, and 60 mM L-leucine
VIII	20 mM glycylglycine; 20 mM glycylglycine + 100 mM glycine
IX	20 mM glycylleucine; 20 mM glycylleucine + 50 mM glycine + 50 mM L-leucine

All the above free amino acids and dipeptides except for L-leucylglycine were obtained from the General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio. L-leucylglycine was obtained from Sigma Chemical Co., St. Louis, Mo.

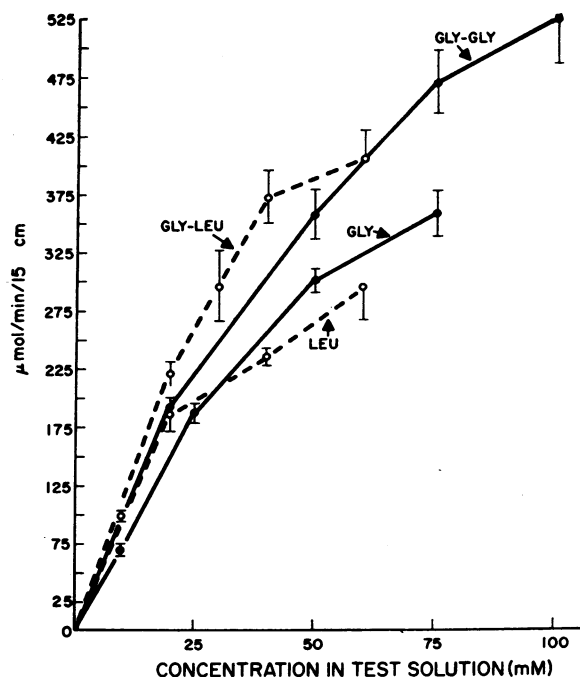


FIGURE 1 Jejunal absorption rates (mean  $\pm$  SEM) of glycylglycine (seven subjects), glycylleucine (five subjects), free glycine (six subjects), and free leucine (five subjects) as a function of concentration in the test solutions. The absorption rates given in this figure are the ones which were used for the calculation of  $K_m$  and  $V_{max}$  values detailed in Tables II and III. For these calculations it was important that the concentrations used did not fully saturate the transport sites. Nevertheless, in a few individuals higher concentrations, not shown here, were used. These studies showed maximal absorption rates that were near the calculated values.

more, amino acid absorption rates are not significantly affected by the changes in water movements (14). Preliminary studies in our laboratory have indicated that the same lack of effect by the above conditions is true for the absorption of dipeptides.

Each test solution was infused at a constant rate of 15 ml/min by a peristaltic pump. After 25 min of equilibration, three 15-min aspirates were obtained. More detail in regard to our perfusion procedures is presented elsewhere (1).

The methods of chemical analysis for free amino acid and dipeptide concentrations as well as the formula for the calculation of their absorption rates have all been described previously (1). For simplicity of presentation, we have used the term absorption to describe the intraluminal disappearance of leucine, glycine, glycylglycine, and glycylleucine. The absorption rates presented for glycylleucine also include hydrolysis, which is a small component (1). The apparent kinetic constants ( $K_m$  and  $V_{max}$ ) of either free amino acid or dipeptide disappearance were calculated in each subject by procedures similar to those proposed by Lineweaver and Burk (16). The reciprocals of the absorption rates were plotted against the reciprocals of the mean intraluminal concentrations. Apparent  $K_m$  and  $V_{max}$  values were determined from the slope and intercept of the line fitted by the method of least squares (17). The mean intra-

TABLE II  
Apparent  $K_m$  and  $V_{max}$  of Glycine or Glycylglycine Absorption

	Glycine		Glycylglycine		Glycylglycine and glycylleucine	
	$V_{max}$	$K_m$	$V_{max}$	$K_m$	$V_{max}$	$K_m$
	$\mu\text{mol}/\text{min}/15\text{ cm}$	$mM$	$\mu\text{mol}/\text{min}/15\text{ cm}$	$mM$	$\mu\text{mol}/\text{min}/15\text{ cm}$	$mM$
G. R.	592	54.1	532	32.5		
G. T.	415	23.5	917	46.8		
B. S.	690	48.2	903	51.6	1,250	442.5
G. L.	485	23.9	1,000	50.0	1,136	301.2
C. E.	752	65.2	917	38.3	725	170.9
B. M.	606	41.4	690	43.3		
J. C.			901	40.4	2,000	709.2
Mean	590	42.7	837	43.3	1,278	406
SEM	56	7.5	62	2.6	265	115
<i>P</i> value			<0.02*	NS*	NS†	<0.02†

\* Compared with glycine  $V_{max}$  and  $K_m$  values.

