Intestinal Transport of Dipeptides in Man: Relative Importance of Hydrolysis and Intact Absorption

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ABSTRACT A 30 cm segment of the duodenum, jejunum, or ileum of normal human volunteers was perfused, on separate occasions, with test solutions containing either glycylglycine, free glycine, glycylleucine, or equimolar amounts of free glycine and free leucine. Luminal fluid contained no hydrolytic activity against glycylglycine and minimal activity against glycylleucine. In each intestinal segment, amino acid absorption rates were significantly greater from the test solutions containing the same amount of amino acids in dipeptide than in free form (as high as 185% increase). Perfusion of each intestinal segment with a test solution containing the equimolar mixture of free glycine and free leucine always resulted in a greater leucine than glycine absorption rate. This preferential absorption of leucine, however, was either diminished (jejunum) or almost abolished (duodenum and ileum) when the glycylleucine solution instead of the equimolar mixture was presented to the intestinal mucosa. Among the three segments, the duodenum exhibited the least potential for the disappearance of dipeptides. The jejunal and ileal dipeptide disappearance rates were either similar for glycylleucine (94% vs. 92%) or slightly different for glycylglycine (92% vs. 79%). Despite lack of a remarkable difference in the disappearance rates, absorption rates of constituent amino acids were markedly greater in the jejunum than in the ileum. This reduced amino acid absorption was brought about by a greater accumulation of free amino acids in the lumen of the ileal segment (3 to 10-fold difference). Inhibition of free glycine absorption by leucine during the perfusion of the intestine with a test solution containing glycylglycine and leucine did not result in any greater concentration of free glycine in the lumen than when the glycylglycine test solution did not contain free leucine. Similarly, inhibition of free glycine and free leucine absorption by isoleucine was not accompanied by any remarkable alteration of absorption rates of the constituent amino acids of glycylleucine. The results of these studies suggest that: (*a*) dipeptide disappearance in the gut lumen is principally accomplished by intact absorption and not by hydrolysis; (*b*) intracellular hydrolysis of dipeptides is markedly greater in the ileum than in the jejunum, while dipeptide absorption rates are either similar or only slightly different in these two segments; (*c*) there is no appreciable hydrolysis of glycylglycine by the membrane-bound enzymes and only a small fraction of glycylleucine is hydrolyzed by these enzymes.

This work was presented in part at the 1971 Annual Meeting of the American Gastroenterological Association, Bal Harbour, Fla.

Received for publication 29 March 1971 and in revised form 5 July 1971.

INTRODUCTION

In recent years, the in vitro and in vivo transport of free amino acids in the intestine of experimental animals (1) as well as of man (2–8) has received considerable attention. In comparison, the intestinal fate of intermediate products of protein digestion (peptides) has not been extensively studied. Enthusiasm in favor of investigation of free amino acid transport was generated principally by earlier studies such as those of Dent and Schilling (9) and Christensen (10) which failed to detect any significant concentration of peptides in the portal blood of experimental animals after ingestion of an adequate protein meal. Therefore, it has been generally believed that "amino acids enter the animal organism from the intestine almost entirely in the free form" (11). We have recently examined the in vivo digestion of dietary proteins in the human small intestine.[1] For several hours after the ingestion of a well-balanced meal, the rises in the luminal concentrations

[1] Unpublished results.

TABLE I

Protocol for Perfusion Studies

Group	Location of perfused intestinal segment	Composition* of test solution
I	Jejunum	10, 20, 25, 50 mM glycylglycine
	Jejunum	20, 50, 100 mM glycine
II	Duodenum, jejunum, ileum‡	50 mM glycylglycine
	Duodenum, jejunum, ileum‡	100 mM glycine
	Jejunum	50 mM glycylglycine + 100 mM L-leucine
	Ileum	50 mM glycylglycine + 50 mM L-leucine
III	Jejunum	20, 50, 100 mM glycyl-L-leucine
	Jejunum	20, 50, 100 mM glycine + L-leucine (the equimolar mixtures)
	Jejunum	20 mM glycyl-L-leucine + 50 mM L-isoleucine
IV	Duodenum, jejunum, ileum‡	50 mM glycyl-L-leucine
	Duodenum, jejunum, ileum‡	50 mM glycine + 50 mM L-leucine
	Ileum	50 mM glycyl-L-leucine + 50 mM L-isoleucine

* All the above free amino acids and dipeptides used in the present study, were obtained from the General Biochemicals, Chagrin Falls, Ohio.

‡ Only one intestinal segment was perfused in each day of study.

were considerably greater for amino acids in peptides than for those in free form. It appeared to us, therefore, that the peptides may be a more important product of protein digestion than free amino acids. In the absence of any knowledge concerning the intestinal fate of peptides when they are introduced into the human intestine, we were prompted to explore this problem.

