FREE AND PEPTIDE AMINO ACID FLUXES ACROSS THE MESENTERIC AND NON-MESENTERIC VISCERA OF SHEEP AND CALVES

BY

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THE CONTRIBUTION OF FREE AND PEPTIDE AMINO ACIDS BY THE STOMACH AND INTESTINAL VISCERA TO THE PORTAL SYSTEM OF SHEEP AND CALVES

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(ABSTRACT)

The flux of free and peptide amino acids across the stomach and intestinal tissues was quantified using sheep and calves maintained in "steady state" conditions by feeding at hourly intervals. Crossbred wethers and Holstein steers were surgically cannulated in the abdominal aorta, mesenteric vein and portal vein. All animals were fed an orchardgrass, corn, SBM-based diet. The steers received three abomasal infusions; a control solution, and an amino acid mixture simulating casein and casein each at a rate equivalent to 25% of daily crude protein intake. Nutrient fluxes from the mesenteric and portal-drained viscera were measured; non-mesenteric flux was calculated as the difference between portal flux and mesenteric flux. Results of this study support the concept that free amino acids are absorbed by the small intestine and not by the stomach. The flux of peptide amino acids across the portal-drained-viscera indicate that a major portion of the amino acids which are absorbed by cattle and sheep are absorbed in the form of peptides from the stomach. The observation that large quantities of peptide amino acids are absorbed from the stomach is unique and it is expected that this most important discovery will revolutionize the feeding of ruminants.

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Chapter I

Introduction

The belief that proteins must be completely hydrolyzed to amino acids prior to absorption went unchallenged for many years. Because of this "classic hypothesis" the mechanism of uptake of free amino acids has received much study over the course of this century, while the possibility of the absorption of other products of protein digestion was largely ignored. Over the past 30 yr this concept of protein digestion has been laid aside. Today scientists generally accept the hypothesis that "under normal circumstances the dietary proteins are almost completely digested to their constitute amino acids and these end products are then rapidly absorbed from the small intestine into the portal blood". This hypothesis allows the concept that peptides as well as free amino acids may be absorbed by the intestine. However, "even in the 1980's, there still appears to be a tendency for one or two reviewers to ignore intestinal transport of peptides as far as possible, as if hoping that this might make it go away" (Matthews, 1987).

Questions on the mechanism of peptide transport, magnitude of peptide versus free amino acid absorption, absorption of intact peptides and the species differences in absorption remain to be answered. It also must be recognized that many small peptides possess biological activities that may be exerted in peripheral tissues. Small peptides may act as neurotransmitters and as integrators between the neural and endocrine regulatory systems. Thus, even if it does become clear that nutritionally significant quantities of amino acids do not enter the circulation in forms larger than free amino acids, the possibility that even trace quantities of biologically active material can enter the circulation needs careful scrutiny, especially

in the light of the high biological potency of many known peptide hormones and other biologically active peptides (Gardner, 1984).

Chapter II

Literature Review

Gastrointestinal Blood Flow

The gastrointestinal tract receives a substantial portion of cardiac output. Hales (1973) estimated blood flows in the resting sheep for rumen, small intestine and large intestine to be about 10, 12 and 11% of cardiac output. Many factors, including stress, environment, and production status of the animal may play a role in altering blood flow to the intestine. The increase in blood flow to the gastrointestinal tract following a meal has been termed postprandial hyperemia and may be of special interest to the nutritionist.

Regulation of Blood Flow During Postprandial Hyperemia. In dogs the shunting of blood flow to the gastrointestinal tract appears to be biphasic in nature. Initially a short, transient increase is noted due to the anticipation and consumption of food suggesting input from the sympathetic nervous system. During this time there is an increase in cardiac output, heart rate and arterial pressure (Fronek and Stahlgren, 1968; Vatner et al., 1970ab, 1974). Thus, blood flow is increased to all parts of the animal. Contraction of the spleen, at this time, may elevate the number of red blood cells and increase circulating blood volume thus increasing the oxygen carrying capacity of the blood. The spleen has been estimated to contain up to one-seventh of the total blood volume and a quarter of the total red blood cells in sheep (Turner and Hidgetts, 1959). Shortly after a meal, blood flow returns to resting

levels. A second, more pronounced increase in blood flow to the gastrointestinal tract follows. Unlike the initial increase in blood flow, no increase in cardiac output is noted. This results in an increase in the percentage of cardiac output directed to the gastrointestinal tract (Fronek and Stahlgren, 1968; Vatner et al., 1970ab, 1974).

In the non-ruminant animal, reported increases in blood flow to segments of the gastrointestinal tract may range from 30 to 300% (Chou et al., 1976; Gallivan et al., 1980; Vatner et al.,1975). Maximum increases are observed to occur between 30 and 90 min (Fronek and Stahlgren, 1968; Vatner et al., 1970ab; 1974). Increases of lesser magnitude have been reported in the ruminant animal. Using sheep, Webster and White (1973) found an average increase in portal blood flow of 400 $ml \cdot min^{-1}$ which peaked 3.5 to 4.5 hr after the consumption of dried grass. Bensadoun and Reid (1962) noted increases in blood flow ranged from 56 to 169% of pre-feeding levels in sheep. Maximum blood flows occurred 5 to 7 hr post feeding. Most studies with ruminants have shown maximum increases in portal blood flow to range from 20 to 39% and these changes generally occur within 7 hr of consumption (Katz and Bergman, 1969; Hume, 1972; Webster and White, 1973).

Mechanisms involved in the regulation of blood flow to the gut, while receiving intensive study, are poorly understood. Currently, evidence suggests a complex system involving the nervous system, gastrointestinal hormones and both local and metabolic vascular regulators. The actions of these regulators are likely directed to the smooth muscle of arterioles and precapillary sphincters which appear to be the major regulatory sites of mesenteric blood flow (Pawlik et al., 1975;

Granger et al., 1984;). Contractile changes in arteriolar smooth muscle determine changes in resistance to the total flow of blood through the gut while alterations in precapillary sphincters regulate tissue perfusion density Thus, capillary surface area and capillary to cell diffusion distance are well regulated. A useful diagram illustrating local control of blood flow was presented by Shepherd (1982). The scheme represented in Figure 1 combines existing evidence supporting the metabolic and myogenic theories of blood flow regulation in the gastrointestinal tract. According to this model any reduction in tissue P_{O_2} availability to demand ratio, due to transport of metabolites or increased concentrations of vasodilator metabolites, evokes increased blood flow and capillary recruitment. Regulation by myogenic inputs, such as increased venous pressure, and neurogenic inputs are also accounted for.

Using microspheres to measure blood flow in the canine gut perfused with various agents, Pawlik et al. (1980) indicated two distinct types of hyperemia exist in the canine intestine. They found that vasodilator drugs or exposure to a hypertonic solution resulted in hemodynamic responses which suggested regulation by both arteriolar and precapillary sphincters. Hyperemia caused by metabolically transported substances, isotonic glucose or L-alanine, caused responses in precapillary sphincters. This may have important nutritional implications. When luminal nutrient concentrations are high (shortly after a meal) both arteriolar and precapillary sphincters are likely regulated resulting in both increased blood flow to the intestinal area and a decrease in the capillary to cell surface ratio. This may allow maximum nutrient uptake by the tissues and also enhance the ability to quickly "flush" the metabolites from the area. When lumen concentrations are low, blood

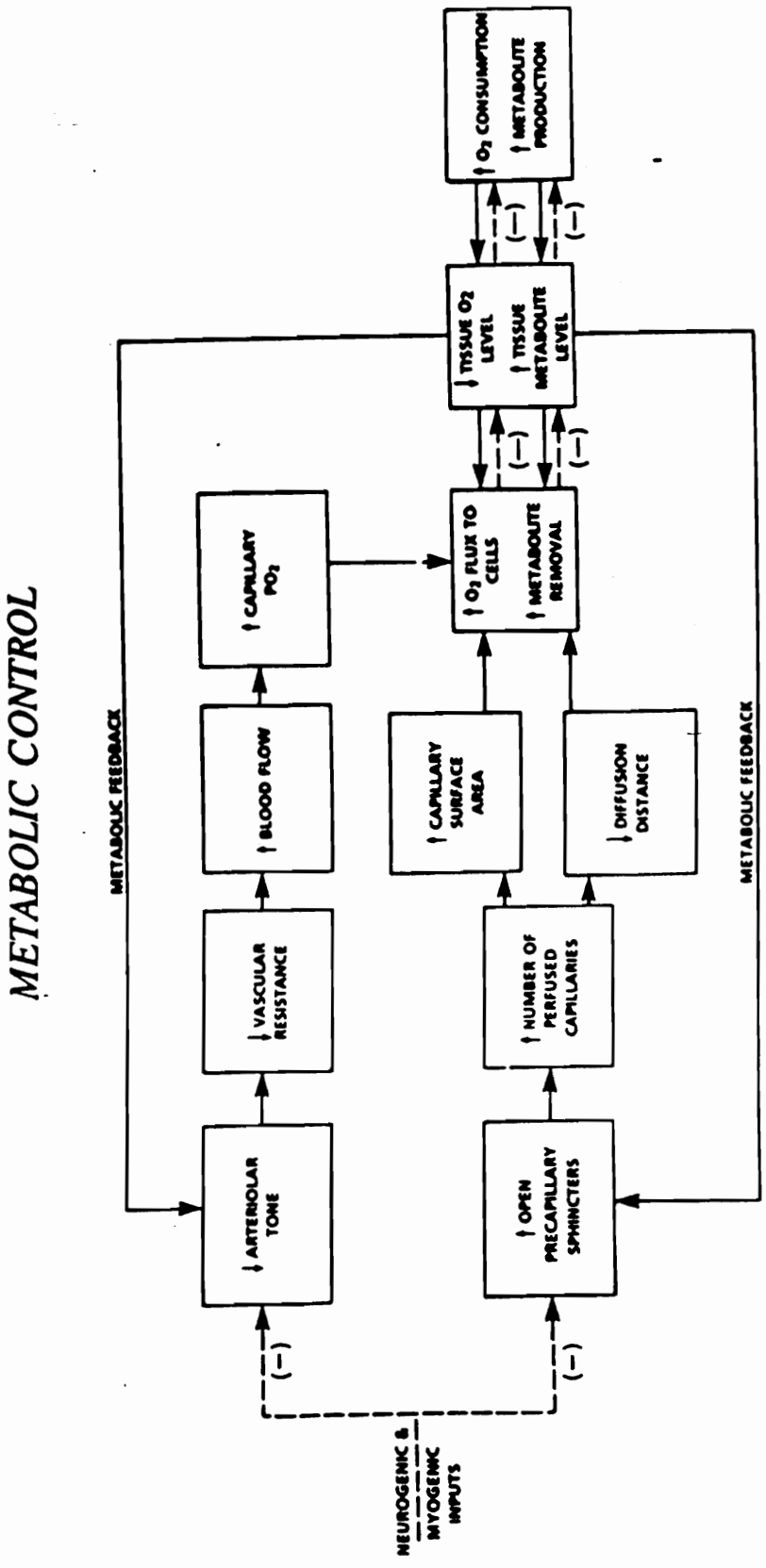


Figure 1. A diagram illustrating the metabolic theory for the control of blood flow to the intestine (Shepherd, 1982).

flow is no longer directed to the gut but the capillary to cell surface ratio may still be minimized when actively absorbed nutrients are present.

Results of numerous studies indicate that there is increased blood flow to regions of the gastrointestinal tract which contain digesta (Gallivan et al., 1980; Vatner et al., 1970ab). However, increased blood flow also has been observed in segments devoid of digesta (Bond et al., 1979). Contradictions also exist as to whether the increased blood flow is directed, within a tissue, to areas of high metabolic activity, ie the mucosa, or whether an increase in blood flow occurs in the region in general. These discrepancies may, in part, be due to the techniques involved in determining blood flow. Additionally, interactions between local, neural, hormonal regulation systems and the effects nutrients have on these systems play a role in regulating blood flow.

The placement of a low fat, low protein diet in segments of the gastrointestinal tract in anesthetized dogs was examined by Chou et al. (1976). Food placed into the stomach resulted in increased blood flow to the stomach within 5 min and to the small intestine within 30 min. Placement of the meal in the duodenum increased blood flow to the small intestine but not to the stomach or to an isolated segment of jejunum. This information would indicate that increased blood flow resulted from concurrent appearance of digesta within the segment and is mediated locally. Partitioning of blood flow, determined with microspheres, showed that the blood was directed to the mucosal layer.

Bond et al. (1979) examined postprandial blood flow in the conscious dog after consumption of a high protein, high fat meal. During blood flow determination the bulk of the meal was in the stomach and proximal small intestine with none in the ileum. Blood flow to the whole body wall of the intestine was reported to

increase 96%, 93% and 153% in the stomach, jejunum and ileum, respectively. A small but insignificant increase was noted in the colon. Increased mucosal blood flow was noted only in the stomach and colon while that to the other segments was shared equally by all tissue layers.

Studies have shown that a variety of anesthetics will alter blood flow within the animal. Portal blood flow has been found to be lower in anesthetized when compared to conscious animals (Katz and Bergman, 1969). Pre-surgical fasting may play a role in this reduction of blood flow since they observed that blood flow to the gastrointestinal tract is reduced in fasted animals. Bensadoun and Reid (1962) reported that portal blood flow for sheep ranked as follows: conscious-fed > conscious-fasted > anesthetized-fasted. Use of anesthetics, in general, results in a decreased cardiac output. The reduced portal blood flow in anesthetized sheep is likely due a general reduction in blood flow through out the animal. Anesthesia, may also influence the red blood cell concentration of blood by maximizing the storage capacity of the spleen (Turner and Hodgetts, 1959). This effect of anesthesia is of concern when plasma and blood flow values are to be utilized in calculating nutrient fluxes.

Blood flow is influenced by the presence of nutrients in the gut as demonstrated by increases in flow in fed versus fasted animals. The composition of the diet also appears to be a major factor in the hyperemic response of the gastrointestinal tract. In general, initiation and maintenance of postprandial hyperemia is influenced by diet composition (i.e. high fat > high protein > high carbohydrate). Moreover, a synergistic effect of these nutrients on gastrointestinal hyperemia has been noted (Siregar and Chou, 1982). The presence of bile also has

been shown to have a significant impact when other nutrients are present in the digestive tract (Sit and Chou, 1984).

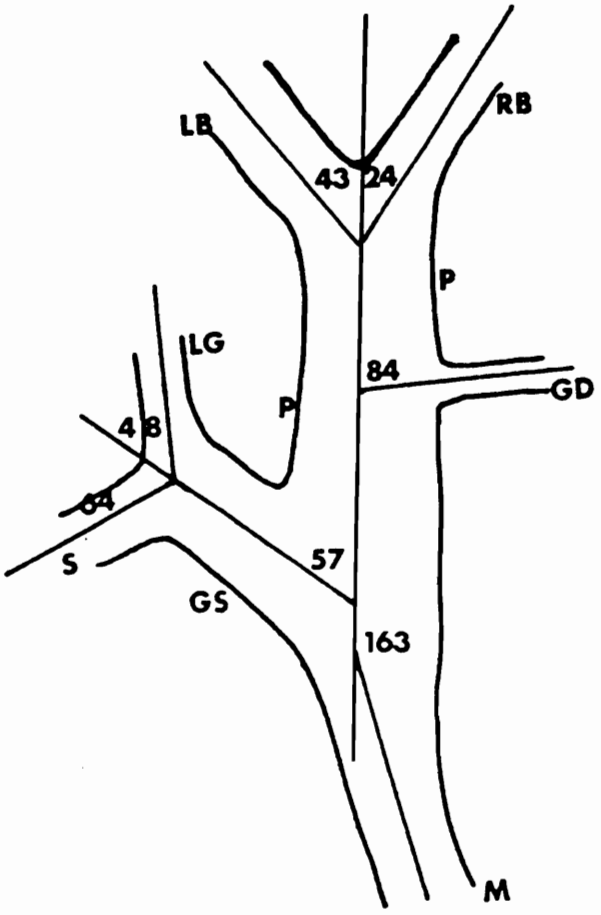
The response of blood flow due to different nutrients presented to the gastrointestinal tract may, in part, be due to the stimulation of the humoral system. Fara et al. (1972) found that placement of oil into the duodenum of cats resulted in increased blood flow in the jejunum. Moreover, when blood from the animal receiving oil was put into a recipient animal in a cross-circulation preparation, blood flow was increased to the gastrointestinal tract of the second animal. This indicates that hormones are likely released due to the presence of different nutrients in the gut lumen and play a role in altering blood flow. Studies to investigate the role of hormones on gastrointestinal blood flow have included investigations of several hormones including pentagastrin, CCK-8, secretin, histamine, glucagon and vasoactive intestinal polypeptide (VIP). While all of the hormones have been shown to produce vasodilation, CCK-8 exerted its effect at physiologic levels (Chou et al., 1977). This is in agreement with the observation that a vasodilator is released into the blood following the placement of fat or acid into the duodenum. Histamine and VIP also may serve to regulate blood flow at physiological levels (Gallivan et al., 1985), however, the status of these as regulators is being debated.

Some general concepts can be developed from the previous discussion. Following the consumption of a meal, blood is directed toward the intestinal region. This redistribution of blood is likely the result of the combined effects of the nervous and hormonal systems and local vasoactive agents. The food consumed will effect the extent and duration of intestinal hyperemia, generally; fat > protein > carbohydrates. Anesthetics may decrease the hyperemic response due to its effect of lowered cardiac output.

Anatomical Structure of the Portal Venous System of Sheep. Extensive detail on the anatomical structure of the portal venous system in sheep (Heath, 1968, Nani et al., 1981) and other domestic animals (Nani et al., 1981) has been reviewed. The portal vein of sheep is formed by the convergence of two large vessels, the mesenteric vein and the gastrosplenic vein. Though variation exists between animals, the mesenteric and gastrosplenic veins converge at about a 140^{o} angle (Fig 2.). A smaller vessel, the gastroduodenal vein, joins the portal vein on its right ventral side at nearly a right angle.

Sources of blood for the portal venous system of sheep are illustrated in Fig. 3. The gastrosplenic vein branches into tributaries which drain areas of the spleen, pancreas, greater omentum, right and left surface of the rumen, reticulum, omasum and the lesser curvature of the abomasum. The mesenteric vein branches into tributaries which drain the jejunum, ileum, caecum, colon, rectum and pancreas. As the name implies sources of blood for the gastroduodenal vein include the abomasum and proximal duodenum.

Collection of blood from catheters inserted into the aorta, mesenteric and portal veins will allow the determination of venoarterial differences of nutrients from the mesenteric (intestinal) and portal (intestinal + stomach) viscera. Concentrations of a nutrient in the mesenteric and portal veins is dependent on the amount of absorption of the nutrient and the blood flow through the vessel. The flux of a nutrient across a tissue is calculated as the product of the venoarterial difference and blood flow. Subtracting the flux of a nutrient determined from mesenteric samples from the flux of a nutrient determined from portal samples gives an estimate of nutrient flux originating from the stomach regions and spleen.



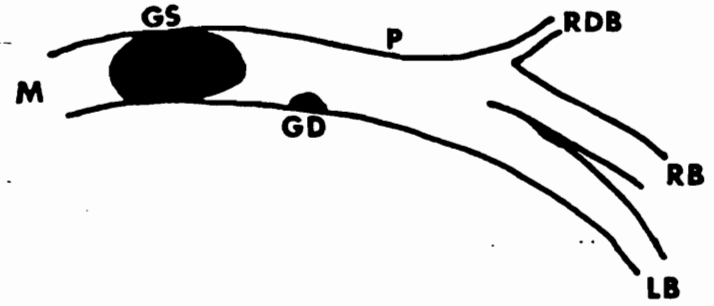
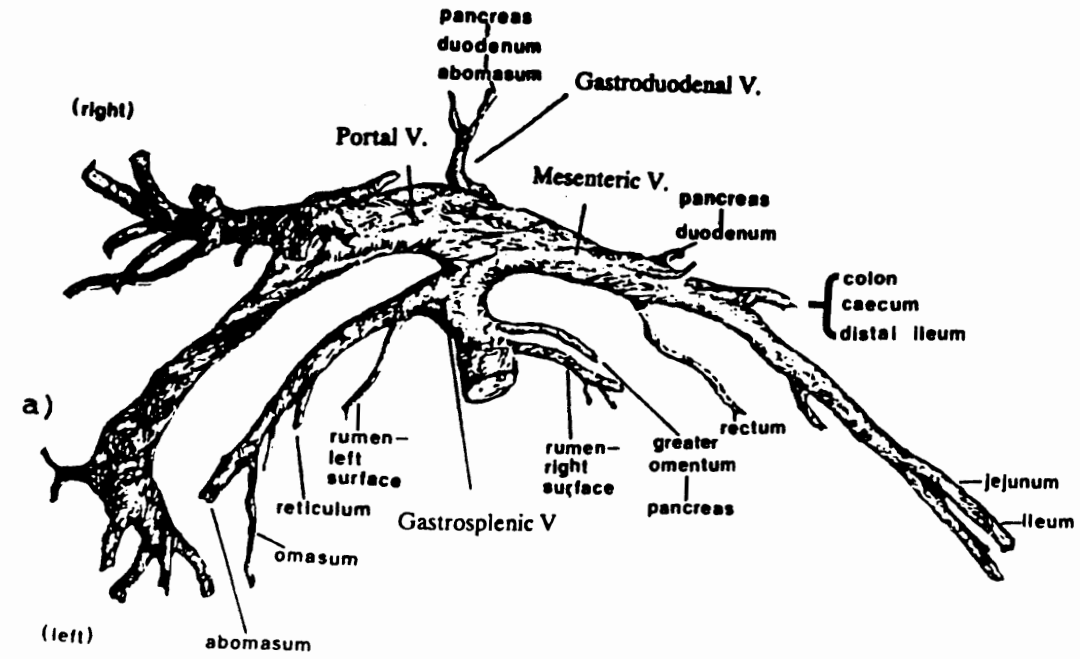


Figure 2a,b. Relative disposition of the tributaries and branches of the portal vein of sheep. Vessels include: P, portal vein; LB, left branch; RB, right branch; RDB, right dorsal branch; GD gastroduodenal vein; GS, gastrosplenic vein; S, splenic vein; LG left gastric vein (Heath, 1968).