† Compared with glycylglycine  $K_m$  and  $V_{max}$  values when no glycylleucine was added to the test solutions.

luminal concentration of a free amino acid or a dipeptide was the arithmetic mean of the infusate and the aspirate. Each Lineweaver-Burk plot was constructed from 6-12 studies in each subject. The kinetic constants were calculated by using the concentration values just below and just above the  $K_m$  values. In a previous publication the problem of selecting the appropriate concentration values in a test segment for the calculation of kinetic constants was discussed (13). This problem has not yet been resolved. Furthermore, the absorption constants have not been corrected for the unstirred water layer (18). Therefore, the absolute values of the absorption constants estimated by our method may not be precise. These values have been used for comparative studies of free amino acid and dipeptide transport systems with the same experimental conditions or within the same subject. The statistical significance of differences

was evaluated either by the paired *t* test or by Student's *t* test (17).

## RESULTS

**Absorption kinetics.** The rates of glycylglycine absorption as a function of concentration followed the kinetics of a saturable transport system (Fig. 1). A limiting absorption rate was approached in the range of glycylglycine concentrations used. Furthermore, Lineweaver-Burk plots of the reciprocals of absorption rates versus the reciprocals of mean intraluminal concentrations showed a straight line relationship in each subject; an example of such a study is presented in Fig. 2. Establishment of this relationship allowed the calculation of absorption constants ( $K_m$  and  $V_{max}$ ) in each subject. These data are detailed in Table II.

TABLE III  
Apparent  $K_m$  and  $V_{max}$  of Leucine and Glycylleucine Absorption

Subjects	Leucine		Glycylleucine	
	$V_{max}$	$K_m$	$V_{max}$	$K_m$
	$\mu\text{mol}/\text{min}/15\text{ cm}$	$mM$	$\mu\text{mol}/\text{min}/15\text{ cm}$	$mM$
G. R.	500	20.5	690	22.6
C. E.	633	41.4	855	47.9
G. L.	277	11.4	502	18.7
P. G.	308	13.4	495	16.8
G. L.	350	15.4	855	28.1
Mean	414	20.4	619	26.8
±SEM	±67	±5.4	±85	±5.9
<i>P</i> value			<0.02*	<0.05*

\* Compared with leucine  $K_m$  and  $V_{max}$  values.

TABLE IV  
Jejunal Absorption Rates of Dipeptides with or without the Addition of Their Natural Digestive Product(s)

Test solutions	Dipeptide absorption rates
	$\mu\text{mol}/\text{min}/15\text{ cm}$
20 mM glycylglycine	208±5
20 mM glycylglycine + 100 mM glycine	203±8
20 mM glycylleucine	228±14
20 mM glycylleucine + 50 mM glycine + 50 mM leucine	232±14

Results are means±SEM of four subjects.

For a comparison between the absorption constants of glycylglycine and free glycine, absorption rates of free glycine at various concentrations were also determined in the same subjects. The mean absorption rates are presented in Fig. 1, and individual kinetic constants are detailed in Table II. There was no significant difference between the  $K_m$  values of glycylglycine and glycine. The  $V_{max}$  values, however, were significantly greater for glycylglycine than for free glycine.

To determine whether the saturation phenomenon was exhibited by another dipeptide, the kinetics of glycylleucine absorption were determined in the jejunum of a separate group of subjects. Again, a limiting absorption rate was approached in the range of glycylleucine concentrations used (Fig. 1). Furthermore, construction of a Lineweaver-Burk plot in each of these subjects showed a straight line relationship between the reciprocals of absorption rates and mean intraluminal concentrations. Individual  $K_m$  and  $V_{max}$  values calculated from these plots are presented in Table III.

