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UPTAKE OF THE COMPONENTS OF PHENYLALANYLPHENYLALANINE AND MALTOSE BY INTESTINAL EPITHELIUM

CHON R. SHOAF, WILLIAM D. HEIZER and MICHAEL CAPLOW

Departments of Biochemistry and Medicine, The University of North Carolina, Chapel Hill, NC 27514 (U.S.A.)

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Summary

The observed rate of phenylalanine absorption into rat intestinal rings with 0.5 or 5.0 mM phenylalanine is greater than that for absorption of phenylalanine from 0.25 or 2.5 mM Phe-Phe, respectively. With the amino acid phenylalanine, V for absorption is the same whether Na^+ is present (149 mM) or absent, but the concentration at which the half-maximal transport rate occurred (K_t) is greater in the absence of Na^+. For Phe-Phe, the V decreases in the absence of Na^+ whilst K_t is not influenced by the Na^+ concentration. The different effect of Na^+ on Phe and Phe-Phe transport indicates that the absorptive mechanism for Phe-Phe is different from that for phenylalanine. Absorption of a mixture of [U-^{14}C]Phe-Phe and Phe-[G-^{3}H]Phe showed identical rates of uptake of the carboxyl and amino terminal amino acids.

Studies of transport of radioactive maltose showed that the rates of uptake of the reducing and non-reducing glucosyl moieties are identical. Radioactive maltose absorption is not inhibited by glucose oxidase.

These results provide evidence that in intestinal epithelium, hydrolysis of Phe-Phe and maltose does not occur on the cell surface with release of the hydrolyzed products to the medium. Rather, hydrolysis and release of the reaction products occur at a point on the cytosol side of a diffusion barrier located in the brush border membrane.

Introduction

Prior to the work of Newey and Smyth [1—3], protein absorption by the intestine was thought to occur by complete intraluminal hydrolysis of pro-

teins followed by absorption of the resulting amino acids. These investigators provided evidence that dipeptides may be absorbed intact by intestinal mucosa and hydrolyzed intracellularly. Using intestinal rings, Cheng et al. [4] observed that in a concentration range where hydrolysis of Met-Met increased only slightly, the rate for methionine uptake from Met-Met was concentration-dependent and exceeded the rate of uptake from free methionine. Two modes of peptide uptake were suggested: (1) luminal surface hydrolysis, followed by uptake of the resultant products by the free amino acid transport mechanism, and (2) intact peptide transport followed by intracellular hydrolysis. Rubino et al. [5] also suggested two mechanisms for [^{14}C]Gly-Pro transport in rabbit ileum based on a biphasic Eadie-Hofstee plot for absorption of this peptide. Recently Matthews [6] reviewed the data concerning peptide absorption including the evidence that luminal or surface hydrolysis followed by absorption of the constituents by the free amino acid systems is of minor importance for the peptides studied. Matthews [6] and Kim [7] propose that intact transport with hydrolysis in the cytosol and hydrolysis by brush border enzymes during the process of transport both occur; the relative rates depend on the particular peptide.

The details of disaccharide digestion-absorption are also unsettled. However, intracellular hydrolysis probably need not be considered, as nearly all of the disaccharidase activity of the intestinal epithelial cell is associated with the brush border membrane [8—11]. The mechanism of digestion-absorption of the disaccharide, maltose, is separate and distinct from the glucose transport mechanism since uptake of glucose from maltose was additive to the uptake of free glucose [12]. The exact site for hydrolysis of maltose and other disaccharides and the relation of hydrolysis to the subsequent mechanism of transport is unclear. It has been suggested [13] that disaccharidases could impart an inward vectorial component to some of the hexose liberated from a glycosidic linkage at the membrane interface and thus directly serve a transport function. Peptide hydrolases might have a similar function in peptide transport.

