Peptide Absorption: A Review of Current Concepts and Future Perspectives

K. E. Webb, Jr., J. C. Matthews, and D. B. DiRienzo

Department of Animal Science, Virginia Polytechnic Institute and State University, Blacksburg 24061-0306

ABSTRACT: Absorption of di- and tripeptides from the gastrointestinal tract is accepted as being an important biological phenomenon. The extent to which peptides are absorbed and the nutritional and metabolic significance of peptide absorption remain unclear. Evidence is strong for the existence of multiple peptide transport systems, including one type that is electrogenic in nature and that requires a protonmotive force and cotransports two \( H^+ \) for every peptide transported. The rate of absorption of peptides can be responsive to level of dietary intake and level of dietary protein. Peptide absorption seems to be an important physiological process in ruminants, and this process may account for a large portion of absorbed amino acids. An important new observation is that the nonmesenteric portion of the portal-drained viscera of the ruminant is a major site of peptide absorption. These new observations may result in a reshaping of the currently accepted theory concerning protein utilization by ruminants.

Key Words: Peptides, Transport, Absorption, Stomach, Small Intestine, Ruminants

Introduction

The concept that small peptides may constitute an important form in which amino acids are absorbed from the gastrointestinal tract is somewhat new among persons involved in livestock production. Among many researchers actively engaged in the study of absorption of protein digestion products, however, the concept that peptides may constitute another form of amino acid absorption has been investigated for some time. For readers interested in exploring this subject to a greater depth, the following recent reviews are suggested: Adibi and Kim, 1981; Smith, 1983; Gardner, 1984; Bender, 1985; Ganapathy and Leibach, 1985; Hoshi, 1985; Silk et al., 1985; Webb, 1986, 1990; Adibi, 1989; Grimble and Silk, 1989; Matthews, 1991a; Webb and Bergman, 1991.

There is little information available about peptide absorption in ruminants in comparison to rats, hamsters, guinea pigs, rabbits, and humans. The purpose of this review is to focus on certain aspects of peptide transport that have been reported, primarily in laboratory species, and to relate these to potential nutritional significance in the ruminant. Further, this review will focus on studies with ruminants that indirectly support the concept of peptide absorption. Finally, some very recent observations from the laboratory of the authors will be highlighted. A companion review also may be of interest (Webb et al., 1982). Unless otherwise defined, the term peptide as used in this review is to be interpreted to mean very small peptides, usually di- or tripeptides.

Characteristics of Carrier-Mediated Peptide Transport

Several models for the absorption of peptides into epithelial cells have been proposed. Because it is likely that the physiological phenomenon of peptide absorption occurs by a combination of events, any model must account for both lumenal...
and cytosolic events. Peptides subjected to luminal hydrolysis are transported as free amino acids after cleavage, whereas peptides resistant to luminal hydrolysis are transported across the enterocytes in the intact state and, subsequent to transport, are hydrolyzed by cytosolic peptidases. The combination of these separate and uncoordinated (as currently understood) processes has been suggested by Matthews (1975) and Ganapathy and Leibach (1982) as being the most viable model due to its relative simplicity and because it "accounts for all the distinctive features of peptide absorption." The model also allows for a high level of cytosolic peptidase activity, which is necessary to maintain a downhill gradient for any diffusion of peptides that might occur.

Research to characterize the uptake of intact peptides by cell membrane permeases has indicated that peptides are transported as zwitterions, that transport is not Na+-dependent, that transport requires an energizing or driving force (most commonly H+ that are symported with peptides into the cell), that transport is an energy-requiring process that ultimately requires the expenditure of ATP, and that the presence of an acidic microenvironment seems to be necessary for most peptide transport.