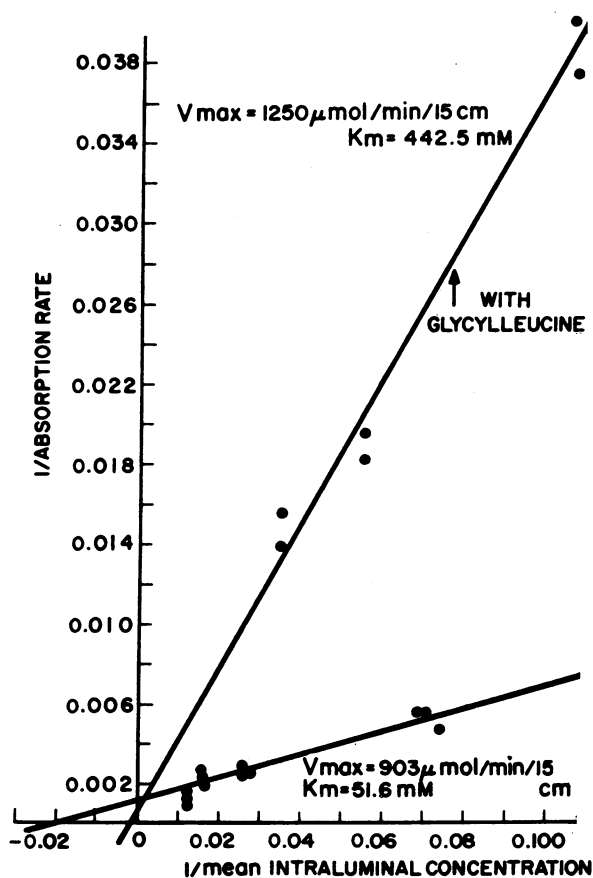


FIGURE 2 Examples of Lineweaver-Burk plots of glycylglycine absorption with or without the addition of glycylleucine to the test solution in one individual (B. S.).

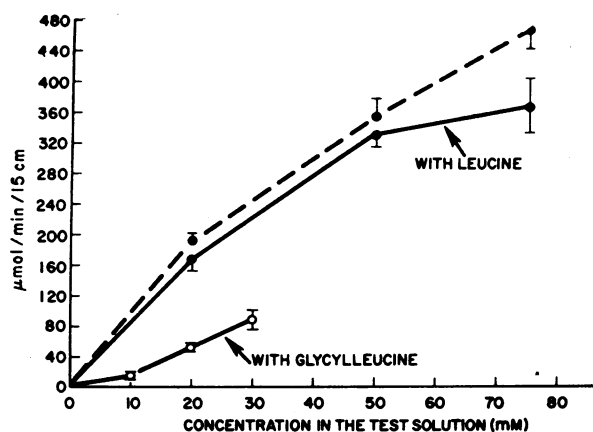


FIGURE 3 Jejunal absorption rates (mean  $\pm$  SEM) of glycylglycine with or without addition of free leucine (100 mM) or glycylleucine (50 mM) to the test solutions. The unlabeled dashed line represents the absorption curve for glycylglycine without the addition of either free leucine or glycylleucine. At each examined concentration, there was no statistically significant difference between absorption rates when leucine was added. Although the curve suggests inhibition of glycylglycine transport by leucine at a concentration of 75 mM, this was not statistically significant. The difference between absorption rates when glycylleucine was added was statistically significant ( $P < 0.01$ , at 20 mM concentration).

For a comparison between the kinetic constants of glycylleucine and leucine absorption, jejunal absorption rates of leucine were determined in the same subjects. The mean leucine absorption rates are presented in Fig. 1 and the individual  $K_m$  and  $V_{max}$  values are detailed in Table III. The glycylleucine  $K_m$  and  $V_{max}$  values were significantly greater than those of leucine.

**Inhibition studies.** To determine whether there is any interaction between the transport of glycylglycine and the transport of either a neutral free amino acid or a dipeptide, the following studies were performed. Test solutions containing varying amounts of glycylglycine (20, 50, and 75 mM) together with a fixed amount of leucine (100 mM) were infused. The amount of leucine in the test solution was well within the range of concentration that saturates the leucine transport sites in the same segment (Fig. 1). The selection of leucine was influenced by the fact that it is a known potent inhibitor of the transport of other neutral free amino acids such as glycine (1). In our previous studies we reported a slight inhibition of glycylglycine absorption by leucine in human jejunum (1). Using a wider range of concentrations of glycylglycine in the present studies, we did not find any significant inhibition of glycylglycine absorption by leucine (Fig. 3).