Experiments to be presented in this report were designed to determine the relative importance of physiological processes, such as hydrolysis by intraluminal or membrane-bound enzymes and intact absorption, which might be involved in disappearance of dipeptides in the intestine. Among all the dipeptides that may have been chosen, the present studies have focused extensively on the hydrolysis, uptake, and transport of glycylglycine and glycylleucine in the jejunum, duodenum, and ileum of normal human subjects. The selection of these two dipeptides offered an opportunity to examine the influence of rapid and slow hydrolyses of dipeptides by the mucosal enzymes on their relative rates of disappearance in the intestine. Previous studies had shown that, comparatively, glycylleucine is an excellent and glycylglycine is a poor substrate for the human mucosal peptide hydrolases (12, 13). Furthermore, microvillous membrane isolated from hamster intestinal epithelial cells demonstrated considerably lower hydrolytic activity against glycylglycine than against other dipeptides (14).

METHODS

18 normal male volunteers whose ages varied between 18 and 26 yr were intubated on 100 different occasions with a double-lumen tube the day before the experiment as described previously (3, 4). The tube was positioned either in the duodenum, jejunum, or ileum of these subjects. The position of the tube was checked in each person by a radiological method as described previously (3). The distance between perfusion opening and the aspiration opening was maintained at 30 cm. The aspiration opening was held at 80, 120, or 200 cm away from the teeth for perfusion studies in the duodenum, jejunum, or ileum respectively. These distances have been previously selected for perfusion studies in man (4, 15). All test solutions were introduced at a constant rate of 15 ml per min by a peristaltic pump.[2] The first 25 min of each perfusion was allowed for the attainment of a steady state in absorption rates. Preliminary studies indicated that this interval was sufficient for this purpose. The fluid collected during the first 25 min of the perfusion was discarded and then four 15-min collections were obtained. Each intestinal aspirate was collected in a polyethylene bottle which was kept chilled in crushed ice. As soon as each 15-min collection was completed, the sample was heated for 15 min in a water bath maintained at 95°C. According to our own preliminary investigation, as well as the report of others (16), the activity of peptide hydrolase enzymes which may be present in the intestinal aspirate is completely destroyed by this treatment. After cooling the intestinal aspirates to room temperature, the samples were filtered through Whatman No. 1 filter paper. The filtrates were then stored at −20°C. Whenever indicated, venous blood was collected in heparinized test tubes before, after 55 min, and just at the termination (85 min) of each perfusion. Each blood sample was immediately centrifuged and plasma was separated. The plasma was then stored at −20°C.

The subjects were divided into four groups, each having five individuals. The anatomical location of the perfused intestinal segments as well as the composition of the test solutions for each study are detailed in Table I. All the test solutions contained 0.4% polyethylene glycol[3] as a nonabsorbable marker (17) and between 100–140 mM sodium

[2] Model 600-1200, the Harvard Apparatus Co., Millis, Mass.

[3] Union Carbide Corp., N. Y.

chloride. Preliminary studies indicated that addition of these amounts of sodium chloride to the test solutions minimized net water movements in the perfused segment. Previous studies have shown that a wide variation in sodium concentration in the test segment does not alter the jejunal absorption rate of either glycine (7) or leucine (5).

The additions of free leucine to glycylglycine solutions and of free isoleucine to glycylleucine solutions were designed to achieve a near saturation of free amino acid absorption sites in the intestine. The selection of appropriate concentrations of these free amino acids was guided by the results of previous studies in this laboratory (4).

In vitro enzyme assay. A segment of the duodenum, jejunum, and ileum of four subjects was perfused on separate occasions with physiological saline solution containing 0.4% polyethylene glycol. Except for the number of intestinal aspirates collected during each perfusion, the method of this perfusion was similar to the one described above. Only two 15-min collections were obtained in this study. After each collection was completed, the intestinal aspirate was immediately centrifuged for 15 min at 3000 rpm in a refrigerated centrifuge maintained at 3°C. ½ ml of either 50 mM glycylglycine or 100 mM glycylleucine was added to 3 ml of the supernatant. The mixture was then incubated for 15 min in a Dubnoff metabolic shaker which was maintained at 37°C. Preliminary studies indicated that there was no further dipeptide hydrolysis after 15 min of incubation. The peptide digestion was terminated by heating the incubated sample for 15 min in a water bath maintained at 95°C.

Chemical analysis. The concentrations of free amino acids in intestinal aspirates and plasma and the concentrations of polyethylene glycol in intestinal aspirates were determined as described previously (3, 18). The purity and identity of glycylglycine and glycylleucine were examined by a combination of paper chromatography, ion-exchange chromatography, and acid hydrolysis of dipeptides to their constituent amino acids. Paper chromatographic analyses using propanol-water (80:20) for glycylglycine and butanol-acetic acid-water (40:10:50) for glycylleucine, resulted in detection of only one ninhydrin-positive spot on the chromatographic paper. Acid hydrolysis of glycylglycine yielded only free glycine and that of glycylleucine yielded only free glycine and free leucine. The following method of ion-exchange chromatography technique[4] was carried out for the dipeptide analysis. A known amount (0.05 μmoles) of either glycylglycine or glycylleucine dissolved in an appropriate volume (0.5 ml) of 0.01 N HCl was applied to the ion-exchange resin.[5] The column was then eluted with 0.20 N sodium citrate buffer (pH 3.20) for 170 min and then with 0.20 N sodium citrate buffer (pH 4.26) for 170 min. The starting temperature was 30°C, but after 70 min it was changed to 62°C. Ion-exchange chromatographic analysis of test solutions containing either glycylglycine or glycylleucine resulted in a single peak. The time of elution from the column for glycylglycine was 235–245 min and for glycylleucine 320–330 min. Test solutions and plasmas were analyzed for dipeptide content by this method. A simpler and shorter ion-exchange chromatography technique was followed for the analysis of dipeptides in the intestinal aspirates. The two dipeptides in the intestinal aspirates were eluted from the column with 0.20 N sodium citrate buffer (pH 4.26) at a column pressure of 150–200 psi and a column temperature of 62°C. The elution time was 90 min for glycylglycine and 160 min for glycylleucine.