Although it is now generally accepted that peptide transport does not occur coupled with Na+, early studies employing whole intestinal tissue (Rubino et al., 1971; Shoaf et al., 1980) showed that the removal of Na+ from the incubation medium resulted in the reduction of peptide uptake. The use of hydrolysis-resistant peptides and the development of brush-border membrane vesicle (BBMV) techniques have enhanced the characterization of peptide transport by eliminating the confounding effects of peptide hydrolysis by cytosolic or brush-border (if papain-treated) peptidases on whole-tissue studies. The preponderance of experiments employing hydrolysis-resistant peptides and the development of brush-border membrane vesicle (BBMV) techniques have enhanced the characterization of peptide transport by eliminating the confounding effects of peptide hydrolysis by cytosolic or brush-border (if papain-treated) peptidases on whole-tissue studies. The preponderance of experiments employing hydrolysis-resistant peptides and the development of brush-border membrane vesicle (BBMV) techniques have enhanced the characterization of peptide transport by eliminating the confounding effects of peptide hydrolysis by cytosolic or brush-border (if papain-treated) peptidases on whole-tissue studies.

The stimulation of peptide transport by an inwardly directed H+ gradient (suggesting H+-coupled transport) was first demonstrated in intestinal epithelial BBMV (Ganapathy and Leibach, 1983) and later in renal BBMV (Ganapathy et al., 1984; Takuwa et al., 1985). The results of these investigations indicated that pH gradients stimulate peptide uptake, that an inward directed proton flow increases peptide uptake even in the absence of Na+, and that peptide transport is an electrogenic process dependent on a transmembrane electrical potential. This last conclusion is supported by research conducted by Boyd and Ward (1982) that showed that an increase in intracellular peptide concentration was accompanied by an increase in membrane depolarization. The observation is consistent with the hypothesized H+/peptide cotransporter model, which states that when positively charged protons cross a relatively negatively charged membrane region, the difference in electrical charges is reduced (depolarized).

The evidence is conflicting as to whether peptide transport is actually energized, or driven, by a H+ gradient. This is due to the lack of evidence for the ability of a H+ gradient to drive the uphill transport of peptides from the relatively low concentration (extravesicular) region into the higher concentration (intracellular) region. This ability, or lack of it, seems to be species-specific and not universal to all mediated transport systems for peptides. The ability of a H+ gradient to drive the uphill transport of peptides has been demonstrated in rabbit (Ganapathy and Leibach, 1983; Hoshi, 1985), rat (Said et al., 1988; Iseki et al., 1989), and guinea pig (Himukai et al., 1983) using BBMV. Uptake studies characterizing dipeptide transport in rabbit, rat, and human intestinal BBMV (Rajendran et al., 1987) and tripeptide transport in human jejunal BBMV (Wilson et al., 1989) indicate that these tissues lack the ability to concentrate peptides against a concentration gradient in the presence of a H+ gradient. Additionally, the glutathione (GSH) transport in rabbit BBMV has been characterized as being pH-dependent with optimal transport reported at pH 7.5 and as being energized by mono- and divalent cations, especially Ca++, but not H+ (Vincenzini et al., 1989).

These conflicting data seem to suggest the existence of at least two classes of carrier-mediated peptide transporters, both of which are dependent on transmembrane electrogenic potential: one class that is energized by a H+ gradient and a second class that is energized by cations other than H+ or Na+. Based on an interpretation of the results of Rubino et al. (1971) and evidence from Ganapathy et al. (1985) and their own cephalosporin uptake experiments, Hori and colleagues (Inui et al., 1988; Kato et al., 1989) have proposed the existence of multiple dipeptide systems in rabbit BBMV. They hypothesize the existence of both an acidic pH-prefering class (uptake driven by an inward H+) and a neutral pH-prefering class (no inward H+ gradient) of peptide transporters.

A transporter for the tripeptide GSH has been characterized in rabbit intestinal tissue using
BBMV (Vincenzini et al., 1988, 1989). This transporter is unique in its ability to be maximally stimulated by divalent cations (especially Ca\(^{2+}\)) and the inability of other peptides to competitively inhibit GSH uptake.

