MOVEMENT OF PLASMA FREE, ERYTHROCYTE FREE, PEPTIDE AND SERUM PROTEIN AMINO ACIDS ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF CALVES

by

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INTRODUCTION

The subject of amino acid requirements of ruminants has received much attention recently. Producers of livestock may wish to manipulate amino acid status of ruminant diets to minimize the wastage of dietary nitrogen sources in relation to production of protein since protein supplementation is extremely expensive. Optimization of performance to produce a product is always desirable. Before we can determine these requirements and utilize our protein resources to their best advantage we must know which amino acids limit production, when this occurs, and we must know the site of action if we are to manipulate amino acid supply to this site with any precision. To accomplish these objectives it is necessary to consider amino acid metabolism at the cellular level. The effects of ruminal fermentation on amino acid supply have received considerable attention but the amounts of amino acids actually made available to the tissues is less well understood.

The question has been asked, "What regulates amino acid concentrations in the circulation?" G.F. Cahill, Jr. and T.T. Aoki, two outstanding researchers in this field, have responded "Everything, more or less!" The liver is the primary organ controlling blood amino acid levels, however, it is the role of the gastrointestinal tract to supply these amino acids to the portal circulation. Absorbed amino acids are subjected to metabolism by the liver even before entering general circulation. The liver is one of the most important sites in the body for amino acid metabolism. It is the site of most plasma protein

synthesis and non-essential amino acid synthesis, the chief site of deamination and the urea cycle. Amino acid metabolism in the ruminant liver is extremely important in terms of gluconeogenesis with the liver accounting for up to 85% of the body glucose turnover in sheep and supplying 30% of the glucose produced.

The relative role of blood free amino acid pools has received considerable attention. The two free amino acids pools, plasma and red blood cells, have been evaluated in several species for their ability to transport amino acids across tissues. Other blood pools such as peptides and blood proteins have received much less attention. Absorption of intact peptides has been suggested through in vitro research but substantial quantities of peptides are not generally found in portal blood. Albumin, which comprises approximately 50% of the serum total protein, is known to transport many compounds in blood. However, other blood proteins have not been considered as interorgan carriers of amino acids until recent work in this laboratory.

It is now known that monitoring only plasma free amino acid levels may lead to incomplete or inaccurate conclusions concerning interorgan movement of free amino acids. Perhaps bound amino acid pools should be considered as well.

LITERATURE REVIEW

FLUXES OF FREE AMINO ACIDS IN PLASMA VS ERYTHROCYTES

Early research into amino acid transport indicated that plasma appeared to play a more quantitative role in movement of amino acids among organs of the dog when compared to the erythrocyte (Elwyn, 1966). However, Elwyn reported that red cell concentrations of amino acids could change essentially independently of those in plasma. GLU is an amino acid that responds very differently in its pattern of transport across tissues in these two pools. In three dogs fed horsemeat Elwyn (1966) observed that concentration changes of GLU across the gut and liver were opposite in direction in these pools. Other amino acids showed a similar but less striking response. This was the first indication of a distinct role for the erythrocyte in interorgan movement of amino acids. In an attempt to further ascertain the roles of plasma and red blood cells in transporting amino acids across the gut and liver of dogs, Elwyn et al. (1972) found evidence for exchange of amino acids between plasma and tissue cells and erythrocytes and tissue cells. The exchange between the two blood pools was very slow while blood pool-tissue exchange was rapid. The independence of the two pools in exchange of amino acids with tissues was suggested as an explanation for the independent and often opposing roles of plasma and red blood cells in interorgan amino acid transport.

Since these early observations in the dog, the erythrocyte has been reported to have a role in amino acid transport in several species

(Felig et al., 1973; Aoki et al., 1976; Heitmann and Bergman, 1980a; Cahill et al., 1981; McCormick and Webb, 1982; Danilson et al., 1983). Several researchers have observed opposing roles for the plasma and erythrocyte pools in amino acid transport (Felig et al., 1973; Aoki et al., 1976; Danilson et al., 1983) while others have reported similar responses suggesting that plasma exchanges simply underestimate total exchange of amino acids across tissues (Heitmann and Bergman, 1980a; Cahill et al., 1981; McCormick and Webb, 1982).

The red blood cell has been considered as a possible indicator of the state of amino acid nutrition of tissues. Blood samples taken from 24 premature infants fed either a high or low protein diet were analyzed for plasma free and erythrocyte free amino acids (Snyderman et al., 1970). The amino acid content of the red blood cell paralleled the content of amino acids in plasma, offering no advantage to monitoring red cell amino acid concentrations.

Additional research comparing amino acid concentrations in plasma and erythrocytes of man was reported by Levy and Barkin (1971). Venous blood obtained from six normal humans and 10 children with metabolic disorders was assayed for amino acid concentrations in plasma and erythrocytes. In both groups erythrocyte concentrations of most amino acids were similar to concentrations in plasma. However, concentrations of ASP, GLU, SER, THR, CYS, MET and ARG were different between the two pools.

Blood taken by heart puncture from 4-wk old chicks fed a 20% casein diet ad libitum, a protein free diet ad libitum or fasted for 24 hr

was analyzed for plasma and erythrocyte free amino acid concentrations (Stephens and Evans, 1971). The erythrocyte contained much greater concentrations of essentially all amino acids under all three nutritional states. However, protein deprivation did reduce the concentrations of most amino acids in both pools.

McCormick (1980) monitored plasma and erythrocyte free amino acid arteriovenous differences across the hindlimb of calves fed a natural diet and following a 72-hr fast. In fed animals, amino acids were generally removed from plasma as blood traversed the hindlimb. Fasting reversed this response resulting in a release of amino acids into venous plasma. Free amino acids in the erythrocyte pool generally showed a nonsignificant response in fed animals with the exception of a release of ASP, GLU and GLY and an uptake of ALA and LYS by the hindlimb of fed calves. The erythrocyte was involved in transport of amino acids from the muscle following a 72-hr fast as was the plasma. These results support the concept of similar responses of amino acids in these two free amino acid pools.

In a study using similar animals, Danilson (1981) monitored plasma and erythrocyte free amino acid exchange across the hindlimb of calves fed either soy protein or urea as the sole nitrogen source. In animals fed soy protein the hindlimb removed amino acids from the plasma pool. Urea feeding reduced amino acid uptake by the hindlimb to a near zero exchange. The erythrocyte free amino acid pool was affected little by transport across the hindlimb of soy protein fed animals. However, a slight tendency for an output into the erythrocyte pool was observed

which was opposite the response for plasma. Urea feeding resulted in little or no exchange of erythrocyte free amino acids across the hindlimb. When plasma and erythrocyte A/V differences were directly compared it was observed that several amino acids responded oppositely between the two free amino acid pools.

Regardless of the relative response of plasma and erythrocyte amino acid transport, it is clear that amino acid flux across tissue beds cannot be accounted for entirely by plasma free amino acid exchanges.

Plasma free amino acids fluxes. Plasma amino acid levels have been monitored in a multitude of protein metabolism studies. These levels have been reported to change with nutritional and physiological conditions, however, less information is available concerning the role of plasma in interorgan amino acid transport.

In the dog amino acid concentration changes in plasma are closely correlated with food intake (Elwyn, 1966). A review which summarizes reported concentrations of amino acids in portal plasma of several monogastrics indicates that the amino acids appearing in portal blood following a meal closely reflect the proportions ingested (Rogers and Harper, 1966). In ruminants portal blood ratios of amino acids may reflect those of rumen microbial protein. A net absorption of amino acids was monitored in three mature wethers fed a diet of chopped alfalfa hay (Hume et al., 1972). Essential amino acids were found in portal blood in similar ratios as those in rumen microbial protein with the exception of MET. ASP and GLU were absorbed in lesser quantities and PRO and ALA in greater quantities than their proportions in

microbial protein. Possible interconversions of these amino acids in the gut wall will be discussed in a later chapter.

Until the work of Elwyn et al. (1968) little information existed concerning the quantitative output or uptake of plasma amino acids by tissues. Three multicatheterized dogs fed a diet of horsemeat were monitored during the absorptive and nonabsorptive period for changes in plasma free amino acid concentrations across the gut and liver. A sharp rise in portal plasma amino acids was observed for 8- to 11-hr postfeeding. A concomitant but smaller rise in arterial concentrations was also observed.

No information of this type was available in ruminants until the work of Wolff et al. (1972) with sheep. Ewes were continuously fed a near maintenance diet of alfalfa pellets. Plasma taken from the aorta, portal vein and hepatic vein was analyzed to determine quantities of amino acids added to plasma by portal drained viscera and quantities metabolized by the liver. Free amino acids in portal plasma were higher than those in arterial blood. These amino acids added to the portal blood served as a source of amino acids for the liver leaving no role for plasma free amino acid transport to peripheral tissues. Elwyn et al. (1972) suggested that plasma carries free amino acids from the gut and periphery to the liver while the erythrocyte carries amino acids from liver to peripheral tissues. An uptake of free amino acids from the erythrocyte pool by the muscle (forearm and hindlimb) has not been observed in man or calves (Felig et al., 1973; Aoki et al., 1976; McCormick 1980; Danilson, 1981).