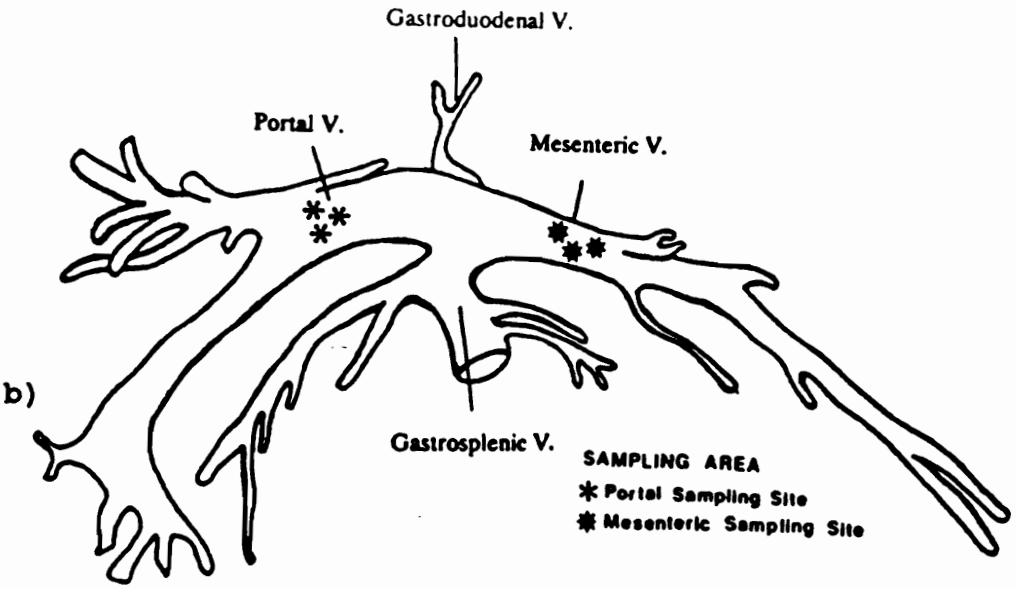


Figure 3. a) Origin and distribution of portal vessels in sheep (Heath, 1968). b) Catheter placement in the portal system of sheep.

Blood Flow in Ruminants. Portal blood flow measures are best compared when they are expressed on a per weight basis (Webster and White, 1973). Portal blood flow ($ml \cdot min^{-1} \cdot kg^{-1}$) in sheep has been reported to range from 28 to 46 (Roe et al., 1966; Katz and Bergman, 1969; Webster and White, 1973; Webster et al., 1975). Values reported for the portal blood flow for different species include: dairy calves, 37-49 (Wangsness and McGilliard, 1973; Dobson et al., 1981; Koeln, 1982; Green et al., 1984; Durand et al., 1988); lactating dairy cows, 42-43 (Huntington, 1984); nonlactating dairy cows, 27-31 (Huntington, 1975; Huntington and Reynolds, 1983); steers, 38-58 (Huntington et al., 1981); beef heifers; 34-37 (Huntington and Reynolds, 1986); and pigs 34-40 (Rerat et al., 1988).

Thus, a fairly consistent range of portal blood flow exists over a number of species. However, when comparing studies one must consider factors which may influence portal blood flow. These factors may include, feed and feed processing methods, amount of feed consumed (ad libitum vs limited feeding) as well as the production state of the animal (lactation, pregnancy).

Janes et al. (1985) fed a dried grass or pelleted corn-based diet in 1-hr intervals to sheep and reported mesenteric blood flows in to be 28.8 and 31.8 $ml \cdot min \cdot kg^{-1}$. There was a tendency for blood flow to be increased in the corn-based diet. Huntington et al. (1981) showed a tendency for steers fed hourly and consuming an 85% concentrate ration to have higher portal blood flow levels than steers consuming alfalfa hay (58 vs 38 $ml \cdot min^{-1} \cdot kg^{-1}$). Portal blood flow for lambs consuming an 85% concentrate diet tended to be higher than portal blood flows in lambs consuming a hay (59 vs 47 $ml \cdot min^{-1} \cdot kg^{-1}$) (Huntington et al., 1980).

Webster et al. (1975) examined portal blood flow in fed and fasted sheep. The mean portal blood flow in six animals following a fasting period of 48 hr was 28.2 $ml \cdot min^{-1} \cdot kg^{-1}$. Food intake influenced blood flow. Sheep fed at maintenance and at 2.5 times maintenance had blood flow values of 36.8 and 61.3 $ml \cdot min \cdot kg^{-1}$, respectively. Blood flow may be related to metabolizable energy intake.

Similar findings were reported by Katz and Bergman (1969) who observed a 12 and 19 $ml \cdot min^{-1} \cdot kg^{-1}$ reduction in portal blood flow in nonpregnant and pregnant ewes, respectively. A reduction in portal blood flow due to fasting has been reported by others for sheep (Bensaduon and Reid, 1962).

Stages of production such as pregnancy or lactation may have important implications on blood flow through the digestive tract. Mean blood flows for twin-pregnant and nonpregnant fed alfalfa grass hay ad libitum sheep were 53 and 43 $ml \cdot min^{-1} \cdot kg^{-1}$, respectively (Katz and Bergman, 1969). Blood flows declined for both groups when fasted for 3 d to 34 and 31 $ml \cdot min^{-1} \cdot kg^{-1}$, respectively. Differences in blood flow due to pregnancy could not be discerned from intake as this was not monitored.

Huntington (1984) reported portal blood flow in lactating cows receiving a corn silage based diet ad libitum was not different 4 through 20 wk post partum. Blood flow ranged from 1183-1357 $liter \cdot hr^{-1}$. Portal blood flow was higher in lactating cows compared to nonlactating cows receiving the same diet. The effect of lactation on portal blood flow could not be separated from the effects of increased feed consumption as dry matter intake was 5.02 and 13.5 $kg \cdot d^{-1}$ for nonlactating and lactating cows, respectively. The increased gastrointestinal blood flow was likely the result of increased feed intake (McGuire et al., 1989).

In view of the evidence presented above, some general concepts can be developed. Portal blood flow values generally ranges from 28 to 50 ml·min·kg^{-1}. Portal blood flow generally is greater in animals fed concentrate diets verses roughage diets and is greater in fed rather than fasted animals. This is consistent with the view that portal blood flow is related to metabolizable energy intake (Webster et al., 1975; McGuire et al., 1989). Pregnancy and lactation may result in increased portal blood flow. This increase in flow is likely the result of increased feed consumption and may only in part be due to the state of production.

Webster and White (1973) measured portal blood flow of sheep fed dried grass ad libitum. They noted that increased blood flow was biphasic in nature when the dried grass was offered once daily. Portal blood flow increased during the consumption of food, then decreased, then it increased again 2 to 6 hr after feeding. This biphasic nature of blood flow may have implications for studies where venoarterial differences are measured. If one assumes that increased blood flow is the end result of increased metabolism and absorption of nutrients, as suggested by the metabolic theory of blood flow, then differences in nutrient flux between studies may simply be due to the time the sample is drawn post feeding. Results can also be confounded by feeds and feed processing methods utilized in the study which may impact on digestibility. Therefore, unless animals in studies being examined are fed to maintain "steady state" conditions, care must be utilized when values of nutrient flux from different studies are compared.

The effects of feeding on the partitioning of blood flow in the gastrointestinal tract of ruminants has been studied. In steers, the mesenteric and non-mesenteric drained viscera account for 42 and 58 percent of portal blood flow (Huntington and Reynolds, 1986). In sheep, blood flow was studied using radioactive microspheres before, during and at two times following the ingestion of a daily meal (Dobson et al., 1981). Increased blood flow was noted in the rumenoreticulum musculature, parotid and submandibular salivary glands during consumption of the meal. Flow to the mucosa of the rumenoreticulum increased during eating and a major increase was noted 2 hr after eating. Elevated blood flow in the mucosa did not return to resting values even after 4 hr. Omasal blood flow decreased during eating but otherwise remained steady throughout the study period. Blood flow to the large intestine, jejunum, pancreas and spleen remained steady while flow to the abomasum, duodenum, ileum, and gut fat was reduced.

Since the liver is the sight of metabolism and catabolism of many nutrients the fraction of blood derived from the portal system is of interest. Naylor et al. (1985) found blood flows of 1.54 and 1.98 $l \cdot min^{-1}$ for portal and hepatic flows, respectively in sheep. This suggests that the portal system and hepatic artery contribute 78 and 22 percent of the blood presented to the liver. These percentages are in agreement with Katz and Bergman (1969) who reported 78 and 82 percent of hepatic blood flow was derived from the portal system in nonpregnant and pregnant sheep.

Protein Metabolism

For the absorption of an amino acid or peptide to occur in the intestines, it must cross into the enterocytes lining the intestine prior to entering the circulatory system. Enterocytes are composed of the brush border and basolateral membranes.

These membranes are distinctive and serve as two barriers (Hopfer et al., 1976). For nutrients in the gut lumen to enter the circulatory system an organism must devise methods to overcome these barriers under various physiological conditions. Knowledge of factors which effect the presentation and uptake of α-amino nitrogen, both at the cellular level and in the intact animal may play an essential role in the improvement of protein utilization.

Amino Acid Absorption. Cells can use energy to actively transport a substance against a concentration gradient. Cells lining the intestine achieve this energy requirement by the formation of an electrochemical gradient. Metabolic energy (ATP) is utilized by the Na^+-K^+/ATPase pump located on the basolateral membrane (Mircheff et al., 1976). This pump maintains a low concentration of Na^+ within the enterocyte. The pump also maintains higher levels of intracellular K^+ which results in a net negative charge inside the cell. Thus, a Na^+ concentration gradient directed into the cell and a electrical membrane potential difference are maintained and utilized to bring amino acids and other substrates into the cell (Mircheff et al., 1980).

Amino acids are transported across the brush border by processes which include: passive diffusion, Na^+-independent transport, and Na^+-dependent transport (Stevens et al., 1982; Stevens et al., 1984). Passive diffusion occurs when the amino acid moves across the membrane down a concentration gradient. Therefore, diffusion will play an increased role in amino acid absorption when lumen concentrations are elevated. However, at lower amino acid concentrations the relative input of carrier mediated transport is increased. Amino acid transport by carrier mechanisms on the brush border membrane are characterized as either Na^+-independent and Na^+-dependent transport pathways. Several transporters are

thought to be present on the brush border. The L system which favors the neutral and aromatic amino acids is Na^+-independent (Oxender and Christensen, 1963). A second Na+-independent system, the Y+, system is specific for the transport of the cationic amino acids including lysine, arginine and histidine (Christensen and Linag, 1966). Three Na^+-dependent carriers have been suggested and include the neutral brush border (NBB), the PHE and the imino systems (Stevens et al., 1982). These carriers transport neutral amino acids, phenylalanine and methionine, and proline and hydroxyproline, respectively.

The relative importance of these systems at various concentrations of amino acids has been demonstrated with the use of brush border membrane vesicles. Stevens et al. (1984) noted in brush border membrane vesicles that the predominance of Na^+-independent transport of phenylalanine for concentrations <1 mM was replaced by Na^+-dependent transport up to the concentration of 17 mM where diffusion became predominant. The contribution of carrier systems on amino acid transport were found to be greater than diffusion up to a concentration of 2.5 mM which suggests that below this concentration, carrier mediated transport is required for sufficient transport of amino acids.

Which method of transport predominates at a given concentration may vary with the amino acid tested, site along the intestine and likely species of animal. Wilson and Webb (1990) examined the relative contribution of Na^+- dependent, Na^+-independent, and diffusion systems on the uptake of methionine and lysine from bovine jejunal and ileal brush border membrane vesicles. Concentration ranges utilized in the study were 0 to 14 mM for methionine and 0 to 7 mM for lysine. Total uptake of methionine by both the ileum and jejunal brush border membrane vesicles was predominantly by diffusion for all concentrations examined

except .125 mM where Na^+-dependent transport was prevalent. For lysine concentrations of .75 mM or less, Na^+-independent > diffusion > Na^+-dependent in jejunal brush border membrane vesicles. In ileal tissue, both Na^+-dependent and Na^+-independent transport were greater than diffusion.

Amino acids are transported across the basolateral membrane by processes which include: passive diffusion, Na^+- independent transport, and Na^+-dependent transport (Hopfer et al., 1976). This membrane is more permeable or "leaky" to amino acids when compared to the brush border membrane. Amino acids concentrated within the cell can readily diffuse through this membrane into the circulatory system. Therefore, diffusion plays a major role in amino acid transport across this membrane. Transport systems found on the basolateral membrane include a Na^+-independent system (L system) and two Na^+-dependent (A, ASC) systems. A third "novel" Na^+-dependent carrier having similar, but not identical, transport capabilities as the A or ASC systems may also be present (Mercheff and Wright, 1980).

Amino acids are carried across the brush border membrane by diffusion and Na^+-dependent and Na^+-independent carriers. The carrier systems are specific in their selection of amino acids for transport. The relative importance of the transport systems appears to be dictated by concentration of amino acids in the lumen. Once transported into the enterocyte, amino acids generally diffuse down a concentration gradient into the circulatory system.

Evidence of Peptide Transport Across the Brush Border Membrane of Enterocytes. The early recognition that α-amino nitrogen may be absorbed in a form other than free amino acids was largely the result of indirect evidence. Silk et al. (1973a) examined the disappearance of amino acids from the human jejunum when a

partial hydrolysate of casein or a equal molar mixture of its constitute amino acids were infused. A double perfusion technique was utilized in the study. Apparent absorption of amino acids was greater and more uniform when the hydrolysate rather than the free amino acid mixture was infused. Amino acids which were poorly absorbed from the free amino acid solution were absorbed to a substantially greater extent from the hydrolysate mixture. A similar study by Silk et al. (1975) showed total absorption of amino acids to be 29% greater and variation of uptake of individual amino acids to be less when a casein hydrolysate mixture was infused compared to an amino acid mixture.

Hara et al. (1984) infused an enzymatic hydrolysate of egg white (composed of 70% di- and tri-peptides and <10% free amino acids) or an equivalent amino acid mixture into rat intestine. Maximum appearance of amino acids in blood were reached sooner (10 to 15 min) for the peptide mixture when compared to the free amino acid mixture (15 to 30 min). Peak responses of the essential amino acids threonine and histidine, and nonessential amino acids serine and tyrosine were especially delayed with the amino acid mixture. Absorptive intensity of amino acids was 70-80% higher for the hydrolysate perfusion when compared to the equivalent mixture of free amino acids. Portal appearance of amino acids was more like the composition of the protein infused when the hydrolysate rather than free amino acid mixture was tested.

Rerat et al. (1988) infused an enzymic hydrolysate of milk or free amino acid mixture into the duodenum of pigs. Analysis of the hydrolysate showed that 80% was in fragments < 1500 MW, 60% <5 amino acids and 8% in the form of free amino acids. Portal samples were analyzed for the appearance of free amino acids. With the exception of methionine, amino acids reached the portal vein more rapidly, in

greater quantities and in a more uniform manner when the hydrolysate was perfused. The data indicated that essential amino acids were preferentially absorbed by the intestine. The observation that amino acids from a peptide mixture are absorbed more rapidly and more uniformly has been noted by others (Crampton et al., 1971; Silk et. al., 1980).

If the peptides were completely hydrolyzed in the intestinal lumen then the rate of appearance of amino acids in the blood could, at best, be similar to that obtained from perfusion of a free amino acid mixture (Matthews, 1975). Moreover, the more uniform uptake of amino acids suggests that competition for amino acid transporters is not similar for the two mixtures. These observations suggest that peptides may be transported across the brush border membrane of the intestine intact.

The uptake of amino acids from a mixture of free amino acids or equivalent mixture of peptides has been examined. A segment of the duodenum, jejunum or ileum of humans was perfused with peptides or their constitute amino acids (Adibi and Morse, 1971). The peptides perfused consisted of glycylglycine and glycylleucine. The tests were performed over a range of concentrations (20 to 100 mM). Hydrolysis by luminal enzymes was absent for glycylglycine and minimal for glycylleucine. Amino acid absorption rates were greatest when the dipeptide rather than the free amino acid mixture was present. When perfused with a free amino acid mixture leucine always was absorbed to a greater extent than lysine. Perfusion of glycylleucine resulted in a more uniform uptake of glycine and leucine. There was no difference in glycine and leucine uptake in the jejunum and preferential absorption of leucine was minimized in the ileum. Results indicated that dipeptide disappearance from the lumen is principally due to absorption of intact peptides

which were subsequently hydrolyzed within the cells lining the gut. Cells lining the ileum appeared to have a greater capacity for intracellular hydrolysis than in the jejunum even though the ability to absorb the peptides was similar.

Silk et al., (1973b) used a double lumen perfusion technique in humans to examine the absorption of glycine and alanine and the dipeptides alanylglycine and glycyl-L-alanine. Glycine was absorbed at a faster rate when the peptide solutions were perfused. Differences in absorption rates of the free amino acids was abolished with infusion of the peptide mixtures. Free amino acids appeared in the lumen and their concentrations could not be accounted for by luminal hydrolysis. These results led the authors to conclude that two modes of uptake may occur, peptide hydrolysis with subsequent uptake of free amino acids and direct uptake of intact peptides.

Hara et al. (1984) examined the absorption of alanine and valine from the duodenum of conscious unrestrained rats after the infusion of a dipeptide, L-alanyl-L-valine or an equilmolar mixture of alanine and valine. Concentrations of both free amino acids in the portal blood were increased with administration of the peptide or free amino acid solution. Alanine and valine were absorbed at equal intensity and absorption of valine was greater than alanine when rats were fed the dipeptide or free amino acids respectively. Adibi (1971) noted a more rapid absorption rate of leucine and glycine in the jejunum of humans when supplied by di- and tri-peptides rather than an equilmolar mixture of free amino acids.

Infusion of a protein compared to a mixture of free amino acids has been observed in several studies to result in a more rapid and more uniform appearance of amino acids in portal blood or a more rapid disappearance of amino acids from the intestinal lumen. Similar observations were obtained when peptides and free amino acid solutions were compared. These results support the concept that amino

acids may be absorbed across the brush border membrane as intact peptides. The more uniform presentation of amino acids to the liver resulting from intact protein or peptide sources may have a significant effect on the efficient production of proteins by the liver.

Evidence for Intact Peptide Absorption into the Portal System. Dawson and Porter (1962) could only account for 41% of the absorbed amino-N from the intestinal lumen of the rat and cat in portal blood. Tagari and Bergman (1978) observed only 30 to 80% of the essential amino acids which disappeared from the lumen could be accounted for in the portal system of sheep. Similar observations in accounting for the disappearance of amino acids from the lumen of sheep were noted by Wolff et al. (1972). Explanations for these discrepancies include the presence of analytical error and the utilization of the amino acids by the intestine for protein production or as a source of energy (Bergman, 1986).
Measurements of amino acid incorporation into rat intestinal tissue suggest that about 10% of absorbed amino acids are utilized for protein production by the mucosa (Bronk and Parsons, 1966).

Adibi and Mercer (1973) noted the presence of large quantities of peptides rather than free amino acids in the intestinal lumen of humans following a high protein meal. As stated by Matthews (1975), early evidence that peptides may be absorbed into the portal system intact was illustrated by Folin and Berglund (1922) who noted a large increase in concentration in the nonprotein, non amino acid, non urea-N fraction of plasma in human subjects following ingestion of a large dose of gelatin. This increase was likely due to peptides containing the amino acids hydroxyproline and proline which are particularly high in gelatin. Unfortunately, this observation went unnoticed or was ignored (Matthews, 1975).

In the early 1960's evidence that peptides may be taken up intact by the enterocyte was reintroduced. This evidence was largely the result of work by Newey and Smith (1959, 1960). They reported that some peptides, glycylglycine and alanylalanine, could be found unhydrolyzed on the serosal surface of the intestine of rats (Newey and Smith, 1959). Two other peptides were completely hydrolyzed to their constituent amino acids. They noted, however, that the hydrolysis of these peptides could not be accounted for in the serosal fluid. They suggested that the peptides were hydrolyzed within the cell. Evidence for intracellular hydrolysis increased when further research demonstrated that peptidase activity in the mucosal fluid was insufficient to account for the amounts of peptide disappearance from the mucosal side (Newey and Smith, 1960). They concluded that peptides could be taken up intact across the mucosal membrane and either hydrolyzed intracellularly to its constitute amino acids or appear on the serosal surface intact.

Peters and MacMahon (1970) collected portal and arterial blood samples from rats before and during intra-duodenal infusion of 100 mM solution of glycine or glycine oligopeptides up to and including tetraglycine. Blood samples obtained prior to infusion contained glycine in the free but not the peptide form. Infusion of peptide solutions resulted in increased glycine concentrations and the appearance of glycylglycine in the portal sample. No tri- or tetraglycine was detected, indicating that they were hydrolyzed to glycylglycine and(or) free glycine prior to appearance in the portal blood. The highest portal concentration of free glycine was noted with the infusion of triglycine suggesting that this peptide was absorbed the fastest.

Slesinger et al. (1977) determined amino acid concentrations in portal blood after duodenal infusions of an amino acid mixture simulating casein or a partial enzymic hydrolysate of casein. Portal plasma was taken prior to infusion and at 5- to

10-min intervals for 1 hr. For some of the amino acids investigated, concentrations of amino acids in portal plasma after the infusion of the hydrolysate solution were much lower than those obtained after the infusion of free amino acids. The authors suggested that the lower amino acid concentrations from the hydrolysate infusion may have been the result of absorption of intact peptides rather than free amino acids. The presence of these peptides in the portal plasma, however, was not determined.

Heading et al. (1979) studied the transport of tyrosylglycylglycine (TGG) and tyrosylalanylglycine (TAG) in rat intestine. In all cases TGG failed to appear in the serosal fluid while TAG was concentrated in serosal fluid. The study also indicated that transport was independent of Na^+. Gardner (1978) examined the uptake of intact peptides from protein digests by rat small intestine. Small amounts of peptide uptake were noted for a soybean digest while substantial quantities of peptides were absorbed form a casein a digest. No evidence of peptide uptake was noted for lactalbumin or Evans peptone hydrolysates. All amino acids investigated except serine, tyrosine, histidine and arginine appeared in the bound fraction.

Bloch et al. (1988) incubated everted jejunal gut sacs prepared from rat intestine with ^{125}I labeled polypeptide fragments (ranging in MW from 6,000 to 25,000 daltons) of bovine serum albumin (BSA-F). Serosal fluid was applied to a Sephedex G-50 column. Large concentrations of radioactivity were reported for elution periods associated with iodide and ^{125}I-labeled free amino acids. A lesser amount of radioactivity was detected in the fraction which corresponded to the elution of the fragments indicating that some amino nitrogen was peptide bound. In vivo studies were also conducted. In the first study, labeled BSA-F were infused into the jejunum of rats and blood samples were taken from either the mesenteric or

portal vein. Blood samples obtained were found to contain labeled fragments. Unlabeled BSA-F were infused into the intestine and fragments were eluded on a sephedex G-100 column in a second study. The fragments were examined for BSA-F by radioimmuno assay procedures. Nanogram amounts of BSA-F were found. Data from these three experiments suggested that potential food derived protein were capable of being transferred to the serosal surface of the intestine.

In the non-ruminant animal perhaps 10 to 33 % of the α-amino-N reaching the portal system is peptide bound (Gardner, 1975; Gardner, 1982; Gardner et al., 1983). Koeln, (1982) determined venoarterial differences of dietary amino acids in calves. Venoarterial differences of peptide amino acids across the gastrointestinal tract were nearly threefold the amount added as free amino acids. Subsequent analysis of the peptide fraction obtained from plasma by gel filtration on Sephedex G-15 showed that peptides with MW ranging from 300 to 1500 were involved (Schlagheck and Webb, 1984). The origin of the peptides, dietary or endogenous, could not be determined due to the design of the study. However, the large quantities of peptide associated amino acids, regardless of origin, would play a substantial role in the protein metabolism of these calves. Large concentrations of peptide bound amino acids have been noted in the peripheral circulation of calves fed natural and purified diets (McCormick and Webb, 1982; Danilson et al., 1987). This large proportion of amino acids associated with peptides may be unique to the ruminant. Thus, the role of peptides in N metabolism may be of special interest to the ruminant nutritionist.