Calculation of data. The absorption rates of amino acids from test solutions containing free amino acids were calculated by the previously published formulas (3, 4). The disappearance rates of dipeptides and the absorption rates of constituent amino acids of the same dipeptides were calculated by the following formulas:

$$P_d = \left(P_p - P_a \times \frac{M_p}{M_a}\right) \times R$$

$$A_a = \left[nP_p - (nP_a + C_a)\frac{M_p}{M_a}\right]R$$

where P_d = dipeptide disappearance rate in μmoles/min per 30 cm segment; P_p = concentration of dipeptide in test solution in micromoles per milliliter; P_a = concentration of dipeptide in intestinal aspirate in micromoles per milliliter; M_p = concentration of polyethylene glycol in test solution; M_a = concentration of polyethylene glycol in intestinal aspirate; R = rate of perfusion in milliliters per minute; A_a = rate of amino acid absorption in μmoles/min per 30 cm segment; n = the number of same amino acid residues in dipeptide linkage, and C_a = concentration of constituent amino acid in free form in intestinal aspirate in micromoles per milliliter. Absorption rates and disappearance rates are synonymous for free amino acids. However, the disappearance rates of dipeptides may include intact absorption as well as hydrolysis. Since there was no hydrolytic activity against dipeptides in test solutions, the average rate of dipeptide hydrolysis by the intraluminal enzymes in the 30 cm intestinal test segments was estimated by the following formula:

$$P_h = \tfrac{1}{2} \times H_a \times \frac{M_p}{M_a} \times R$$

where P_h = average rate of dipeptide hydrolysis in micromoles per minute; H_a = maximal amount of dipeptides (micromoles) that can be hydrolyzed by 1 ml of intestinal aspirate in the described in vitro enzyme assay conditions. The statistical significance of differences in disappearance and absorption rates, concentration values, and hydrolytic activities was evaluated by the paired *t* test in the same group and by Student's *t* test in different groups of subjects (19).

In previous studies, we reported the excellent reproducibility of amino acid absorption rates in the same subjects (3, 4). In the present study there was also excellent agreement in each subject among the four dipeptide disappearance rate determined from each test solution. Each dipeptide disappearance rate did not vary more than 4% from the mean value. For the sake of brevity, all the disappearance rates and absorption rates are presented as mean values for each group. Each mean value was calculated from 20 separate determinations. However, the results of the studies as shown for each group were similar also for each member of that group.

RESULTS

Amino acid and dipeptide disappearance rates in the jejunum. As shown in Fig. 1, at each concentration the rate of absorption of glycine was significantly greater from the glycylglycine solutions (bound glycine) than from the solution containing an equivalent amount of free glycine ($P < 0.01$). For example, the absorp-

[4] Model 120 C amino acid analyzer, Beckman Instruments, Inc., Palo Alto, Calif.

[5] UR-30, Beckman Instruments, Inc., Palo Alto, Calif.

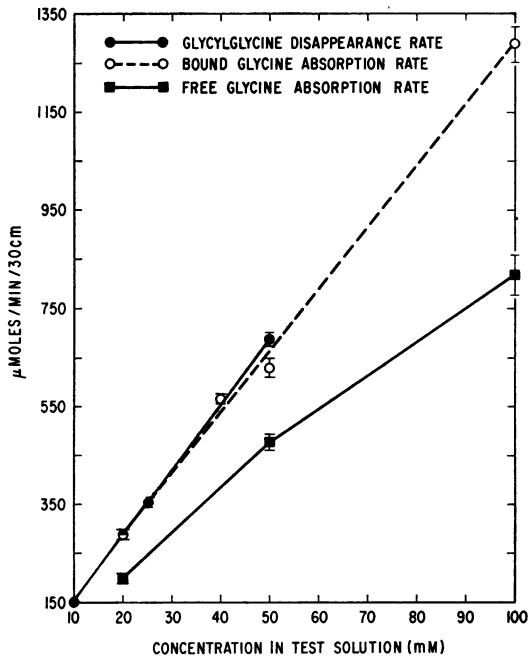

FIGURE 1 Glycine absorption rates and glycylglycine disappearance rates in jejunum of five subjects (mean ±SEM). Please note that in this and the following figures the vertical scales do not begin at zero.

tion rate of glycine from a 100 mM free glycine solution was 760 μmoles/min per 30 cm, while the absorption rate from a 50 mM glycylglycine solution was 1284 μmoles/min per 30 cm. In the range of concentrations used, there was almost complete disappearance of glycylglycine in the perfused segment (92–99% of the initial amounts). In addition, the disappearance rates of glycylglycine from the 20 and 50 mM test solutions were significantly greater than the absorption rates of free glycine from the 20 and 50 mM test solutions of glycine ($P < 0.01$).