Erythrocyte free amino acid fluxes. The blood cells constitute a large portion of the total blood volume thus their potential role as carriers of amino acids may be considerable. Unlike plasma, erythrocyte amino acid concentration changes appear to be less related to food intake (Elwyn, 1966). However, the chicken may be an exception. Chicks were fed a basal diet supplemented with varying levels of a test protein (Stephens and Evans, 1972). Blood taken via heart puncture revealed that increased protein intake led to an accumulation of ASP and GLU in red cells with no change in the plasma concentrations of these two amino acids. The red cell concentration of ASP and GLU increased with increasing protein levels and the magnitude of the increase was enhanced as biological value of the test protein decreased. These authors suggest that red cell concentration changes in these two amino acids may be developed as a rapid test for protein quality.

Red blood cell amino acid concentrations have also been reported to change with varying degrees of protein energy malnutrition (El-Shobaki et al., 1982). One hundred thirty nine malnourished Egyptian children were evaluated for essential and nonessential amino acid content of red blood cells. Moderate to severe Kwashiorkor resulted in decreased essential and nonessential amino acid concentrations in the red blood cell while Marasmus resulted in elevated essential and nonessential amino acids over controls.

The mechanism for amino acid entry into red blood cells has not

been elucidated. Yunis and Arimura (1965) sought to differentiate between mechanisms of amino acid transport into reticulocytes and those of the mature red cells. Reticulocyte production was stimulated in rats by repeated bleeding. Reticulocytes and mature cells were incubated for 90 min in vitro with several ^{14}C-amino acids and varying concentrations of sodium and potassium. Following incubation, amino acids were extracted from cells and assayed. A similar experiment was conducted with human mature red cells and reticulocytes from patients with hemolytic anemia. GLY and ALA transport into rat and human reticulocytes required sodium and potassium. LYS and HIS were transported against a concentration gradient without sodium or potassium. Transport of these four amino acids was much less in erythrocytes, however, sodium and potassium did stimulate GLY and ALA uptakes. Thus maturation of red cells appeared to result in a decreased ability to take up amino acids. Shortly after this report of limited amino acid uptake by mature red cells, Elwyn (1966) reported transport of amino acids by erythrocytes in dogs. Particularly large changes in GLU concentrations in red cells crossing the gut and liver were observed. The mechanism for this transport was unknown but Elwyn speculated that the dog erythrocyte may be capable of concentrating amino acids to a greater extent than human erythrocytes.

The red blood cell appears to be quantitatively more important in the transport of certain amino acids than others. In humans, GLU and ASP are found in higher concentrations in the erythrocyte while ARG is in lower concentations than in plasma (Levy and Barkin, 1971; Cahill et

al., 1981). Human research indicates the differences which may exist between concentrations of certain amino acids in plasma and erythrocytes (Levy and Barkin, 1971). Venous blood taken from three adults and three children revealed the presence of ASP in erythrocytes while none could be detected in plasma. GLU, SER and THR were also much more concentrated in the erythrocyte than in plasma while CYS, MET and ARG were more concentrated in plasma. These researchers suggest that "intracellular trapping" may account for the high concentration of acidic amino acids in red blood cells. Also intracellular deamination of ASN and GLN may occur. The low concentrations of ARG may be attributed to the catabolism by arginase within the cell.

The erythrocyte appears to function in the transport of ALA from peripheral tissue and gut to the liver (Felig et al., 1973). Twelve men were fasted for 10 to 14 hr prior to placement of catheters in the brachial artery, hepatic vein and femoral artery. Portal samples were taken via needle puncture. Blood samples were taken from all catheters while the subjects were under anesthesia. Under these conditions, 22 to 32% of the net movement of ALA to and from tissues was via blood cells. ALA movement is vital to the transport of ammonia from peripheral tissues to the liver via the ALA cycle. Thus the degree of transport via red cells which exceeds that of all other amino acids is unique and further emphasizes the role of erythrocytes as carriers of amino acids.

The role of the erythrocyte in ruminants was not considered until recently. Twenty experiments were conducted on eight ewes fed hourly

to compare concentrations of amino acids in whole blood and plasma and to compare rates and direction of amino acid transport in these pools across tissues (Heitmann and Bergman, 1980a). The results of this study support the concept of red cell amino acid transport. At least one-half of the GLN and GLU transport was by red blood cells. However, the direction of that transport paralleled the response of plasma free amino acids. Thus plasma amino acid exchanges simply underestimated total exchange across the splanchnic bed.

The mechanism of erythrocyte amino acid transport continues to elude scientists. Since the early work of Yunis and Arimura (1965) research interest in this area has continued, however, the information available on the permeability of red cells to amino acids is still limited. It has been suggested that the gamma-glutamyl cycle may be involved in erythrocyte amino acid transport. Glutathione is found almost exclusively within cells and is known to exist in erythrocytes. It is translocated across cell membranes via the gamma-glutamyl cycle (Meister, 1981). This illustrates another interorgan amino acid transport mechanism for red cells. Glutathione is believed to function as a storage form of CYS and may constitute a portion of interorgan sulfur transfer. The mechanism of amino acid translocation into cells via membrane gamma-glutamyl transpeptidase has been extensively studied in Meister's laboratory while other systems have received less attention.

Winter and Christensen (1964) were the first to study permeability of human erythrocytes to neutral amino acids. Rate of

uptake was affected by structural factors including the size of side chains and the presence of polar groups. Young and Ellory (1977) suggested that a considerable variation in red blood cell permeability to amino acids existed between species. Red blood cells of ruminants (cattle, sheep and goats) appeared to have a lower permeability to large neutral amino acids than did human, rat and rabbit cells.

The presence of amino acid transport systems across the gut and other tissues led researchers to consider the possible existence of such systems for red blood cells. No sodium dependent transfer of amino acids has been reported, with the exception of GLY and ALA (Yunis and Arimura, 1965). Kinetic analyses have not been done thus only direct sodium dependence has been evaluated.

Winter and Christensen (1964) suggested that human erythrocytes have three neutral amino acid transport systems: (1) a low capacity system for GLY and ALA, (2) a saturable system for amino acids with large hydrocarbon side chains and (3) a high capacity system for amino acids with large side chains. Similar systems may exist for dibasic amino acid transport as well.

Perhaps one of the most interesting aspects of amino acid transport by red blood cells is the difference that exists between reticulocytes and erythrocytes. Research on age dependence of amino acid transport in red blood cells of pigs indicated that uptake rates in adult and newborn pig red cells and induced reticulocytes differed (Grey and Lee, 1974). Uptakes were much faster in induced cells and those from newborn pigs than in erythrocytes. Reticulocytes appear to

possess sodium-dependent transport systems similar to those reported in other cells (Young and Ellory, 1977). The maturation of these cells appears to result in a loss of these transport systems. Additional systems which have not been elucidated may exist in reticulocytes which are not lost during maturation thus supporting continued ability to transport amino acids.

Recent research (Hider and McCormack, 1980) suggests that some amino acids may enter red blood cells via a chelate facilitated transfer possibly involving copper found in the red cell membrane while others support the concept of a simple carrier system (Rosenberg, 1981).

Much of this specific transport research has been conducted in vitro, while observations of interorgan amino acid transport were observed in vivo. Cahill et al. (1981) has reserached red cell transport in vitro and in man and has often observed discrepancies in his observations between the two techniques. This researcher has stated that "Somehow the red cell knows whether it is in a capillary or test tube".

Species, experimental conditions, dietary and physiological conditions appear to affect the role that the erythrocyte pool plays in free amino acid transport. While several researchers have reported similar responses for amino acids in this pool and in the plasma pool, others contend that the roles are often opposite.

SPLANCHNIC METABOLISM OF FREE AMINO ACIDS

The gastrointestinal tract (GIT) and liver are highly involved in

amino acid metabolism. These tissues along with muscle and kidney have high requirements for amino acids even in adults. While the gut supplies amino acids to the body through absorption into portal circulation, extensive amino acid metabolism may occur in gut tissues. These absorbed amino acids are subjected to metabolism by the liver even before entering general circulation. The liver is one of the most important sites in the body for amino acid metabolism. It is well established that it is the site of most plasma protein and nonessential amino acid synthesis and the chief site of deamination and the urea cycle. In ruminants the liver is also extremely important in terms of gluconeogenesis with the liver of sheep supplying as much as 30% of the glucose produced from amino acids (Wolff and Bergman, 1972). Thus the GIT and liver (splanchnic bed) have a vital function in regulating amino acid movement to peripheral tissues for protein synthesis and other productive functions.