Adibi et al. (1977) noted that the administration of glycylleucine when compared to the administration of an equivalent mixture of glycine and leucine resulted in a 1.6 fold increase in insulin secretion. Since insulin increases uptake of

amino acids by muscle and decreases protein breakdown, this may play a role in increasing N balance. Evidence of biologically active peptides crossing the intestinal barrier when given orally in large doses has been reported. Bowers et al. (1970) observed that large oral doses of porcine thyrotropin releasing hormone (TSH) were biologically active. This would suggest that TSH, a tripeptide; (pyro) glycylhistidylproline (NH_2), is released intact into the bloodstream after absorption by the intestine. Other examples of biologically active peptides crossing the intestine include insulin (Danforth and Moore, 1959), leutalizing hormone-releasing hormone (Humphry et al., 1973) and immunoglobulins (Hemmings and Williams, 1978).

Certain peptides resulting from the partial digestion of casein are thought to be absorbed intact and these have been termed "casomorphins". These peptides may be of special interest to nutritionists since milk consumption in humans and the use of casein in both feeding programs and nutritional studies of animals is common. Casomorphins are the result of enzymatic hydrolysis of the β-casein and include the following: Tyr-Pro-Phe-Pro-Gly-Pro-Ile (β-casomorphin 7), Tyr-Pro-Phe-Pro-Gly (β-casomorphin 5) Tyr-Pro-Phe-Pro (β-casomorphin 4) Tyr-Pro-Phe (β-casomorphin 3) (Brantl et al., 1981; Hannelore et al., 1990). Schusdziarra et al. (1983) demonstrated that IV infusions of casomorphin fragments (4 to 7) potentiate the glucose-amino acid induced insulin release in dogs. This effect was lost with addition of naloxone suggesting the actions of casomorphins result from activation of opioid receptors. Tome (1987) suggested that casomorphins must reach the serosa in order to be effective. Casomorphins result in decreased motility of the intestine (Hannelore et al., 1990). This would be consistent with increasing the potential for the absorption of polypeptide fragments especially in the neonate where absorption of immunoglobulins prior to gut closure is essential for the survival of the animal.

Intact peptides which have been shown to cross various intestinal preparations or appear in the system as intact peptides include, peptides containing a D isomer of an amino acids, glycylglycine, glycylphenylalanine, glycylproline, proline-L-glycine, carnosine (β-alanylhistidine), anserine (β-Alanylmethylhistidine), sarcosine and proline and hydroxyproline containing peptides (Adibi and Morse, 1971; Gardner, 1984). Generally high doses of peptides were utilized in these studies and increases in free amino acids were substantially greater than that of the peptides.

A major factor governing the entry of a peptide into the portal system intact is the resistance of the peptide to hydrolysis (Asatoor, 1973). Factors influencing resistance to hydrolysis include heat damage, cyclization of n-terminal glutamine, presence of phosphopeptides, proline or hydroxyproline residues, and gamma-glutamyl linkages (Gardner, 1983). It is of interest to note the high proline content of the casomorphins listed above. The hydrolysis procedure used to prepare mixtures of partial protein hydrolysates also may influence uptake of peptides. Crampton et al. (1971) reported an increase in α-amino N uptake when a pancreatic hydrolysates were compared with acid hydrolysates for casein (62 vs 46%) and for bovine serum albumin (62 vs 33%).

The addition of intact peptides into the portal system has been documented. The uptake of intact peptides may help account for the discrepancies between the quantity of amino acids disappearing from the intestinal lumen and appearing in the portal blood. The relative importance of free and peptide amino acid additions to the circulation may vary between the non-ruminant and ruminant animal. The significance of intact peptide absorption is under study in both species from both a nutritional and pharmocological aspect.

Mechanism of Peptide Uptake by the Enterocyte: Evidence for Independence of Peptide and Amino Acid Transport. Studies have shown that peptides can be concentrated within tissue cells of the small intestine and kidney. This process is inhibited by metabolic inhibitor thus indicating an energy requiring process (Matthews et al., 1974). Work by Matthews et al. (1979) has showed that carrier mediated transport and diffusion play a role in peptide uptake. Using the peptides glycylsarcosine and glutamylglytamine they estimated the relative contributions of these two transport mechanisms on peptide transport. They found that carrier mediated transport was dominate and diffusion only played a minor role in uptake. Similar results were reported by Sleisenger et al. (1976). The extent of the diffusion component exerting an effect on peptide uptake may be influenced with the peptide investigated (Ferraris et al., 1988)

Early investigators examining the driving force of the carrier mediated transport of peptides suggested, like in amino acid transport, that Na^+ was transported with the peptide across the membrane (Matthews, 1975). Other investigators found little or no involvement of this ion on peptide uptake (Cheeseman and Develin, 1985; Burston and Matthews, 1987). Differences between these studies may have been due to the differences in the peptides or techniques utilized. Peptides may be transported across a membrane intact or hydrolyzed prior to transport. In the event of the latter, the resultant amino acids would be transported across the membrane with Na^+. Thus, differences in the susceptibility of peptides to surface hydrolysis and determination of the extent of hydrolysis may be an issue. Intact intestinal cells further pose the problems of intracellular hydrolysis and back diffusion as well as the Na^+-K^+/ATPase mechanism on the basolateral membrane. Gardner (1978) reported that an average of 23% of the amino acids

absorbed in the peptide form would pass back into the intestinal lumen. Heading et al. (1977) also noted a significant amount of free amino acid flow back to the lumen but this varied with the individual amino acid (glycine 33%, proline 12.5%). The site in the small intestine may have implications on this as ileal tissue appears to have higher intracellular peptidase activity than the jejunum (Adibi and Morse, 1971).

The use of brush border membrane vesicles would remove cellular metabolism and minimize Na^+-K^+/ATPase activity giving this system an advantage for studying the effect of Na^+ on peptide transport. This system can be enhanced if a poorly hydrolyzed peptide or peptide hydrolase inhibitor such as bestatin also is incorporated. Using this methodology, it has clearly been demonstrated that peptide uptake is independent of the presence of Na^+. Transport of glycyl-L-proline occurred in both the presence of Na^+ or K^+ while alanine transport only occurred in the presence of Na^+ (Ganapathy et al., 1981). The independence of peptide uptake from Na^+ using other hydrolytic resistant peptides and hydrolysis susceptible peptides in the presence of peptidase inhibitors has been demonstrated (Cheeseman and Develin, 1985).

Today, peptides are thought to be cotransported with protons across the brush border membrane. This was first proposed by Ganapathy and Leibach (1985). Takuwa et al. (1985) demonstrated the pH dependency of peptide uptake using brush border membrane vesicles from rabbit small intestine. Peptide transport was found to be entirely independent of Na^+ but significantly stimulated by lowering extravesicular pH (maximum pH 5.5). The effect of low extravesicular pH was abolished with addition of a protonaphore. An inward directed pH gradient resulted in a decrease in K_t for the peptide but not affecting the V_{max}. This would suggest a

stimulation of rate of absorption of the peptide without an increase in the number of transport sites.

Miyamoto et al. (1985) studied the uphill transport of intact glycylsarcosine in rabbit renal brush border membrane vesicles. Dissipation of the proton gradient with an ionophore, carbonylcyanide p-trifloromethoxyphenylhydrazone, abolished transport of the peptide against a concentration gradient. Generation of an inside negative membrane potential doubled uphill uptake of the peptide. These results demonstrate that uptake was electrogenic and driven by an inward proton gradient.

A microclimate, high in proton concentration, exists in the unstirred layer due to a Na^+/H^+ exchanger on the brush border membrane. The proton gradient is maintained by the flow of Na^+ down a concentration gradient. Thus, the proton required as a driving force to move peptides across the brush border membrane is indirectly dependent on the Na^+-K^+/ATPase pump on the basolateral membrane. This indirect dependency on Na^+ may explain discrepancies of Na^+-dependent peptide transport in intact cells from its independence found in membrane vesicles. It is of interest to speculate why a mechanism of this type evolved. Since protons result from gastric secretions this system of transport may have developed to increase the energy efficiency of uptake. Less energy may be needed to maintain the proton concentration at the brush border membrane in the proximal parts of the intestine.

Evidence has clearly shown the independence of free amino acid and peptide transport systems. Free amino acids are cotransported with Na^+ while peptides are cotransported with H^+. Both Na^+ and H^+ are transported across the membrane down a concentration gradient ultimately maintained by the Na^+-K^+/ATPase pump located on the basolateral membrane. Two independent systems may have evolved to insure the survival of the animal in the event one system fails.

Site of Amino Acid and Peptide Absorption in the Non-ruminant. In the non-ruminant animal the bulk of amino acid and peptide absorption appears to be in the jejunum (Heading et al., 1977). Direct comparisons of amino acid absorption in intestinal segments suggests the jejunum > ileum > duodenum. Absorption of the peptides including; glycylproline, glycylsarcosine, glycylphenylalanine and carnosine has been found to be greatest in the jejunum (Heading et al., 1977; Schedl et al., 1979; Ferraris et al., 1988). The site of absorption may depend on the individual peptide present in the lumen. Adibi and Morse (1971) reported that duodenum consistently had the least amount of absorption of peptides in humans. The jejunal and ileal dipeptide disappearance rates were either similar as for glycylleucine (94 and 92%) or were slightly different for glycylglycine (92 vs 79%).

Competition of Mucosal Uptake of Peptides and Amino Acids. The independence of peptide and amino acid transport systems clearly has been established. Jejunal uptake of carnosine was inhibited by equilmolar mixtures of other peptides including; Glycylglycine, tri-glycine, glycylsarcosine, glycylproline, methylmethionine and prolylhydroxyproline but not by an equivalent mixture of free amino acids in the hamster (Addison et al., 1974). Addition of basic (lysyllysine) and acetic (glutamylglutamine) peptides were not shown to inhibit the neutral peptide carnosine or cause inhibition between each other. Separate transport systems for basic, acidic and neutral peptides similar to the cationic, anionic and neutral systems described for free amino acids are suggested to be present. Similar observations of the lack of competition between amino acids and peptides have been noted by others (Rubino et al., 1971; Cheeseman and Develin, 1973; Matthews et al., 1974). The lack of competition between free amino acids and peptides during transport across

the brush border membrane is consistent with the concept that amino acids and peptides are transported by independent systems.

Factors Affecting The Uptake of Peptides by the Brush Border Membrane. Peptide size and composition influence uptake. Peters and MacMahon (1970) infused free glycine or equilmolar mixtures of di-, tri-, and tetraglycine into the duodenum of rats. A marked increase in free glycine and minimal amounts of the dipeptide were found in the plasma. No other oligopeptides were detected. Plasma concentrations of free glycine and the dipeptide were observed following infusion of the tripeptide and tetrapeptide, respectively. Heading et al. (1979) reported that the tripeptide tyrosylglycylglycine was absorbed intact from the intestine of rats. Under normal circumstances, in the adult mammal, tetrapeptides, like larger peptides and proteins need to be hydrolyzed to amino acids and di- and tripeptides prior to absorption (Silk et al., 1985). Substitution of an acetyl group on the terminal end or an amide group on the c terminal end of glycylglycine prevented its inhibitory effect on carnosine (Addison et al., 1974). Adibi et al. (1986) administered peptides or amino acids i.v. to rats and dipeptide and amino acid concentrations were measured in plasma, tissues and urine. Glycine substituting for alanine on the terminal of three peptides tested, resulted in a significant decrease in their hydrolysis in plasma. A decreased hydrolysis rate was noted for glycyl-L-alanine when compared to alanylglycine in human intestine (Silk et al., 1973b). Thus, both size and composition of a peptide may effect intact uptake into the circulatory system.

Peptide Utilization by the Peripheral Tissues. Krzysik et al. (1975) reported that glycylglycine was not hydrolyzed in the blood and minimal hydrolase activity of glycylleucine was associated with blood. Thus, the rapid clearance of these peptides must be the result of uptake by the peripheral tissues.

Krzysik and Adibi (1977) investigated the hydrolase activities against glycylleucine and glycylglycine using soluble fractions of blood, liver, kidney cortex skeletal muscle and jejunal and ileal mucosa of rats. The dipeptide glycylleucine was removed more rapidly from the plasma of intact animals than glycylglycine. Maximal tissue hydrolase activity was observed in the kidney and intestine with less activity observed in the other tissues investigated. The order of V_{max} for glycylleucine was found to be kidney > ileum > jejunum > liver > muscle > blood, while the V_{max} in the kidney and ileum switched for glycylglycine. No hydrolysis activity was noted for this dipeptide in the blood. They reported that, when calculated on a tissue weight basis V_{max} values for the liver and muscle were comparable to the other tissues.

The metabolic fate of three dipeptides, glycylleucine, glycylglycine, and glycylsarcosine were investigated
after i.v. administration into rats (Adibi et al., 1977). The composition of the peptide had a major affect on the fate of the peptide. Glycylsarcosine had the slowest plasma clearance, and the peptide could be found intact in the urine (13% of that administered) and in the liver, muscle, intestinal mucosa and renal cortex. Neither of the other two peptides were detected in the tissues of the animal nor were they excreted intact in the urine.

Lochs et al. (1988) utilized an organ balance technique in dogs to investigate the rate at which individual organs removed the dipeptides glycylleucine and glycylglycine from plasma. Though infused at identical rates, glycylleucine plasma concentrations were twofold less than glycylglycine, suggesting a greater extraction from plasma. The extraction of glycylleucine by organs could be accounted as follows; liver (25%), kidney (24%), muscle (12%) and gut tissue (10%). A similar order of extraction was not noted for the glycylglycine as the kidney accounted for

37% while the muscle, liver and gut tissue accounted for 18, 15 and 11% extraction, respectively.

These studies suggest that plasma has a limited ability to hydrolyze the peptides. Therefore, their rapid removal must be explained by the rapid transport of peptides from the blood into the peripheral tissues. Uptake is evidenced by the demonstration of the presence of intact carnosine in peripheral tissues (Adibi et al., 1977). With the exception of carnosine, no other intact peptides were found in these tissues. Hydrolysis of these peptides must occur in the membrane of tissues or more likely via intracellular hydrolysis. Current thinking on the synthesis of proteins and the participation of codons would require the hydrolysis of peptides prior to incorporation into protein.

Protein Metabolism in the Ruminant

The study of protein nutrition in the ruminant animal is lagging behind our knowledge of protein metabolism in other animals. This may be due to both inherent characteristics of the animal as well as early studies in ruminant N utilization. The ruminant animal poses considerable complications to the study of protein metabolism due to the interrelationships between the animal, rumen bacteria and protozoa (Huntington, 1986). Detailed reviews of N metabolism in the rumen have been published (Baldwin and Denham, 1979; Smith, 1979; Tamminga, 1979; Baldwin and Allison, 1983; Leng and Nolin, 1984). Research using non-protein N led to the conclusion that the ruminant animal required only a source of ammonia and carbon skeleton for the production of microbial protein. The microflora within the rumen could synthesize all essential amino acids (Loosli, 1949). The animal

could obtain its protein needs by the digestion of the microflora. Thus, the importance of protein quality was not of concern to the ruminant nutritionist for a number of years.

However, subsequent studies have demonstrated increased production when protein sources rather non-protein N sources are provided in ruminant diets (Oltjen, 1969; Young et al., 1973). The increase in production may, in part, be due to a more efficient production of microflora. Bacteria may obtain 50 to 60 % of the N incorporated into their proteins as a result of direct uptake of amino acids and peptides (Pittman and Bryant, 1964).

Peptide Accumulation in the Rumen. Generally the concentration of free amino acids in the rumen are low (Wright and Hungate, 1967; Broderick et al., 1981). Russell et al. (1983) hypothesized that peptide uptake rather than amino acid uptake is the rate limiting step in ruminal protein degradation. This hypothesis is supported in work by Chen et al. (1987a). They reported that sufficient protease activity occurs in the rumen but peptidase enzymes may be saturated. Thus, peptides may accumulate in the rumen. A preferential uptake of hydrophillic peptides by bacteria was noted (Chen et al., 1987b). While the K_m values for the uptake of hydrophillic and hydrophobic peptides were similar, the V_{max} was nearly twofold greater for the latter. This supports the concept that peptides containing long lipophillic side chains may have increased affinity for transport (Adibi and Soleimanpour, 1974). Broderick and Wallace (1988) reported increased peptide concentrations in the rumen when casein, but not ovalbumin was fed to sheep. They suggested that peptide accumulation in the rumen will only occur when a rapidly degraded protein, such as casein, is fed.

Peptides have been reported to accumulate in the rumen when proteins prone to rapid ruminal degradation are provided to the rumen microbes. The ability of the peptides to be utilized by the microorganisms appears to be dictated, in part, by the hydrophobic and hydrophillic properties of the peptide.

Bypass Protein and Post Ruminal N Supplementation. Post ruminal infusions of proteins, such as casein, have resulted in increased animal performance (Clark, 1975; Cohick et al., 1986; Gow et al., 1979; Rogers et al., 1984). This observation has stimulated interest in by-passing proteins from the rumen. Some dietary protein escapes ruminal degradation, passing directly to the small intestine. Factors contributing to the by-pass of dietary protein is a area of major interest to the ruminant nutritionist. Generally, but not always, the more soluble a protein is the more extensively it will be degraded in the rumen (Crawford et al., 1978; Chen et al., 1987). Treatment of feeds with heat or chemicals have been shown to reduce rumen degradation of protein and sometimes results in increased performance by the animal. If carried too far, these treatments also may decrease the digestibility of the protein in the small intestine.

Increasing the amount by-pass protein to the small intestine may result in an increased quantity of protein but not a good balance of amino acids. A single amino acid deficiency prevents protein synthesis beyond the point of that "limiting amino acid". Individual amino acid deficiencies would result in the deamination of the remaining amino acids, loss of the ammonia and urea and use of the carbon chain for energy (Church and Pond 1982).

Theoretically, the by-pass of limiting amino acids would be the most efficient method of increasing animal performance. The first three limiting amino acids in growing steers when protein is supplied by rumen microbes are, in order,

methionine, lysine and threonine (Richardson and Hatfeild, 1978). These amino acids have been by-passed from the rumen by protective coatings (Smith et al., 1984; Papas et al., 1984ab; Oke et al., 1986; Rogers et al., 1987; Wright and Loerch, 1988) and by feeding amino acid analogs (Lundquist et al., 1983; Polan et al., 1970; Winfschitl and Stern, 1988). These treatments have been shown to increase production, at times, but unpredictable benefits of their use have precluded regular supplementation in animal production systems. Use of microbes to produce amino acids through use of biotechnology may allow the feeding of individual amino acids to be very economically feasible.

The importance of peptides as a source of amino acids to be absorbed by the intestine is under investigation. Peptides rather than amino acids may be the form of choice to be supplemented in the future. Based upon the discussion already presented, the advantages of supplementing peptides rather than amino acids would include a more rapid and uniform absorption rate of amino acids, lower competition of free amino acids being absorbed at the brush border membrane, and a lower energy expenditure for the transport of equivalent quantities of amino acids across the intestinal barrier.

Improved performance reported with postruminal supplementation of proteins or amino acids has resulted in efforts to alter protein nutrition in the ruminant to be centered on minimizing ruminal degradation or by-passing the rumen altogether. The effects of by-passing proteins or limiting amino acids are often unpredictable reflecting the complex nature of protein metabolism in the ruminant.

Amino Acid and Peptide Absorption in the Rumen. Leibholtz (1971a) examined amino acid transport in the rumen of sheep and observed that only 6% of the N leaving the rumen was in the form of amino acids while the bulk of N leaving

was in the form of ammonia. Thus, amino acid absorption from the rumen appears to play a minor role of amino acid absorption of the ruminant. The absorption of histidine by rumen epithelium has been studied in vitro (Leibholtz, 1971b). Using isolated rumen epithelium histidine uptake was found to be unidirectional, steriochemically selective, pH dependent, competitively inhibited and reduced in the presence of 2,4-dinitrophenol and iodoacetate. This indicates that histidine was taken up by carrier mediated transport which required energy. Epithelium obtained from starved animals was shown to have a greater capacity for the uptake of amino acids. Whether the increase in uptake was due to the carrier mechanisms or to diffusion was not differentiated. The absorption of minimal levels of amino acids by the rumen have been noted by others (Cook et al., 1965; Chand et al., 1968)

Karasov et al. (1987) reported that populations of amino acid transporters are altered by different protein levels in the diet in the rat intestine. When protein levels are high, both essential and nonessential amino acids are taken up efficiently. However, when the diet is deficient in protein, little change or an increase in the number of transporters for the essential amino acids occurs while transporters for the nonessential amino acids are down regulated. A similar occurrence in altering the amino acid transporter population may occur in the rumen. This phenomenon is of interest to the ruminant nutritionists for it may have implications for an increased role of N transport in during compensatory growth and in both fasting and subsequent refeeding of animals which is common in studies examining the absorption of amino acids.

The potential existence for peptide transport in the rumen is an intriguing idea. Peptides accumulate in the rumen (Russell et al., 1983; Chen et al., 1987), hydrogen is readily available and protons are known to be cotransported with

peptides across the brush border membranes (Ganapathy and Leibach, 1985; Takuwa et al., 1985) and transporters have been found for amino acids in the rumen (Leibholtz, 1971b). If peptides are absorbed from the rumen or other region of the ruminant stomach they may be utilized as a tool for supplying limiting amino acids for the animal. The advantages of supplementing peptides rather than amino acids have previously been discussed.

Use of hydrolysis resistant peptides, such as proline containing peptides (Heizer and Laster, 1969) with amino acids such as methionine or lysine may decrease hydrolysis of the peptide and may allow intact peptides to be taken across the rumen. Proline, which is not considered an essential amino acid, would serve as a carrier to get the more desirable amino acid across the membrane. It should be noted, however, that proline may not be wasted for it could be utilized by the body or for milk production and this would be more cost efficient from an energy perspective because the body would not have to produce it.

The presence of transporters in the rumen have been demonstrated. However, the current concept is that the intestine is the primary site of amino acid uptake with little or no absorption of amino acids in the rumen. The absorption of peptides by the stomach region has not been studied.

Postruminal Protein Digestion. It generally is assumed that digestion of protein in the abomasum and small intestine of ruminants is similar to digestion of protein in the stomach and small intestine of non-ruminants. Notable differences between the non-ruminant and ruminants are that, in ruminants, there is an attenuated buffering of the digesta in the small intestine and the secretion of large amounts of nucleases by the pancreas. Microbial N in the form of nucleic acids accounts for 12 to 18% of the N entering the duodenum and 75 to 90 % of these

nucleic acids disappear in the small intestine (Bergen, 1978). In the stomach of non-ruminants and abomasum of ruminants, proteins are broken down to oligo peptides and peptides by actions of HCl and pepsins. These protein fractions are then released into the duodenum. Pancreatic enzymes further digest the luminal contents to peptides and some free amino acids. Intestinal peptidases further hydrolyze peptides to free amino acids. Most of these peptidases are imbedded in the intestinal wall, however, some peptidase activity can be detected within the lumen. The origin of the latter is presumed to be the result of sloughed mucosal cells. The mucosa of the small intestine contains uptake systems for free amino acids, peptides, nucleotides, and nucleosides (Berger, 1978).