As shown in Fig. 2, at each concentration both leucine and glycine absorption rates from the glycylleucine solutions (bound glycine and bound leucine) were markedly greater than the absorption rates of these two amino acids from the equimolar mixtures of free glycine and free leucine ($P < 0.01$). At each concentration, also, the rate of absorption was significantly greater for leucine than for glycine whether dipeptide or the equimolar mixture was used for perfusion ($P < 0.01$). However, the differences between the absorption rates of these two amino acids were considerably less pronounced when the dipeptide instead of the equimolar mixture was used. There was nearly complete disappearance of glycylleucine (94–99% of initial amounts) during the perfusion of the 30 cm segment when the concentration of this dipeptide in test solutions ranged from 10 to 50 mM. Comparing Fig. 1 and Fig. 2, one should note that when the concentrations ranged from 10 to 50 mM, there was no significant difference between disappearance rates of glycylglycine (uppermost line, Fig. 1) and glycylleucine (uppermost line, Fig. 2). Furthermore, free glycine absorption (lowermost line, Fig. 1) was markedly inhibited by free leucine (lowermost line, Fig. 2).

Amino acid and dipeptide disappearance rates in different segments of the small intestine. The results of perfusion studies with test solutions of either 50 mM glycylglycine or 100 mM free glycine are summarized in Fig. 3. The rates of glycine absorption from either dipeptide or free amino acid solution as well as the rate of glycylglycine disappearance was greatest in the jejunum and smallest in the duodenum. However, in each segment absorption of glycine was markedly greater from the glycylglycine solution than from test solution containing an equivalent amount of free glycine. The concentrations of free amino acids and dipeptides in intestinal aspirates during the perfusion studies in each segment are summarized in Table II. These data demonstrate that the concentration of free amino acids was lowest in the duodenum and highest in the ileum. On the other hand, the concentration of dipeptides was highest in the duodenum and lowest in the jejunum. It should be noted that luminal concentrations of the free amino acids were greater during the perfusion of intestinal seg-

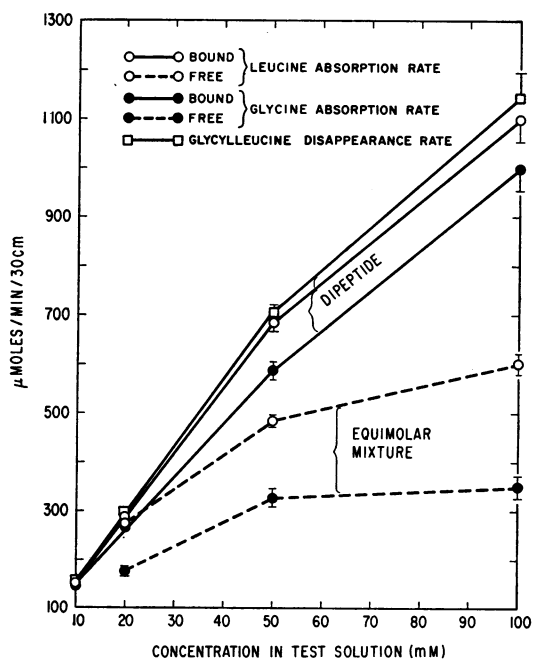

FIGURE 2 Glycine and leucine absorption rates and glycylleucine disappearance rates in jejunum of five subjects (mean ±SEM).

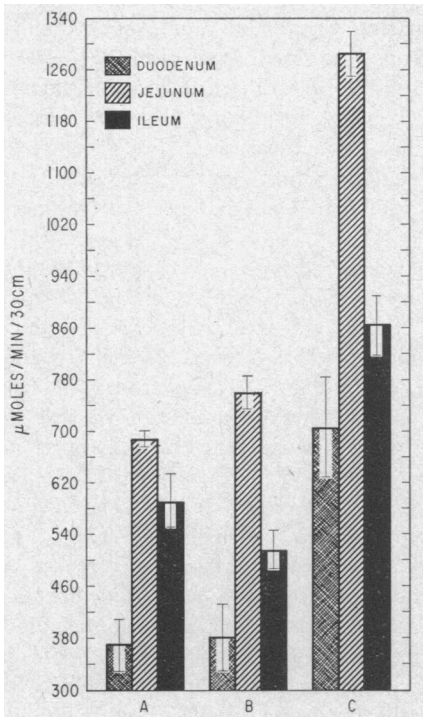

FIGURE 3 Glycylglycine disappearance rates and glycine absorption rates in various intestinal segments of five subjects (mean ±SEM). A, glycylglycine disappearance rates from a 50 mM solution of glycylglycine. The difference between jejunal and ileal disappearance rates was significant ($P < 0.01$). B, glycine absorption rates from a 100 mM solution of free glycine. The difference between duodenal and ileal absorption rates was significant ($P < 0.01$). C, glycine absorption rates from a 50 mM solution of glycylglycine. The difference between duodenal and ileal glycine absorption rates was significant ($P < 0.01$).

ments with glycylleucine solutions than with glycylglycine solutions.