In considering the possible role of hydrolases in transport it is important to note that with a large number of peptide hydrolytic enzymes the carboxyl and the amino components of the substrate are not simultaneously released. This results from the fact that the catalytic mechanism involves an active serine of cysteine residue which forms a covalent bond with a portion of the substrate [14,15]: the carbonyl moiety of the peptide bond is covalently bound to the enzyme in an acyl-enzyme while the amino component of the peptide bond dissociates. Deacylation occurs in a separate hydrolytic step. In the case of a dipeptide, the amino acid contributing the carbonyl moiety to the peptide bond will form the covalent bond and consequently be the last amino acid to leave the enzyme. The amino acid contributing the amino group to the peptide bond will not be bound to the enzyme covalently and will leave the enzyme complex first. Similarly, there is evidence that the two moieties of maltose are released from the hydrolyzing enzyme at different rates as a result of the formation of a glycosyl-enzyme intermediate [16,17].

Based on these mechanisms, it is possible that when Phe-Phe or maltose is hydrolyzed in the brush border membrane the components may be released at different locations in the membrane with different propensities for back

diffussion to the luminal surface. Thus, depending on the location of the enzyme in the membrane when each component is released, a different rate of transport of the two components may be observed. In the present study this concept has been used to probe and further define the site of hydrolysis of Phe-Phe and maltose.

Materials and Methods

Synthesis of labeled Phe-Phe. Labeled derivatives of Phe-Phe were synthesized by reaction of dicyclohexylcarbodiimide with *t*-butyloxycarbonyl phenylalanine synthesized as previously described [18], and phenylalanine ethyl ester [19]. Deblocking of the dipeptide amino group by hydrogen bromide/acetic acid and of the carboxyl group by NaOH saponification was as described by Greenstein and Winitz [19]. [U-^{14}C]Phe-Phe and Phe-[G-^{3}H]Phe were both synthesized.

Fluorescent derivatives [20] of both radioactive Phe-Phe compounds and commercially available Phe-Phe were chromatographed on thin-layer micropolyamide sheets by using the method of Zimmer et al. [21] and found to comigrate. The radioactive purity of the synthesized dipeptides was determined by an application of the Gibbs phase rule [22] in which the percent increase in radioactivity of a saturated solution of radioactive Phe-Phe was determined when more labeled dipeptide was added. Maximum solubilization of Phe-[G-^{3}H]Phe or [U-^{14}C]Phe-Phe was accomplished by dissolving 1 mg of solute in 1.0 ml of water. After centrifugation at 500 × *g* for 10 min, a 5-μl aliquot of the supernatant was counted for radioactivity. Following the addition of another 5.0 mg of the synthesized radioactive dipeptide and vortexing to attempt additional solubilization, the suspension was again centrifuged at 500 × *g* for 10 min and 5-μl of the supernatant were counted for radioactivity. The additional counts are derived from radioactive impurities in 5 mg of the radioactive Phe-Phe; from this the percent purity was calculated. From these measurements, the estimated purity of Phe-[G-^{3}H]Phe and [U-^{14}C]Phe-Phe was 99.7 and 99.4%, respectively.

Radioactive maltose synthesis. Maltose with the tritium label in only the reducing glycosyl moiety was prepared as described by Shoaf et al. [23].

Absorption by everted intestinal rings. The small intestine from Sprague-Dawley rats, excluding the proximal 30 cm, was everted and 0.5-cm sections were cut taking care to avoid Peyer's patches. The rings were kept in 4° C oxygenated Krebs-Ringer phosphate buffer [24], pH 7.4, until needed. When Na^+-free incubation conditions were required, the Krebs-Ringer phosphate buffer was modified by substituting choline chloride for NaCl and K_2HPO_4 for Na_2HPO_4. Incubation of the rings was performed in 25-ml Ehrlenmeyer flasks with each flask having one ring in 10 ml of Krebs-Ringer phosphate containing the stated concentrations of substrate. The flasks were incubated at 37° C in a Dubnoff shaker (100 strokes/min) and aereated with 99.5% O_2. After 8 min the rings were removed, carefully blotted on Whatman No. 50 filter paper over a Buchner funnel, weighed (wet weight), and boiled for 5 min in 0.5 ml water to elute the cell contents. Aliquots (400-μl) were added to 5 ml of Biofluor scintillation fluid and counted for ^{3}H and ^{14}C, with appropriate

corection for spillover to determine the ^{3}H : ^{14}C ratio. The boiled rings were dried at 100° C to constant weight (dry weight).