Transport of specific amino acids across the GIT. GLN is one of few amino acids removed from plasma in large amounts by portal drained viscera. Elwyn et al. (1968) fed three cannulated dogs a diet of horsemeat while monitoring plasma free amino acid exchange across the GIT. GLN was the only amino acid removed from plasma by the gut. Later research in man, rats and sheep has revealed GLN uptake as well (Marliss et al., 1971; Windmueller and Spaeth, 1974; Heitmann and Bergman, 1978). At least one-half of the carbon of GLN removed by the rat gut is used for energy while the nitrogen is utilized for ALA,

CIT and ammonia production (Windmueller and Spaeth, 1974).

Not only does the gut utilize GLN but the intestinal brush border cells have the ability to synthesize GLN. Brush border cells were isolated from 4- to 7-wk old chick small intestine and incubated in vitro (Porteous, 1980). GLN produced under these conditions accounted for 60% of the GLU uptake by the cells. GLN and ASP were also converted to ALA under these conditions. Evidence for similar conversions have been reported in vivo. When portal concentrations of amino acids in sheep fed alfalfa were compared with amino acid ratios in microbial protein there is a close similarity with the exception of ASP, GLU, ALA and PRO (Hume et al., 1972). ASP and GLU are found in smaller quantities while ALA is greatly elevated. Bergman and Heitmann (1980) have frequently observed that ALA was absorbed into portal blood of sheep in larger amounts than any other amino acid while GLU absorption was negligible. ALA has been reported to account for 19% of the alpha-amino nitrogen appearing in portal blood of mature sheep (Wolff et al., 1972). Associated with this large absorption of ALA was a gut uptake of GLN and GLU and only a small gut output of ASP. Approximately 35% of the amino acid release from the gut of five men fasted overnight was in the form of ALA (Felig et al., 1973). Exchanges of GLU were not reported, however, GLN was removed from whole blood by the gut while a nonsignificant gut output of ASP was observed. In five dogs fed raw lean beef, ALA constituted 23% of the gut amino acid output into portal blood (Bloomgarden et al., 1981). These data suggest production by the gut or preferential absorption. Concentration

changes for GLU, GLN and ASP were not reported.

With the exception of GLU and in some cases GLN, amino acids are usually added to the portal circulation as blood traverses the GIT due to free amino acid absorption.

Transport of specific amino acids across the liver. Early research on amino acid uptake and metabolism by the liver was conducted in the dog (McMenamy et al., 1962). Dog livers were perfused in vivo with dog blood and an amino acid mixture through the portal vein and blood returned by the vena cava was oxygenated and brought to a delivery reservoir. Plasma and liver amino acids were determined by paper chromatography. The uptake of amino acids by the liver was calculated from the amount of amino acid infused, concentration of amino acid in blood, blood volume and amount of blood exchanged. During the perfusion period, plasma amino acid concentrations rose steadily. The results indicated that the liver did not remove all of the added amino acids on a single passage of blood through the liver although the addition of amino acids to plasma did cause a rapid uptake of amino acids by the liver. This research further suggested that if amino acids were stored for short periods in the liver, they were not present as the free amino acids since a rise in liver free amino acids was not observed during the addition of amino acids to plasma. These researchers suggested a storage as protein which may be reconverted to amino acids when needed for use by tissues.

However, generally the liver is believed to remove similar quantities of amino acids from blood as the GIT adds. This was established in the early work of Elwyn et al. (1968) using dogs. These researchers reported that "the liver took up what the gut put out." Few amino acids have been reported in higher quantities in hepatic venous blood than blood entering the liver. Hepatic output of GLU and ORN was observed in mature ewes (Wolff et al., 1972). The liver of these ewes (fed a near maintenance diet) was able to remove essentially the entire gut output of all other amino acids. More recent work in Bergman's laboratory has revealed a release of GLU, CIT and ORN by the liver into the plasma pool of ewes (Heitmann and Bergman, 1980b). The hepatic production of CIT and ORN was associated with a concomitant hepatic uptake of ARG. This transfer would be indicative of amino acid fluxes assoicated with the urea cycle. Present research by Bergman and Pell (1982) confirmed this response of urea cycle amino acids. The hepatic production of GLU may result from transamination and ammonia detoxification in the liver.

Other amino acids are generally removed by the liver especially the glucogenic amino acids which include ALA, GLN, GLY and SER (Heitmann and Bergman, 1980b). Sheep were infused in the vena cava with a single U-^{14}C amino acid and U-^{14}C-glucose to measure glucose turnover (Wolff and Bergman, 1972). Plasma glucose and plasma amino acid specific activities were determined on samples from the aorta, portal and hepatic veins. ALA and GLU contributed more to glucose turnover than did the other amino acids monitored.

GLY is removed by the liver in larger quantities than any other amino acid (Bergman and Heitmann, 1978). Heitmann and Bergman (1980b) suggested that all of the GLY removed by the liver is not used for glucose production since GLY is not highly glucogenic in sheep when compared to other amino acids. These researchers suggest that a portion of the GLY is utilized for conjugation of bile salts and detoxificaiton of benzoic acid to form hippuric acid. GLY, ALA and GLN have been reported to account for one-half of the alpha-amino nitrogen removed by the liver of sheep fed continuously at a maintenance level (Wolff et al., 1972). ALA and GLN are major amino acids involved in interorgan amino acid transport in ruminants (Bergman and Heitmann, 1978). GLN uptake by the liver has been reported in several studies with sheep fed at hourly intervals (Bergman and Heitmann, 1980). These two amino acids are released by muscle and function in transporting ammonia to the liver via the GLU-GLN conversion and the ALA cycle where it can be converted to urea. GLN and ALA may account for as much as one-half of the amino acids used for hepatic gluconeogenesis in sheep under certain conditions (Bergman and Heitmann, 1978). Thus these amino acids taken up by the liver may be catabolized or used for liver or plasma protein synthesis.

A method for evaluating the net gut and liver effects on amino acid metabolism as well as what amino acids are available for peripheral tissues is termed net splanchnic output and has been defined as the algebraic sum of liver and gut output (Elwyn, 1972). Research with dogs fed horsemeat has revealed no net splanchnic output of amino

acids in plasma (Elwyn et al., 1968). Exceptions included the branched chain amino acids which are believed to be catabolized primarily in muscle (Goldberg and Chang, 1978). Similarly no net splanchnic output of amino acids, except CIT, in plasma or red blood cells of fasted man has been observed (Felig et al., 1973). A review of research by Cahill et al. (1981) indicated that the splanchnic region always appeared to release GLN. Early research in the laboratory of Bergman supported the contention of no net splanchnic output of amino acids in plasma to peripheral tissues (Wolff et al. 1972). These researchers along with Elwyn began to consider the question of how amino acids are supplied to peripheral tissues. If amino acids are not transported from the splanchnic bed as free amino acids then transport must occur via a bound form such as plasma proteins.

FREE AMINO ACID FLUXES DURING FASTING

Several researchers have considered the effects of fasting on amino acid transport and metabolism. Fasting appears to result in decreased portal absorption of amino acids which is countered by a release of amino acids from muscle to sustain a continued hepatic uptake of these amino acids.

Following a 10- to 14-hr fast in men a net release of THR, CIT, GLY, ALA, VAL, MET, LEU and PHE from the gut into whole blood was observed (Felig et al., 1973). ALA was responsible for 35% of this total release. This was accompanied by a release of ALA, THR, MET and LEU into the red blood cell. A net splanchnic uptake of 10 amino

acids was observed while a consistent output of CIT was reported. Research with mature sheep fasted for 72-hr has also indicated a continued but reduced uptake of amino acids into portal blood (Heitmann and Bergman, 1980b). GLN was the only amino acid removed by the gastrointestinal tract under these conditions. Fasting resulted in an increased removal of two gluconeogenic amino acids, GLY and ALA. This is supported by observations of decreased plasma ALA in fasted man (Adibi, 1976). ALA transport in isolated rat hepatocytes was recently reported to increase as a result of starvation and may reflect an increased activity of one or more sodium-dependent transport systems in the liver (Hayes and McGivan, 1982). The increased removal of gluconeogenic amino acids may suggest increased hepatic gluconeogenesis from amino acids during this time of need for increased glucose synthesis. As discussed earlier, the liver may account for up to 85% of the glucose turnover in sheep (Wolff and Bergman, 1972). While propionate is responsible for a large portion of the glucose produced in fed animals its role is reduced with fasting at which time glucose production from amino acids is enhanced.

In a recent review of their research, Bergman and Heitmann (1980) report decreased arterial plasma concentrations of GLN and increased concentrations of GLY and the branched chain amino acids in sheep fasted for 72-hr. The same response was observed in calves fasted for the same period (McCormick, 1980). Similarly, in pigs fasted 18- to 42-hr arterial plasma levels of branched chain amino acids increased, however, GLY levels were decreased by fasting (Ohshima et

al., 1982). The branched chain amino acids are affected most dramatically and promptly in response to fasting in man (Adibi, 1976). Starvation for only 1 d was shown to increase plasma branched chain amino acid concentrations. The increased plasma levels of branched chain amino acids may result from output by the liver or muscle as a result of protein breakdown. Livesay and Lund (1980) evaluated the effects of 24-hr starvation on the branched chain amino acid content of rat tissues. Fasting resulted in a decreased liver content while skeletal muscle was affected little. Felig et al. (1969) reported a muscle release of branched chain amino acids in man during starvation. An earlier study with rats force fed an adequate diet or a protein free diet revealed that liver concentrations of free amino acids were significantly reduced during the first 2 d of this feeding reguime (Adibi et al., 1973). However, the fall in hepatic concentrations tended to plateau by about the second day while the decrease in muscle amino acids continued during the 8-d trial.