Hypotheses have been proposed for models of peptide transport that include the integrated but separate functioning of H\(^{+}\)/peptide symporters and Na\(^{+}\)/H\(^{+}\) exchangers (Ganapathy and Leibach, 1985; Hoshi, 1985), possibly ATP-driven H\(^{+}\) transporters (Hoshi, 1986), and Na\(^{+}\)/K\(^{+}\) ATPases. In these models, an existing membrane potential would drive two protons (Hoshi, 1986) across the brush-border membrane with one peptide. As the intracellular pH drops, the Na\(^{+}\)/H\(^{+}\) exchanger would be stimulated to exchange an intracellular H\(^{+}\) for an extracellular Na\(^{+}\). Thus, both the intracellular pH and the transmembrane electrical potential would be restored to basal levels. The Na\(^{+}\)/K\(^{+}\) ATPases of the basolateral membrane would then pump the transported Na\(^{+}\) out of the cell, thereby reestablishing the high extracellular Na\(^{+}\) gradient, at the cost of ATP hydrolysis.

The above discussion, at least for the H\(^{+}\)/peptide transporter, requires that a pH gradient exist across the apical border of intestinal enterocytes separating the lumen from the cell cytosol in order for peptide transport to occur. Additionally, the enterocytes require the biological mechanisms necessary to maintain normal cytosolic pH when protons are transported into the cell along with the peptides and to regenerate the inwardly-directed pH gradient. The presence of a pH gradient of a 1-log magnitude has been measured in rat proximal jejunal tissue (Lucas, 1983). Exchangers of Na\(^{+}\)/H\(^{+}\) that function to pump H\(^{+}\) out of the cell in exchange for Na\(^{+}\) in the presence of a high intravesicular proton concentration have been shown to exist in intestinal brush-border membranes in the rat (Murer et al., 1976), rabbit (Knickelbein et al., 1983), and human (Kikuchi et al., 1988). That peptide uptake stimulated Na\(^{+}\) uptake, but that Na\(^{+}\) failed to stimulate peptide uptake, provides evidence for the coordinated functioning of the H\(^{+}\)/peptide symporter and the H\(^{+}\)/Na\(^{+}\) antiporter (Himukai et al., 1983; Cheese-man and Devlin, 1985).

Recently, attempts have been made to characterize the chemical structure of peptide transporters with particular emphasis on the chemical groups responsible for substrate binding. Using photoaffinity labeling techniques, Kramer et al. (1988) have identified a 127-kDa putative binding-protein constituent of the carnosine and glycylproline transporter in rabbit intestinal BBMV that is H\(^{+}\)-dependent. In the same tissue, Kato et al. (1989) suggest that histidine residues in the transporter are essential for H\(^{+}\)-coupled peptide transport because of their role as peptide binding sites under acidic conditions (pH 6.5). These researchers also report that their investigations suggest that thiol and sulfhydryl groups on the transporter are not essential for peptide binding.

In contrast, Miyamoto et al. (1986, 1989) reported that maximal peptide transport in rabbit renal cortex BBMV requires the reduction of constitutive dithiol groups present on the transporter at or near the peptide binding site. These authors suggested that an interchange between dithiol and sulfhydryl groups may catalyze and/or regulate the functioning of the renal peptide transporter.

Although the actual isolation of peptide transporters has not been reported, the transporter responsible for glycylproline and glycylsarcosine transport recently has been expression-cloned in *Xenopus laevis* oocytes (Miyamoto et al., 1991). These researchers observed a threefold increase in the ability of the oocytes to take up these peptides, but not the constitutive amino acids (glycine or sarcosine), following microinjection of rabbit intestinal mucosal cell poly(A\(^{+}\)) mRNA. This uptake was determined to be H\(^{+}\)-dependent.