Extracellular fluid corrections were calculated according to the method of Cheng et al. [4] using [^{14}C]inulin or [^{3}H]inulin, as appropriate. When double-labeled substrate was used, extracellular fluid corrections were approximated by previously determined values of 0.310, or 0.991 μl/mg dry wt., depending on the method used for blotting the tissue. Intracellular fluid volume was then calculated [4] and transport of individual substrates is recorded as μmol/ml of intracellular fluid per min or nmol/ml of intracelular fluid per min.

Intracellular metabolism of glucose releases some of the hydrogen atoms of glucose into water, which readily diffuses from the cell (Shoaf, C.R., Heizer, W.D. and Caplow, M., unpublished observations). Therefore, when the absorption of 0.25 mM [U-^{14}C]maltose and [6-^{3}H]maltose (labeled only in the reducing end) was studied, the incubation time was reduced to 2 min to minimize the extent of glycolysis, and 30 mM unlabeled glucose was added to the medium to saturate glucose metabolic pathways.

Lineweaver-Burk determinations of V and K_t. The apparent V and K_t for [G-^{3}H]phenylalanine and [U-^{14}C]Phe-Phe transport in the presence and absence of Na^+ were determined by using Lineweaver-Burk plots (see Fig. 1). The modified (Na^+-free) Krebs-Ringer buffer was used for absorption studies in the absence of Na^+. Rings were chosen randomly from the small intestine since the rate of absorption of phenylalanine varied along the length of the intestine from a mean of 0.8 μmol/ml per min at 30 cm from the pylorus to 0.29 μmol/ml per min at 80 cm from the pylorus. Ring segments used in the experiments were from a 10 cm section of the intestine and in this range a very small variability in absorbtion was found. The kinetic parameters for phenylalanine and Phe-Phe were determined by using the method of Wilkinson [25], which utilizes a weighted average linear-regression analysis.

Effect of glucose oxidase on maltose uptake. The specific activity of each glucose oxidase preparation used was determined by the colorimetric method provided by the Sigmal Chemical Co. The conditions described by Rutloff et al. [26] were duplicated by using a 15 min incubation time, 5 mM maltose, and 424 units of glucose oxidase.

Materials. D-[6-^{3}H]Glucose (33.99 Ci/mmol), [U-^{14}C]phenylalanine (448 mCi/mmol), [^{14}C]inulin (1.82 mCi/gm) and Biofluor cocktail were from New England Nuclear. β-D-[U-^{14}C]Maltose (7.9 mCi/mmol), [G-^{3}H]phenylalanine (1.0 Ci/mmol) and [^{3}H]inulin (1.9 Ci/mmol) were from Amersham Corp. Glucose oxidase was from Sigma Chemical Co.

Results

Comparison of phenylalanine and Phe-Phe absorption rates by everted intestinal rings

The absorption of amino acids by intestinal epithelium is more rapid from many dipeptides than from equivalent concentrations of the free amino acid components [4]. However, not every dipeptide tested demonstrates this phenomenon [4], and we did not observe it in the case of Phe-Phe. The rate of absorption in 0.5 and 5 mM [U-^{14}C]phenylalanine was compared with the

TABLE I

RATE OF PHENYLALANINE UPTAKE FROM [U-^{14}C]PHENYLALANINE AND Phe-[G-^{3}H]Phe

Individual 0.5 cm everted intestinal rings were used for simultaneous absorption of 0.50 mM [U-^{14}C]-phenylalanine and 0.25 mM Phe-[G-^{3}H]Phe or simultaneous absorption of 5.0 mM [U-^{14}C]phenylalanine and 2.50 mM Phe[G-^{3}H]Phe. Incubation was for 8 min at 37°C. Absorption rates expressed as nmol of phenylalanine taken up per ml of intracellular fluid per min of incubation.

Substrate	N	Concentration (mM)	Absorption rate (nmol/ml per min)
[U-^{14}C]Phenylalanine	11	0.50	156 ± 17.6
Phe-[G-^{3}H]Phe	5	0.25	66.0 ± 5.2
[U-^{14}C]Phenylalanine	9	5.00	787 ± 46
Phe-[G-^{3}H]Phe	5	2.50	288 ± 3.1

absorption of 0.25 and 2.5 mM Phe-[G-^{3}H]Phe, respectively. The different radioactive labels permitted comparison of absorption of the two compounds simultaneously in the same intestinal ring. At both concentrations the rate of absorption of phenylalanine from the free amino acid was approx. 3-fold greater than from the peptide (Table I). Higher concentrations could not be tested because of the limited solubility of Phe-Phe.