Fasting of sheep for 72-hr had a profound affect on the transport of carbon and amino groups away from muscle during this period of protein mobilization (Heitmann and Bergman, 1980b). ALA, GLN and GLY were released by the hindlimb in greater quantities after a 72-hr fast.

Ballard et al. (1976) fasted five wethers for 6 d and observed a release of amino acids, especially ALA, from the hindlimb. Following a 72-hr fast of calves, McCormick (1980) also observed a release of amino acids by the hindlimb into plasma and suggested this to indicate muscle

catabolism. Amino acids released in the greatest quantities were ALA and GLN. This researcher observed a substantial involvement of the erythrocyte in transporting amino acids from the hindlimb. ALA and GLY were released in substantial quantities by the hindlimb into the erythrocyte pool. These results were similar to those observed in post-absorptive man by Felig et al. (1973) who reported a release of amino acids by the leg into the erythrocyte pool. Similarly ALA was released in large quantities.

PEPTIDE ABSORPTION AND TRANSPORT

It is generally believed that proteins must be completely hydrolyzed to free amino acids before absorption can occur. However, peptides are found in the gut lumen and do enter the intestinal mucosa. Whether intact peptides can escape hydrolysis and enter portal circulation under normal conditions remains unclear.

Hume et al. (1972) was unable to account for 10% of daily nitrogen intake in sheep fed alfalfa when plasma free amino acid absorption, ammonia absorption and fecal excretion were monitored. In sheep the appearance rate of VAL, ILE, LYS and HIS in portal blood was only 56 to 67% of the disappearance rate from the gut lumen (Wolff et al., 1972). Tagari and Bergman (1978) reported that only 30 to 80% of the amino acids leaving the intestinal lumen could be accounted for in portal plasma of sheep. These researchers suggest that other nitrogen containing compounds may be absorbed. The role of peptide amino acid transport received little attention until the work of McCormick (1980)

and Danilson (1981).

Intestinal absorption. Failure to detect peptides in portal blood caused many researchers to abandon this area of research for several years (Christensen, 1949; Dent and Schilling, 1949). The development of new analytical techniques has led researchers to reinvestigate the role of the peptide. During the next decade, several researchers found evidence for absorption of di-GLY in vitro. Agar et al. (1953) investigated the absorption of di- and tri-GLY and LEU-GLY by rat small intestine in vitro. All peptides were hydrolyzed on the mucosal side and constituent amino acids were transfered to the serosal side, however, a small amount of GLY-GLY was detected in the serosal fluid from the di- and tri-GLY. The classical work of Newey and Smyth (1959) was the first to indicate that the form in which protein digestion products enter mucosal cells and the form in which they enter the portal blood may differ. GLY-LEU, GLY-TYR, GLY-GLY and ALA-ALA were investigated using everted sacs of rat small intestine. Only constituent amino acids of GLY-LEU and GLY-TYR entered serosal fluid. Small amounts of unhydrolyzed peptides and free amino acids appeared in serosal fluid when GLY-GLY and ALA-ALA were incubated. The researchers concluded that although the peptidase activity of serosal fluid was high, the appearence of only free amino acids and little peptide in serosal fluid could not be accounted for by hydrolysis in serosal fluid. These researchers speculated that hydrolysis may occur in the mucosal cells. Later research by Newey and Smyth (1960) revealed that the peptidase activity in mucosal fluid was insufficient to

account for the amounts of peptides disappearing from the mucosal side. These researchers described an "intracellular hydrolysis". This term indicated "that the site of hydrolysis is such that the amino acids are not set free quantitatively in the mucosal fluid but are set free in the cell, although some of them may later diffuse back into the mucosal fluid from the interior of the cell".

Peptides may be found in large quantities in the gut lumen of humans following a meal. Four humans were fed a test meal which provided 50 g bovine serum albumin, 120 g carbohydrate and 40 g fat (Adibi and Mercer, 1973). Intestinal aspirates obtained before and after the meal were analyzed for free and peptide amino acid concentrations. Concentrations of both free and peptide amino acids increased following the meal. The concentration of peptide amino acids in the jejunum was approximately fivefold greater than the free amino acid concentrations during a 1-hr period following the meal. These peptides are cheifly a result of pancreatic endo- and exo-peptidases. The intestinal epithelium has high peptidase activity and may result in the final digestion of these peptides to amino acids. Matthews (1975a,b) has studied peptide transport across the intestine largely in vitro and concludes that small peptides resulting from protein digestion are taken up by the mucosa where they are hydrolyzed to amino acids within absorptive cells. Several peptidases have been identified in the intestinal mucosa of the rat (Shoaf et al., 1976).

Adibi (1980) supports these concepts and suggests that there are three phases of dietary protein breakdown to free amino acids: (1)

intraluminal phase, (2) surface membrane phase and (3) cytoplasmic phase. The intraluminal phase involves the digestion of proteins by pancreatic enzymes to amino acids and small peptides. In the second phase the brush border is presented with a mixture of amino acids and peptides. These are cleared by independent uptake mechanisms and hydrolysis of peptides. During the cytoplasmic phase in the enterocyte, small peptides are hydrolyzed to free amino acids by cytoplasmic peptide hydrolases.

Peptide carrier systems are responsible for transporting peptides into the enterocytes (Adibi and Kim, 1981). The molecular structure of the peptide influences the affinity for uptake as is true with other carrier mediated systems. Other structural properties that influence dipeptide absorption are the number of amino acid residues, the length of the side chain and sterioisomerism. However, it appears that neutral, basic and acidic dipeptides are transported by a common carrier mediated system (Adibi and Kim, 1981).

Gamma-glutamyl transpeptidase found in the brush border membrane catalyzes the transfer and hydrolysis of gamma-glutamyl groups. Activity of this enzyme is high in the brush border of intestinal epithelial cells. A competitive inhibitor of this enzyme, bromosulfophthalein, has been shown to inhibit uptake of amino acids by intestinal cells (Garvey et al., 1976). This may indicate that gamma-glutamyl transpeptidase does have a role in the uptake of amino acids at the brush border, however, the potential role in peptide absorption remains unclear.

Wolff et al. (1972) could account for only 24% of the CYS disappearing from the lumen of sheep by monitoring portal plasma. Glutathione may be serving as a transport and conservation mechanism for CYS by way of the red blood cell thus providing a mode of uptake from the gut.

Pocius et al. (1981) calculated the uptake of glutathione and its constituent amino acids for milk protein synthesis from whole blood in producing Holstein cows. The possible contribution of CYS and GLY from glutathione was greater than the output of CYS and GLY in milk. This suggested that glutathione degradation in the mammary gland may supply constituent amino acids for synthesis of milk proteins. Although the mode of glutathione utilization by the tissue is not clear, this tripeptide may serve as a protective transport form of amino acids to tissues.

Since peptide hydrolase activity is present in the mucosa it is questionable whether peptides can escape hydrolysis and appear intact in portal blood. There is evidence that such absorption does occur. Peters and MacMahon (1970) infused di-, tri- and tetra-GLY into the duodenum of rats. GLY and di-GLY were detected in portal blood, however, infusion of tetra-GLY resulted in the highest GLY concentration. These investigators suggest that cleavage of the longer peptides to di-GLY and the free amino acid occurs at the brush border. It was estimated that less than 10% of the di-GLY passed directly into the portal circulation.

Adibi (1971) perfused the human intestine with several GLY

containing peptides or the constituent free amino acids. The concentration and rate of absorption of GLY was greater for di-GLY than the free amino acid. The concentration and rate of absorption of LEU and GLY from the dipeptide was greater than from the free amino acids. A greater quantity of LEU than GLY was absorbed from the equimolar mixtures of the free amino acids. This difference was not observed when dipeptides were perfused.

Adibi and Morse (1977) perfused human jejunum with equivalent amounts of GLY as the free or peptide (di, tri or tetra) form. Uptake was greatest and similar for di- and tri-GLY and lowest and similar for the free GLY and tetra-GLY.

Sleisenger et al. (1977) infused female guinea pigs intraduodenally with an amino acid mixture simulating casein and an enzymic hydrolysate of casein (containing 2 to 3 residues). Portal plasma was taken prior to infusion and at 5- to 10-min intervals for 60 min. Plasma amino acids were determined on portal samples. Infusion of the hydrolysate resulted in a lower rate of amino acid entry into portal blood than did the infusion of free amino acids. These researchers speculated that peptide absorption accounted for the decreased rate of amino acids appearing in portal blood, however, peptide appearance in portal blood was not directly monitored.