It is now well established that peptide transport does not occur by Na\(^{+}\)-coupled transport. Instead, there is a large body of evidence indicating that peptides are cotransported with one type of symporter that is electrogenic in nature, that requires a protonmotive force, and that cotransports two H\(^{+}\) for every peptide translocated into epithelial cells. It would seem that conditions within the gastrointestinal tract of ruminants are highly favorable for the existence of this type of peptide transporter. It is not unusual for the pH of the digesta to remain acidic throughout the first one-half to two-thirds of the length of the small intestine in the ruminant (Ben-Ghedalia et al., 1974). Also, the digesta within the ruminant stomach are essentially always at least slightly acidic in nature.

Evidence also continues to accumulate for the existence of a second peptide transporter that is pH-dependent but that is not energized by H\(^{+}\) or Na\(^{+}\) (Kato et al., 1989). Additionally, the GSH tripeptide transporter seems to represent a third type of transporter that apparently transports only GSH (Vincenzini et al., 1988, 1989). The physiological significance of these transport types is unknown, but one may speculate that the H\(^{+}\)-energized transporter would function and be found to be more proximally located in the intestinal tract than the hypothesized pH-neutral transporter or the GSH transporter. In addition, one may also speculate that the proposed GSH transporter has complete substrate specificity for GSH because of the important antioxidant function of GSH in membranes.
Adaptive Responses in the Absorption of Peptides

Digestion of proteins and absorption of digestion products are complex processes that may be controlled, or at least influenced, by such factors as diet composition, level of intake, production state, age of the animal, administration of metabolism modifiers, and numerous other factors, some of which may as of yet be undefined. Interestingly, alterations in the absorptive responses of amino acid and peptides in reaction to many of these factors are not the same and sometimes are totally different.

Long-term restriction of dietary nutrients (50% of ad libitum) resulted in an increased ability of rat jejunal tissue to absorb both L-methionine and L-methionyl-L-methionine (Lis et al., 1972a). Similar results were obtained when comparing an amino acid mixture simulating casein and a pancreatic partial hydrolysate of casein: dietary restriction resulted in an increased ability of rat intestinal tissues to absorb both substrates (Lis et al., 1972b). Fasting rats for 15 h resulted in a doubling of the appearance rate of \(^{14}\)C in the portal blood from \([14\text{C}]\text{glycylglycine}\) infused into the duodenum (Gallo-Torres and Ludorf, 1973).

Conversely, results from some studies show that dietary restriction results in a diminished ability of intestinal tissues from hamsters to absorb amino acids and peptides. The ability of intestinal tissues to absorb glycylsarcosine and L-leucine was decreased over a wide range of concentrations when dietary restriction of 50 and 100% were imposed (Schedl et al., 1979). Absorption of glycylsarcosine was more severely decreased than absorption of L-leucine. The responses between the two levels of restriction were similar. There was a decrease in \(V_{\text{max}}\) for L-leucine transport and a larger decrease for glycylsarcosine. Decreases in \(V_{\text{max}}\) may be explained by a reduction in the number of transport sites, and this may be associated with a reduced protein synthesis caused by dietary restriction. Dietary restriction did not alter the \(K_{\text{m}}\) for L-leucine uptake, but, for glycylsarcosine, the \(K_{\text{m}}\) was increased nearly threefold. This marked decrease in the affinity of the transporter(s) for glycylsarcosine suggests that dietary restriction altered the transporter(s) at the molecular level, such as by modifying the binding site(s). Glycine and glycylglycylglycine absorption from the jejunum of humans starved for 2 wk were decreased (Vazquez et al., 1985). Even though absorption of both the free amino acid and the tripeptide was decreased by starvation, total glycine absorption was greater with the tripeptide. The authors suggest that peptides may better meet the needs for amino acids during such periods of deprivation.

Responses to dietary protein levels have been shown to be equally as variable in influencing absorption of protein digestion products as has been the response to overall dietary intake. Extended feeding (40 to 84 d) of a protein-free diet resulted in a decreased ability of rat jejunal tissues to absorb methionine, whereas the ability to absorb L-methionyl-L-methionine was increased (Lis et al., 1972a). Feeding restricted amounts of protein for the same time period had little effect on the ability to absorb either methionine or L-methionyl-L-methionine. Protein deprivation resulted in a decreased ability of intestinal tissues of rats to absorb free amino acids from a mixture, whereas the ability to absorb peptides from a mixture changed very little (Lis et al., 1972b). Feeding a high-protein diet (72 vs 18%) to mice resulted in an increased ability of intestinal tissues to absorb the dipeptide carnosine (Ferraris et al., 1988).