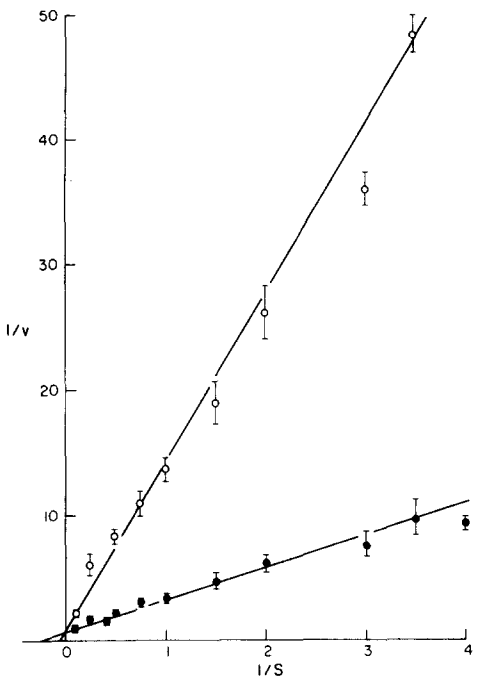
Effect of Na^+ concentration of V and K_t of phenylalanine and Phe-Phe absorption

Elimination of Na^+ from the incubation medium affects the transport kinetics of phenylalanine and Phe-Phe differently. Lineweaver-Burk plots for free phenylalanine absorption are shown in Fig. 1. In the presence and absence of Na^+ the K_t value was 3.5 ± 0.28 mM and 16.9 ± 2.5 mM ($P < 0.0025$), respectively. V was not significantly affected by Na^+ concentration; it was 1.35 ± 0.07 μmol/ml per min in the presence of Na^+ and 1.25 ± 0.14 μmol/ml per min in the absence of Na^+. In contrast, similar plots for Phe-Phe absorption (Fig. 2) show that in the presence and absence of Na^+, K_t is unchanged at 1.56 ± 0.45 mM and 1.54 ± 0.11 mM, respectively. V was 0.236 ± 0.042 μmol/ml per min in the presence of Na^+ and 0.114 ± 0.014 mol/ml per min in the absence of Na^+ ($P < 0.01$).

Rate of transport of the components of Phe-Phe and maltose: Phe-Phe transport

Intestinal transport of a mixture of Phe-[G-^{3}H]Phe (labeled only in the carboxyl terminus with ^{3}H and [U-^{14}C]Phe-Phe (labeled only in the amino terminus with ^{14}C) was studied and the intracellular ^{3}H : ^{14}C ratio was compared with the isotope ratio in the incubation medium. Any difference in the rate of transport of the amino and carboxyl terminal amino acids would be manifested as a difference in the intracellular fluid and medium isotope ratios. Radioactivity was counted to an error of ±1% and it is estimated that a 2% difference in the transport of the amino or carboxyl terminal amino acid could be detected using this method.

8-min incubations of intestinal rings in a [U-^{14}C]Phe-Phe and Phe-[G-^{3}H]Phe mixture were carried out in seven experiments, and as shown in Table II there



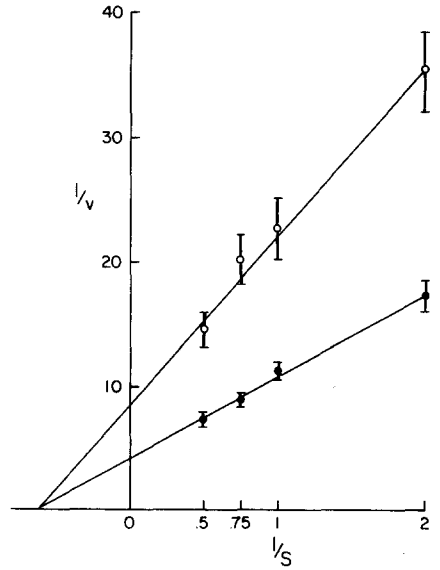


Fig. 1. Lineweaver-Burk plot of [G-^{3}H]phenylalanine absorption in the presence (•) and absence (○) of Na^+. Everted intestinal rings (0.5 cm) were incubated 8 min at 37°C. Each point and the error bar show the mean and the range of eleven determinations. Transport velocity, v, is in μmol/ml per min and substrate concentration, S, is in mM.