Nitrogen Composition of Digesta Reaching the Small Intestine. In the non-ruminant animal, the amino acid profile reaching the duodenum is similar to that of the diet (Elwyn et al., 1968). The nitrogenous compounds entering the small intestine of the ruminant are composed of microbial and undegraded dietary proteins, nucleic acids of microbial origin, constitutes of bacterial cell walls, ammonia-N and in addition endogenous N sources including the gastric proteolytic enzymes (Armstrong et al., 1977) .

The relatively stable amino acid composition of ruminal microflora tends to attenuate changes in the amino acid composition of digesta reaching the duodenum. In the functioning ruminant animal, the amino acid profile reaching the duodenum resembles that of the rumen microbes (Hume et al., 1972; Wolff et al., 1972). Thus, low gut outputs of histidine and methionine into the portal system of the ruminant can be attributed to their low concentration in rumen microbes. Oldham and Tamminga (1980) reported the N profile of duodenal contents in cows to be essential

amino acids, 35%; nonessential amino acids, 30%; amides 4%; nucleic acids, 11%; ammonia, 6% and unknown, 14%.

The amount and quality of dietary crude protein reaching the duodenum may vary with diet due to actions of microflora in the rumen. Microbial action can reduce the quantity of crude protein reaching the small intestine, when high protein diets are fed, or increase the level when diets contain low crude protein levels. Generally, when the crude protein level in the diet is below 13 to 15%, the amount of crude protein reaching the duodenum exceeds dietary intake (Owens and Zinn, 1988).

The composition of N reaching the duodenum in the ruminant largely reflects the N composition of rumen microbes. The impact of the rumen microbes on the protein reaching the duodenum may be beneficial when dietary sources are low in quality or quantity of crude protein and detrimental when high quality protein diets are consumed.

Sources of Amino Acids and Peptides in the Gastrointestinal Tract. Sources of amino acids and peptides in the gastrointestinal tract include dietary proteins, microbial proteins and endogenous sources proteins. Endogenous sources on N may add substantial amounts of N into both the stomach and intestine of the animal. As summarized by Egan et al. (1986) the non-urea N entering the lower esophagus in the form of mucoproteins has been estimated to contribute .2 to .4 g $N \cdot d^{-1}$. Nitrogen addition by ruminoreticular epithelium may range from 2 to 10 g $N \cdot d^{-1}$. Abomasal secretions by the fundic and pyloric regions may contribute .48 to 2.76 g $N \cdot d^{-1}$. Thus, between 3 and 12 g of non-urea N (endogenous protein) can be added to the dietary intake prior to reaching the duodenum per day. Additional N likely is added by sloughed cells of the oral and nasal cavities and upper respiratory tract.

Nitrogen, in the form of urea, is extensively recycled to the rumen via the saliva and diffusion across the rumen wall or intestine. Kennedy and Milligan (1980) estimated that from 23 to 90 % of urea-N is recycled back to the digestive tract depending on the amount of dietary N. Generally, the lower the crude protein in the diet the greater the amount of urea recycled back to the rumen. Norton et al. (1978) estimated that urea-N was added by saliva at a rate of .5 to 2 g urea N·kg dry matter^{-1} while diffusion of urea into the rumen may add 1 to 13 g N·d^{-1} (Nolen and Leng, 1972). With typical diets, N recycling to the rumen via urea and ammonia pools equals 10-15 % of dietary N (Owens and Zinn, 1988).

Recycled urea can be utilized as a source of free amino acids or peptides only if it is utilized by the microbes. Thus, factors other than N availability may play a role in the amount of N reaching the duodenum. The amount of crude protein reaching the duodenum also may be influenced with the amount of energy consumed (Oldham and Tamminga, 1980). The biological value of protein may also be increased or decreased in the rumen.

In sheep, the quantity of N reaching the duodenum ranges from 16 to 25 g·d^{-1} (Ben-Ghedalia et al., 1974; Tagari and Bergman, 1978). The flow of N in the intestine increases in the duodenum due to additions of bile, pancreatic and duodenal juices. Ben-Ghedalia et al. (1974) noted the addition of .21 g·hr^{-1} of total N in lumen contents occurred between .05 and 1 m from the pylorus in sheep weighing from 50 to 60 kg. Tagari and Bergman (1978) reported a 4.4 g·d^{-1} increase in total N in the duodenum.

These measurements are consistent with values reported by others. Bile and pancreatic flows have been estimated to be .62 ml·kg^{-1}·hr^{-1} and 9.5 ml·kg^{-1}·d^{-1} (Harrison and Hill, 1960; Magee, 1961; Taylor, 1962). The N content has been

estimated at 76.5 $mg \cdot dl^{-1}$ for bile (Harrison, 1962) and between .48 and .72 $gm \cdot dl^{-1}$ for pancreatic juice (Taylor, 1962). Thus, for a 38 kg animal N additions from bile and pancreatic enzymes can be expected to be between 2.2 to 3 g $N \cdot d^{-1}$. Tamminga et al. (1979) reported that the endogenous protein contribution in the duodenum was 4 g N per kg DM ingested. Mucus secretions along the length of the intestine also add endogenous protein to the gastrointestinal tract. Additional sources of endogenous proteins include plasma albumin (Campbell et al., 1961) and desquamation of the intestinal mucosa. In the dog estimates of 5 to 10% of the plasma protein turnover can be attributed to hydrolysis of plasma albumin by the gut (Waldman et al., 1967) The contribution of N by mucosa cells remains unclear. However, estimates of the total contribution of endogenous proteins by the intestine are likely at least equal to the amounts leaving the abomasum. (Nolen, 1975).

Large quantities of endogenous proteins are added to the gastrointestinal tract in both the stomach and large intestine. Quantities added to the lumen likely are equal to or exceed amounts added by the diet. If additions of these sources of amino acids are taken into account, the discrepancies in accounting for the amounts of amino acids disappearing from the intestinal lumen and concomitant appearance of amino acids in the portal blood are greater than values reported.

Absorption of Amino Acids in the Intestine. Ben-Ghedalia et al (1974) reported that the most intensive rate of α-amino N uptake occurred between 7 and 15 m distant from the pylorus. Similar observations were made in studies by Tagari and Bergman (1978) and Ben-Ghedalia et al., (1982). In the latter two studies the percentage of amino acids absorbed by the lower small intestine increased with additional protein supplied to the small intestine. A preference for the uptake of

essential rather than nonessential amino acids has been observed (Armstrong et al., 1977).

While the highest rate of absorption occurs in the jejunum the highest capacity for transporting amino acids is in the ileum. Philips et al. (1976) investigated the absorption of methionine, valine and threonine in sheep. Both threonine and valine were absorbed in greater quantities in the ileum when compared to the jejunum. No preferential uptake for methionine was noted. Similar observations for lysine have been reported by Johns and Bergen (1973). Using bovine brush border membrane vesicles, Wilson and Webb (1990) reported that total lysine and methionine uptake by ileal tissues tended to be greater than uptake by jejunal tissues. In the ruminant the highest rate of amino acid absorption is in the jejunum while the ileum appears to have a higher capacity for amino acid absorption.

Amino Acid and Peptide Fluxes in the Gastrointestinal Tract. Amino acids generally are added to the portal system through absorption from the gut. In sheep, additions of amino acids to the portal system by the gastrointestinal tract range from .1 to 2.3 mmoles·hr^{-1} (Wolff et al., 1972; Heitmann and Bergman, 1980). Common fluxes in the portal system of ruminants and non-ruminants include; extraction of glutamine from the blood into the gastrointestinal tract, elevated alanine concentrations and low cystine levels. The latter may be the result of synthesis of glutathione by red blood cells (Elwyn et al. 1968).

Glutamine is continuously extracted from blood by the intestine. The extent of glutamine extraction can be significant. Wolff et al. (1972) noted glutamine was extracted by the gastrointestinal tract at a rate of 1.45 mmol·hr^{-1} . Glutamine serves as a vehicle for moving excess ammonium ions from the peripheral tissues and as a source of urinary ammonium ion for carrying away hydrogen excesses (Anonymous,

1989). The release of this amino acid from muscle occurs in both fed and fasted animals (Ballard et al., 1976). Glutamine can then be taken up by the gut tissues where it is utilized as an energy source. Glutamine may also be utilized by the gut for the production of proline, citrulline, organic acids, alanine and ammonia. (Windmueller and Spaeth,1974;1980). The small intestine also can produce glutamine. Porteous (1980) accounted for 60% of glutamate uptake by brush border cells of chicks as glutamine.

The addition of glutamate and aspartate to the portal system by the gastrointestinal tract is minimal though high concentrations of these amino acids are often found in the gut lumen (Tagari and Bergman. 1980). Moreover, the extraction of glutamate from the blood by the intestine has reported (Heitmann and Bergman, 1980). Burston et al. (1972) reported extensive transamination of aspartic and glutamic acids by the intestine, whether presented as free amino acids or in peptides to form alanine. The extensive transamination of glutamate and aspartate to glutamine and asparagine would explain their low concentrations in portal blood.

Alanine is produced from the metabolism of glutamate, asparagine and glutamine by the gut tissues (Burston et al., 1972; Porteous 1980). Alanine also serves as a primary transporter of amino N from the peripheral tissues to the liver (Ballard et al., 1976) Thus, the amount of alanine added to the portal system during absorption from the intestine generally is greater than any other amino acid. Wolff et al. (1972) noted alanine reaching the portal vein, accounted for 19% of the total amino N reaching the liver. Similar observations were noted by Koeln (1982) who reported that alanine comprised 19.3% of the free amino acids in portal plasma of calves.

Current data supports the concept that peptides may play a major role in amino acid metabolism in the ruminant. Koeln (1982) observed an increase in plasma peptide bound amino acids concentrations as blood traversed the gastrointestinal tract. Portal appearance of peptide amino acids was about threefold greater than for free amino acids. The largest increases in peptide amino acid concentrations were noted for aspartate, serine, glycine, leucine and lysine.

The liver removes most of the amino acids added to portal blood by the gut. This concept was established by Elwyn et al. (1968) who reported the liver of dogs removed the amino acids added to the portal system by the intestinal tract. Wolff et al. (1972) noted hepatic uptake exceeded gastrointestinal tract input for glycine, alanine, glutamine, tyrosine, arginine and phenylalanine, suggesting that these amino acids were also released by the peripheral tissues. Glycine, alanine and glutamine accounted for about 50% of the -amino N extracted by the liver. A net release from the splanchnic tissues was observed for the branched chain amino acids (isoleucine, leucine and valine), glutamate, aspartate, asparagine and the urea cycle amino acids ornithine and citrulline. Extraction of the amino acids alanine, glutamine and arginine and net splanchnic output of glutamate, ornithine, citrulline and the branched chained amino acids also were reported by Heitmann and Bergman (1980).

Bergman (1986) reported that the liver extracts 40% of the branched chained amino acids for protein production while the remainder are utilized by the peripheral tissues. The extraction of the branched chain amino acids by the liver appears to be unique to the ruminant since minimal removal of these amino acids by the liver occurs in the non-ruminant animal (Wahren 1982). Koeln (1982) reported a small but positive splanchnic output for essentially all free amino acids except glutamine in calves.

Fluxes of amino acids across peripheral tissues has been summarized by Bergman (1986). Arginine is released by peripheral tissues and kidneys with a concomitant extraction of the urea acid cycle anion acids citrulline and ornithine. The peripheral tissues release alanine, glutamine and glycine. The latter two also are released by the kidney. Alanine is extracted from the blood by the liver and kidney for gluconeogenesis. Glutamine is extracted by the liver and intestine for energy. In the small intestine alanine released from the catabolism of glutamine is released and extracted by the liver for gluconeogenesis.

In general, amino acids are added to the portal circulation by the mesenteric tissues. Most of these amino acids added to the portal circulation are removed by the liver. Glutamine and alanine serve to remove amino-N from the peripheral tissues. Glutamine is extracted from the blood by the intestine for a source of energy. Alanine is an important gluconeogenic precursor. Specific amino acids are released by the peripheral tissues and taken up by the liver while others are released by the liver to be utilized by the peripheral tissues. Peptides may play a yet undefined role in inter-organ cycling of amino acids.

Factors Affecting Plasma Amino Acid Levels. Plasma amino acid homeostasis results from the combined effects of tissue turnover and dietary inputs. Protein tissues of the body are continuously in a state of turnover. The constant breakdown and rebuilding of protein tissues may seem to be energetically inefficient, however this process has evolved to insure survival of the organism under constantly changing conditions (Schimke, 1977). The magnitude of amino acid release or uptake by any organ depends on the balance between protein synthesis and degradation. Factors which may influence tissue turnover and thus amino acid levels in the blood include dietary imbalances and hormone levels. Dietary imbalances

which have distinct effects on the amino acid profile in blood include; protein-energy deficiencies, protein deficiency, and amino acid imbalances. A nutritional state which results in the deficiency of both energy and protein is generally characterized by an increase in the essential:nonessential amino acid ratio. Body tissue proteins are mobilized to provide amino acids for a source of energy and amino acids for processes vital for the animals survival The alteration of the ratio between these two classes of amino acids results from the preferential utilization of the nonessential amino acids for gluconeogenesis (Felig et al., 1969; Erikson, 1988).

When energy is adequate but protein is deficient there is a concomitant narrowing of the essential:nonessential amino acid ratio (Felig et al., 1969; Lunn et al., 1976). Since energy is not a limiting factor, the body may continue to produce nonessential amino acids. Protein turnover will add both essential and nonessential amino acids to the blood. Thus, the relative amount of essential amino acids is decreased because the addition of these amino acids from dietary sources is limited. In an effort to maximize the additions of essential amino acids by the diet, alterations in the population of amino acid transporters has been reported where essential amino acid transporters are "upgraded" while those for the nonessential amino acids are "downgraded" (Karasov et al., 1987).

Amino acid imbalances can result in altered absorption due to either insufficient or surplus of an amino acid(s). Factors associated with a limiting amino acid have been previously discussed. The surplus of an individual amino acid may also exhibit detrimental effects through competition for transport at the brush border membrane.

The body's hormonal environment can alter amino acid levels. Two hormones which can greatly influence these levels in the ruminant include insulin

and growth hormone. Insulin is an anabolic hormone which is the main regulator of the disposition of absorbed nutrients in the "fed state". This hormone facilitates diffusion of glucose across membranes insulin sensitive tissues, other than the liver. Insulin increases activity of hepatic enzymes, thus indirectly increasing glucose absorption (Dickson, 1984). Plasma concentrations of insulin may be effected by production status of the animal. This is very evident in negative energy balances associated with lactation. Insulin concentration is depressed at the onset of lactation and increases as days in milk increase (Herbein et al., 1975). In ruminants, insulin secretion is correlated with dry matter and protein intakes but not plasma glucose concentration (Basset et al., 1971). Increased plasma insulin concentrations resulted from i.v. administration of amino acids in cattle (McAtee and Trenkle, 1971) and lambs (Call et al., 1972). Protein metabolism in the ruminant is effected by insulin concentration. Insulin has been shown to stimulate the uptake of valine, isoleucine, leucine, tyrosine, lysine and alanine in extrahepatic tissues of sheep (Brockman et al., 1975). Low insulin levels associated with fasting result in release in amino acids from the peripheral tissue (Ballard et al., 1976; Istasse et al., 1987).

Growth hormone functions to maintain (or increase) body protein. This role is especially critical during periods of low energy intake when muscle is being catabolized for the production of gluconeogenic precursors. Growth hormone increases uptake of amino acids, primarily those affected by the A system, by the peripheral tissues. A concurrent inhibition of glucose and fatty acids occurs in these tissues, thus partitioning these metabolites to the production of energy. These anabolic effects of growth hormone appear to the an indirect effect via insulin-like growth factors. In the liver, growth hormone increases the absorption of amino acids transported on the A and L systems. Albumin synthesis is increased due to growth

hormone's effect of increasing the number of MRNA involved in protein synthesis. This hormone exerts a sparing effect on plasma amino acids by mobilizing fatty acids for energy production. Because of this, numerous studies are presently investigating the exogenous administration of this hormone for increasing growth rate and leanness in meat animals and in enhancing milk production in dairy animals (Bines and Hart, 1981; Trenkle, 1981; Bauman et al., 1982). Concentrations of growth hormone can vary dramatically. Driver and Forbes (1981) noted growth hormone concentrations in sheep ranged from 6-82 $ng \cdot ml^{-1}$. In one animal concentrations reached 150^{+} $ng \cdot ml^{-1}$. Hormone concentrations suggested regular interval release in some, but not all animals. Of interest was the high percentage (71%) of spontaneous feedings which occurred 1 hr after individual peaks of blood growth hormone levels. Concentrations of growth hormone in the blood are negatively correlated with nutrient intake and plasma insulin concentrations (Basset et al., 1971). Concentrations may also be influenced by the production status of the animal. This is especially evident in the lactating animal as plasma growth hormone concentrations steadily decrease concurrently with increased d in milk (Herbein et al., 1975).

Plasma amino acid levels can be effected by both nutritional and humoral status of the animal. Diets deficient in both energy and protein result in a widening of the essential to nonessential amino acid ratio while a protein deficiency results in a narrowing of this ratio. Two important hormones involved in protein metabolism are insulin and growth hormone. Insulin promotes amino acid uptake and utilization by in the absorptive state while growth hormone appears to limit tissue catabolism during post absorptive states.

Oxygen Utilization and Blood Gas Analysis

As described previously, the active transport of amino acids and other metabolites is directly dependent on the maintenance of a Na^+-K^+-gradient. The transport of peptides is indirectly dependent on the maintenance of this gradient. A study by Milligan and McBride (1985) indicated that greater than 20% of the energy expenditure of skeletal muscle, duodenal epithelium and liver of ruminants was required to transport Na^+ and K^+ across the plasma membrane. Factors affecting oxygen consumption by these tissues included dry matter intake and lactation. Total respiration of intestinal epithelium was reduced when animals were fasted and increased when animals were fed above maintenance. On a per weight basis oxygen utilization by Na^+-K^+/ATPase-dependent systems of fed animals was 2.5 times greater than in fasted animals. An increase in O_2 consumption by Na^+-K^+/ATPase-dependent systems was also noted for liver and intestinal epithelium due to lactation.

Oxygen flux across a tissue bed, determined by arteriovenous differences times blood flow, may be utilized to calculate the energy consumption of that tissue (Huntington and Tyrell, 1985). Using this method, Huntington and Reynolds (1987) suggested the portal drained viscera of cattle accounted for 8-10% of body tissue but 18-25% of whole animal O_2 consumption. The liver also consumed O_2 at a rate disproportionate from its mass. Presumably, the high rate of O_2 utilization by these tissues is due to high rates of metabolism. Reported values for oxygen utilization in steers and dairy cows range from 918 to 1014 and 1296 to 2494 $mmol \cdot hr^{-1}$, respectively. Reported O_2 utilization by portal drained viscera of sheep range from 120 to 137 $mmol \cdot hr^{-1}$ (Gross et al., 1990). In steers mesenteric and non-mesenteric

drained viscera accounted for 51 and 49% of the oxygen consumption of portal drained viscera.

Heat production in ruminants can be calculated directly from oxygen consumption. The equation for converting oxygen utilization to heat production described by McLean (1972) is as follows:

$$H = 4.89\ V_O * X \quad \text{where}$$

H = heat in kcal

V_O = volume of expired air

$X = [O_2]_{in} - [O_2]_{out}$

From this equation Huntington and Reynolds (1987) estimated 110 kcal of heat are produced per mole of oxygen consumed. Thus, the measurement of oxygen consumption by a tissue bed can be used to estimate the amount of energy utilized for heat production by those tissues.

Oxygen utilization by a tissue is an indicator of the energy requirements of that tissue. On basis of mass, the visceral tissues in general utilize a disproportionate amount of energy. The intestine requires large amounts of energy for the transport of Na^+ and K^+. In ruminants the mesenteric and non-mesenteric viscera have similar energy requirements. The high energy requirements of the non-mesenteric tissues may be due to the constant muscular activity in these tissues but the possibility of active transport by the non-mesenteric tissues should not be lightly discarded.

Blood constituents in sheep previously reported are given in table 2.1

Table 2.1. Common values for blood parameters in sheep[a,b]

Haemoglobin ($g \cdot dl^{-1}$)	9-15
Hematocrit (%)	27-45
P_{O_2} (mmHg)	
Arterial	77-107
Venous	1-41
P_{CO_2} (mmHg)	
Venous	37-41
Osmolarity ($mOsmol \cdot liter^{-1}$)	293-304
Sodium (mM)	145-163
Potassium (mM)	4.6-5.2
Lactate (mM)	950-1600

[a]Smith, 1978.
[b]Hecker, 1983.

Chapter III

OBJECTIVES

The broad objective of this study was to elucidate the involvement of the transport of free and peptide amino acids by different gastrointestinal tissues in the nitrogen metabolism of the ruminant animal.

Specific objectives included;

1) To determine of amino acid and peptide fluxes across mesenteric and non-mesenteric tissues in sheep

2) To examine the effect of abomasal infusions of casein and a mixture of amino acids simulating casein on the flux of free and peptide amino acids in growing calves.

3) To estimate energy utilization of mesenteric and non-mesenteric tissues by determining oxygen consumption.

4) To examine blood flow, pH and plasma fluxes of lactate, Na^+ and K^+.

Chapter IV

THE CONTRIBUTION OF FREE AND PEPTIDE AMINO ACIDS BY THE STOMACH AND INTESTINAL VISCERA TO THE PORTAL SYSTEM OF SHEEP[1]

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Abstract

Nutrient fluxes were determined for mesenteric and non-mesenteric regions of portal drained viscera in six crossbred wethers (37.5 kg) surgically fitted with abdominal aorta, and mesenteric and portal vein cannulae. Wethers were kept near steady state conditions by feeding at hourly intervals under continuous lighting. Arterial, mesenteric and portal samples were obtained 10 to 15 d post-surgery. Nutrient fluxes from mesenteric and portal-drained viscera were measured. The non-mesenteric flux was calculated as the difference between portal flux and mesenteric flux. Blood pH was lower ($P<.001$) and PCO_2 and HCO_3 concentrations higher ($P<.03$) in venous blood when compared to arterial blood. Portal blood tended to be lower in pH ($P<.13$) than mesenteric blood. Arterial, mesenteric and portal plasma osmolarities were similar. Blood flow from the mesenteric and non-mesenteric viscera was .93 and 1.24 liters·min^{-1}, respectively. Oxygen consumption

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[3]Send reprint requests to this author.
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by the portal drained viscera averaged 121 mmol·hr^{-1}. Non-mesenteric and mesenteric tissues contributed equally to oxygen consumption by the portal drained viscera (49.8 and 50.2%, respectively). The appearance of free amino acids was essentially limited to the blood draining the intestinal region. Total net absorption of free amino acids from the intestinal region was 35.74 g·d^{-1}. Peptide amino acids were observed in plasma draining both the mesenteric and non-mesenteric viscera. The flux of peptide amino acids across the non-mesenteric viscera was nearly six-fold greater than the flux of peptide amino acids across the mesenteric viscera indicating a possible major role of the stomach in the absorption of peptides.