A rather complex assay is required to completely deproteinize blood samples and determination of amino acid content before and after hydrolysis must be accomplished to ascertain whether peptides appear in portal blood. Such a method was described by Lindblad et al. (1979).

Whole blood and plasma were deproteinized with sulfosalicyclic acid and further subjected to Sephadex G-25. Samples were analyzed before and after acid hydrolysis using ion-exchange chromatography. Treatments and infusion solutions used by Lindblad et al (1979) were the same as those used by Sleisenger et al. (1977). Results of the study indicated that only amino acids enter portal blood during absorption of a casein hydrolysate. Extremely low and often unmeasurable concentrations of peptides were found in blood and plasma.

Kinetics of dipeptide disappearance from the lumen, inhibition studies with free amino acids vs peptides, and inhibition of peptide hydrolase suggest that absorption of intact peptides may be a major mode of disappearance from the human intestinal lumen (Adibi, 1980). The absorption of dipeptides from the intestinal lumen has been observed in a number of studies but whether this occurs under normal conditions in appreciable quantities is unclear. The discrepancies observed in these studies may reflect the very low levels of peptides present in portal blood. Perhaps improved analytical techniques will allow the more precise monitoring of blood peptide levels.

Role in interorgan amino acid transport. Elwyn (1972) suggests that at least two peptides have a role in interorgan amino acid transport. Glutathione which is transported solely in the erythrocyte may serve as a reservoir and protected form of CYS. This tripeptide may carry more of its constituent amino acids between organs than are transported in the free form. Carnosine is synthesized in the liver and

released for transport via the plasma to peripheral tissues. This appears to occur in a cyclic manner with carnosine synthesis occurring in the liver followed by transport to peripheral tissues and gut where it is broken down to HIS which is transported back to the liver.

Interorgan transport of amino acids via peptides has received little attention. However, certain peptides have been reported to be released by the gut and synthesized by the liver. Vasoactive intestinal peptide (VIP) is present in the intestinal mucosa of several species and is removed from portal circulation by rat liver where degradation products are rapidly released (Misbin et al., 1982). GIP is also a gastrointestinal peptide of similar size but is not taken up by the liver.

McCormick (1980) investigated the role of peptides in transport of amino acids across the hindlimb of calves fed a natural diet and following a 72-hr fast. Amino acids in the peptide pool were generally removed as blood crossed the hindlimb. Exceptions were a release of basic amino acids into venous blood. The peptide fraction appeared to be an important source of ASP, SER, GLU, ALA and PHE for the hindlimb. Fasting reversed this response to an output of amino acids by the hindlimb into the peptide pool. The responses of amino acids in this pool were similar in direction to those in plasma and erythrocyte free amino acid pools but the magnitude of response was much greater. Similarly, Danilson (1981) observed an uptake of amino acids from the peptide pool as blood traversed the hindlimb of calves fed soy protein or urea as the sole nitrogen source. Results of these two studies lend evidence to a role of peptide amino acid transport across tissue beds.

Quantitatively the peptide pool appears to be highly involved in interorgan transport when compared to free amino acid movement.

Whether peptides are absorbed intact and function in transporting constituent amino acids to tissues remains a point of discussion. Research with several species in vivo and in vitro supports these concepts yet the mechanism of entry into portal circulation remains unclear. Peptides may provide a means of presenting amino acids to peripheral tissues thus offering an explanation for the lack of net splanchnic output of free amino acids. The implications of peptide absorption would profoundly change our thinking in terms of amino acid absorption. The absorption of peptides may be a means of overcoming amino acid antagonisms which are known to occur during absorption. Matthews and Payne (1975) suggested that peptides may lead to a more simultaneous presentation of amino acids to tissues after hydrolysis and result in a more efficient protein synthesis.

BLOOD PROTEINS AS CARRIERS OF AMINO ACIDS

Although it has been speculated that blood proteins carry amino acids from the splanchnic bed to peripheral tissues (Elwyn, 1970) little research has been conducted to specifically monitor blood protein amino acid transport across tissues. Maintenance of protein turnover in the body requires a continual supply of amino acids. Although free amino acids are supplied to the gastrointestinal tract and liver by absorption little evidence exists for a net splanchnic output of free amino acids to peripheral tissues. Several researchers, whose work was previously

described, have observed no net splanchnic free amino acid output in several species (Elwyn et al., 1968; Felig and Wahren, 1971; Wolff et al., 1972; Heitmann and Bergman, 1980b). The potential role of blood proteins as carriers of their constituent or bound amino acids certainly exists. These proteins are synthesized primarily in the liver and degraded in other tissues. This in itself constitutes a form of amino acid transport (Elwyn, 1972). In vitro research with rat liver suggests that only 10% of albumin degradation occurs in the liver (Gordon, 1957). Others suggest that 50% of albumin breakdown may occur in the rat liver while 20% occurs in the gastrointestinal tract (Katz et al., 1961). Plasma proteins like tissue proteins certainly play an important role in maintaining the blood amino acid pool (Kawai, 1973). Changes in blood proteins occur during altered nutrition and have been monitored as indicators of nutritional adequacy.

Albumin. Albumin is the major protein synthesized by the liver and exported into plasma (Rothschild et al., 1980). This compound may account for as much as 50% of the protein produced by the liver (Rothschild et al., 1972) but others (Peters, 1970) suggests this albumin synthesis accounts for one-third of protein produced by the liver. Many years ago albumin was shown to function in the transport of tryptophan (McMenamy and Onely, 1958). Albumin has an extensive ability to non-specifically bind many components (Kawai, 1973). Along with its function in the maintenance of osmotic pressure, albumin carries several compounds such as fatty acids and bilirubin to the kidney and liver.

Albumin was speculated to serve as a circulating reservoir of amino acids for use by tissues in times of depletion (Peters, 1970). Hoffenberg et al. (1966) reported that a degree of body albumin stores may be depleted before a lowering of serum albumin concentrations is observed. Dietary restriction of proteins appears to result in both decreased albumin synthesis and breakdown. Decreased albumin catabolism may occur as an adaptive mechanism to preserve the body pool. Yap and Hafkenscheid (1981) suggested that decreased albumin synthesis may occur because of inability of polyribosomes to take up amino acids for synthesis during starvation. Rothschild et al. (1974) suggest that albumin synthesis may be decreased by as much as 50% in rabbits following a 24-hr fast. Normal albumin concentrations may be maintained during fasting because the synthesis of albumin responds similarly as other liver proteins to adrenal hormones and to an increased supply of amino acids from catabolism of muscle proteins (Elwyn, 1972).

Extensive turnover, movement throughout the body and binding capability of the albumin molecule lend support for a role in movement of amino acids across tissues.

Other proteins. Other blood proteins include globulins, glycoproteins, lipoproteins, fibrinogen and prothrombin (Kawai, 1973). These proteins are synthesized chiefly in the liver and reticulo-endothelial (RE) cells. RE cells are primarily responsible for immunoglobulin synthesis. The synthesis of these proteins to maintain turnover requires a substantial supply of amino acids. Lobley et al.

(1980) reported that 1.6 kg of protein were synthesized per day in 240 kg heifers. Of this synthesis, 7 to 8% occurred in the liver, 12 to 16% in muscle and 38 to 46% in the gastrointestinal system. Fasting alters both the synthesis and degradation rates of liver and tissue proteins. McNurlan et al. (1979) reported decreased protein synthesis in the liver and small intestine of starved rats. Gan and Jeffay (1967) fasted mice for 7 d and observed a decrease in total liver and muscle protein. During the first 3-d period, approximately one-half of the liver protein lost was due to increased protein catabolism while synthsis rate was unaffected. These researchers suggest that during early starvation, the liver degraded protein stores to supply amino acids to the body. As the fast progressed, muscle protein breakdown supplied these amino acids. Following 8 d of protein deprivation in rats, 30% of the liver mass was lost (McNurlan and Garlick, 1981). Adibi et al. (1973) reported a 25% reduction in skeletal muscle protein and a 66% decrease in liver protein of rats following an 8-d fast.

The most detailed studies designed to specifically evaluate interorgan exchange of amino acids in serum proteins were conducted by McCormick (1980) and Danilson (1981). These researchers monitored the exchange of amino acids in serum protein fractions across the hindlimb of growing calves under differing nutritional regiments. McCormick (1980) monitored amino acid exchange in four protein fractions. Fraction I was primarily globulins; fraction II was largely albumin; fraction III was almost exclusively albumin and fraction IV was a mixture of proteins. An output of amino acids into the globulin

fraction of venous blood was observed in calves fed a natural diet and following a 72-hr fast. Fasting resulted in an increased magnitude of amino acid exchange. Amino acids were generally removed by the hindlimb from the two albumin pools of fed calves and released into these pools following a 72-hr fast. A variable response was observed for amino acids in the mixed protein fraction. The large magnitude of exchange across tissues and the response to nutritional stress suggests that these fractions are functioning in interorgan amino acid transport.