Absorption of leucine was decreased after feeding a low-protein (5%) diet to rats for 1 wk, and after 3 wk absorption was decreased even further (Table 1; Cheeseman, 1986). After feeding the low-protein diet for 10 wk, leucine uptake had returned to control levels. Lysine absorption also was decreased by feeding the low-protein diet, but unlike with leucine, the decrease in absorption still existed after 10 wk. The response of the peptide absorptive mechanisms seemed to be quite different. Glycyl-L-leucine absorption was increased after 1 wk, and after 3 wk the increase was nearly threefold the rate in the controls. At 10 wk the effect was far less pronounced, even though absorption was still elevated. These results clearly point out that when the effects of protein restriction (and likely dietary restriction as well) are studied, length of restriction period and different substrates may influence the results obtained. Furthermore, the diet fed to an animal during growth and development may exert some general effects on intestinal transport in the adult (Karasov et al., 1985). These effects may be mostly due to differences in body weight and gut size rather than to specific irreversible effects on specific transport systems.

Table 1. Uptake of 10 mM L-leucine, L-lysine, or glycyl-L-leucine by jejunal epithelial mucosa from rats fed a 23 or 5% protein diet\(^a\)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>23% Protein control</th>
<th>5% Protein</th>
<th>1 wk</th>
<th>3 wk</th>
<th>10 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leucine</td>
<td></td>
<td></td>
<td>51</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>L-Lysine</td>
<td></td>
<td></td>
<td>51</td>
<td>25</td>
<td>41</td>
</tr>
<tr>
<td>Glycyl-L-leucine</td>
<td></td>
<td></td>
<td>15</td>
<td>24</td>
<td>44</td>
</tr>
</tbody>
</table>

\(\text{From Cheeseman, 1986.}\)
Whether these or similar processes occur in the ruminant is not known. It would be of interest to investigate adaptive mechanisms in the ruminant gastrointestinal tract, particularly as this may relate to meeting amino acid needs through amino acid and peptide absorption during such critical periods as lactation or during increased performance as influenced by the administration of agents such as anabolic steroids, bovine somatotropin, and β-agonists. As presented in several reviews, metabolic changes that may influence efficiency of protein utilization are known or suspected to accompany the use of these agents (Boyd et al., 1991; Breier et al., 1991; McDowell, 1991; McDowell and Annison, 1991).

Portal/Mesenteric Appearance of Amino Acids and Peptides in Ruminants

Efforts to determine the quantities of amino acids being absorbed from the gastrointestinal tract have been numerous and have involved the application of in vitro, in situ, and in vivo techniques. The results obtained, or not obtained, with each technique employed must be interpreted with full awareness of what is being measured. We now know that previous assumptions that amino acids are absorbed exclusively in the free form are not correct. These assumptions, however, dictated the nature of the development of biological and analytical approaches for quantifying amino acid absorption.

The lack of convenient analytical methods is a hindrance to furthering our knowledge of peptide absorption. Chromatographic separation followed by quantification of 40 to 60 free amino acids can be done routinely, but not without a certain degree of difficulty. Separating and quantifying the large number of possible di- and tripeptides (8,400 if we assume 20 amino acids are involved) looms as a task that will challenge even the best chromatographers. It seems likely, therefore, that even with the obvious limitations of compounded errors and not knowing exactly what is being measured the "difference method" of measuring peptide amino acids, which involves measuring amino acids before and after acid or proteolytic hydrolysis, will continue to be used pending development of more satisfactory methods.