(Key Words: Amino Acids, Peptides, Oxygen Consumption, Nutrient Uptake, Mesenteric Viscera, Non-mesenteric Viscera, Sheep.)

Introduction

Since the classical work by Newey and Smith (1959, 1960) evidence has clearly shown that peptides play a role in the uptake of protein digestion products from the intestinal lumen (See reviews by Gardner 1984; Matthews, 1975; 1976; Silk et al., 1985). Current doctrine suggests that di- and tripeptides can be transported intact across the brush border membrane. The driving force of peptide transport appears to be a proton gradient and is separate from Na^+-dependent transport of free amino acids (Ganapathy et al., 1985; Takawa et al., 1985, Miyamoto, 1985). Generally, it is believed that the major portion of these peptides are hydrolyzed within the enterocyte to free amino acids prior to entering the portal system but there is some passage of intact peptides.

In the nonruminant animal, perhaps 10 to 30 % of the α-amino nitrogen reaching the portal system may be in the form of intact peptides (Gardner, 1975; 1982; Gardner et al., 1983). Koeln (1982) reported that two thirds of the amino acids

in the portal blood of calves were associated with peptides. Most of these peptides had molecular weights between 300 and 500 daltons (Schlagheck and Webb, 1984). Large concentrations of peptide amino acids have been noted in the peripheral circulation of calves fed natural and purified diets (McCormick and Webb, 1982; Danilson et al., 1987). This suggests that peptides play a more dramatic role in protein metabolism in the ruminant.

Numerous studies have utilized catheters placed in an artery and the portal vein to obtain estimates of arterovenous differences and blood flow to determine nutrient fluxes across the gastrointestinal tract of sheep (Gross et al., 1990; Hume et al., 1972; Heitmann and Bergman, 1980; Wolff et al., 1972) and cattle (Koeln, 1982). In the case of amino acids and peptides, portal samples were assumed to be indicative of amino acid and(or) peptide absorption occurring in the small intestine because it had been reported that the rumen plays a minimal role in the absorption of amino acids (Leibholtz, 1971ab). Recently it has been observed that peptide concentrations become elevated in the rumen because of the action of bacteria (Chen et al., 1987; Broderick and Wallace, 1988). The present study was undertaken to measure quantities of free and peptide amino acids reaching the portal system of sheep and to determine whether these were coming from the mesenteric (intestinal) or the non-mesenteric (stomach) regions of the gastrointestinal tract.

Materials and Methods

Animals Care and Management. The experiment was conducted using six crossbred wethers which averaged 38 kg. At least 1 wk prior to surgery animals were tethered in individual pens on non-flattened expanded metal floors. All animals

received Ivermectin[5] (.02 ml·kg^{-1}) for control of parasites and were injected with vitamins A, D, E and selenium (Table 4.1). Wethers were managed so that they would have near "steady state" digestive and metabolic conditions (Minson and Cowper, 1966; Wolff and Bergman, 1972). Wethers were fed 24 meals of equal portion (hourly) via an automatic feeder under constant light. The animals were fed an orchardgrass, corn and soybean based diet (Table 4.1) in quantities sufficient to produce a gain of 150 g·d^{-1}. Feed refusals were collected daily. Fresh water was available ad libitum. Wethers were fasted for 48 hr prior to surgery and following surgery were reacclimated to the diet over a 3- to 5-d period.

Surgical Preparation. Chronic catheters were surgically implanted in the abdominal aorta, portal vein, and mesenteric veins under strict aseptic conditions. Anesthesia was induced with an i.m. injection of a Ketamine [6] (5 mg·kg^{-1}) and Xylazine[7] (.1 mg·kg^{-1}) mixture. The animal was placed on a surgical table in left lateral recumbency. A tracheal tube was inserted and anesthesia was maintained with 1 to 3% Fluothane[8] in oxygen (flow 25 ml·min^{-1}·kg^{-1}) on a semi-open circuit. A jugular catheter was inserted to allow i.v. administration of an isotonic mixture of glucose and lactated ringers solution[9]. The i.v. administration rate was 10 ml·min^{-1}·kg^{-1} for the first hour and 5 ml·min^{-1}·kg^{-1} until the end of the procedure. Heated water[10] (38^{o}C) was circulated in a pad[11] placed beneath the animal to maintain body temperature during the procedure. The

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[5]Ivomec, MSDAGVET Division of Merck and Co. Inc. Rahway, NJ.
[6]Ketamine, Veterinary Products Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, NY
[7]Rompun, Mobay Corporation, Animal Health Div., Shawnee, KA.
[8]Bromochlorotriflouethane, Ayerst Laboratories, New York, NY.
[9]Travenol Laboratories, Inc., Deerfield, IL
[10]Aquamatic K-Module, Baxter Hospital Supply, Norcross Ga.
[11]American Reusable K-Pad, Baxter Hospital Supply, Norcross Ga.

TABLE 4.1. COMPOSITION OF THE EXPERIMENTAL DIET UTILIZED IN THE SHEEP STUDY[a]

ITEM	%[b]
Ingredient composition	
Corn, ground	50.00
Orchardgrass hay	30.00
Soybean meal	13.30
Molasses	5.00
Deflourinated rock phosphate	.42
Limestone	.78
Trace mineralized salt	.50
Coccidiostat[c]	++
Chemical composition	
Dry matter	91.06
Crude protein[d]	13.92

[a] All animals received intramuscular injections of Vitamin A, 500,000 IU; Vitamin D, 75,000 IU; Vitamin E 3.7 $IU \cdot kg^{-1}$; and selenium $55ug \cdot kg^{-1}$.
[b] As fed basis.
[c] Cocci-Control [ix] Crumbles Medicated, Southern States, Inc., Richmond, VA. ($.5\ mg \cdot kg^{-1} \cdot d^{-1}$).
[d] Dry matter basis.

arterial catheter was inserted into the abdominal aorta via the right femoral artery using techniques described by McCormick and Webb (1982). For placement of portal and mesenteric vein catheters, a 22-cm incision was made 2 cm caudal and parallel to the last rib and chondral cartilage. When necessary, the rumen was deflated to allow better access to the portal system. The small intestine was externalized and kept moistened with warm sterile physiological saline solution ($38^{o}C$) throughout the procedure. Duplicate portal and mesenteric sampling catheters and mesenteric infusion catheters were implanted via mesenteric branches. During the catheterization procedure small mesenteric branches were exposed and .5 ml xylocaine was injected into neighboring fascia for maintenance of vessel tonicity (McCormick and Webb, 1982). Entry was through these small mesenteric branches and the catheters were inserted until the tips were appropriately positioned. Portal and mesenteric veins were palpated to insure that catheter tips, for sampling, were located near the portal-liver junction and caudal to the mesenteric-gastrosplenic junction, respectively. Catheters for the infusion of para aminohippuric acid were placed in small distal mesenteric veins. All catheters were passed under the skin and exteriorized on the lumbar region and attached to luer-lock valves. Prior to closing the incision, an abdominal lavage was preformed with heparinized physiological saline (10 USP units$\cdot ml^{-1}$) to minimize adhesion formation.

Cannula were flushed initially, 5 d post surgery and daily thereafter with sterile physiological saline. Following flushing, all catheters were filled with a sterile heparinized saline solution (30 USP units$\cdot ml^{-1}$) containing 2.4 mg penicillin$\cdot ml^{-1}$. Following surgery, the animal received injections of cyanocabolamine[12] (.5 ml) and B vitamins[13] (5 ml). An analgesic, Butorphranol[14] (.1 mg$\cdot kg^{-1}$), was administered

[12]Cyanocobalamin Injection, Vedco Inc., Overland Park, KS.
[13]Vitamin B Complex Forte, Vedco Inc., St. Joseph, MO.

i.m. 1 to 2 d post surgery. Daily i.m. antibiotic injections[15] (20 $mg \cdot kg^{-1}$) were given for 3 to 5 d postsurgery.

Catheter description. All venous catheters were made of silastic medical grade tubing[16] (.97 mm i.d., 1.65 mm o.d.). Lengths of catheters were 80, 77.5 and 84 cm for portal, mesenteric sample and mesenteric infusion catheters respectively. A 1 cm piece of sponge[17] was glued to catheters with silastic adhesive distal to the tip so that the portion of the catheter which was inserted into the vessel was 19, 14 and 7.5 cm for portal, mesenteric sampling and mesenteric infusion, respectively . The arterial catheters were produced as follows: 1) a 76 cm piece of Silastic[18] tubing (1.57 mm i.d. 3.18 mm o.d.) was split open to a length of 60 cm.; 2) a 99 cm piece of teflon[19] (.97 mm i.d. 1.58 mm o.d.) was inserted into the split in the silastic tubing; 3) The unsplit portion of silastic was expanded with xylene to allow the insertion of 7 cm of the teflon resulting in a 32 cm piece of uncovered teflon; 4) the silastic tubing was sealed with Silastic medical grade adhesive[20]; 5) the silastic tubing at the end where the teflon exited was covered with a 7.6 cm piece of Ivalon sponge. Prior to implantation all catheters were treated with a 2% ($wt \cdot wt^{-1}$) heparin complex solution[21].

Sampling. Lambs were allowed to recover from surgery until they consumed amounts of the diets which exceed maintenance requirements for at least 5 d.

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[14]Butorphranol, Fort Dodge, Edgemont, PA.
[15]Liquamycin 100, Pfizer, Inc. New York, NY.
[16]Silastic Medical Grade Tubing, Dow Corning Corp., Midland, MI.
[17]Ivalon sponge, Unipoint Industries, Inc., High Point, NC.
[18]Silastic Medical Grade Tubing, Dow Corning Corp. Midland, MI.
[19]AIN Plastics Inc. Norfolk, VA.
[20]Silastic Medical Adhesive, Dow Corning Corp. Midland, MI.
[21]TDMAC-Heparin Complex Coating, Polysciences, Inc., Warrington, PA.

Arterial and venous samples were obtained for determination of nutrient flux 10 to 15 d after surgery. Samples of blood from the aorta, mesenteric and portal veins were simultaneously collected in heparinized syringes at 0, 15, 30 and 45 min during a 1-hr feeding period. Mesenteric and portal blood flow were determined by procedures adapted from Katz and Bergman (1969). Blood flow was determined by dilution of a primed (.08 $ml \cdot kg^{-1}$ 10% $wt \cdot v^{-1}$ p-aminohippuric acid (PAH) solution) continuous infusion of sterile 1.5% PAH solution into the mesenteric infusion catheter. Blood plasma was obtained by harvesting the liquid phase following centrifugation. Separate samples of whole blood were collected into 1 ml heparinized syringes for blood gas analysis. Samples of whole blood and plasma were immediately placed on ice.

Laboratory Analyses. Para-aminohippuric acid concentrations in whole blood were assayed by methods described by Katz and Bergman (1969). Whole blood was analyzed for pH and oxygen saturation (O_2Sat) using a blood gas analyzer[22]. Blood hematocrits (Hct) were obtained using microhematocrit procedures. Hematocrits were utilized to correct plasma flow (PF) by the following equation: PF = BF * ((1-Hct) + .2(Hct)) where BF equals blood flow. Hematocrits also were utilized to calculate oxygen content (O_2Ct) in whole blood using the following equation: O_2Ct = 1.39 (Hb) * $O_2Sat/100$; where Hemoglobin (Hb) = Hct/3. Total carbon dioxide, bicarbonate, base excess, Na^+ and K^+ concentrations also were determined by blood gas analysis. Osmolarity of plasma was determined using an osmometer[23].

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[22]Stat Profile 2 Blood Gas Analyzer, Nova Biomedical, Waltham, MA.

[23]Osmette A Automatic Analyzer, Precision Systems, Inc. Sudbury, MA.

Plasma lactate concentrations were determined using immobilized enzyme analysis procedures[24].

The plasma samples obtained at 0, 15, 30 and 45 min were composited for each vessel and deproteinized by filtration in preparation for amino acid analyses. A 1:1 mixture of plasma and internal standard was placed into a micropartition system[25] and filtered through a membrane filter[26] at 1,500 x *g* for 15 min. The filter excluded protein and peptide fragments of a molecular weight greater than 10,000 daltons. The filtrate was stored at -20^{o}C until analysis. An aliquot of the filtrate was hydrolyzed in HCl as described by Cohen et al. (1989). The filtrate and the hydrolyzed filtrate were analyzed for free and total amino acid concentrations, respectively on a PICO·TAG Amino Acid Analysis System[27]. The concentration of peptide amino acids was calculated as the difference between total and free amino acids.

Mesenteric and portal-drained visceral fluxes of nutrients were calculated as the product of the difference between venous and arterial concentrations and plasma flow. The non-mesenteric contribution to portal flux was calculated as the difference between portal flux and mesenteric flux. The non-mesenteric contribution to portal flux represents tissue beds drained by the gastrosplenic vein (rumen, reticulum, omasum, abomasum, pancreas and spleen) and gastroduodenal vein (abomasum, pancreas and proximal duodenum) (Heath, 1968). A positive flux indicated net

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[24]YSI Model 27, YSI Scientific, Yellow Springs, OH.
[25]Micropartition System MPA-1, Amicon Division, W. R. Grace & Co.-Conn Danvers, MA.
[26]Millipore Type PLGC Ultrafiltration Membrane, 10,000 NMWL, Millipore Corp., Bedford, MA.
[27] Pico.Tag Amino Acid Analysis System, Waters Div. of Millipore, Millipore Corp. Milford, MA.

release of a nutrient by the gut, whereas a negative flux indicated net utilization of a nutrient by gut tissues.

Statistical Analyses. Differences between blood flow, between nutrient fluxes across the mesenteric and non-mesenteric viscera and between free and peptide bound amino acids were tested by the paired T-test procedures of SAS (1985). Differences in pH, Hct, HCO_3, CO_2, osmolarity and lactate were evaluated by analysis of variance using the general linear model procedure of SAS and orthogonal contrasts were used to test for significant differences between means.

Results and Discussion

All six animals responded well to surgery and returned to a normal feeding schedule within 2 to 3 d post surgery. Rectal temperatures were normal within 4 d post surgery. The average daily dry matter intake 5 d prior to sampling was 852 g. Feeding habits varied with animals. Though feed was presented at hourly intervals, it was not consistently consumed during individual feeding periods. Hume et al. (1972) reported that net absorption of amino acids into portal blood could be altered by feeding frequency. Some variation in nutrient fluxes between animals, thus, may be attributed to differences between the last meal consumed and time of sampling.

Presented in Table 4.2 are blood parameters investigated for samples obtained from the aorta, mesenteric vein and portal vein. Blood pH ranged from 7.49 in arterial blood to 7.42 in samples obtained from the portal vein. Difference between arterial and venous blood pH ($P<.001$) may, in part, be due to the increase in CO_2 concentration. Total CO_2 content was increased from 26.51 mM in arterial blood to 27.36 mM ($P<.01$) in venous blood. Bicarbonate concentrations were

TABLE 4.2. ARTERIAL, MESENTERIC AND PORTAL BLOOD pH, HEMATOCRIT, GASSES AND MINERAL CONCENTRATIONS AND PLASMA OSMOLARITY, AND LACTATE CONCENTRATIONS OF FED SHEEP

	Arterial	Mesenteric	Portal	p^a	p^b	SE^c
pH	7.49	7.44	7.42	.001	.15	.008
Hematocrit (%)	29.6	29.7	29.4	1.00	.07	.142
Oxygen (mmHg)	82.94	43.20	45.44	.001	.52	2.33
Oxygen saturation (%)	96.98	80.15	81.49	.001	.51	1.34
Carbon dioxide (mmHg)	33.2	38.4	40.3	.001	.18	.887
Total carbon dioxide (mM)	26.5	27.3	27.4	.01	.70	1.90
Bicarbonate (mM)	25.5	26.0	26.1	.03	.18	.168
Base excess (mM)	2.93	2.46	2.21	.007	.20	.121
Sodium (mM)	147.8	148.6	148.5	.02	.66	.169
Potassium (mM)	4.09	4.06	4.18	.62	.10	.044
Osmolarity	282	283	284	.28	.14	.624
Lactate ($mg \cdot dl^{-1}$)	10.38	12.72	13.33	.001	.34	.432

[a] Probability that a difference between arterial blood and venous blood this large or larger could have occurred by chance.
[b] Probability that a difference between mesenteric and portal blood this large or larger could have occurred by chance.
[c] Standard error of the treatment mean.

higher in venous blood when compared to arterial blood ($P<.03$), but were similar between blood obtained from mesenteric and portal veins ($P<.18$). Base excess was lower for venous blood when compared to arterial blood ($P<.007$). Base excess is defined as the number of millimoles of strong acid or base needed to titrate 1 liter of blood to pH 7.4 while the P_{CO_2} is held constant at 40 mmHg. The decrease in base excess indicates that P_{CO_2} level was not the only factor contributing to the decrease in pH. Other factors contributing to the lower venous pH may include the absorption of organic acids including acetate, propionate, beta-hydroxybutyrate and lactate from the portal-drained viscera. (Stevens, 1970; Annison and Armstrong, 1970). Plasma L-lactate concentrations were higher in venous plasma ($P <.001$) when compared to arterial plasma.

Hematocrits were similar between arterial and venous blood. A small decrease in hematocrit was noted between the mesenteric and portal blood ($P <.07$). This may be attributed to the absorption of large quantities of water by the non-mesenteric viscera. Average plasma osmolarity was similar for all vessels. Values observed for the blood pH, hematocrit, oxygen, oxygen saturation, carbon dioxide, bicarbonate, and osmolarity are in agreement with previously reported values for sheep (English et al., 1969; Smith et al, 1978).

Average blood flow and oxygen consumption by the mesenteric and non-mesenteric visceral tissues are presented in Table 4.3. Portal blood flow for the six sheep averaged 58.7 $ml \cdot min^{-1} \cdot kg^{-1}$ which is similar to flows obtained by others (Huntington et al., 1980; Gross et al., 1990). On a percentage basis, the non-mesenteric and mesenteric viscera accounted for 58 and 42 percent of portal blood flow, respectively. Blood flow from the two regions were similar ($P<.17$) and in four animals the ratio of blood flow from the two tissue beds was near 1:1. Huntington

TABLE 4.3. BLOOD FLOW AND OXYGEN, L-LACTATE, SODIUM AND POTASSIUM FLUXES ACROSS THE MESENTERIC AND NON-MESENTERIC VISCERA OF FED SHEEP

	Tissue					
	Mesenteric	P^a	Non-mesenteric	P^a	P^b	SE^c
Blood flow ($ml \cdot min^{-1} \cdot kg^{-1}$)	27.7	.001	33.57	.001	.17	3.47
Oxygen ($mmol \cdot hr^{-1}$)	60.25	.001	61.63	.01	.92	5.01
L-Lactate ($mmol \cdot hr^{-1}$)	11.42	.01	23.27	.03	.18	5.96
Sodium ($mmol \cdot hr^{-1}$)	.83	.02	.63	.58	.81	.62
Potassium ($mmol \cdot hr^{-1}$)	-.03	.46	.24	.20	.13	.14

[a] The probability that the flux did not differ from zero.
[b] Probability that a difference between mesenteric and non-mesenteric flux this large or larger could have occurred by chance.
[c] Standard error of the vessel mean.

and Reynolds (1986) noted the non-mesenteric and mesenteric viscera accounted for 58 and 42 % of the blood flowing to the portal system of steers. Hales (1973) reported that the percentage of cardiac output directed toward the spleen, rumen, small intestine and large intestine of sheep was 4.66, 10, 11 and 9 percent, respectively. Thus, in their study these tissues accounted for approximately 35 % of the cardiac output. Cardiac output of sheep has been estimated to be between 115 to 143 $ml \cdot min^{-1} \cdot kg^{-1}$ (Hales, 1973; Bowers et al., 1979). Using these figures the cardiac output for the sheep in our study would be between 4.37 and 5.43 $liters \cdot min^{-1}$. Thus, it is estimated that in the present study, there was a cardiac output directed to the portal drained viscera of between 41 and 50%.

Oxygen consumption by the portal drained viscera (PDV) averaged 121 $mmol \cdot hr^{-1}$. Gross et al., 1990 reported that oxygen consumption by PDV of sheep fed alfalfa or infused with three different levels of energy to be 137, 120, 61 and 126 $mmol \cdot hr^{-1}$. Net oxygen consumption differed from zero for blood crossing the mesenteric (60.25 $mmol \cdot hr^{-1}$, $P < .001$) and non-mesenteric (61.63 $mmol \cdot hr^{-1}$, $P < .01$) tissues and each contributed about equally, 49.8 and 50.2% to PDV oxygen consumption (Table 4.2.).

Based on tissue mass, Huntington and Reynolds (1987) reported that the PDV and liver consume a disproportionate share of oxygen. Glucose and amino acids are actively cotransported across the brush border with Na^+. The driving force of this transport is an electrochemical gradient maintained by the Na^+-K^+/ATPase pump. Milligan and McBride (1985) reported that more than 20% of the energy expenditure of skeletal muscle, duodenal epithelium and liver in ruminants is required to transport Na^+ and K^+ across basal membranes. Thus, a high oxygen consumption rate in the small intestine is expected. In steers, oxygen consumption

by the mesenteric viscera accounted for only 51 % of the consumption by the portal drained viscera (Huntington and Reynolds, 1986). The high oxygen requirements observed in the present study for non-mesenteric viscera may be due to the tissue mass and to muscle activity of the ruminant stomach. The stomach of these lambs likely accounted for 39 to 49 % of the alimentary canal (Dziuk, 1984). The possibility of active transport of substances by non-mesenteric tissues, however, should be considered.

There was a net flux of L-lactate (Table 4.3) across mesenteric ($P<.01$) and non-mesenteric ($P<.03$) visceral tissues. Net fluxes of L-lactate across the non-mesenteric and mesenteric tissues were 23.7 and 11.42 $mmol \cdot hr^{-1}$, respectively and the difference was not significant. Lactate can be absorbed along the entire alimentary tract. Ward et al. (1961) reported that lactic acid accounted for 18, 26, 26 and 25 % of the organic acids present in the abomasum, small intestine, caecum and colon, respectively. Generally, the absorption of lactate is considered to be via diffusion, however, a Na^+-coupled transport has been suggested to exist on the brush border membrane (Giesecke and Stangassinger, 1980). The amount of lactate absorbed by the gastrointestinal tract is dependent on the diet consumed by the animal. In the present study, the net flux of L-lactate across the PDV was larger than measurements obtained in studies which utilized a high forage diets (Huntington et al., 1980.; Gross et al., 1990). A high concentrate diet may result in elevated rates of lactate production by the direct metabolism of carbohydrates to lactate or indirectly by the conversion of propionate to lactate.

The flux of Na^+ was different from zero in the case of mesenteric ($P<.02$) but not non-mesenteric ($P<.58$) tissues, but when these were compared they were

not statistically different (Table 4.3). Potassium flux did not differ from zero for either the mesenteric or non-mesenteric viscera.