Danilson (1981) evaluated amino acid exchange in a primarily globulin fraction and a primarily albumin fraction. Exchange of amino acids in the globulin fraction was variable with both uptake and release of amino acids as blood traversed the hindlimb of calves fed soy protein as the sole nitrogen source. Urea feeding resulted in a consistently large uptake of most amino acids by the hindlimb. The magnitude of this response led this researcher to suggest an important role of this protein fraction in providing amino acids to the hindlimb of urea fed animals. A similar response was observed for amino acids in the albumin fraction, with this fraction supplying amino acids to the hindlimb under conditions of urea feeding.

These observations support the concept of a splanchnic output of serum proteins to supply consituent or bound amino acids to peripheral tissues especially during times of protein inadequacy. It remains to be shown directly whether this net splanchnic output of amino acids in proteins occurs.

The plasma and erythrocyte free amino acid pools appear to have

distinct roles in interorgan transport of amino acids across tissue beds. The relative roles of these two pools appear to vary with nutritional state, species and individual amino acids. It has become evident that monitoring only plasma free amino acids is not sufficient to obtain a quantitative evaluation of amino acid exchange. Peptides remain an area of intensive research. The lack of ability to observe appreciable quantities of peptides in blood may simply be a reflection of analytical techniques since in vitro research suggests absorption of these molecules. The role of serum proteins in transporting amino acids in the body has been eluded to but not pursued in depth. Hindlimb research in cattle and the role of albumin as a transport molecule certainly suggest a potential role for these proteins in interorgan transport.

OBJECTIVE

The objective of this study was to quantify the movement of plasma peptide, plasma free, erythrocyte free and serum protein amino acids to and from the gastrointestinal system and liver of growing calves in a "steady state" metabolism and following a 72-hr fast.

PEPTIDE, PLASMA AND ERYTHROCYTE AMINO ACID MOVEMENT ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF CALVES

Summary

Arteriovenous differences in peptide, plasma free and erythroctye (RBC) free amino acids were quantified across the gastrointestinal tract (GIT) and liver of six growing Holstein steer calves (136 kg). Animals were cannulated in the abdominal aorta, portal vein and hepatic vein and maintained in a "steady state" metabolism by hourly feeding. Blood was obtained simultaneously from the three cannulae at 9 to 12 d postsurgery and again following a 72-hr fast. The peptide fraction accounted for the greatest concentration of amino acids in arterial blood of fed and fasted calves. Most amino acids were added to plasma by the GIT but fasting reduced the magnitude of this addition. GLN and GLU were the only plasma amino acids removed by the GIT. ALA accounted for 19% of the total GIT output of plasma free amino acids. The liver removed a large portion of amino acids in portal plasma but a net splanchnic output of plasma free amino acids occurred in fed and fasted calves. Hepatic removal of amino acids, particularly VAL, ILE and LEU, increased after fasting. The GIT generally removed amino acids from the RBC. A small hepatic output of amino acids resulted in no net splanchnic output into the RBC pool with the exception of ASP in fed calves and a net uptake of GLY in fasted calves. Peptide amino acids increased as blood crossed the GIT. Responses paralleled those

of plasma free amino acids in direction but were threefold greater in magnitude. A net output of peptide amino acids from the splanchnic bed was observed in fed and fasted calves. Quantitatively peptides appear to be highly involved in interorgan transport and may be a substantial form for absorption of amino acids in calves.

Introduction

Plasma free amino acids have traditionally been monitered in protein and amino acid metabolism studies with many species and have been reported (Elwyn, 1966) to be quantitatively most involved in interorgan amino acid transport in the dog. More recently it has become evident that other blood pools including erythrocyte free amino acids function in amino acid movement through the body. Research has shown that monitoring only plasma free amino acid fluxes across tissue beds may underestimate total amino acid transport in man (Felig et al., 1973; Cahill et al., 1981) sheep (Heitmann and Bergman, 1980a) and cattle (McCormick and Webb, 1982; Danilson et al., 1983). Amino acid fluxes across various tissue beds do not necessarily respond the same between plasma and erythrocyte pools (Elwyn, 1966; Felig et al., 1973; Aoki et al., 1976; McCormick and Webb, 1982; Danilson et al., 1983). The relative importance and role of these two pools may vary with species, dietary and physiological conditions and amino acid.

For years it was generally agreed that proteins must be hydrolyzed to free amino acids before absorption from the intestine could occur. It is now known that an active transport system for di-

and tripeptides exists in the intestinal brush-border (Adibi, 1975). It is still believed that peptides are completely hydrolyzed before entering portal circulation and thus free amino acids are the mayor protein digestion products in portal blood (Adibi and Kim, 1981). During intestinal infusions of high concentrations of peptides, small peptides have been found in portal blood of rats (Peters and MacMahon, 1970; Boullin et al., 1973). Peptide amino acid exchanges across the hindlimb of calves have been reported (Danilson, 1981; McCormick and Webb, 1982). Amino acids in this peptide pool often responded differently than those in either the plasma or erythrocyte pools but the relative importance of this pool varied with nutritional state and amino acid. Whether peptides entering mucosal cells can escape hydrolysis and enter portal circulation is unclear. The absorption of biologically active peptides (Matthews, 1975a), results of infusion studies and in vitro research would indicate that this can occur. However, limited information is available on peptide appearance in portal blood under normal conditions. Further, the relative role of the peptide pool as an interorgan carrier of amino acids has not been defined. It was the objective of this research to quantify the movement of plasma free, erythrocyte free and plasma peptide amino acids across the gastrointestinal system (GIT) and liver of calves in a "steady state" metabolism and following a 72-hr fast.

Experimental Procedure

Animals and feeding. Six Holstein steer calves weighing an

average of 136 kg were fed a natural diet (table 1) on an equal metabolic weight basis to gain .9 kg/d. The diet provided 77 kcal net energy (NE) for maintenance plus 53 kcal NE gain per $W^{.75}$ (Lofgreen and Garrett, 1968). Each animal received a total of $.1(W^{.75})$ kg feed per day. The average age of the calves was 175 d. All calves were castrated, vaccinated for blackleg, treated for internal parasites and injected with vitamins A, D and E and selenium (table 1) prior to use. Animals were housed in confinement on slotted floors under constant light and fed 24 (hourly) equal meals per day via automatic feeders. This feeding system appears to result in near steady state conditions of ruminal fermentation, passage and absorption rates and concentrations of blood metabolites (Minson and Cowper, 1966). Fresh water was available at all times. Calves were fasted for 36 h prior to surgery and following surgery were reacclimated to the diet over a 2- to 3-d period.

Surgical preparation. Cannulae were surgically implanted in the abdominal aorta, portal vein and hepatic vein to determine amino acid exchange across the GIT and liver. Animals were injected intramuscularly with .75 ml Rompun[1] to induce anesthesia. Once the initial state of anesthesia was achieved, the trachea was intubated and anesthesia was maintained with halothane[2] in oxygen. A complete description of cannulae materials and preparation is presented in Appendix A. The aortic cannula was inserted through the right femoral

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[1]Xylazine, Cutter Laboratories, Inc., Shawnee, Kansas.
[2]Bromochlorotrifluorethane. Ayerst Laboroatories, New York, NY.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIET[a]

ITEM	%[b]
Ingredient composition	
Corn, ground (IFN 4-02-931)	50.00
Orchardgrass hay (IFN 1-03-438)	30.00
Soybean meal (IFN 5-04-604)	13.30
Molasses (IFN 4-04-696)	5.00
Defluorinated rock phosphate (IFN 6-01-780)	.42
Limestone (IFN 6-02-632)	.78
Trace mineral salt	.50
Chemical composition	
Dry matter	91.06
Crude protein[c]	13.92
Acid detergent fiber[c]	17.40

[a] All animals received intramuscular injections of Vitamin A, 1 million IU; Vitamin D, 150,000 IU; Vitamin E, 510 IU and selenium 7.5 mg.
[b] As fed basis.
[c] Dry matter basis.

artery into the abdominal aorta using techniques similar to those described by McGilliard (1972) and modified by McCormick and Webb (1982).