Fig. 2. Lineweaver-Burk plot of Phe-[G-^{3}H]Phe absorption in the presence (•) and absence (○) of Na^+. Everted intestinal rings (0.5 cm) were incubated at 37° for 8 min. Each point and the error bar show the mean and the range of six determinations. Transport velocity, v, is in μmol/ml per min and substrate concentration, S, is in mM.

is no significant difference between the ^{3}H : ^{14}C ratio in the intracellular contents, and the initial incubation medium. The mean Phe-[G-^{3}H]Phe absorption rate for these experiments was 382 ± 40 nmol/ml per min, and the mean intracellular phenylalanine concentration after incubation was 3.06 ± 0.32 mM. Free phenylalanine in the medium following incubation of the rings was determined by the method of Shoaf et al. [27], and from this measurement it was determined that 4.8% of the initially present 1.4 mM dipeptide had been hydrolyzed during the incubation. Based on the measured V and K_t values for the Phe and Phe-Phe transport (Fig. 1), it can be calculated from the equation: rate = $[V(S)]/[K_t + S]$ (assuming that the Phe concentration was 0.067 mM, the mean of the zero initial concentration and the final 0.134 mM concentration, and the Phe-Phe concentration was 1.4 mM throughout the experiment) that at least 81% of the Phe transported was derived from Phe-Phe.

Attempts to perturb the hydrolytic mechanism by adding free phenylalanine (1.4 and 14 mM) to preferentially displace one of the hydrolyzed components did not alter the intracellular ^{3}H : ^{14}C ratio, although uptake from the dipeptide was inhibited 45% by 14 mM phenylalanine. Five of six phenylalanine

TABLE II

INTRACELLULAR ^3H : ^{14}C RATIO AFTER INCUBATION WITH Phe-[G-^3H]Phe AND [U-^{14}C]Phe-Phe AND EFFECT OF VARIOUS INHIBITORS

Everted intestinal rings (0.5 cm) were incubated at 37°C for 8 min in 10 ml of a 1.4 mM mixture of Phe-[G-^3H]Phe and [U-^{14}C]Phe-Phe. Concentrations of inhibitors are 14 mM unless stated otherwise.

Expt.	N	Phe-[G-^3H]Phe : [U-^{14}C]Phe-Phe		Inhibitor	% Inhibition
		Medium	Intracellular		
1	7	1.02 ± 0.01	1.02 ± 0.01		
2	4	1.03 ± 0.03	1.02 ± 0.01	phenylalanine 1.4 mM	0
3	2	1.01	1.00	phenylalanine	45
4	2	1.02	1.02	phenylalanine amide	49
5	2	0.996	0.983	D-phenylalanine	24
6	2	0.988	0.988	*N*-acetylphenylalanine	79
7	2	0.983	0.985	*p*-fluorophenylalanine	49
8	2	0.990	0.970	hydroxyphenylalanine	0
9	2	0.990	0.988	phenylalanine ethyl ester	29

derivatives tested also inhibited uptake of the dipeptide, but none altered the intracellular ^3H : ^{14}C ratio (Table II).

Maltose transport

A discrepancy in the literature [26,28] makes it unclear as to whether free glucose released during intestinal digestion-absorption of maltose is accessible to glucose oxidase. Miller and Crane [28] showed that glucose oxidase does not inhibit uptake of glucose from maltose and proposed that the site of maltose hydrolysis is 'deep' in the membrane where it is inaccessible to the enzyme. However, Rutloff et al. [27] reported inhibition of glucose uptake from maltose by glucose oxidase, but the type of enzyme used was not stated. We tested the effect of equally active amounts of three glucose oxidase preparations on glucose absorption from maltose under conditions described by Rutloff et al. [26]. The results in Table III show that in the presence of purer glucose oxidase preparations the rate of maltose absorption is not significantly

TABLE III

EFFECT OF THREE GLUCOSE OXIDASE PREPARATIONS ON MALTOSE UPTAKE BY RAT INTESTINAL RINGS

Glucose oxidase (424 units) was added to 10-ml incubation mixtures of 5 mM β-D-maltose, β-D-[U-^{14}C]-maltose (15.1 μCi) and [^3H]inulin (37.5 μCi). Everted rings (0.5 cm) were incubated 15 min at 37°C. Absorption rates are in units of nmol maltose/ml intracellular volume per min; results are an average from six experiments.