Presented in Table 4.4 are the fluxes of plasma free amino acids across the mesenteric and non-mesenteric viscera. Generally all of the amino acids were added to blood as the mesenteric viscera was traversed. The net flux of free amino acids across the mesenteric tissues was observed to be different from zero ($P<.10$) for ten amino acids investigated with a number of others approaching significance ($P<.20$). Total additions of amino acids which normally would be considered to be constitutes of dietary protein by the small intestine was 36.74 $g \cdot d^{-1}$. This represents approximately 31 % of the daily intake of 118.6 g crude protein per day. Essential and nonessential amino acids contributed equally to the daily total free amino acid addition to the mesenteric blood. The preferential uptake of essential amino acids by the small intestine has been previously reported (Armstrong et al., 1977). Alanine flux was greatest for free amino acids and contributed 13% of the amino acid addition to the mesenteric blood. Wolff et al. (1972) noted that alanine comprised 19% of the α-amino acid N addition to portal blood of sheep. The high contribution of alanine to the portal system may be attributed to the combined addition from intestinal absorption and from catabolism of glutamine by the intestine.

Glutamine was extracted from the blood at a rate of 5.87 $g \cdot d^{-1}$ ($P < .08$). The extraction of glutamine by the intestinal tissue has been reported for ruminants and non- ruminants and occurs in both fed and fasted animals (Ballard et al., 1976). Glutamine serves as a vehicle for moving excess ammonium ion from the peripheral tissues and as a source of urinary ammonium ion for carrying away hydrogen excesses (Anonymous, 1989). Intestinal tissues may utilize glutamine as a source of

TABLE 4.4. PLASMA FREE AMINO ACID FLUXES ACROSS MESENTERIC AND NON-MESENTERIC DRAINED VISCERA OF SHEEP

	Mesenteric flux		Non-mesenteric flux			
	$g \cdot d^{-1}$	P^a	$g \cdot d^{-1}$	P^a	P^b	SE^c
Dietary Protein amino acids						
Alanine	4.93	.04	2.76	.16	.41	1.72
Arginine	.95	.64	-1.33	.65	.48	.45
Asparagine	3.86	.05	1.70	.47	.51	2.15
Aspartic acid	.89	.02	.68	.36	.78	.51
Glutamic acid	3.06	.05	-.01	1.00	.32	1.98
Glutamine	-5.87	.08	-6.67	.07	.87	3.34
Glycine	2.18	.06	-2.10	.11	.008	.71
Histidine	.77	.20	-.06	.94	.14	.33
Isoleucine	2.21	.14	.19	.91	.42	1.61
Leucine	3.76	.21	1.40	.66	.64	3.32
Lysine	2.98	.15	1.85	.46	.74	2.26
Methionine	.85	.23	.56	.55	.79	.72
Phenylalanine	2.33	.13	.36	.80	.42	1.60
Proline	2.79	.04	2.14	.43	.83	2.03
Serine	2.37	.05	1.72	.40	.78	1.53
Threonine	1.36	.31	-1.67	.72	.56	3.40
Tryptophan	.66	.38	-.55	.46	.19	.57
Tyrosine	2.23	.12	-.13	.92	.31	1.47
Valine	2.43	.18	-.22	.91	.38	1.96
Essential[d]	18.30	.21	.53	.97	.46	15.81
Nonessential[e]	18.74	.04	5.04	.64	.33	8.88
Total[f]	36.47	.11	5.57	.83	.41	24.48
Other amino acids						
1-Methylhistidine	-.40	.24	.18	.51	.33	.38
3-Methylhistidine	.02	.96	-.20	.69	.55	.24
Carnosine	-.62	.38	-.56	.60	.97	1.07
Citrulline	.94	.44	3.00	.27	.08	1.25
Hydroxylysine	.18	.37	-.01	.99	.51	.19
Hydroxyproline	.38	.15	-.19	.43	.11	.21
α-Aminoadipic Acid	-2.64	.48	1.16	.79	.29	2.28
Ornithine	.10	.70	.96	.30	.24	.45
Phosphoethanolamine	.23	.10	-.24	.46	.20	.23
Phosphoserine	6.97	.41	-1.71	.82	.27	4.94
β-Aminoisobutyric acid	.36	.14	-.36	.14	.14	.29
Taurine	.54	.08	.53	.76	1.00	1.15

[a] Probability that the flux did not differ from zero.
[b] Probability that a difference between mesenteric and non-mesenteric flux this large or larger could have occurred by chance.
[c] Standard error of the vessel mean.
[d] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+ Methionine+ Phenylalanine+Threonine+Tryptophan.
[e] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+ Alanine+Proline+Tyrosine.
[f] Total=Essential+Nonessential.

energy or it may be utilized for the production of proline, citrulline, organic acids, alanine and ammonia (Windmueller and Spaeth, 1974,1980).

The nitrogenous compounds entering the small intestine of the ruminant consist of microbial and undegraded dietary proteins, nucleic acids of microbial origin, constituents of bacterial cell walls, ammonia-N and endogenous N sources including the gastric proteolytic enzymes (Armstrong et al., 1977). Thus, unlike the non-ruminant, amino acid profiles entering the duodenum of the ruminant resemble that of the rumen microbes. Of the amino acids commonly considered as part of a dietary source, tryptophan, histidine and methionine were absorbed at the lowest daily rates. The low absorption of methionine and histidine is likely due to their low concentrations in rumen microflora (Hume et al.,1972; Wolff et al.,1972; Bergman,1986).

The values obtained for free amino acid flux across non-mesenteric tissues generally were small, equally distributed between positive and negative values and not significantly different from zero. An exception was glutamine which was extracted ($P < .07$) from plasma as the non-mesenteric tissues were traversed. Glutamine may serve as an energy source for these tissues as it does in intestinal tissues.

These data support the concept that little or no amino acid absorption occurs from the stomach. This thinking was pioneered by the work of Leibhotz (1971a). She reported that amino acid absorption was minimal from the rumen and represented only 6% of the N passing into the mucosa. It seems, therefore, that the data from the present study support the current concept that free amino acids are absorbed from the intestine and that little or no absorption occurs in the stomach. Even though sizable differences were observed between mesenteric and non-

mesenteric fluxes, the large amount of variation encountered precluded detection of statistical significance.

For peptide amino acids which normally would be considered to be constitutes of dietary proteins, flux was positive and amounted to a total flux of 52.01 $g \cdot d^{-1}$ (Table 4.5). With the exception of tyrosine ($P < .08$) and glycine ($P < .01$), none of these fluxes were determined to be statistically different from zero. Glycine flux was greatest for plasma peptide amino acids and contributed 19% of the amino acids associated with peptides. Transport of intact, glycine- containing peptides has been demonstrated both in vitro and in vivo (Newey and Smith 1959,1960; Peters and MacMahon, 1970).

Of the other amino acids measured, the fluxes were distributed between positive and negative values. A large extraction of phosphoserine was observed. The reason for this was not apparent. The positive flux of taurine was likely the result of hydrolysis of bile salts in an effort to recycle bile secretions.

Flux of peptide amino acids which normally would be considered to be constituents of dietary protein across the non-mesenteric viscera averaged nearly six-fold the flux of peptide amino acids across the mesenteric viscera. It would appear that the non-mesenteric tissues is a site of a substantial addition of peptide amino acids to plasma. The two most obvious means by which these peptide amino acids would be added to plasma as these tissues are traversed are absorption and tissue degradation (protein turnover). The amino acids, 1- and 3-methylhistidine and hydroxylysine, are formed within animal tissues by the methylation of histidine and lysine after these amino acids are incorporated in proteins. Upon degradation of the protein, 3-methylhistidine cannot be reabsorbed and is often utilized to estimate protein turnover (Young et al., 1972, Nashizawa et al., 1979). While low quantities

TABLE 4.5. PLASMA PEPTIDE AMINO ACID FLUX ACROSS MESENTERIC AND NON-MESENTERIC DRAINED VISCERA OF SHEEP

	Mesenteric flux		Non-mesenteric flux			
	$g \cdot d^{-1}$	P^a	$g \cdot d^{-1}$	P^a	P^b	SE^c
Dietary protein amino acids						
Alanine	2.88	.26	16.77	.31	.33	9.18
Arginine	1.16	.55	20.36	.24	.23	9.97
Aspartic acid	4.06	.29	28.22	.33	.34	16.20
Glutamic acid	.63	.93	50.62	.31	.25	26.87
Glycine	9.48	.01	9.63	.41	.99	8.03
Histidine	1.72	.31	11.04	.30	.31	5.80
Isoleucine	.97	.43	12.66	.32	.31	7.39
Leucine	5.15	.30	29.76	.33	.34	16.60
Lysine	6.06	.32	24.94	.35	.38	13.76
Methionine	.34	.54	-0.82	.41	.29	.69
Phenylalanine	1.89	.42	18.48	.34	.33	10.95
Proline	2.14	.42	20.92	.31	.30	11.55
Serine	3.52	.34	17.15	.29	.29	8.13
Threonine	5.69	.18	12.76	.54	.68	11.54
Tyrosine	3.09	.08	15.77	.36	.42	10.21
Valine	3.01	.30	20.36	.34	.36	12.07
Essential[d]	26.20	.30	149.31	.35	.36	86.22
Nonessential[e]	25.81	.26	159.09	.32	.33	88.21
Total[f]	52.01	.28	308.40	.33	.35	174.35
Other amino acids						
1-Methylhistidine	.07	.21	-.05	.83	.66	.17
3-Methylhistidine	.41	.41	-2.01	.47	.36	1.69
Citrulline	-1.23	.46	4.84	.08	.11	2.19
Hydroxylysine	-.80	.30	.13	.90	.53	.97
Hydroxyproline	-.67	.57	-.30	.85	.69	1.60
α-Aminoadipic Acid	2.82	.44	.14	.97	.50	2.61
α-Aminobutyric acid	.11	.36	.17	.61	.88	.27
Ornithine	.02	.96	-1.84	.39	.43	1.49
Phosphoethanolamine	-.01	.98	1.07	.34	.40	.84
Phosphoserine	-17.24	.31	-10.33	.39	.55	7.53
β-Alanine	-.07	.54	.40	.16	.13	.19
β-Aminoisobutyric acid	-.36	.14	.36	.14	.14	.29
Taurine	1.50	.05	-2.27	.14	.03	.85

[a] Probability that the flux did not differ from zero.
[b] Probability that a difference between mesenteric and non-mesenteric flux this large or larger could have occurred by chance.
[c] Standard error of the vessel mean.
[d] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+ Methionine+ Phenylalanine+Threonine+Tryptophan.
[e] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+ Alanine+Proline+Tyrosine.
[f] Total=Essential+Nonessential.

of 3-methylhistidine are present in the gastrointestinal tissues, the negative uptake of this amino acid in this study indicates that the increase in peptide amino acid flux across the gut is not associated with protein turnover. The most likely explanation for the flux of these peptide amino acids, therefore, seem to be absorption.

Free and peptide amino acid flux across the mesenteric and non-mesenteric viscera are compared in Table 4.6. Fluxes of amino acids across the mesenteric viscera were similar for most of the free and peptide amino acids investigated. An exception was glycine which was found to appear in greater quantities in the peptide form ($P < .02$). While the flux of essential, nonessential and total amino acids was greater for peptide amino acids, these were not significantly different from the flux of free amino acids in these categories. Hume et al. (1972) reported that the portal appearance of amino acids was less than 50% of the protein intake in sheep. After attempting to estimate other fates of amino acids, including absorption as ammonia, fecal excretion of undigested residues of feed and microbial protein, they still were not able to account for 100% of protein intake. If they would have included a consideration of the absorption of endogenous protein, their estimate of recovery of protein as absorbed amino acids would be even less. Only one-half to two-thirds of the quantity of amino acids disappearing from the lumen of the intestine were found to appear in the portal plasma of sheep (Wolff et al., 1972). Additionally, only 30 to 80% of individual amino acids disappearing from the intestinal lumen could be accounted for in the portal plasma of sheep (Tagari and Bergman, 1978). All authors generally attributed these lower recoveries to either tissue metabolism or analytical error. Mucosal tissue metabolism of amino acids is known to be considerable (Bergman, 1986), but the possibility of peptide appearance in the portal blood was not suggested. Earlier work at Virginia Tech showed that about 70% of

TABLE 4.6. PLASMA FREE AND PEPTIDE AMINO ACID FLUX ACROSS PORTAL DRAINED VISCERA OF SHEEP

	Mesenteric flux				Non-mesenteric flux			
	Free	Peptide			Free	Peptide		
	---g·d^{-1}---		P[a]	P[b]	----g·d^{-1}----		P[a]	SE[b]
Dietary protein amino acids								
Alanine	4.93	2.88	.51	2.03	2.76	16.77	.37	9.95
Arginine	.95	1.16	.94	1.83	-1.33	20.36	.18	9.87
Asparagine	3.86				1.70			
Aspartic acid	.89	4.06	.40	2.42	.68	28.22	.33	18.01
Glutamic acid	3.06	.63	.75	5.04	-.01	50.62	.29	30.09
Glutamine	-5.87				-6.67			
Glycine	2.18	9.48	.02	1.44	-2.10	9.63	.32	7.51
Histidine	.77	1.72	.56	1.07	-.06	11.04	.31	6.99
Isoleucine	2.21	.97	.57	1.44	.19	12.66	.30	7.60
Leucine	3.76	5.15	.83	4.43	1.40	29.76	.32	18.21
Lysine	2.98	6.06	.65	4.48	1.85	24.94	.36	16.13
Methionine	.85	.34	.62	.67	.56	-.82	.41	1.07
Phenylalanine	2.33	1.89	.89	2.14	.36	18.48	.34	12.20
Proline	2.79	2.14	.90	1.96	-2.14	20.92	.30	12.05
Serine	2.37	3.52	.77	2.64	1.72	17.15	.29	9.16
Threonine	1.36	5.69	.38	3.21	-1.67	12.76	.49	13.56
Tryptophan	.66				-.55			
Tyrosine	2.23	3.09	.68	1.38	-.13	15.77	.34	10.54
Valine	2.43	3.01	.88	2.69	-.22	20.36	.32	13.24
Essential	18.30	26.20	.81	21.76	-.53	149.31	.33	97.02
Nonessential	18.44	25.81	.74	15.22	5.04	159.09	.31	95.94
Total	36.74	52.01	.78	36.75	5.57	308.40	.32	192.81
Other amino acids								
1-Methylhistidine	-.40	.07	.23	.24	.18	-.05	.64	.33
3-Methylhistidine	.02	.41	.62	.51	-.20	-2.00	.53	1.91
Carnosine	-.62				-.56			
Cittruline	.94	-1.23	.34	1.46	3.00	4.84	.15	3.31
Hydroxylysine	.18	.80	.23	.51	-.01	.13	.92	.89
Hydroxyproline	.38	-.67	.45	.89	-.19	-.30	.77	1.11
α-Aminoadipic Acid	-2.64	2.82	.46	4.82	1.16	.14	.91	5.92
Ornithine	.10	-.02	.43	.10	.96	-1.84	.34	1.86
Phosphoethanolamine	.23	-.01	.55	.27	-.24	1.07	.29	.79
Phosphoserine	6.97	-17.24	.34	16.14	-1.71	-10.33	.64	12.23
β-Alanine		-.07				.40		
β-Aminoisobutyric acid	.36	-.36	.14	.29	-.36	.36	.14	.29
Taurine	.54	1.50	.21	.47	.53	-2.27	.36	1.97

[a] Probability that a difference between free and peptide amino acid fluxes this large or larger could have occurred by chance.
[b] Standard error of sample mean.
[d] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+ Methionine+ Phenylalanine+Threonine+Tryptophan.
[e] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+Alanine +Proline+Tyrosine.
[f] Total=Essential+Nonessential.

the amino acids appeared as peptides (Koeln, 1982). At the University of Newcastle Upon Tyne, similar appearances of peptides were recorded in mesenteric plasma of sheep (Read and Parker, unpublished).

In the non-mesenteric tissues (Table 4.6), the near zero flux of free amino acids is contrasted to the very large flux of peptide amino acids. This observation would indicate that the stomach is an important site of peptide absorption. This is noteworthy because it has been thought that ammonia is the only N-containing compound absorbed from the stomach. If these observations are confirmed, then a serious reshaping of the thinking concerning protein utilization by the ruminant will be forthcoming. 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 protein. Further experimentation to examine these observations is imperative.

Implications

Results from this study support the concept that free amino acids are absorbed from the intestine of sheep and not from the stomach. Peptide amino acids appear to be absorbed from both the intestine and the stomach. The quantity of amino acids absorbed in the form of peptides from the stomach was about six-fold greater than from the intestine. These observations will have a major impact on our understanding of the process of protein digestion and absorption in ruminants. Further, these observations will dramatically alter many strategies used in providing for the proper protein nutrition of ruminants.

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Chapter V

THE CONTRIBUTION OF FREE AND PEPTIDE AMINO ACIDS BY THE STOMACH AND INTESTINAL VISCERA TO THE PORTAL SYSTEM OF CALVES[28]

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Abstract

Free and peptide amino acid fluxes were measured across the mesenteric and non-mesenteric drained viscera of three growing Holstein steer calves (108 kg) receiving abomasal infusions of casein, an amino acid mixture simulating casein and a control solution. Two calves, were surgically cannulated in the abdominal aorta and mesenteric and portal veins. In a third calf the gastrosplenic vein was cannulated rather than the portal vein. The rate of abomasal infusion was calculated to provide 25% of dietary crude protein when infusing amino acids or Na-caseinate. Additions of free and peptide amino acids occurred as blood traversed the mesenteric viscera. Infusion of amino acids or casein tended to result in an increased appearance of free amino acids with little effect on peptide amino acid appearance. Large additions of peptide amino acids occurred as blood traversed the non-mesenteric viscera for all infusion treatments. Free amino acids were extracted from the blood by non-mesenteric viscera when abomasal treatments were the

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control and casein infusions while substantial quantities were added to the blood when free amino acids were infused.

(Key Words: Amino Acids, Peptide Amino Acids, Mesenteric Viscera, Non-mesenteric Viscera, Calves.)

Introduction

For many years the quantity of N contained in the diet rather than quality of protein was the major concern in protein nutrition for the ruminant animal. Rumen microbes could use the N provided and carbon skeletons to form microbial protein (Loosli, 1949; Allison, 1969) which would provide the protein requirements of the animal upon digestion. However, numerous reports of increased production by feeding intact sources of protein (Oltjen, 1969; Young et al., 1973), postruminal infusions of protein (Gow et al., 1979; Rogers et al., 1984; Cohick et al., 1986) and the identification of limiting amino acids (Richardson and Hatfield, 1978) has renewed interest in protein quality. The rumen has limited capacity for the absorption of free amino acids (Leibholtz 1971a,b) and protein quality may be lowered by catabolism of amino acids by rumen microbes to ammonia. Therefore, efforts to regulate protein utilization in the ruminant have centered on limiting protein degradation in the rumen or bypassing the rumen entirely. These methods have, at times, shown increased production, but their benefits are often unpredictable.

The current belief is that amino acids are absorbed by the intestine as free amino acids or as small peptides with the major portion of the peptides being hydrolyzed to free amino acids by the intestinal mucosa prior to entry into the portal system. However, to date, studies have been unable to account for the discrepancies between amino acid disappearance from the intestinal lumen and appearance in portal blood (Hume et al., 1972; Wolff et al., 1972; Tagari and Bergman, 1978)

Accumulation of peptides has been reported in the rumen (Chen et al., 1987; Broderick and Wallace, 1988) and large quantities of peptides may be formed by digestion of protein in the small intestine (Adibi and Mercer, 1973). Previous studies have reported the appearance of large quantities of peptide amino acids in portal blood of calves (Koeln, 1982) and mesenteric blood of sheep (Read and Parker, unpublished). DiRienzo and Webb (1990) reported that the majority of the peptides in the portal of sheep blood originated from the non-mesenteric viscera. The present study was undertaken to measure the quantities of free and peptide amino acids reaching the portal system of calves, to determine whether these were coming from the mesenteric (intestinal) or the non-mesenteric (stomach) regions of the gastrointestinal tract and whether these could be influenced by abomasal infusions of amino acids or proteins.

Materials and Methods

Animal Care and Management. The experiment was conducted using three growing Holstein steers which averaged 108 kg. All animals were castrated, received Levamisole phosphate[32] (.044 $ml \cdot kg^{-1}$) for control of parasites and injected with vitamins A, D, E and selenium (Table 5.1). Calves were managed so that they would have near "steady state" digestive and metabolic conditions as described by McCormick and Webb (1982). Calves were fed 24 meals of equal portion (hourly) via an automatic feeder under constant light. The animals were fed an orchardgrass, corn and soybean based diet (Table 5.1) in quantities to produce a gain of .9 $kg \cdot d^{-1}$. Each animal received a total daily feed allotment of .1 kg/kg body $wt^{\cdot .75}$. Calves were fasted for 48 hr prior to surgery and following surgery were reacclimated to the diet over a 3- to 5-d period.

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[32]Tramisol, Cyanamid Agricultural De Puerto Rico, Inc. Manati, Puerto Rico.

TABLE 5.1. COMPOSITION OF THE EXPERIMENTAL DIET FED TO CALVES[a]

ITEM	%[b]
Ingredient composition	
Corn, ground	50.00
Orchardgrass hay	30.00
Soybean meal	13.30
Molasses	5.00
Deflourinated rock phosphate	.42
Limestone	.78
Trace mineralized salt	.50
Coccidiostat[c]	++
Chemical composition	
Dry matter	91.06
Crude protein[d]	13.92

[a] All animals received intramuscular injections of vitamin A, 1 x 10^6 IU; vitamin D, 150,000 IU; vitamin E, 510 IU; and 7.5 mg selenium.
[b] As fed basis.
[c] Cocci-Control [ix] Crumbles Medicated, Southern States, Inc., Richmond, VA. (.5 $mg \cdot kg^{-1} \cdot d^{-1}$).
[d] Dry matter basis.

Surgical Preparation. An abomasal infusion cannula and chronic catheters in the abdominal aorta, mesenteric vein, and portal or gastrosplenic veins were surgically implanted under strict aseptic conditions. Anesthesia was induced by injection of 1 ml Xylazine[33] intramuscularly. The animal was placed on a surgical table in left lateral recumbency. A tracheal tube was inserted and anesthesia was maintained with 1 to 3% Fluothane[34] in oxygen on a closed circuit. A jugular catheter was inserted to allow i.v. administration of an isotonic mixture of glucose and lactated ringers solution[35]. The i.v. administration rate was 10 $ml \cdot min^{-1} \cdot kg^{-1}$ for the first hour and 5 $ml \cdot min^{-1} \cdot kg^{-1}$ until the end of the procedure.