In addition to leucine, glycine, the natural digestive product of glycylglycine, also failed to affect the jejunal absorption rates of this dipeptide (Table IV). The lack of

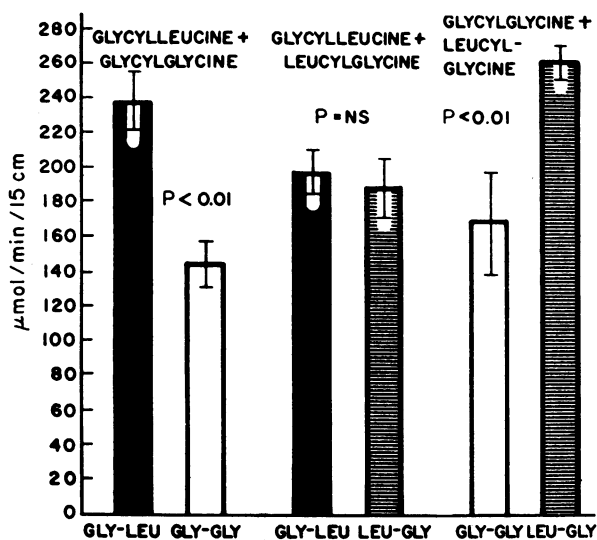


FIGURE 4 Jejunal dipeptide absorption rates (mean  $\pm$  SEM in five subjects) from equimolar mixtures (20 mM each) of two dipeptides.

interaction between the transport of free amino acids and a dipeptide was not unique to glycylglycine, since an equimolar mixture of free glycine and free leucine also did not alter significantly the jejunal absorption rate of glycylleucine.

The effect of glycylleucine on glycylglycine absorption examined at several concentrations is shown in Fig. 3. The concentration (50 mM) of glycylleucine in each test solution was based on the saturation of absorption of this dipeptide at this concentration (Fig. 1). In contrast to the lack of inhibition by leucine or glycine, glycylglycine absorptions were markedly inhibited by glycylleucine. The kinetic analysis in each subject revealed that the inhibition is either totally or partially competitive in nature; an example of such a study is presented in Fig. 2. Competitive inhibition was apparent from the lack of a significant change in  $V_{max}$  values, but a large increase in the  $K_m$  values when glycylleucine was added to the test solutions (Table II).

**Selective absorption.** Jejunal absorption rates of dipeptides from equimolar mixtures of two dipeptides are presented in Fig. 4. Both glycylleucine and leucylglycine were better absorbed than glycylglycine. There was no difference between the absorption rates of glycylleucine and leucylglycine when these dipeptides were presented as an equimolar mixture.

## DISCUSSION

The criteria for the transport of dipeptides by a carrier-mediated mechanism in human jejunum appear to be fulfilled by the results of the present studies. Furthermore, the data suggest that the carrier system for dipeptides is

separate from the carrier system for neutral free amino acids. This suggestion is supported by the observations that there were interactions between the transport of the dipeptides studied, but the transport of dipeptides was uninfluenced by free amino acids.

The dipeptide carrier system discriminates between dipeptides. The amino acids in both the COOH-terminal and NH<sub>2</sub>-terminal positions of the peptide linkage appear important in determining the characteristics of absorption. In both positions an amino acid with a longer side chain renders the dipeptide a more preferred substrate for the membrane absorption sites. This phenomenon is reminiscent of the factors governing the selectivity of free amino acid absorption (12, 13, 19, 20). Based on the established heterogeneity of carrier systems for free amino acids (21), it may be anticipated that future investigation might reveal several distinct species of carrier systems for dipeptides.

The  $K_m$  values are considered to be indices of the apparent affinities of substrates for the same membrane transport sites; the lower the  $K_m$ , the higher the apparent affinity (13, 21). Studies from various laboratories, using different techniques of investigation, have all indicated that leucine has a higher affinity than glycine for the neutral free amino acid carrier system (21–23). This phenomenon was further confirmed in the results of the present studies by showing a lower  $K_m$  value (mean  $\pm$  SEM) for leucine than for glycine ( $20.4 \pm 5.4$  vs.  $42.7 \pm 7.5$ ,  $P < 0.05$ ). By similar reasoning, glycylleucine appears to have a higher affinity than glycylglycine for the dipeptide carrier system, since the  $K_m$  value of glycylleucine is smaller than that of glycylglycine ( $26.8 \pm 5.9$  vs.  $43.3 \pm 2.6$ ,  $P < 0.05$ ). In this context it is interesting to note that there is overlapping in the range of  $K_m$  values for dipeptides and in those for free amino acids (Tables II and III). However, one important difference distinguishes the function of the carrier system for free amino acids from that of dipeptides. The rates of transport are generally greater for dipeptides than for free amino acids. This ability is evident from the noticeable differences in  $V_{max}$  values (Tables II and III) and absorption rates (Fig. 1). Glycylglycine and glycylleucine  $V_{max}$  values appeared greater than the  $V_{max}$  value of either leucine or glycine. The differences were all statistically significant ( $P$  values  $< 0.02$ ), except for the difference between  $V_{max}$  values of glycylleucine and glycine.