Duodenal, jejunal, and ileal absorption rates of glycine and leucine from test solutions of either 50 mM glycylleucine or 50 mM free leucine together with 50 mM free glycine (the equimolar mixture) are summarized in Fig. 4. There was a greater absorption of leucine than glycine from the test solution containing the equimolar mixture of these two amino acids in each of the three intestinal segments ($P < 0.01$). This difference in absorption was much more pronounced in the jejunum than in the other two segments. In comparison, the difference was either significantly diminished (jejunum) or almost abolished (duodenum and ileum) when the glycine and leucine absorption rates were determined from the test solution containing these two amino acids in dipeptide form. Again, in all three segments, the absorption rates of both these amino acids were greater from the solution containing glycylleucine than from the free amino acid mixture, the difference being more dramatic in the jejunum than in the other two segments.

The jejunal and ileal disappearance rates of glycylleucine were similar, but they were considerably greater than the disappearance rate of the same dipeptide from the duodenum (Fig. 4). 94 and 91% of the initial amounts of glycylleucine introduced into the jejunal and ileal segments disappeared respectively, while only 42% of the initial amount of the same dipeptide disappeared in the duodenum.

Plasma-free amino acid and dipeptide levels during intestinal perfusions. There was no significant difference in plasma levels of free glycine during the perfusion of the jejunum of the same subjects with the solution of either 100 mM free glycine or 50 mM glycylglycine (Table III). On the other hand, a greater increase in plasma-free glycine levels was observed during perfusion of the ileum of the same subjects using a 50 mM glycylglycine solution than when using a 100 mM

TABLE II

Concentrations (millimolar) of Dipeptides and*

Composition of test solution	Duodenum		
	Free glycine	Free leucine	Dipeptide
50 mM glycylleucine	1.82 ±0.15	0.42 ±0.06	28.00 ±1.38
50 mM glycylleucine + 50 mM isoleucine			
20 mM glycylleucine			
20 mM glycylleucine + 50 mM isoleucine			
50 mM glycylglycine	2.29 ±0.11		25.85 ±1.71
50 mM glycylglycine + 100 mM leucine			
50 mM glycylglycine + 50 mM leucine			

* Each value represents the mean ±SEM determined in five subjects.

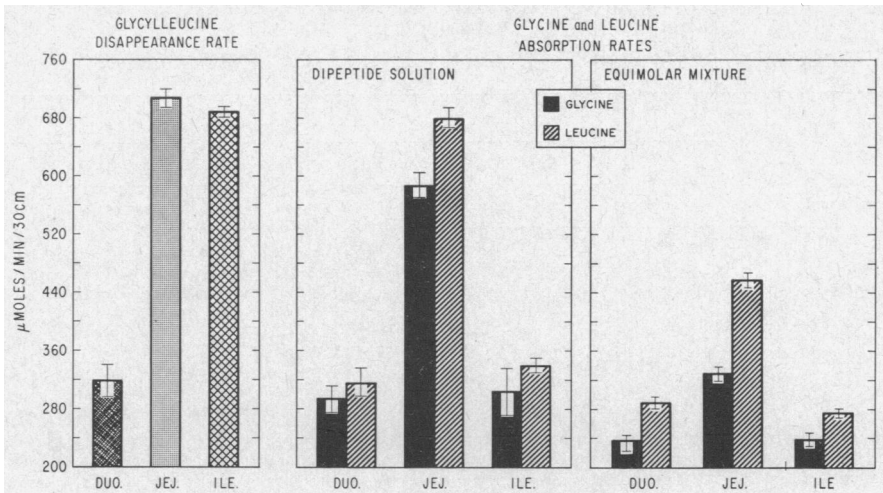

FIGURE 4 Glycylleucine disappearance rates, and glycine and leucine absorption rates from duodenal (DUO), jejunal (JEJ), and ileal (ILE) segments of five subjects (mean ±SEM). Test solutions contained either 50 mM glycylleucine (dipeptide solution) or 50 mM leucine together with 50 mM free glycine (equimolar mixture).

glycine solution. However, the elevation of plasma-free glycine levels during the perfusion of the ileum was considerably smaller than the increase in plasma glycine levels during the perfusion of the jejunum of the same subject with these solutions. Although glycylglycine was not detected in the plasma after an overnight fast or during the perfusion of the intestine with free glycine solutions, there was a considerable accumulation of this dipeptide during the perfusion studies using glycylglycine solutions. The plasma levels of dipeptide were significantly greater during the perfusion of jejunum than ileum.