Several investigators have attempted to quantify amino acid absorption by estimating the amounts of individual amino acids or α-amino N arriving in the portal circulation or ruminants. Generally, these procedures have involved some variation of measuring anteriovenous concentration changes and blood flow through the portal-drained viscera. A common observation from these studies, though not necessarily pointed out as such by all authors, was that the quantity of free amino acids appearing in the portal circulation was only a fraction of the quantity that was contained in the diet.

Chopped alfalfa was fed to sheep, and the amino acids that appeared in the portal circulation amounted to < 40% of crude protein intake (Hume et al., 1972). They reported that absorption of NH₃ N accounted for approximately 20% of N intake, and they calculated that fecal excretion accounted for approximately 30% of N intake. Amino acids appearing in the portal circulation accounted for only 26.1% of N intake in sheep fed alfalfa pellets (Wolfe et al., 1972). Ammonia N appearance was equivalent to approximately 48% of N intake.

Individual amino acids disappearing from the small intestine (pyloric-ileal differences) and portal appearance of amino acids were measured in sheep (Tagari and Bergman, 1978). They reported that only 30 to 80% of individual amino acids disappearing from the intestinal lumen could be accounted for in the portal plasma. Calculations derived from their data show that an equivalent of approximately 13 and 18% of the intake N from a medium- and high-protein diet, respectively, appeared in the portal circulation as amino acids. Appearance of NH₃ N for these two diets was 83 and 44% of intake N.

In the same laboratory, portal appearance of amino acids was studied under a variety of metabolic conditions (Heitmann and Bergman, 1980). The authors did not report portal appearance of amino acids with reference to dietary intake, but from the data they presented it can be calculated that an equivalent of only 12% of dietary crude protein appeared as amino acids in portal plasma. The authors suggested that their plasma data may underestimate total appearance in the blood by approximately 22%, because the concentration of amino acids contained in blood cells was not included. This would increase the appearance to approximately 15.4%.

Amino acid absorption was compared in cattle and sheep fed either alfalfa hay or a concentrate (78% corn) diet (Prior et al., 1981). The amounts of essential amino acids required to meet the needs for whole-body protein synthesis in both sheep and cattle were calculated. These researchers concluded that the amino acids appearing in the portal vein could meet only a fraction of the needs for essential amino acids. From their data, it can be calculated than an equivalent of approximately 15 to 35% of crude protein intake was represented by amino acids appearing in the portal blood. Net absorption of amino acids was only 31 and 88% of crude protein intake, respectively, for steers fed
diets composed of grass hay and grass hay plus concentrate (Seal et al., 1992).

Reports in recent years of \( \alpha \)-amino N appearance in the portal circulation largely confirm the results of previous reports in which free amino acids were measured. The amount of \( \alpha \)-amino N appearing in the portal blood expressed as a percentage of intake N was only approximately 36% in lactating cows (Huntington, 1984; Reynolds et al., 1988), 20% in steers fed alfalfa, and 49% in steers fed concentrate (Reynolds and Huntington, 1988), 43% in sheep fed alfalfa (Gross et al., 1990), 24% in steers fed a concentrate diet (Guerino et al., 1991), and 50 to 60% in lambs fed a concentrate diet to appetite or at maintenance (Burrin et al., 1991).

Contrary to the studies already cited, Sniffen and Jacobson (1975) reported that the net absorption of amino acids was greater than the amount consumed. When good-quality alfalfa (18.8% CP) was fed to steers, they found that nearly twice the quantity of amino acids consumed appeared in portal blood. However, only 61% of the quantity of amino acids consumed was accounted for as amino acids appearing in the portal blood when poor-quality alfalfa (13.9% CP) was fed. If the data that are presented are used to calculate amino acid appearance in portal blood expressed on the basis of crude protein intake, values of 133 and 40% are obtained for the good-quality and poor-quality alfalfas, respectively. Corresponding values calculated for \( NH_3 \) N appearance are 9.6 and 18.4%, respectively, for the good-quality and poor-quality alfalfas. Huntington and Prior (1985) calculated amino acid intake of heifers fed a pelleted concentrate diet and measured portal appearance of amino acids. They reported that portal appearance of essential amino acids ranged from –2 to 113% and averaged 62.7% of amino acid intake.