In some animal species two separate mechanisms for the intestinal transport of free glycine have been described (21). This problem has not yet been fully investigated in man. Assuming that free glycine uses two different pathways for its transport, the absorption kinetics of free glycine presented in Fig. 1 would be the composite of two systems. This would further dramatize

that the potential for absorption through the dipeptide transport system is greater than for each of the glycine transporting mechanisms.

It is perhaps pertinent to point out that the data presented in the present report provide additional support for our earlier conclusions that intact absorption is the major mode of dipeptide disappearance in the gut lumen (1, 2). The biochemical characteristics of the hydrolytic system, as observed in vitro by others, are not reflected in the functional characteristics of the dipeptide transport system as shown in the present investigation. First, the rate of hydrolysis of glycyllucine is markedly greater than the rate of hydrolysis of glycyglycine, either by the intracellular (24, 25) or by the brush border peptide hydrolases.<sup>1</sup> The order of absorption constants of these dipeptides is the reverse of their order of hydrolysis;  $V_{max}$  of glycyglycine is significantly greater than that of glycyllucine ( $837 \pm 62$  vs.  $619 \pm 85$   $\mu\text{mol}/\text{min}/15$  cm,  $P < 0.01$ ). Second, according to Kania, Santiago, and Gray, the human intestinal surface peptidase is an amino oligopeptidase (26). In our experiments the switching of the position of amino acid residue in the  $\text{NH}_2$ - and  $\text{COOH}$ -terminal positions did not make any difference in the selectivity of absorption rates: glycyllucine and leucylglycine were absorbed at equal rates (Fig. 4). Third, the available evidence indicates that in general dipeptidases are substrate-specific. For example, the enzyme that hydrolyzes glycyglycine does not appear to hydrolyze dipeptides such as glycyllucine and leucylglycine (27). In contrast, the intestinal transport of glycyglycine is markedly inhibited by glycyllucine (Fig. 3). Fourth, Newey and Smyth found that the intestinal hydrolysis of glycyglycine is reduced by the addition of free amino acids, such as methionine and methionine analogue, to the incubation media (28). Unlike hydrolysis, intestinal absorption of glycyglycine is not affected by free amino acids (Fig. 3 and Table IV).

The present studies do not include absorption rates of amino acid constituents of either glycyglycine or glycyllucine. The previously described phenomena of greater absorption and less transport interaction of amino acids in dipeptides than in free amino acid forms were also observed in the present studies (1). Furthermore, these phenomena have been recently confirmed and extended to other dipeptides by Hellier, Holdsworth, Perrett, Thirumalai, and McColl (3, 4), Cook (5), and Silk, Perrett, and Clark (6).

Finally, recent studies in microorganisms (29, 30) as well as in mammalian intestine (31) have all shown that dipeptide transport is a widespread biological distribution. Measurements by Rubino, Field, and Shwachman of influx of the glycine residue of glycyproline in an in vitro preparation of rabbit ileum have provided evi-

dence for a selective influx process that is shared by dipeptides but not by free amino acids (32). In addition, Addison, Burston, and Matthews, using jejunal rings of hamster intestines, have shown active transport for a dipeptide (glycylsarcosine), which is slowly hydrolyzed (33). The clinical importance of the dipeptide transport system in human nutrition is underscored by the following recent observations. First, after a protein meal there is a considerably greater amount of small peptides than of free amino acids in the gut lumen (34). Second, absence of protein malnutrition in patients with hereditary deficiencies of either neutral or basic free amino acid transport systems may be accounted for by their unimpaired ability to absorb amino acids from dipeptides (8, 11). Third, when jejunal absorption is markedly reduced by an inflammatory disease of the mucosa, the carrier system for dipeptide appears to be less affected than the carrier system for free amino acid (35).

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