In contrast to glycylglycine, glycylleucine was not detected in the plasma of subjects either before or during the perfusion studies. The failure to detect glycylleucine in plasma persisted when either the jejunum or ileum was perfused with test solutions containing as high as 100 mM glycylleucine. The plasma levels of free glycine and leucine, however, were markedly increased during perfusion with this dipeptide solution. These levels were greater during perfusion of the jejunum than the ileum. As shown in Table III, perfusion of the jejunum with the dipeptide solution did not result in a greater rise in plasma amino acid concentrations than the perfusion of the jejunum with the free amino acid mixture. On the other hand, during the perfusion of the ileum, a greater rise in plasma concentrations of both glycine and leucine was attained with glycylleucine solution than with the equimolar mixture of these two free amino acids.

Effect of free amino acids on disappearance of di-

Free Amino Acids in the Intestinal Aspirates

Jejunum			Ileum		
Free glycine	Free leucine	Dipeptide	Free glycine	Free leucine	Dipeptide
9.77 ±1.85	2.44 ±0.79	4.05 ±1.01	25.94 ±2.06	23.37 ±0.50	4.10 ±0.46
			27.81 ±3.13	26.01 ±2.09	6.96 ±1.01
2.65 ±0.16	0.29 ±0.11	0.38 ±0.14			
4.04 ±0.46	3.88 ±0.16	0.29 ±0.16			
8.16 ±1.46		5.96 ±1.23	24.79 ±3.41		12.74 ±3.20
5.19 ±0.76		12.90 ±1.17			
			19.43 ±1.32		26.25 ±2.14

TABLE III

Dipeptide Levels and Changes in Free Amino Acid Levels* in Plasma (micromoles/liter)*

Composition of test solution	Jejunum				Ileum			
	Free glycine		Free leucine		Free glycine		Free leucine	
	55 min	85 min	55 min	85 min	55 min	85 min	55 min	85 min
50 mM glycylleucine	348 ±48	435 ±43	514 ±30	712 ±30	114 ±17 ($P < 0.01$)	140 ±11	298 ±20 ($P < 0.01$)	327 ±32
50 mM leucine + 50 mM glycine	300 ±67	482 ±51	558 ±44	740 ±73	67 ±5	101 ±9	220 ±17	263 ±21
			Glycylglycine				Glycylglycine	
50 mM glycylglycine	868 ±68	1148 ±49	47 ±6	69 ±12	606 ±25 ($P < 0.01$)	707 ±52	18 ±2	24 ±2
100 mM glycine	921 ±108	1127 ±98	0	0	140 ±19	250 ±33	0	0

* Each value represents the mean ±SEM determined in five subjects. Each value for free glycine or free leucine represents the difference between the levels before and after 55 or 85 min of perfusion. Glycylglycine levels are the actual plasma concentrations after 55 and 85 min of perfusion, since this dipeptide was not found in plasma before the perfusions. Glycylleucine was not detected in plasma either before or during the perfusions.

peptides and their amino acid constituents. Jejunal and ileal disappearance rates of glycylglycine and glycine before and after addition of free leucine to the test solutions of 50 mM glycylglycine are summarized in Fig. 5.

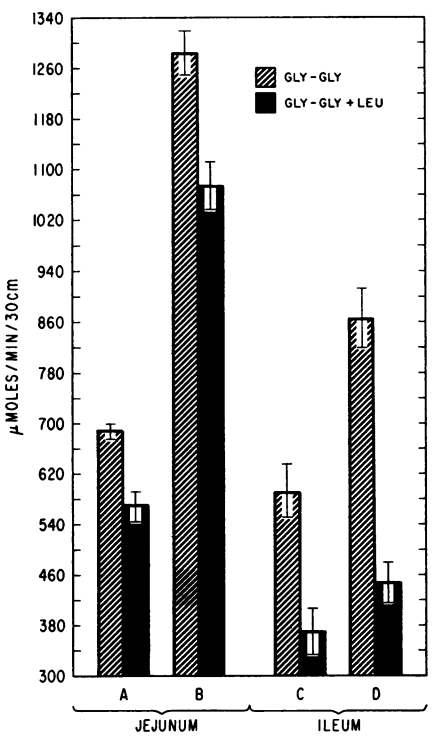

FIGURE 5 Glycylglycine disappearance rates, and glycine absorption rates in jejunum and ileum of five subjects before and after addition of leucine to a test solution containing 50 mM glycylglycine (mean ±SEM). A, jejunal glycylglycine disappearance rates ($P < 0.01$); B, jejunal glycine absorption rates ($P < 0.01$); C, ileal glycylglycine disappearance rates ($P < 0.01$); D, ileal glycine absorption rates ($P < 0.01$).

The disappearance rates of glycylglycine as well as its constituent amino acid were significantly reduced by free leucine in both segments. This was much more pronounced in the ileum than in the jejunum. It should be noted that the addition of free leucine did not increase the concentration of free glycine in aspirate either from the jejunum or from the ileum (Table II).

The effect of isoleucine on jejunal and ileal disappearance rates of glycylleucine and its constituent amino acids is shown in Fig. 6. Addition of isoleucine to the test solutions containing glycylleucine did not alter disappearance rates of this dipeptide either in the jejunum or ileum. However, the absorption rates of both constituent amino acids were significantly reduced in the jejunum. This reduction was more marked for leucine (13%) than for glycine (6%). Similarly, addition of isoleucine to the test solutions resulted in significant alteration in the concentrations of free glycine and free leucine only in aspirates from the jejunum. The increase in the concentration was more pronounced for leucine than for glycine (Table II).