The arterial catheters were inserted into the abdominal aorta via the right femoral artery and into the right ileac artery using techniques similar to those described by McCormick and Webb (1982). For placement of the venous catheters, an incision was made 2 cm caudal and parallel to the last rib. When necessary, the rumen was deflated to allow better access to the portal system. Duplicate mesenteric sampling catheters and mesenteric infusion catheters were implanted via mesenteric branches as described in the previous chapter. Duplicate catheters also were implanted into the portal vein of two calves via mesenteric branches and into the gastrosplenic vein via the right ruminal vein in a third calf. An ultrasonic flow probe[36] was placed around the mesenteric vein of all 3 calves for measurement of blood flow. Anatomical limitations in the length of the portal vein precluded the placement of a flow probe around this vessel. A flow probe was also placed around the gastrosplenic vein of the third calf but was damaged by the animal shortly after

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[33]Rompun, Mobay Corporation, Animal Health Div. Shawnee, KA.
[34]Bromochlorotriflourethane, Ayerst Laboratories, New York, NY.
[35]Travenol Laboratories, Inc., Deerfield, IL
[36]Transonics Systems Inc., Cornell Research Park, Ithica, NY.

recovery. Catheters for the infusion of para aminohippuric acid (PAH) were placed in small distal mesenteric veins. All catheters were passed under the skin and exteriorized on the lumbar region and attached to luer-lock valves. Prior to closing the incision, the intestine was deluged with heparinized physiological saline (10 USP units·ml^{-1}) to minimize adhesion formation.

Cannula were flushed initially, 5 d post surgery and daily thereafter with sterile physiological saline. Following flushing, all catheters were filled with a sterile heparinized saline solution (30 USP units·ml^{-1}) containing 2.4 mg penicillin·ml^{-1}. Daily intramuscular antibiotic injections[37] (20 mg·kg^{-1}) were given for 3- to 5-d postsurgery. Aspirin (325 mg) was provided with each hourly feeding for 1- to 2-d post surgery as an analgesic.

Catheter Description. Arterial and venous catheters were composed of teflon (.97 mm i.d., 1.58 mm o.d.) inserted into silastic medical grade tubing[38] (1.57 mm i.d., 3.18 mm o.d.) as described in the previous chapter. Tips of the teflon catheters were rounded and the teflon was treated with a 2% (wt/wt) heparin complex solution[39] prior to implantation. Lengths of teflon and silastic tubing, respectively for the catheters in cm were as follows: femoral artery (112,76); ileac artery (107,71); mesenteric sampling (91.5,84); mesenteric infusion (86,84); portal (91.5,76) and gastrosplenic (86,68.5). Resulting tip lengths of free teflon for insertion into blood vessels were 45, 45.7, 17.5, 12, 17.5 and 27.5 cm for the femoral, ileac, mesenteric sampling, mesenteric infusion, portal and gastrosplenic veins, respectively. The

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[37] Liquamycin 100, Pfizer, Inc. New York, NY.
[38] Silastic Medical Grade Tubing, Dow Corning Corp., Midland, MI.
[39] TDMAC-Heparin Complex Coating, Polysciences, Inc., Warrington, PA.

silastic tubing on arterial and venous catheters, at the end where the teflon exited, was covered with a 1 or 9 cm piece of Ivalon sponge[40], respectively.

Infusions. Two calves received abomasal infusions for 10 d and a third calf for 5 d prior to sampling. Infusion mixtures consisted of a control solution (the solvent for the other infusions), 2.5 % ($w \cdot v^{-1}$) Na-caseinate or an equal molar mixture of the constituent amino acids of casein. Sodium chloride was added to the blank and amino acid solutions (.773 $g \cdot liter^{-1}$) to obtain equilmolar concentrations of Na^+. The composition of the amino acid mixture is listed in Table 5.2. Casein and free amino acid mixtures were infused at a rate equivalent to 25% of the daily dietary crude protein intake.

Sampling. Arterial and venous samples were collected at 0, 15, 30 and 45 min during a 1-hr feeding period. Two calves were sampled on d 10 of each infusion treatment period. The third calf was sampled on d 5 of each treatment period. Mesenteric blood flow was measured every minute during the 1-hr feeding period by ultrasound. Average blood flow for individual sample periods was calculated as the average of mesenteric blood flow during the time interval the samples were being collected. Portal blood flow was determined by procedures adapted from Katz and Bergman (1969). Blood flow was determined by dilution of a primed infusion of a sterile 10% ($wt \cdot v^{-1}$) sterile PAH solution (.08 $ml \cdot kg^{-1}$) followed by a continuous infusion of sterile 1% PAH solution in .9% saline at a rate of 6.72 $ml \cdot min^{-1}$ into a distal mesenteric vein. The PAH was infused for 30 min prior to sampling and during the sampling period by means of a screw driven syringe constant-infusion pump[41]. Samples of blood from the aorta, mesenteric vein and portal vein of two

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[40]Ivalon sponge, Unipoint Industries, Inc., High Point, NC.
[41]Infusion-withdrawal pump. Harvard apparatus Co., Millism MA.

TABLE 5.2. COMPOSITION OF AMINO ACID MIXTURE INFUSED INTO THE ABOMASUM OF HOLSTEIN STEERS

Amino acid	$g \cdot kg^{-1}$	(%)
L-Alanine	27.62	2.76
L-Aspartic acid	63.72	6.37
L-Arginine (Free base)	33.98	3.40
L-Cystine	3.65	.37
L-Glutamic acid (Free base)	201.74	20.17
L-Glycine	20.08	2.01
L-Histidine (Free base)	24.92	2.49
L-Isoleucine	47.10	4.71
L-Leucine	86.43	8.64
L-Lysine HCl	89.39	8.94
L-Methionine	25.07	5.50
L-Phenylalanine	47.54	4.75
L-Proline	117.52	11.75
L-Serine	55.25	5.53
L-Threonine	37.96	3.80
L-Tryptophan	11.19	1.12
L-Tyrosine	49.82	4.98
L-Valine	57.02	5.70

calves and the aorta and mesenteric vein of a third calf were simultaneously collected at the end of each infusion period. Only mesenteric and arterial blood were taken from the third calf after each infusion period due to gastrosplenic catheter malfunction. Additionally, two sets of samples from the third calf were obtained from the aorta, mesenteric vein and gastrosplenic vein prior to any abomasal infusion. Samples of whole blood and plasma were immediately placed on ice.

Laboratory Analyses. Para-aminohippuric acid concentrations in whole blood were assayed by methods described by Katz and Bergman (1969). Blood hematocrits (Hct) were obtained using microhematocrit procedures. Hematocrits were utilized to correct plasma flow (PF) in whole blood using the following equation: PF = BF * (1-Hct) + .2(Hct); where BF = blood flow.

Arterial, mesenteric and portal plasma samples obtained from two calves and collected at 0, 15, 30 and 45 min were processed individually for amino acid analysis. Samples from the third calf were composited prior to processing. Plasma samples were deproteinized by filtration. A 1:1 mixture of plasma and internal standard was placed into a micropartition system[42] and filtered through a membrane filter[43] at 1,500 x *g* for 15 min. The filter excluded protein and peptide fragments of a molecular weight greater than 10,000 daltons. The filtrate was stored at -20°C until analysis. An aliquot of the filtrate was hydrolyzed in HCl as described by Cohen et al. (1989). Both the filtrate and the hydrolyzed filtrate were analyzed for free and total amino acid concentrations, respectively on a PICO·TAG Amino Acid Analysis

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[42]Micropartition System MPA-1, Amicon Division, W. R. Grace & Co.-Conn Danvers, MA.

[43]Millipore Type PLGC Ultrafiltration Membrane, 10,000 NMWL, Millipore Corp., Bedford, MA.

System[44]. The concentration of peptide amino acids was calculated as the difference between total and free amino acids.

For two calves, mesenteric and portal-drained visceral fluxes of nutrients were calculated as product of the difference between venous and arterial concentrations and plasma flow. The non-mesenteric contribution to portal flux was calculated as the difference between portal flux and mesenteric flux. The non-mesenteric contribution to portal flux represented tissue beds drained by the gastrosplenic vein (rumen, reticulum, omasum, abomasum, pancreas and spleen) and gastroduodenal vein (abomasum, pancreas and proximal duodenum) (Heath, 1968). A positive flux indicated net release of a nutrient by the gut, whereas a negative flux indicated net utilization of a nutrient by gut tissues.

Statistical Analyses. Differences of free and peptide amino acid flux across the mesenteric and non-mesenteric viscera due to infusion treatment were tested using the General Linear Model procedures of SAS (1985) and orthogonal contrasts were used to test for significant differences between means. Differences between free and peptide fluxes within a treatment were tested by the paired T-test procedures of SAS (1985).

Results

All three calves responded well to surgery and returned to a normal feeding schedule within 4 to 6 d post surgery. Rectal temperatures were slightly elevated from 1 to 7 d post surgery. Average blood flow across the mesenteric tissues of all calves was 2.67, 2.33 and 2.43 liters·min^{-1} during the infusion of .05 N HCl, amino acids and casein, respectively. For two calves, portal blood flow averaged 3.00, 3.22 and 4.38 liters·min^{-1} during the corresponding infusions.

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[44] Pico.Tag Amino Acid Analysis System, Waters Div. of Millipore, Millipore Corp. Milford, MA.

Free amino acid flux across the mesenteric viscera of animals receiving the control, amino acid and casein infusions are presented in Table 5.3. Generally, dietary protein amino acids (DPAA) were added to the blood as the mesenteric viscera was traversed for all infusion treatments. A high variation in the amino acid fluxes existed when animals received the control infusion. This primarily was due to a limited addition of free amino acids to the mesenteric blood during the sampling period of one of the calves. Methionine was added to the mesenteric blood ($P<.05$). Glutamine tended to be extracted from the blood by the intestine ($P<.16$). The variation in amino acid fluxes was reduced when either amino acids or casein were infused into the abomasum. When the amino acid mixture was infused, DPAA fluxes were substantial and tended ($P<.20$) to be different from zero for more than half of the amino acids. The infusion of casein also resulted in substantial fluxes of DPAA across the mesenteric tissues.

There was a general tendency for DPAA fluxes across the mesenteric tissues to be greater when the amino acid mixture or casein was infused ($P<.35$). The flux of DPAA was similar between amino acids infused with the amino acid mixture and casein. For the total DPAA, fluxes across the mesenteric viscera were 58.05 ($P<.37$), 156.27 ($P<.14$) and 195.39 ($P<.19$) with the control, amino acid and casein infusions, respectively. These values amount to 12, 30 and 37% of the CP provided by the diets and infusion treatments. The flux of essential amino acids appeared to be greater than the flux of nonessential amino acids.

Peptide amino acid fluxes across the mesenteric viscera are reported in Table 5.4. Generally, most peptide amino acids were added to the blood flowing through the mesenteric viscera. However, methionine, glutamate, citrulline, hydroxyproline and hydroxylysine appeared to be extracted from the blood. Neither total, essential

TABLE 5.3. FREE AMINO ACID FLUX ACROSS MESENTERIC VISCERA OF GROWING HOLSTEIN STEERS RECEIVING A CONTROL, AMINO ACID AND CASEIN INFUSION INTO THE ABOMASUM[a]

Item	Infusion								
	Control		Amino acid		Casein				
	$g \cdot d^{-1}$	P^b	$g \cdot d^{-1}$	P^b	$g \cdot d^{-1}$	P^b	P^c	P^d	SE^e
Dietary protein amino acid									
Alanine	8.75	.31	17.19	.02	25.78	.22	.38	.59	10.43
Arginine	9.46	.38	13.55	.38	13.46	.08	.63	.99	6.42
Aspartic acid	-.24	.87	2.49	.17	1.61	.02	.21	.64	1.23
Glutamic acid	-1.40	.83	2.27	.17	1.24	.64	.39	.80	2.65
Glycine	-1.42	.81	5.28	.13	10.45	.23	.21	.51	5.06
Histidine	-.20	.89	3.76	.32	2.87	.08	.16	.73	1.66
Isoleucine	3.84	.42	10.08	.19	12.92	.26	.44	.80	7.30
Leucine	5.62	.52	20.57	.13	23.37	.20	.31	.87	11.54
Lysine	1.74	.81	15.00	.03	12.61	.16	.17	.79	5.96
Methionine	3.40	.05	7.35	.17	8.27	.15	.36	.86	3.47
Phenylalanine	8.56	.24	12.41	.11	15.79	.12	.49	.71	5.90
Proline	4.55	.29	12.75	.32	12.21	.18	.48	.97	8.26
Serine	4.16	.27	9.18	.11	13.15	.22	.36	.64	5.58
Threonine	.49	.93	2.67	.54	11.61	.27	.47	.41	6.81
Tryptophan	1.34	.50	-.56	.85	1.46	.02	.66	.40	1.51
Tyrosine	6.96	.20	11.31	.14	15.05	.16	.47	.70	6.41
Valine	2.45	.57	10.97	.27	13.54	.34	.42	.85	8.98
Essential[f]	36.70	.43	95.81	.18	115.90	.17	.38	.82	57.14
Nonessential[g]	21.35	.42	60.46	.08	79.49	.21	.31	.71	34.21
Total[h]	58.05	.37	156.27	.14	195.39	.19	.35	.78	90.62
Other amino acids									
Asparagine	4.30	.56	5.28	.20	18.14	.28	.56	.39	9.42
Citrulline	3.71	.34	2.60	.59	6.99	.04	.80	.40	3.27
Glutamine	-23.12	.16	-35.73	.22	-16.03	.25	.88	.35	13.26
Hydroxylysine	5.76	.19	2.55	.45	-.17	.30	.15	.41	2.09
Hydroxyproline	-1.33	.30	-1.30	.62	.19	.81	.52	.30	.89
Ornithine	-2.26	.42	3.50	.41	.48	.75	.29	.49	2.84
Phosphoserine	40.68	.33	4.30	.35	14.95	.40	.22	.69	17.32
Taurine	-.85	.60	1.00	.57	-.28	.46	.53	.56	1.42

[a] Values are means of three calves.
[b] Probability that the flux did not differ from zero.
[c] Probability that the amino acid flux during the control infusion differs from the infusion of an amino acid source.
[d] Probability that the amino acid flux during the amino acid infusion differs from the infusion of casein.
[e] Standard error of treatment mean.
[f] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+ Methionine+ Phenylalanine+Threonine+Tryptophan.
[g] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+ Alanine+Proline+Tyrosine.
[h] Total=Essential+Nonessential.

TABLE 5.4. PEPTIDE AMINO ACID FLUX ACROSS MESENTERIC VISCERA OF GROWING HOLSTEIN STEERS RECEIVING A CONTROL, AMINO ACID AND CASEIN INFUSION INTO THE ABOMASUM[a]

	Infusion								
	Control		Amino acid		Casein				
	g·d^{-1}	P[b]	g·d^{-1}	P[b]	g·d^{-1}	P[b]	P[c]	P[d]	SE[e]
Dietary protein amino acid									
Alanine	.16	.98	.74	.75	1.96	.58	.85	.86	4.71
Arginine	-2.71	.65	3.35	.68	1.09	.53	.38	.72	4.08
Aspartic acid	3.65	.62	4.16	.28	-3.07	.86	.80	.61	9.30
Glutamic acid	-6.28	.64	-19.76	.23	-24.86	.17	.03	.40	3.84
Glycine	31.73	.32	9.90	.31	42.49	.16	.68	.08	10.10
Histidine	.83	.64	-.17	.92	1.16	.42	.88	.60	1.65
Isoleucine	.80	.80	1.71	.41	.28	.54	.94	.65	2.03
Leucine	6.39	.45	7.01	.14	4.93	.28	.96	.81	5.63
Lysine	.84	.93	4.41	.20	3.09	.30	.69	.87	5.44
Methionine	-5.09	.22	-2.75	.11	-5.28	.08	.68	.42	1.99
Phenylalanine	1.79	.52	2.23	.39	1.02	.54	.94	.63	1.66
Proline	3.70	.05	2.83	.55	.81	.82	.52	.55	2.19
Serine	-2.06	.66	3.16	.33	2.02	.59	.31	.82	3.29
Threonine	4.68	.56	5.84	.14	3.96	.50	.98	.84	6.07
Tyrosine	5.06	.28	4.64	.14	-1.82	.65	.37	.20	2.96
Valine	6.00	.34	5.35	.20	4.49	.24	.85	.89	4.32
Essential[f]	13.52	.76	26.99	.34	14.76	.31	.85	.79	30.04
Nonessential[g]	35.96	.50	5.66	.85	17.53	.52	.33	.67	18.04
Total[h]	49.48	.61	32.65	.52	32.29	.28	.79	1.00	47.62
Other amino acids									
Citrulline	-4.16	.14	-9.73	.26	-3.71	.31	.51	.22	2.93
Hydroxylysine	-4.90	.13	-.91	.76	2.07	.01	.03	.20	1.36
Hydroxyproline	-3.84	.54	-.49	.70	-2.50	.47	.59	.69	3.29
Ornithine	-.41	.90	1.45	.14	1.55	.26	.42	.97	1.75
Phosphoserine	-11.05	.42	-1.85	.51	3.84	.42	.29	.64	8.06
Taurine	.40	.84	.40	.64	.31	.70	.98	.96	1.42

[a] Values are mean of three animals
[b] Probability that the flux did not differ from zero.
[c] Probability that the amino acid flux during the control infusion differs from the infusion of an amino acid source.
[d] Probability that the amino acid flux during the amino acid infusion differs from the infusion of casein.
[e] Standard error of treatment mean.
[f] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+Methionine+Phenylalanine+Threonine+Tryptophan.
[g] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+Alanine+Proline+Tyrosine.
[h] Total=Essential+Nonessential.

nor nonessential peptide amino acid flux appeared to be influenced by infusions. Individually, peptide amino acids affected by the infusion treatment include glutamate, glycine and hydroxylysine. The infusion of a blank solution resulted in a greater addition of hydroxylysine ($P<.03$) and lower extraction of glutamate ($P<.03$) when compared to the infusion of free amino acids or casein. The latter two treatments resulted in different fluxes for glycine ($P<.08$).

Free and peptide amino acid fluxes for the different infusions are compared in Table 5.5. Within all treatments, the flux of free amino acids numerically was greater than the flux of peptide amino acids for most amino acids investigated. Glycine may be an exception, with fluxes of 31.73, 9.90 and 42.49 $g \cdot d^{-1}$ as a peptide amino acid during the control, amino acid and casein treatment periods, respectively.

The values obtained for the flux of free amino acids across the non-mesenteric viscera during the control and casein infusions were generally not significantly different from zero (Table 5.6). However, the infusion of free amino acids into the abomasum resulted in a large flux of most of the amino acids investigated. Free amino acid flux was different from zero for arginine ($P<.03$), isoleucine ($P<.07$), phenylalanine ($P<.03$), and tyrosine ($P<.02$) with the flux of numerous other amino acids approaching significance.

The flux of peptide amino acids across non-mesenteric viscera is presented in Table 5.7. Generally, fluxes of all peptide amino acids investigated were positive. For amino acids which normally would be considered to be constituents of dietary proteins, total flux amounted to 427.74, 230.35 and 140.84 g.d-1. With few exceptions, none of these fluxes were determined to be statistically different from zero.

TABLE 5.5. FREE AND PEPTIDE AMINO ACID FLUXES ACROSS MESENTERIC VISCERA OF GROWING HOLSTEIN STEERS

Item	Control				Amino acid				Casein			
	Free	Peptide	P[a]	SE[b]	Free	Peptide	P[a]	SE[b]	Free	Peptide	P[a]	SE[b]
	---g·d^{-1}---				----g·d^{-1}----				----g·d^{-1}---			
Dietary protein amino acids												
Arginine	9.46	-2.71	.39	7.90	13.55	3.35	.64	13.36	13.46	1.09	.14	3.61
Histidine	-.20	.83	.32	.56	3.76	-.17	.44	2.90	2.87	1.16	.17	.57
Isoleucine	3.84	.80	.09	.70	10.08	1.71	.30	4.29	12.92	.28	.29	6.22
Leucine	5.62	6.39	.91	4.25	20.57	7.01	.29	6.73	23.37	4.93	.19	6.76
Lysine	1.74	.84	.72	1.51	15.00	4.41	.09	2.36	12.61	3.09	.11	2.42
Methionine	3.40	-5.09	.06	1.59	7.35	-2.75	.15	3.09	8.27	-5.28	.11	3.55
Phenylalanine	8.56	1.79	.26	3.04	12.41	2.23	.19	3.71	15.79	1.02	.12	3.93
Threonine	.49	4.68	.25	1.86	2.67	5.84	.64	4.09	11.61	3.96	.45	5.84
Tryptophan	1.34				-.56				1.46			
Valine	2.45	6.00	.29	1.78	10.97	5.35	.56	5.71	13.54	4.49	.42	6.43
Alanine	8.75	.16	.18	3.01	17.19	.74	.01	1.23	25.78	1.96	.18	8.19
Aspartic acid	-.24	3.65	.62	4.69	2.49	4.16	.68	2.50	1.61	-3.07	.78	10.48
Glutamic acid	-1.40	-6.28	.57	5.09	2.27	-19.76	.19	8.09	1.24	-24.86	.15	8.07
Glycine	-1.42	31.73	.27	15.31	5.28	9.90	.47	3.71	10.45	42.49	.34	18.21
Proline	4.55	3.70	.79	1.96	12.75	2.83	.53	9.35	12.21	.81	.06	2.01
Serine	4.16	-2.06	.06	1.09	9.18	3.16	.08	1.29	13.15	2.02	.14	3.27
Tyrosine	6.96	5.06	.53	1.79	11.31	4.64	.38	4.21	15.05	-1.82	.24	7.30
Essential[d]	36.70	13.52	.10	5.76	95.81	26.99	.39	44.56	115.90	14.76	.16	32.05
Nonessential[e]	21.35	35.96	.66	19.92	60.46	5.66	.17	18.53	79.49	17.53	.33	34.17
Total[f]	58.05	49.48	.81	21.92	156.27	32.65	.27	57.72	195.39	32.29	.22	64.93
Other amino acids												
Asparagine	4.30				5.28				18.14			
Citrulline	3.71	-4.16	.12	2.08	2.60	-9.73	.19	4.46	6.99	-3.71	.12	2.95
Glutamine	-23.12				-35.73				-16.03			
Hydroxylysine	5.76	-4.90	.13	2.97	2.55	-.91	.59	3.80	-.17	2.07	.03	.25
Hydroxyproline	-1.33	-3.84	.62	3.05	-1.30	-.49	.83	2.35	.19	-2.50	.39	1.73
Ornithine	-2.26	-.41	.60	2.11	3.50	1.45	.66	2.81	.48	1.55	.60	1.24
Phosphoserine	40.68	-11.05	.19	18.83	4.30	-1.85	.36	3.68	14.95	3.84	.60	12.75
Taurine	-.85	.40	.70	1.99	1.00	.40	.81	1.57	-.28	.31	.30	.30

[a] Values are mean of three animals.
[b] Probability that differences this large or larger between free and peptide amino acids could have occurred by chance.
[c] Standard error of sample treatment.
[d] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+Methionine+Phenylalanine+Threonine+Tryptophan.
[e] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+Alanine+Proline+Tyrosine.
[f] Total=Essential+Nonessential.