Glucose oxidase	Absorption (nmol/ml per min)	*P*
None	456 ± 38	
Sigma Type II	419 ± 37	>0.25
Sigma Type V	399 ± 25	>0.10
Sigma Crude	112 ± 24	<0.0005

decreased by added enzyme. However, a highly impure Sigma 'Crude' glucose oxidase preparation caused 75% inhibition of maltose uptake. Since the inhibition of transport is only seen with impure glucose oxidase preparations, it may be concluded that the inhibition is not caused by glucose oxidase reaction with maltose-derived glucose, and that maltose hydrolysis occurs at a point which is inaccessible to glucose oxidase, as has been previously concluded [28]. The inhibition by impure glucose oxidase may be caused by a contaminating maltase activity.

To study the rate of transport of the two glycosyl moieties of maltose by intestinal rings, the incubation medium contained [U-^{14}C]maltose, [6-^{3}H]-maltose (labeled only in the reducing end), 0.25 mM unlabeled maltose, and 30 mM glucose. The use of [U-^{14}C]maltose instead of maltose labeled in only the non-reducing end has the effect of reducing by half any change in the intracellular ^{3}H : ^{14}C ratio that might occur due to differential transport of the two glucose moieties of maltose, since [U-^{14}C]maltose is labeled in both positions. In these studies 30 mM glucose was added to minimize intracellular glucose metabolism which could lead to efflux of the ^{3}H label as $^{3}H_2O$ (Shoaf, C.R., Heizer, W.D. and Caplow, M., unpublished observations). When rings were incubated for 2 min in the doubly-labeled maltose mixture having a ^{3}H : ^{14}C ratio of 0.326, the mean intracellular ^{3}H : ^{14}C ratio in duplicate determinations was 0.329. A second similar experiment gave a mean intracellular ^{3}H : ^{14}C ratio of 0.458 when the initial incubation medium ^{3}H : ^{14}C ratio was 0.459. Treatment of these data by comparison of paired sets showed no significant difference between incubation medium and intracellular ^{3}H : ^{14}C ratios ($P > 0.45$, $n = 4$). After a 2 min incubation of a reaction initially containing maltose, but no glucose, the amount of glucose detected [29] in the medium corresponded to hydrolysis of less than 5% of the maltose. This means that the radioactivity taken into the tissue was primarily derived from maltose rather than from glucose. In a control experiment with [6-^{3}H]glucose and [U-^{14}C]glucose in place of double-labeled maltose the mean intracellular ^{3}H : ^{14}C ratio of duplicate determinations was 1.09 ± 0.01 compared to a ratio of 1.13 in the incubation medium. The slight difference has been demonstrated to be caused by loss of intracellular ^{3}H as $^{3}H_2O$ due to glucose metabolism (Shoaf, C.R., Heizer, W.D. and Caplow, M., unpublished observations).

Discussion

Several reported experiments have shown that intestinal uptake of amino acids is more rapid from the dipeptides than from comparable equimolar concentrations of the constituent amino acids [6]. These observations require that there be separate uptake systems for amino acids and dipeptides, for if the dipeptide were first hydrolyzed and the amino acid product subsequently taken up by the amino acid systems, the rate of uptake from the dipeptide could not exceed uptake from the amino acid mixture. However, we observed that phenylalanine is less rapidly transported from Phe-Phe than from free phenylalanine (Table I). Thus, a single system for uptake of free phenylalanine, and uptake of phenylalanine released from Phe-Phe cannot be excluded on this basis, and neither can absorption of Phe-Phe by a dipeptide system separate

from the free amino acid system and of lower velocity.