In vitro assay of peptide hydrolase activities in the intestinal aspirates. There was no hydrolysis of glycylglycine when this dipeptide was incubated with the aspirate obtained from either the duodenum, jejunum, or ileum. There was minimal hydrolytic activity against glycylleucine in the aspirates obtained from all three segments (Table IV). Estimation of hydrolytic rates of glycylleucine by the intraluminal enzymes showed that only a few micromoles of this dipeptide could have been hydrolyzed during each minute by this enzyme activity in each segment (Table IV). As shown in this Table, ileal fluid had a greater hydrolytic activity against glycylleucine than did the other fluids.

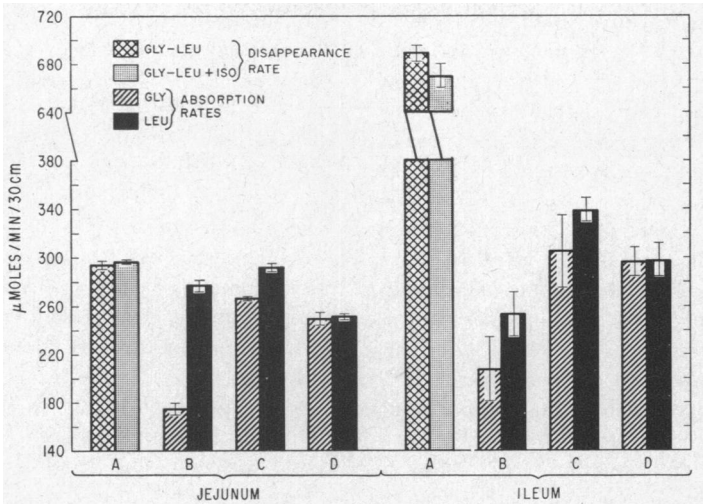

FIGURE 6 Glycylleucine disappearance rates, and glycine and leucine absorption rates in jejunum and ileum of five subjects before and after addition of isoleucine to glycylleucine solutions (mean ±SEM). The concentration of glycylleucine for jejunal and ileal studies were 20 and 50 mM respectively. A, glycylleucine disappearance rates; B, glycine and leucine absorption rates from the equimolar mixtures. The difference between absorption rates of these amino acids was significant in each segment ($P < 0.01$). C, glycine and leucine absorption rates from glycylleucine solution. The difference between absorption rates of these amino acids was significant only in jejunum ($P < 0.01$). D, glycine and leucine absorption rates after addition of isoleucine to dipeptide solution. The absorption rate of each of these amino acids was significantly reduced only in jejunum ($P < 0.01$).

DISCUSSION

The present studies have provided evidence for the absorption of unhydrolyzed dipeptides in man by demonstrating the accumulation of glycylglycine in peripheral plasma during the perfusion of the small intestine with solutions containing this dipeptide. Perfusion of the intestine with solutions containing free glycine was not accompanied by the appearance of any glycylglycine in the plasma. In addition, the present data, as will be discussed later, strongly suggest that glycylleucine is also absorbed in this fashion in the human intestine. The failure to demonstrate the presence of the former dipeptide in plasma in the present study may have been due to the much greater peptide hydrolase activity against glycylleucine than against glycylglycine in tissues such as intestine (12, 13). All available findings considered, this transport phenomenon appears not only to encompass the above two dipeptides, but also to apply to a substantial range of dipeptides (20–22). Although several years ago we suggested the intact absorption of dipeptides (23), in the present study, we have proceeded further to obtain the evidence for differences in the intestinal fate of the same dipeptide in different segments of the small intestine and different dipeptides in the same segment. The following discussion will highlight these differences for each of the dipeptides investigated in the present study.

The absence of peptide hydrolase activity in the luminal fluids, the small concentration of free glycine in the jejunal aspirates, and the failure to increase the concentration of free glycine in the jejunal aspirate by the addition of an inhibitor of free glycine absorption

TABLE IV

Peptide Hydrolase Activity of the Luminal Fluids and the Average Rates of Hydrolysis of Dipeptides by these Enzymes in the Test Segments

	Duodenum	Jejunum	Ileum
Peptide hydrolase activity, *μmoles/ml per 15 min**			
Glycylglycine	0	0	0
Glycylleucine	0.32 ±0.09	0.47 ±0.10	1.53 ±0.33
Hydrolytic rate, *μmoles/min**			
Glycylleucine	2.01 ±0.42	3.29 ±0.66	11.07 ±1.68
		($P < 0.01$)	

* Each value represents mean ±SEM determined in four subjects.