For cannulation of the portal and hepatic veins, an incision was made over the twelfth rib. Once the periosteum was separated from the bone, a portion of the rib was removed to allow access to the portal vein and liver. The portal vein was located by blunt dissection and cannulae were inserted 7.5 cm directly into the portal vein. A sharp tip made of bone (bone point) placed in the end of the cannulae allowed tubing to be pushed through the vessel wall into the vein with a minimum of blood loss. Cannulae were then threaded into the vein and the bone points were dislodged by flushing the cannulae with .9% saline. Cannulae were then sutured to the vein via a 2 cm^2 piece of polyvinyl sponge[3] and a sleeve of silastic[4] tubing. Two cannulae were implanted to increase the preparation patency and avoid loss of animals due to cannula malfunction. The hepatic vein cannula was inserted by placing a trochar with a syringe attachment into the liver, beginning near the attachment of the gall bladder and extending into the center of the liver. The liver was palpated and the trochar manipulated until a constant flow of blood could be drawn into the syringe indicating that the trochar was in a hepatic vein. The cannula was then inserted through the trochar tube approximately 12.5 cm into the lobe. The

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[3]Ivalon sponge. Unipoint Industries, Inc., High Point, NC.
[4]Silastic Medical Grade Tubing, Dow Corning Corp., Midland, MI.

tube was removed and the cannula was secured to the liver in a similar manner as the portal vein cannulae. A mesenteric artery was exposed and cannulated using procedures similar to those for implanting aortic cannulae to allow for infusion of p-aminohippuric acid (PAH). All cannulae were passed under the skin from the point of entry into the artery or vein and exteriorized on the lumbar region of the calf. Cannulae were composed of Teflon[5] within the artery or vein and silastic under the skin. Outside diameters of Teflon cannulae were 1.8, 2.5 and 2.8 mm for mesenteric, aorta and portal cannulae, respectively. In all cases 1.57 by 3.18 mm silastic tubing was used. Prior to implantation all cannula materials were treated with a 2% (wt/wt) heparin complex[6] solution. Several combinations of tubing were tested throughout the study and are discussed in Appendix A. Cannulae were flushed initially, 5 d postsurgery and daily thereafter with 10 ml .9% saline. Following flushing all cannulae were filled with 5 ml heparinized (40 units/ml) .9% saline containing 2.4 mg penicillin/ml. Rectal temperatures were monitered daily. Daily intramuscular antibiotic[7] injections of 10 ml were given for 3 to 5 d postsurgery. Following the final sampling, cannulae positions were verified by dissection during postmortem examination.

Sampling. Blood samples were taken 9 to 12 d after surgery from animals in a "steady state" metabolism. Samples of heparinized and

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[5]TFE tubing, Kaufman Glass Co., Wilmington, DE.
[6]TDMAC-Heparin complex Coating, Polysciences, Inc.
[7]Combiotic, Pfizer Agricultural Div., New York, NY.

non-heparinized blood were simultaneously withdrawn from the aorta, portal and hepatic veins at the midpoint of a 1-hr feeding interval (0930 hr). Twenty-four hours later, animals were fasted for 72 hr and re-sampled. Infusion techniques and analyses for PAH are discussed in Appendix B.

Laboratory analyses and calculations. Plasma free amino acids were determined on filtrates obtained following deproteinization of 8 ml of plasma with 2 ml of 20% sulfosalicyclic acid (SSA). The plasma filtrate was then subjected to a 22-hr hydrolysis with 4 N methanesulfonic acid at 115 C (Simpson et al., 1976) to generate a fraction referred to as plasma peptide amino acids. Hydrolyzed samples were adjusted to pH 2 with .7 N LiOH prior to analysis. Amino acid concentrations recorded for the hydrolyzed samples were corrected for contributions of free amino acids present before hydrolysis. Erythrocyte free amino acids were determined on filtrates resulting from protein precipitation from 8 ml of whole blood with 8 ml of 10% SSA. Filtrates were freeze-dried and reconstituted in .01 N HCl prior to amino acid analysis. Corrections were made for plasma contribution based on microhematocrit to obtain a measure of erythrocyte free amino acid concentrations from whole blood concentrations. Twenty percent of the packed cell volume was assumed to be plasma (Elwyn, 1966). A diagrammatic representation of these procedures is presented in Appendix C. Amino acid concentrations were determined by ion exchange chromatography[8]. Concentrations and arteriovenous (A/V) differences for each amino acid are reported on a whole blood basis.

Formulas used for A/V difference computations were:

GIT A/V difference = Aorta concentration - Portal vein concentration

Liver A/V difference = {.2(Aorta conc) + .8(Portal vein conc)} - Hepatic vein conc

Liver A/V difference computation is based on 20% of the blood entering the liver from the hepatic artery and an 80% contribution by the portal vein (Katz and Bergman, 1969). Net splanchnic output is reported as the algebraic sum of liver and gut outputs (Elwyn, 1972). Reduced blood glutathione (Beutler et al., 1963) was determined on all samples.

Statistical analyses. Differences between tissue beds and between fed and fasted values within a blood pool were tested using least squares procedures (Barr et al., 1979). Arteriovenous differences between paired samples were evaluated by the Student's t-test. An example of the analysis of variance used is presented in Appendix D.

Results and Discussion

All animals responded well to surgery and returned to a normal feeding schedule within 3 d postsurgery. Rectal temperatures were generally elevated for only 1 to 2 d after surgery and in no case for more than 5 d. Cannula patency continued to be a problem throughout the experiment. Clotting of blood in or around cannulae appeared to be eliminated by the heparin treatment of cannula materials prior to implantation, however, formation of a fibrous sheath around the tip

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[8]Model TSM Amino Acid Analyzer, Technicon Instruments, Tarrytown, NY.

rendered several cannulae nonfunctional. The major loss of patency appeared to result from location of the cannula tip against the vessel wall so that the cannula could be infused but no blood could be drawn. Several cannulae remained patent for up to 2 mo after surgery.

Arterial concentrations. The peptide fraction accounted for the greatest absolute concentration of amino acids in arterial blood in both fed and fasted animals while the erythrocyte fraction generally accounted for the least amount (table 2). Similar responses in growing calves were observed by McCormick and Webb (1982) and Danilson et al. (1983). Total amino acid concentration in the peptide fraction was approximately twofold greater than that for the plasma fraction and sixfold greater than that of the erythrocyte fraction. Fasting significantly decreased the concentrations of most amino acids in arterial plasma. Exceptions included increases in the concentrations of CYS ($P<.01$) and the branched chain amino acids ($P<.03$). Arterial plasma concentrations of branched chain amino acids have been reported to substantially increase following a 72-hr fast in sheep (Heitmann and Bergman, 1980b) and in calves (McCormick and Webb, 1982). Heitmann and Bergman (1980b) suggest that branched chain amino acid concentrations depend on the duration and type of fast. The total essential amino acid (EAA) concentration was unaffected by fasting, however, total nonessential amino acids (NEAA) were decreased by approximately 20% ($P<.01$) resulting in an increase in the essential/nonessential (E/NE) ratio ($P<.01$) and a decrease in the total plasma free amino acid concentration ($P<.07$).

TABLE 2. PLASMA FREE, ERYTHROCYTE FREE AND PLAMA PEPTIDE AMINO ACIDS IN ARTERIAL BLOOD OF FED AND FASTED CALVES

	Plasma[a]				Erythrocyte[a]				Peptide[a]			
	Fed	Fast	P[b]	EMS[c]	Fed	Fast	P[b]	EMS[c]	Fed	Fast	P[b]	EMS[c]
ASP	1.68	.94	.01	.01	2.73	2.90	.68	.49	30.78	32.58	.55	23.20
THR	5.69	4.72	.18	1.17	2.26	2.08	.73	.70	20.61	23.34	.25	13.40
SER	7.32	3.69	.01	.91	2.02	2.55	.28	.57	27.71	30.90	.25	18.44
ASN	3.70	1.50	.01	.30								
GLU	12.15	6.80	.01	1.56	2.36	3.80	.35	3.41	46.50	49.13	.51	40.99
GLN	12.96	11.27	.11	2.28								
GLY	28.42	30.05	.40	9.66	11.76[f]	20.50[g]	.01	9.30	38.84	53.27	.01	40.60
ALA	13.59	8.00	.01	3.13	12.34[f]	13.01[g]			23.16	27.16	.10	11.32
CIT	1.38	1.44	.59	.04								
VAL	16.86	21.25	.01	2.63	1.79	3.65	.01	.35	12.52	15.26	.15	7.62
CYS	1.15	1.36	.01	.01								
MET	1.58	1.08	.01	.03	.89	1.40	.02	.08	.88	.97	.72	.14
ILE	9.19	9.94	.32	1.43	1.02	1.68	.02	.12	9.15	12.05	.11	6.63
LEU	12.65	14.91	.08	3.12	2.52	3.98	.01	.18	29.12	31.99	.27	15.92
TYR	3.97	2.43	.01	.17	1.35[d]	1.57	.04	.02	5.73	6.90	.17	1.66
PHE	4.52	4.04	.18	.29	.38[d]	.73	.06	.04	15.60	18.02	.18	7.35
ORN	5.88	6.53	.61	4.41	3.37[e]	3.12[e]	.80	1.54				
LYS	7.15	8.13	.34	2.60	3.15[e]	3.56	.74	2.50	24.28	30.19	.11	28.36
HIS	5.15	5.16	.98	1.08	7.12	7.90	.57	4.85	9.84	8.25	.14	2.52
ARG	11.33	5.15	.01	3.77					9.58	12.17	.06	3.59
BCAA	38.70	46.10	.03	19.63	5.32	9.32	.01	1.20	50.79	60.06	.16	94.56
EAA	74.12	74.38	.96	74.39	18.00	24.98	.02	11.77	131.58	152.24	.17	484.15
NEAA	92.20	74.01	.01	42.02	28.55	34.14	.17	37.32	172.72	199.94	.10	526.84
TOTAL	166.32	148.39	.07	184.79	46.55	59.12	.06	78.32	304.30	352.18	.11	1884.17
E/NE	.80	1.00	.01	.01	.71	.76	.31	.01	.76	.76	.86	.01

[a]Mean concentrations are expressed as um/dl blood and represent six observations each.
[b]Probability that fed and fasted mean differences this large or larger could occur by chance.
[c]Error mean square.
[d,e,f,g] Least square mean based on five, four, three or one observations, respectively.