It is obvious from these reports that absorbed free amino acids constitute a variable proportion of amino acid intake. It would not necessarily be expected that all amino acids that are consumed are absorbed, but, often, when other components such as \( NH_3 \) N, nucleic acid N, and undigested N are considered as well, the balance does not always tally. Additionally, contributions of recycled N and endogenous N to net absorption usually are not quantified. It may be possible that some of the discrepancy would be accounted for if the potential contribution of absorbed peptides is considered. Matthews (1991a) defined the situation fairly well in a recent review when he suggested that "if we continue to look only for free amino acids, we shall find only free amino acids: peptides cannot be expected to declare their presence." We are aware of work from only two laboratories in which estimations have been made of peptide appearances in portal or mesenteric blood. We reported that approximately 79% of the amino acids appearing in portal blood of calves were associated with peptides (Koeln and Webb, 1982). The size of a high proportion of these peptides was estimated to be < 300 Da (Schlagheck and Webb, 1984). Read (1988) observed a large mesenteric appearance of peptides in sheep fed perennial ryegrass silage either alone or supplemented with fish meal. Seal and Parker (1992) used a reverse-phase HPLC procedure to partially separate the circulating, low-molecular-weight peptides in portal and peripheral blood of steers, sheep, and rats. In contrast to our work, they reported for steers and sheep that small peptides were not the most prevalent form, but rather that the major component of the peptide mixture in plasma was composed of pentapeptides and above. Net portal appearance of peptide amino acids varied with species and relative size of the peptides. Overall, they reported a net negative portal appearance of peptide amino acids in steers and sheep and a positive portal appearance in rats. The high level of larger peptides they observed likely were not the result of absorption because it is thought, as discussed earlier, that absorption of peptides larger than tripeptides is minimal. Also, although they report a negative portal appearance of peptides, the chromatogram they present in the article indicate a positive portal appearance of small peptides in steers.

Recently, we have completed additional studies that substantiate and further define the nature of this peptide appearance. We have quantified the in vivo flux of both free and peptide amino acids across the mesenteric and nonmesenteric portions of the portal-drained viscera of both calves and sheep (DiRienzo, 1990). Six wethers (38 kg) and three Holstein steer calves (108 kg) had sampling catheters implanted in the abdominal aorta, portal vein, and mesenteric vein prior to its convergence with the splenic vein. Mesenteric and portal blood flows were determined by measuring the dilution of \( p \)-aminohippuric acid that was infused constantly into a distal mesenteric vein. Nonmesenteric blood flow was calculated as the difference between portal and mesenteric blood flow.

The wethers and steers were fed quantities of diet to support gains of 150 and 900 g/d, respectively. The diet was composed of 30% orchardgrass hay, 50% ground corn grain, 13.3% soybean meal, 5% molasses, and 1.7% mineral supplements. Blood plasma was obtained from blood collected during a 1-h feeding period from the aorta, mesenteric vein, and portal vein. The plasma was deproteinized and analyzed for free amino acids. An aliquot of deproteinized plasma
Table 2. Mesenteric and nonmesenteric fluxes of free and peptide amino acids in sheep and calves

<table>
<thead>
<tr>
<th>Item</th>
<th>Animal&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mesenteric flux</th>
<th>Nonmesenteric flux</th>
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<tr>
<td></td>
<td></td>
<td>Free</td>
<td>Peptide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g/d</td>
<td></td>
</tr>
<tr>
<td>Essential amino acids</td>
<td>Sheep</td>
<td>18.30</td>
<td>26.20</td>
</tr>
<tr>
<td>Nonessential amino</td>
<td>Sheep</td>
<td>18.44</td>
<td>25.81</td>
</tr>
<tr>
<td>acids</td>
<td>Calves</td>
<td>38.70</td>
<td>35.96</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are from six crossbred wethers and three Holstein steer calves.