(leucine), suggest that glycylglycine disappearance in the gut lumen is accomplished principally or exclusively by intact dipeptide absorption. In contrast to a single mode of disappearance for glycylglycine, namely intact absorption, the present data suggest that disappearance of glycylleucine is accomplished by several mechanisms: (*a*) hydrolysis by the luminal enzymes; (*b*) hydrolysis by membrane-bound enzymes; (*c*) intact absorption. The hydrolysis of glycylleucine by the intraluminal enzymes accounts for a very small fraction of disappearance rates of this dipeptide. The greater absorption rate of leucine than glycine from the glycylleucine solution in jejunum suggests that a portion of glycylleucine is hydrolyzed at the cell surface and then an equimolar mixture of free amino acids is presented to the free amino acid carrier systems. In this situation, free amino acid carrier systems are able to express their selectivity. Furthermore, when isoleucine, in a concentration sufficient to saturate the free amino acid absorption sites (4), is added to a glycylleucine test solution, this selectivity is entirely abolished. The magnitude of the dipeptide disappearance rate due to hydrolysis can be estimated from the difference in leucine absorption rates during the perfusion with test solutions containing only glycylleucine or glycylleucine together with isoleucine (Fig. 6). There is only a 13% reduction in leucine absorption rates when the free amino acid absorption sites are nearly saturated with isoleucine. Therefore, intact absorption rather than hydrolysis appears to be the principal mechanism involved in the luminal disappearance of glycylleucine. The absorption of glycylleucine before its hydrolysis is further supported by the facts that absorption rates of glycine and leucine from dipeptide solutions are markedly greater than absorption rates of these amino acids from the equimolar mixtures of free glycine and free leucine, and the preferential absorption of leucine is diminished when the dipeptide solution instead of the equimolar mixture is presented to the jejunal mucosa. These differences could have been brought about only if the constituent amino acids of glycylleucine entered the mucosal cells by a mechanism other than the carrier systems for free amino acids.

Additional support for the hypothesis, that intact absorption is the principal mechanism for the disappearance of dipeptides from the intestinal lumen, may be provided by the comparison of jejunal disappearance rates of glycylglycine and glycylleucine. If these dipeptides, when they are in the lumen, were exposed to the action of mucosal enzymes (12, 13), a marked difference in the disappearance rates of glycylglycine and glycylleucine would have been expected. But the disappearance rates of these two dipeptides are similar in the jejunum (uppermost lines of Figs. 1, 2). Our data, therefore, suggest that the cell membrane denies a contact between the extracellular dipéptides and intracellular enzymes. Once the dipeptides are transported inside the cells, they hardly escape the hydrolytic action of peptide hydrolases. Indeed, recent in vitro studies have shown that the bulk of these enzymes are concentrated predominantly in the cytosol, and only a small amount of enzyme activity against dipeptides are found in the brush border region (24).

During the perfusion of intestinal segments with solutions containing either glycylglycine or glycylleucine, the luminal fluid aspirated from the ileum contained markedly greater amounts of free amino acids than the fluids aspirated from either the jejunum or duodenum. Does this finding indicate, that in comparison to other segments, the hydrolysis of dipeptides in the lumen or at the mucosal surface of the ileum is a major mechanism for dipeptide disappearance? As in other segments, the ileal fluid lacked any hydrolytic activity against glycylglycine and had only minimal hydrolytic activity against glycylleucine. Addition of free amino acids in concentrations sufficient to saturate the free amino acid absorption sites in the ileum (4) to dipeptide solutions, failed to further increase the concentrations of amino acid constituents of dipeptides in the lumen. If the hydrolysis of dipeptides by the membrane-bound enzymes accounted for the accumulated free amino acids, then a greater concentration of free amino acids during the perfusion of the ileum with solutions containing dipeptides together with free amino acids than dipeptides alone should have been observed. In contrast to the lack of evidence for the extracellular hydrolysis, a greater intracellular hydrolysis of dipeptides in the distal than in the proximal segment may adequately explain the high concentration of free amino acids in the ileal lumen. The greater intracellular hydrolysis may lead to the build up of a greater intracellular concentration of constituent amino acids which then favors a greater back-diffusion. This suggestion is supported further by comparing the results of determinations of free glycine and glycylglycine levels in plasma during the perfusion studies. This type of comparison could be made only for glycylglycine, since glycylleucine was not found in peripheral plasma. While the absorption rate of glycylglycine is greater by only 14% in jejunum than in ileum (Fig. 3), there is a threefold difference in plasma concentration of glycylglycine attained during the perfusion of these two segments with the same solution of glycylglycine (Table III). In comparison to the marked differences in plasma concentrations of glycylglycine, the rise in plasma concentration of free glycine during the perfusion of ileum with glycylglycine was only 30% smaller than the rise in plasma concentration of free glycine during the perfusion of the jejunum (Table III). These additional data, therefore, indicate that a markedly greater

intracellular hydrolysis together with a slightly smaller cellular uptake of glycylglycine in the ileum than in the jejunum may have accounted for the difference in plasma levels.

Finally, it is important to point out the potential application of the information gained in the present study to the therapy of clinical disorders involving either impaired protein digestion or reduced amino acid absorption by hereditary or acquired factors. In these situations, a more efficient absorption of amino acids or their absorption by a mechanism other than free amino acid carrier systems, e.g. dipeptide absorption may be desired. Feeding of dipeptides instead of a free amino acid mixture may accomplish this task at a lower osmotic cost and a greater kinetic advantage.

ACKNOWLEDGMENTS

This work was supported in part by a grant (N-63) from the Health Research and Services Foundation.

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