TABLE 5.6. FREE AMINO ACID FLUX ACROSS THE NON-MESENTERIC VISCERA OF GROWING HOLSTEIN STEERS RECEIVING A CONTROL, AMINO ACID AND CASEIN INFUSION INTO THE ABOMASUM[a]

Item	Infusion								
	Control		Amino acid		Casein				
	$g \cdot d^{-1}$	P^b	$g \cdot d^{-1}$	P^b	$g \cdot d^{-1}$	P^b	P^c	P^d	SE^e
Dietary protein amino acids									
Alanine	6.63	.67	22.92	.40	6.63	.27	.43	.23	6.77
Arginine	-3.35	.12	7.96	.03	-7.95	.20	.15	.01	1.22
Aspartic acid	-1.52	.33	8.02	.19	-.10	.97	.13	.09	1.84
Glutamic acid	-16.15	.37	-2.61	.83	-8.45	.18	.36	.63	7.33
Glycine	-2.08	.34	34.21	.15	.85	.90	.14	.07	6.78
Histidine	6.88	.14	5.11	.41	-2.50	.44	.30	.25	3.31
Isoleucine	4.01	.72	10.37	.07	1.65	.73	.77	.33	4.87
Leucine	-1.85	.92	13.71	.27	-.33	.66	.58	.45	10.56
Lysine	7.02	.53	26.64	.23	7.61	.51	.03	.01	1.41
Methionine	1.67	.83	5.24	.32	5.27	.50	.61	1.00	4.96
Phenylalanine	.13	.98	11.92	.03	-.08	.98	.29	.13	3.33
Proline	2.08	.76	9.44	.30	-2.15	.67	.84	.28	5.54
Serine	5.80	.61	14.87	.23	8.74	.25	.17	.21	2.35
Threonine	1.89	.65	-7.09	.05	-1.17	.94	.48	.54	5.72
Tryptophan	2.68	.54	-3.91	.44	-3.57	.42	.27	.95	3.43
Tyrosine	-.50	.95	11.45	.02	2.23	.14	.22	.20	3.41
Valine	3.17	.56	22.84	.20	2.73	.78	.42	.21	7.83
Essential[f]	22.26	.72	92.78	.003	1.65	.96	.48	.11	23.84
Nonessential[g]	-5.73	.82	98.30	.02	7.74	.55	.05	.03	11.59
Total[h]	16.52	.85	191.08	.009	9.39	.83	.19	.07	35.43
Other amino acids									
Asparagine	8.58	.63	-28.20	.68	6.77	.47	.59	.42	24.70
Citrulline	7.39	.35	-5.88	.12	1.31	.17	.12	.23	2.95
Glutamine	20.79	.61	-21.67	.52	7.79	.71	.08	.10	6.98
Hydroxylysine	-4.69	.63	-.80	.50	-.98	.70	.53	.98	4.08
Hydroxyproline	-1.77	.05	-.22	.74	-.70	.62	.30	.70	.77
Ornithine	3.93	.25	11.62	.32	6.12	.19	.27	.28	2.67
Phosphoserine	-52.40	.42	3.36	.50	5.80	.78	.13	.93	18.68
Taurine	2.93	.52	11.52	.54	-3.66	.10	.92	.26	6.99

[a] Values are means of two animals.
[b] Probability that the amino acid flux did not differ from zero.
[c] Probability that the amino acid flux during the control infusion differs from the infusion of an amino acid source.
[d] Probability that the amino acid flux during the amino acid infusion differs from the infusion of casein.
[e] Standard error of the treatment mean.
[f] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+Methionine+Phenylalanine+Threonine+Tryptophan.
[g] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+Alanine+Proline+Tyrosine.
[h] Total=Essential+Nonessential.

TABLE 5.7. PEPTIDE AMINO ACID FLUX ACROSS THE NON-MESENTERIC VISCERA OF GROWING HOLSTEIN STEERS RECEIVING A CONTROL, AMINO ACID AND CASEIN INFUSION INTO THE ABOMASUM[a]

Item	Infusion								
	Control		Amino acid		Casein				
	$g \cdot d^{-1}$	P[b]	$g \cdot d^{-1}$	P[b]	$g \cdot d^{-1}$	P[b]	P[c]	P[d]	SE[e]
Dietary protein amino acids									
Alanine	16.52	.22	14.16	.53	2.90	.15	.62	.55	11.15
Arginine	28.79	.08	10.37	.62	2.94	.24	.22	.66	10.29
Aspartic acid	31.08	.28	14.14	.71	19.54	.49	.66	.88	22.63
Glutamic acid	71.97	.09	-7.40	.91	-2.24	.93	.18	.92	30.74
Glycine	25.02	.57	10.18	.89	9.43	.13	.71	.99	29.50
Histidine	11.81	.29	9.31	.60	1.95	.82	.69	.68	10.91
Isoleucine	12.35	.38	7.51	.67	4.24	.47	.69	.86	11.42
Leucine	34.35	.47	33.82	.60	9.92	.38	.82	.71	39.95
Lysine	38.92	.26	26.53	.60	33.44	.39	.82	.88	27.71
Methionine	2.50	.76	-4.79	.38	1.56	.84	.60	.50	5.47
Phenylalanine	17.65	.44	14.58	.64	5.78	.37	.78	.78	19.48
Proline	19.26	.45	10.80	.71	8.18	.24	.72	.93	19.21
Serine	29.16	.44	25.06	.56	4.97	.66	.72	.66	28.22
Threonine	33.11	.45	24.79	.66	27.60	.28	.89	.96	36.41
Tyrosine	25.70	.42	21.04	.55	9.95	.34	.75	.77	22.97
Valine	29.56	.45	20.26	.68	.67	.49	.67	.70	31.52
Essential[f]	209.04	.38	142.37	.65	88.10	.13	.72	.86	188.40
Nonessential[g]	218.71	.17	87.98	.77	52.75	.31	.50	.88	148.93
Total[h]	427.74	.29	230.35	.70	140.84	.04	.62	.87	337.30
Other amino acids									
Citrulline	-6.70	.58	12.73	.28	.80	.91	.29	.39	7.68
Hydroxylysine	8.96	.27	3.17	.71	6.20	.09	.58	.73	5.39
Hydroxyproline	-1.18	.73	2.14	.82	6.27	.11	.50	.65	5.43
Ornithine	5.95	.31	-.81	.88	5.15	.65	.70	.61	7.03
Taurine	.32	.76	2.20	.21	-.67	.54	.72	.15	.89

[a] Values are means of two animals
[b] Probability that the amino acid flux did not differ from zero.
[c] Probability that the amino acid flux during the control infusion differs from the infusion of an amino acid source.
[d] Probability that the amino acid flux during the amino acid infusion differs from the infusion of casein.
[e] Standard error to treatment mean.
[f] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+Methionine+Phenylalanine+Threonine+Tryptophan.
[g] Nonessential=Aspartic acid+Glutamic Acid+Serine+Glycine+Alanine+Proline+Tyrosine.
[h] Total=Essential+Nonessential.

Comparisons of free and peptide amino acid flux across the non-mesenteric viscera are reported in Table 5.8. Generally, flux across the non-mesenteric viscera was greater for most of the peptide amino acids investigated compared to free amino acids but statistically significant differences were limited to a few amino acids. For amino acids which normally would be considered to be constituents of dietary proteins, total flux as a free or peptide amino acid appeared to be influenced by infusion treatments. Control and casein infusions resulted in a 19- and 15-fold greater flux as peptide rather than free amino acids, respectively. The infusion of free amino acids into the abomasum, however, resulted in nearly equal fluxes for free and peptide amino acids.

Discussion

Free and peptide amino acid fluxes obtained in this study support the concept that amino acids enter the blood from the intestine primarily in the free form. The addition of postruminal N in the form of free amino acids resulted in an apparent increase in the flux of free amino acids with little effect on the flux of peptide amino acids. Similarly, the infusion of casein increased the flux of free amino acids with little effect on peptide amino acid flux across the mesenteric tissue. Unlike the infusion of free amino acids, casein digestion in the intestine would likely result in a large release of peptide amino acids in addition to free amino acids in the intestinal lumen. The absorption of free amino acids can occur from the intestinal lumen and(or) the absorption of small peptides followed by hydrolysis to constituent amino acids prior to release into the mesenteric blood (Matthews and Adibi, 1976). It seems therefore, that the similar observations for free and peptide amino acids, which resulted from the infusion of free amino acids and casein, was likely due to extensive hydrolysis of peptides either prior to entry into the intestinal mucosa or

TABLE 5.8. FREE AND PEPTIDE AMINO ACID FLUXES ACROSS NON-MESENTERIC VISCERA OF GROWING HOLSTEIN STEERS

Item	Control				Amino acid				Casein			
	Free	Peptide	P[b]	SE[c]	Free	Peptide	P[b]	SE[c]	Free	Peptide	P[b]	SE[c]
	---g·d^{-1}----				----g·d^{-1}----				----g·d^{-1}----			
Dietary protein amino acids												
Alanine	6.63	16.52	.68	12.49	22.92	14.16	.05	.46	6.63	2.90	.36	1.65
Arginine	-3.35	28.79	.06	2.27	7.96	10.37	.90	11.01	-7.95	2.94	.08	1.01
Aspartic acid	-1.52	31.08	.28	10.93	8.02	14.14	.88	21.81	-.10	19.54	.53	15.09
Glutamic acid	-16.15	71.97	.004	.35	-2.61	-7.40	.95	42.96	-8.45	-2.24	.80	13.33
Glycine	-2.08	25.02	.55	22.62	34.21	10.18	.77	46.00	.85	9.43	.45	5.23
Histidine	6.88	11.81	.46	3.04	5.11	9.31	.72	6.19	-2.50	1.95	.51	3.21
Isoleucine	4.01	12.35	.71	11.95	10.37	7.51	.88	10.25	1.65	4.24	.79	5.27
Leucine	-1.85	34.35	.58	32.73	13.71	33.82	.77	37.21	-.33	9.92	.39	5.14
Lysine	7.02	38.92	.41	17.14	26.64	26.53	1.00	18.75	7.61	33.44	.35	11.13
Methionine	1.67	2.45	.96	8.80	5.24	-4.79	.35	4.32	5.27	1.56	.80	8.05
Phenylalanine	.13	17.65	.51	12.74	11.92	14.58	.93	15.96	-.08	5.78	.07	.45
Proline	2.08	19.26	.57	15.34	9.44	10.80	.97	18.93	-2.15	8.18	.38	4.93
Serine	5.80	29.16	.60	22.89	14.87	25.06	.75	17.78	8.74	4.97	.81	8.52
Threonine	1.89	33.11	.50	21.88	-7.09	24.79	.58	28.84	-1.17	27.60	.45	17.26
Tryprophan	2.68				-3.91				-3.57			
Tyrosine	-.50	25.70	.50	18.27	11.45	21.04	.77	17.93	2.23	9.95	.44	4.45
Valine	3.17	29.56	.53	20.78	22.84	20.26	.96	31.48	2.73	.67	.84	5.82
Essential[d]	22.26	209.03	.50	133.48	92.78	142.37	.86	161.71	1.65	88.10	.30	31.11
Nonessential[e]	-5.73	218.71	.22	56.94	98.30	87.98	.97	164.95	7.74	52.75	.26	13.48
Total[f]	16.52	427.74	.37	190.42	191.10	230.35	.95	326.66	9.39	140.84	.12	17.63
Other amino acids												
Asparagine	8.58				-28.20				6.77			
Citrulline	7.39	-6.70	.48	9.30	-5.88	12.73	.23	4.92	1.31	.80	.95	4.44
Glutamine	20.79				-21.70				7.79			
Hydroxylysine	-4.69	8.96	.44	7.95	-.80	3.17	.68	5.16	-.98	6.20	.24	1.97
Hydroxyproline	-1.77	-1.18	.85	1.76	-.22	2.14	.79	4.91	-.70	6.27	.01	.07
Ornithine	3.93	5.95	.42	1.11	11.62	-.81	.11	1.56	6.12	5.15	.94	7.33
Phosphoserine	-52.4				3.36				5.8			
Taurine	2.93	.32	.46	1.64	11.52	2.20	.59	8.64	-3.66	-.67	.04	.13

[a] Values are mean of two animals.
[b] Probability that differences this large or larger between free and peptide amino acids could have occurred by chance.
[c] Standard error of the sample.
[d] Essential=Arginine+Histidine+Isoleucine+Leucine+Lysine+Methionine+ Phenylalanine+Threonine+ Tryptophan.
[e] Nonessential=Aspartic acid+Glutamic Acid+ Serine+ Glycine+ Alanine+Proline+Tyrosine.

intracellularly prior to release into the mesenteric circulation. Glycine containing peptides may be an exception. A much greater flux of glycine occurred as a peptide rather than as a free amino acid during the blank and casein infusion. The transport of intact glycine containing peptides across the intestine has been previously reported (Peters and MacMahon 1970; Burston and Matthews 1987)

It would appear that some non-mesenteric tissues are capable of additions of free amino acids to the blood when large concentrations of free amino acids are present in the lumen. Minimal uptake of free amino acids by the rumen previously have been reported (Leibholtz 1971a,b) and this has led to the current concept that little or no amino acid transport occurs from the stomach. However, free amino acid concentrations in the rumen generally remain low due to actions of rumen microbes (Wright and Hungate, 1967; Broderick et al. 1981) and substantial release of amino acids by digestion of proteins generally does not occur until the mid to lower intestine (Ben-Ghedalia et al., 1972,1984). Karasov et al. (1987) reported that populations of amino acid transporters are altered by different protein levels in the diet. It is possible that the long term infusion of free amino acids may have resulted in changes in the transport capabilities for free amino acids by some non-mesenteric tissues or increased diffusion of free amino acids across these tissues.

The data presented in Table 5.7 indicates that the non-mesenteric tissues are a site of substantial addition of peptide amino acids to the plasma. Similar observations have been reported for sheep (DiRienzo and Webb, Chapter IV). In their previous study, a low flux of 3-methylhistidine across the non-mesenteric viscera was reported which implies that peptide additions as a result of protein turnover was minimal. Thus, the most likely explanation for the source of the flux of peptide amino acids across the non-mesenteric tissues would be absorption.

Values for the flux of free and peptide amino acids for calves and sheep (DiRienzo and Webb, unpublished data) were indirectly quantified as the difference between portal flux and mesenteric flux. A direct quantification of free and peptide amino acids in blood draining the gastrosplenic viscera is more desirable since it would reduce sources of variation.

Reported in Table 5.9 are the average venoarterial differences from two collection d for concentrations of free and peptide amino acids across mesenteric and non-mesenteric viscera. Values reported in this table are in agreement with the general observation that flux of both free and peptide amino acids occurs as the mesenteric viscera is traversed. Fluxes across these tissues appears to be predominantly as free amino acids.

Direct quantification of venoarterial concentration differences across the gastrosplenic viscera indicates that there is extraction rather than addition of free amino acids by these tissues. However, a substantial addition of peptide amino acids by the gastrosplenic viscera seems to occur as is indicated by large venoarterial differences which occurred across these tissues. This is in agreement with values reported in the previous discussion by indirect measurements of peptide amino acid additions which occurred across the non-mesenteric viscera and supports observations of peptide amino acid additions by the non-mesenteric viscera of sheep

TABLE 5.9. VENOARTERIAL CONCENTRATION DIFFERENCES IN PLASMA FREE AND PEPTIDE AMINO ACIDS ACROSS THE MESENTERIC AND SPLENIC VISCERA OF A FED HOLSTEIN STEER[a]

	Mesenteric		Splenic	
	Free	Peptide	Free	Peptide
	----------uM·dl^{-1}----------			
Dietary protein amino acids				
Arginine	4.49	.67	-1.23	2.64
Histidine	1.20	.47	-.31	1.66
Isoleucine	6.59	1.02	-3.27	3.79
Leucine	10.57	2.07	-3.58	9.02
Lysine	6.34	.12	-.29	3.32
Methionine	4.57	.23	1.10	1.64
Phenylalanine	4.79	1.82	-.58	3.13
Threonine	9.74	-8.41	-3.62	4.90
Tryprophan	.23		-.97	3.19
Valine	8.46	2.24	-6.88	9.26
Alanine	22.82	2.66	-.82	10.16
Aspartate	.47	5.99	-.32	4.79
Glutamate	-.62	7.32	-5.38	20.72
Glycine	14.01	10.43	-1.36	10.10
Proline	28.81	17.88	-8.76	36.55
Serine	9.14	-.30	-2.53	6.43
Tyrosine	5.06	-1.91	-1.14	3.19
Essential	56.95	.22	-19.63	38.27
Nonessential	79.67	42.07	-20.30	91.94
Total	136.62	42.28	-39.92	130.20
Other amino acids				
Asparagine	12.93		-.76	-4.91
Glutamine	-5.24		-1.59	-20.65
Hydroxy Proline	.01	2.19	-.90	1.54
Ornithine	.83	-.15	-.08	.13
Phosphoserine	1.72	-1.61	-1.41	-4.81
Taurine	.14	1.01	-.65	-.37

[a] Values are means of two observations on one steer.

Implications

Results from this study support the concept that free amino acids are absorbed from the intestine and not from the stomach. However, long term post ruminal additions of free amino acids may alter the capabilities of the stomach and(or) duodenum to absorb free amino acids. Peptide amino acid appearance was demonstrated in plasma traversing both the intestinal and stomach tissues. The quantity of amino acids added to the blood by the stomach was substantially greater than additions by Results from this study support the concept that free amino acids are absorbed from the intestine and not the stomach. However, long term post ruminal additions of free amino acids may alter the capabilities of the stomach and (or) duodenum to absorb free amino acids. Peptide amino acid appearance was demonstrated in plasma traversing both the intestinal and stomach tissues. The quantity of amino acids added to the blood by the stomach was substantially greater than additions by the intestine and support reported peptide fluxes across the non-mesenteric viscera of sheep. If these observations are confirmed, dramatic alterations in the feeding strategies for the protein nutrition of ruminants are imminent.

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Chapter VI

Epilogue

Nutritionists, in general have been hesitant to accept the role of peptide absorption in protein metabolism. Over the years, however, evidence has accumulated, primarily in nonruminant species, which indicates that there is substantial uptake of peptides across the brush border membrane of the intestine. Most of these peptides are believed to be hydrolyzed either during transport across the brush border membrane or, more likely, within the enterocyte prior to release in the blood. The quantity of intact peptides reaching the circulation and the significance of these peptides, however, remains in question.

Gardner estimated intact peptides may have contributed 10 to 30% of the total α-amino nitrogen entering the portal system of rats (Gardner, 1975, 1982; Gardner et al., 1983). Koeln (1982) reported that venoarterial differences of peptide amino acids across the gastrointestinal tract of calves were nearly threefold the amount added as free amino acids. Thus, a large discrepancy for the estimated relative additions of free and peptide amino acids to the gastrointestinal circulation existed between nonruminant and ruminant animals. Characteristics peculiar to the digestive system of the ruminant animal include actions of the microbial population on protein in the stomach region and the attenuated buffering of digesta in the gastrointestinal tract. Peptides are believed to be cotransported with hydrogen ions across membranes (Miyamoto et al., 1985; Takuwa et al., 1985). Thus, one might speculate that preferential uptake of peptides in the intestine of the ruminant evolved as a result of the attenuated buffering capacity of the gastrointestinal. This line of thinking is enhanced by reports of minimal uptake of free amino acids by the

rumen (Leibholtz 1971a) or other non-mesenteric tissues (Huntington and Reynolds, 1986).

The possibility of peptide uptake by the non-mesenteric viscera, however, was never addressed.

In general, results of studies in Chapters IV and V support the current concept that end products of protein digestion absorbed by the intestine enter the circulation primarily, but not exclusively, as free amino acids. Our findings also support the concept that little or no uptake of free amino acids occurs in the non-mesenteric viscera but a net extraction of a number of free amino acids may occur in these tissues. The most significant finding of these studies is evidence in both sheep and cattle that indicates that large amounts of peptide amino acids may be absorbed from the stomach region of the ruminant animal. Because of the catabolic properties of the rumen microbes, most strategies for manipulating protein nutrition have centered around minimizing protein degradation in the rumen or bypassing the rumen altogether. If the absorption of peptides from the stomach region is confirmed, there will be far reaching consequences for strategies utilized by nutritionists and industry in providing the proper protein nutrition of the ruminant animal.

Where to go from here.

Obviously, more work is required to confirm the findings in the present studies. Large variation between animals in the appearance of peptide amino acids and to a lesser extent free amino acids was evident in these studies. Individual animal variation may have played a substantial role, but other factors contributing to the variation may include the amount of time since the last meal was consumed (in the sheep study), and the indirect methods utilized to determine the quantities of

free and peptide amino acids added by the non-mesenteric tissue. However, the finding of large concentrations of peptide amino acids in blood directly obtained from the gastrosplenic blood supported our observations based on indirect measurements.

Future studies need to be conducted to confirm the present findings. If these findings are confirmed questions to be addressed in the future include the identification of the specific tissue or tissues in the stomach (rumen, etc.) involved in the transport of peptides and the nature of the transport mechanism(s) located on that tissue.

Future studies should be conducted both in vitro and in vivo. Initially, transport studies of tissues in vitro may prove to be more beneficial. Transport of peptides by tissues from the rumen, reticulum, omasum and abomasum can be studied under controlled conditions using a Ussing chamber. If the transport of peptides is demonstrated it would both support our findings and identify which of the non-mesenteric tissue(s) were involved in the transport of peptides. Additional studies should also be conducted to characterize the components of uptake; carrier mediated transport vs diffusion.

Future studies also should include in vivo studies of peptide transport by the non-mesenteric viscera. Blood could be collected from anesthetized animals via syringe which would alleviate problems of non-functioning catheters. Peptide appearance by non-mesenteric tissues could be measured as the differences in venous and arterial concentrations. Blood flow through the vessel may be obtained by placing ultrasound probes around the vessels and filling the body cavity with saline to remove air between the probe and vessel. The quantity of peptides then could be determined as the product of the venoarterial difference and blood flow. In

this study, diets for the animals could include the following: a) a diet high in nonprotein-N, b) a diet high in bypass protein and c) a diet with a protein readily degraded in the rumen. By feeding these diets the expectation would be for low concentrations of peptides in the rumen with the first two diets and increased concentrations for the third diet.

A second in vivo study should utilize animals surgically implanted with chronic catheters for sampling blood and a cannula for infusing treatments. The researcher may want to choose which vessels are to be catheterized and site for the infusion cannula based on the in vitro studies described above. The animals could be infused with control, amino acid and intact protein similar to the present calf study. The best procedure would be to infuse the total daily allotment of protein because this would minimize differences between animals due to protein intake and the time since the last meal consumed by the animal. This requirement may be met using a semi-purified diet. Blood samples could then be processed and analyzed as in the present study.

At the end of the two above studies tissue samples should be obtained for in vitro studies. Characterization of the components of transport of these tissues may indicate that the aggregate of peptide transporters may be altered by the diet.

In addition to infusing the treatments listed in the preceding paragraph, the researcher could infuse radioactive peptides and amino acids into different segments of the gastrointestinal tract. Blood samples obtained from the various segments of the gastrointestinal tract which were infused could be analyzed for free amino acids and peptides. These could be isolated via chromatographic procedures and the radioactivity of these components measured.

The results of the present studies indicate that a major portion of the amino acids which are absorbed by cattle and sheep are absorbed in the form of peptides from the stomach. The observation that peptides are absorbed from the stomach may be the most important discovery ever made related to protein utilization by ruminant animals. Because this concept will revolutionize the formulation of diets for ruminant animals it is imperative that studies designed to critically evaluate this phenomenon be initiated as soon as possible. As the results of these studies become available, we then can begin the process of rewriting the textbooks.

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