<sup>b</sup>Probability that difference this large or larger between free and peptide amino acids could have occurred by chance.

was hydrolyzed in HCl and analyzed for total amino acids. Peptide amino acids were calculated as the differences between total and free amino acids. The flux of free and peptide amino acids across the mesenteric- and nonmesenteric-drained viscers were calculated as the product of venoarterial differences and blood flow.

Drainage into the mesenteric vein is from the jejunum, ileum, cecum, colon, and pancreas, whereas the nonmesenteric drainage comes from the rumen, reticulum, omasum, abomasum, duodenum, and spleen. Presented in Table 2 are the fluxes of free and peptide amino acids across the mesenteric- and nonmesenteric-drained viscers. In both species, the mesenteric flux of free and peptide amino acids was approximately equal. As anticipated, the flux of free amino acids across the nonmesenteric-drained viscers was minimal. Particularly noteworthy is the observation that the flux of peptide amino acids across the nonmesenteric-drained viscers accounted for a large portion of total portal-drained viscers amino acid flux in both calves and sheep.

The ability for absorption of peptides to occur across the mucosal tissues of the rumen and omasum has been confirmed with in vitro procedures employing both radiolabeled and nonradiolabeled peptides (Matthews, 1991b). Rumenal and omasal epithelia collected from four and seven sheep were used to study absorption of carnosine and methionylglycine, respectively. The isolated epithelia were placed in parabiotic chambers with the mucosal surface exposed to a buffered solution (pH 6.0) containing various concentrations of the respective dipeptide and the serosal surface was exposed to a buffered solution (pH 7.4) with no peptide. The chambers were continuously oxygenated and incubated at 39°C. Samples of chamber buffers were taken at regular intervals for 240 min. Carnosine was quantified by HPLC analysis. Methionylglycine was quantified using <sup>35</sup>S-methionylglycine as a representative marker by liquid scintillation counting of <sup>35</sup>S. Because the effect of time on the appearance of peptides in the serosal buffer solutions was linear, the data presented in Tables 3 and 4 are for the 60-min sampling only. For both peptides, there was a linear increase in serosal appearance as concentration increased. That peptide appearance was an apparently nonsaturable process suggests that absorption occurred by diffusion and not by carrier-mediated transport. Omasal epithelia seemed to have a greater ability than ruminal epithelia to absorb the peptides evaluated, when peptide uptake was expressed on an equal mucosal tissue weight basis.

If subsequent studies continue to substantiate our current observations about peptide absorption from the rumen and omasum, then a serious reshaping of the currently accepted tenet concerning protein utilization by the ruminant will be necessary. The concept that the small intestine is the only site of amino acid absorption will be changed and efforts to influence dietary protein passage through the stomach will need to be reevaluated. If the stomach is confirmed as the site of absorption of a major portion of total amino acids in the form of peptides, this will explain many of the unpredictable responses observed with different sources of dietary proteins. Further experimentation to examine these observations is imperative.

Table 3. Influence of various concentrations of carnosine on the mucosal side of sheep ruminal and omasal epithelia on carnosine appearance on the serosal side of these tissues after 60 minutes of in vitro incubation

<table>
<thead>
<tr>
<th>Tissue&lt;sup&gt;bc&lt;/sup&gt;</th>
<th>Concentration, mmol/L&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>nmol/mg of dry tissue</td>
</tr>
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<table>
<thead>
<tr>
<th>Tissue&lt;sup&gt;bc&lt;/sup&gt;</th>
<th>Concentration, mmol/L&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Rumen</td>
<td>.09</td>
</tr>
<tr>
<td>Omasum</td>
<td>4.48</td>
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</tbody>
</table>

<sup>a</sup>Linear concentration effect (P < .02).

<sup>b</sup>Tissues differed (P < .01).

<sup>c</sup>Tissue × concentration interaction (P < .06).