A comparison of amino acid and dipeptide absorption was also made by studying their Na^+ dependence. Rubino et al. [5] showed that when the Na^+ concentration was reduced from 140 to 40 mM, the K_t of free glycine transport by rat intestine increased and V was unchanged. On the other hand, the K_t for glycine transport from Gly-Pro was unchanged while V was decreased in the reduced Na^+ medium, indicating separate transport mechanisms for glycine and Gly-Pro. Our results are similar and show that when Na^+ was reduced K_t changed and V remained the same for phenylalanine absorption while V changed and K_t remained the same for Phe-Phe absorption. Since a decreased Na^+ concentration has different effects on transport of phenylalanine and Phe-Phe, we conclude that neither Phe-Phe nor its constituent amino acids are taken up by the Na^+-coupled system responsible for uptake of free phenylalanine.

To date, published experiments do not indicate unequivocally how the uptake system for Phe-Phe and similar dipeptides operates. One possibility is that the dipeptide is transported intact through the brush border membrane and hydrolyzed intracellularly. Alternatively, Phe-Phe may be taken up by the brush border and hydrolyzed by membrane peptide hydrolases during the process of transport with release of free phenylalanine in or on the membrane. In the latter case several mechanisms are possible including: (1) the peptide hydrolase may function both as the hydrolytic catalyst and as the transport protein as postulated for sucrase by Storelli et al. [30] (mechanism A of Matthews [6] or Kim [7]); or (2) the peptide hydrolase may act as the hydrolytic catalyst for the dipeptide where the amino acids components are released to transmembrane carriers different from those utilized by the transport system for free amino acids (mechanism B of Matthews [6]).

If hydrolysis of Phe-Phe occurs in the membrane, most of the resulting free phenylalanine must be released on the cytosol side of a membrane diffusion barrier since release into the lumen with subsequent uptake is not compatible with our observed effect of NaCl on transport. Also, the free phenylalanine in the incubation medium corresponded to hydrolysis of less than 5% of the Phe-Phe present in the incubation medium, which suggests that the extent of surface hydrolysis of Phe-Phe followed by release into the medium or of back diffusion of free phenylalanine is relatively small. However, it should be noted that the amount of free phenylalanine released into the medium is significant when compared to the quantity of Phe-Phe absorbed, which was approx. 3% of the total present in the medium.

The double-label experiment was designed to determine whether the phenylalanine taken up by the tissue was derived disproportionately from the carboxyl or amino terminal moieties of Phe-Phe. The finding that the amino and carboxyl components of Phe-Phe are transported at identical rates (Table II) indicates that the uptake mechanism must involve either intact transport, followed by intracellular hydrolysis, or hydrolysis in the brush broder membrane with release of both components on the cytosol side of a diffusion barrier.

Prior to conducting double-label experiments with maltose similar to those described for Phe-Phe, it was important to establish whether the glucose released during intestinal digestion-absorption of maltose is or is not accessible

to glucose oxidase in the medium. Our observation that pure glucose oxidase does not inhibit maltose absorption confirms the results of Miller and Crane [28] and provides evidence that the liberated glucose is not released into the luminal medium and that maltose hydrolysis occurs at a location in the membrane that is inaccessable to luminal glucose oxidase. Results of the double-label maltose experiments, in which the ^{3}H : ^{14}C intracellular ratio is equal to that in the incubation medium indicate that both glucose moieties of maltose are released on the cytosol side of a brush border diffusion barrier for glucose.

In this investigation we have compared the rates of absorption of the constituents of a symmetrical dipeptide, Phe-Phe, and a symmetrical disaccharide, maltose. Surface hydrolysis of the dipeptide or disaccharide followed by transport of the hydrolysis products has been shown to be insignificant. The hydrolysis products from both the dipeptide and disaccharide are released from the hydrolyzing enzyme at a location that is on the cytosol side of a membrane diffusion barrier. The release site could be in the membrane or in the cytosol. For maltose, the hydrolyzing enzyme is known to be primarily in the brush border membrane, as there is little activity elsewhere in the cell. For Phe-Phe, hydrolyzing activity is present in the membrane and in the cytosol, and these results are equally compatible with hydrolysis at either site.

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