Concentrations of erythrocyte free amino acids tended to increase with fasting. Most notable was the substantial increase in the branched chain amino acid concentrations ($P<.01$). Large quantities of GLY and ALA were present in the erythrocyte in both fed and fasted animals. These two amino acids accounted for 52 and 57% of the total erythrocyte free amino acid concentration in fed and fasted calves, respectively. The transport of ALA by blood cells has been reported to exceed that of any other amino acid in man (Felig et al., 1973). The basic amino acids in the erythrocyte free amino acid pool were not significantly affected by fasting. Fasting resulted in a 39 and 27% increase in EAA and total amino acid concentrations, respectively. These concentration changes indicate that the plasma free and erythrocyte free amino acid pools respond differently to nutritional stress.

GLY ($P<.01$), ALA ($P<.10$) and ARG ($P<.06$) increased in the peptide fraction following a 72-hr fast. Other peptide amino acid concentrations tended to increase with fasting but the responses were nonsignificant ($P>.10$).

Plasma free amino acid A/V differences. Amino acids were generally added to plasma as blood traversed the GIT of fed and fasted animals (table 3). GLU and GLN were the only amino acids with concentrations higher in arterial than in venous blood of fed calves. The same response for GLU and GLN in plasma of mature ewes has been reported (Wolff et al., 1972). Several studies with many species have shown that GLN is removed from plasma by portal-drained viscera (Elwyn et al., 1968; Marliss et al., 1971; Windmueller and Spaeth, 1974;

TABLE 3. PLASMA FREE AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Amino acid	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
ASP	-.52	(.01)	-.28	(.11)	.15	(.37)	.43	(.04)	.01	.16	.16
THR	-1.26	(.01)	-.21	(.53)	.13	(.69)	1.18	(.01)	.01	.01	.65
SER	-1.95	(.01)	-.66	(.08)	.69	(.07)	1.88	(.01)	.01	.01	.73
ASN	-1.85	(.01)	-.54	(.21)	.31	(.47)	1.02[g]	(.07)	.01	.04	1.01
GLU	.67	(.35)	1.09	(.14)	-3.18	(.01)	-.95	(.24)	.01	.08	2.83
GLN	1.10	(.13)	1.78	(.02)	-.12	(.87)	-.08	(.92)	.05	.63	2.87
GLY	-3.69	(.01)	-1.87	(.04)	2.10	(.02)	5.24	(.01)	.01	.01	4.06
ALA	-5.04	(.01)	-1.32	(.05)	1.98	(.01)	5.18	(.01)	.01	.01	2.27
CIT	-.44	(.01)	-.30	(.01)	.08	(.42)	.02	(.84)	.01	.71	.06
VAL	-1.96	(.01)	-.35	(.45)	.10	(.83)	.80	(.13)	.01	.02	1.19
CYS	-.14	(.01)	-.04	(.33)	.10	(.04)	.15	(.01)	.01	.14	.01
MET	-.29	(.01)	-.10	(.33)	.10	(.32)	.35	(.01)	.01	.06	.06
ILE	-1.60	(.01)	-.24	(.48)	-.18	(.59)	.74	(.06)	.01	.01	.62
LEU	-2.54	(.01)	-.50	(.34)	-.10	(.84)	1.04	(.09)	.01	.01	1.54
TYR	-1.22	(.01)	-.32	(.21)	.56	(.04)	.92	(.01)	.01	.03	.36
PHE	-1.32	(.01)	-.37	(.19)	.58	(.05)	.96	(.01)	.01	.03	.44
ORN	-.76	(.23)	.05	(.93)	-.14	(.82)	-.70	(.32)	.92	.85	2.14
LYS	-2.08	(.01)	-1.25	(.06)	.07	(.91)	1.53	(.04)	.01	.09	2.15
HIS[h]	-.80	(.02)	.90	(.01)	-.22	(.48)	-.18	(.59)	.42	.01	.52
ARG	-1.18	(.12)	-1.48	(.06)	.69	(.35)	1.27	(.14)	.01	.85	3.05

[a] Values represent the concentration difference between arterial and venous samples expressed as uM/dl whole blood.
Negative values indicate greater concentrations in venous blood.
[b] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in () indicate the probability that the A/V difference does not differ from zero.
[f,g] Least square mean based on five or four observations, respectively.
[h] Tissue x state interaction (P<.02).

Lindsay et al., 1975; Heitmann and Bergman, 1978, 1980b). The role of GLN may be for energy but its carbon is also used for CIT, PRO and organic acid production and the nitrogen for synthesis of CIT, ALA, PRO and ammonia (Windmueller and Spaeth 1974, 1978; Heitmann and Bergman, 1980b). Fasting significantly reduced the magnitude of amino acid uptake into plasma. The continued uptake of amino acids into portal plasma after the 72-hr fast would tend to indicate that the fast was not sufficiently long for the animals to be in a completely post absorptive state. GLU ($P<.14$), GLN ($P<.02$) and HIS ($P<.01$) were removed from plasma across the GIT of fasted calves. Substantial outputs of SER, GLY, ALA, CIT and the basic amino acids into portal plasma were observed. The concentration change for ALA was large under both nutritional states and accounted for 19% of the total amino acid output from the GIT into portal plasma in fed calves and 13% in fasted calves. Similarly, Wolff et al., (1972) found that ALA accounted for 19% of alpha-amino nitrogen appearing in portal plasma of sheep and Bloomgarden et al. (1981) reported that 23% of the gut amino acid output in the dog was ALA. In fasted men, 35% of the amino acid release from the gut was in the form of ALA (Felig et al., 1973).

GLU was the only amino acid released in significant quantities by the liver of fed calves. A release of GLU from the liver has been reported in sheep (Heitmann and Bergman, 1980b). Hepatic GLU production in this study and in studies with man (Felig, 1975) may result from hepatic metabolism of amino acids, transamination and detoxification of ammonia (Wolff et al., 1972). The liver tended to

remove all other amino acids from the plasma pool in fed and fasted animals. However, only a portion of the amino acids added to portal blood was removed. Fasting resulted in an increased hepatic uptake of amino acids regardless of a decreased uptake from the gut. The absolute amount removed was greater ($P<.09$) for most amino acids following the 72-hr fast.

GLY and ALA were removed by the liver in greater quantities than other amino acids, accounting for 53 and 46% of the total liver uptake in fed and fasted animals, respectively. Similarly, GLY, ALA and GLN have been reported to account for about 50% of the alpha-amino nitrogen removed by the liver of sheep (Wolff et al., 1972). GLY is removed by the liver of fed sheep in larger quantities than other amino acids (Bergman and Heitmann, 1978). A portion of this GLY functions in the detoxification of benzoic acid in ruminants (Heitmann and Bergman, 1980b). ALA functions through the alanine cycle to transport nitrogen from peripheral tissues to the liver where it can be converted to urea (Marliss et al., 1971) and may explain the large hepatic uptake of this amino acid under both nutritional states. ALA transport in isolated rat hepatocytes increases with starvation and is believed to result from increased activity of a transport system for this amino acid (Hayes and McGivan, 1982). ALA, along with GLU and GLN, is also one of the most gluconeogenic amino acids. Considerable glucose synthesis must occur in the liver of ruminants since glucose absorbed from the gastrointestinal system cannot always meet the needs of the animal. GLN and ALA may account for nearly 50% of the amino

acids used for hepatic glucose production (Bergman and Heitmann, 1978) while these amino acids and GLU may each account for 3 to 6% of total glucose production in sheep (Heitmann et al., 1973). The present study supports these observations through increased hepatic uptake of these gluconeogenic amino acids. During fasting, a time when there is an increased need for hepatic gluconeogenesis, liver uptake of amino acids increased.

While amino acids were added to portal plasma and a portion of these was removed by the liver, a net splanchnic output ($P<.05$) occurred for essentially all amino acids in plasma of fed calves (table 4). GLN was the exception showing a tendency for net uptake ($P<.14$). GLN is also removed by the splanchnic bed of man where it is believed to be converted to ALA by nonhepatic tissues (Felig, 1975). Branched chain amino acids accounted for 26% of the net splanchnic output in the plasma fraction of fed calves. This output is a result of the large uptake across the GIT and the lack of uptake or release by the liver. A net flow of branched chain amino acids from the liver to peripheral tissues has been reported in the dog (Elwyn, 1972; Bloomgarden et al., 1981). In man branched chain amino acids are not utilized by the liver and in some catabolic states are produced (Cahill et al., 1981).

Fasting changed ($P<.08$) the net splanchnic response from an output to an uptake for most amino acids. Net uptakes of these amino acids may reflect the increasing utilization of amino acids for energy during a time of nutritional stress. A net uptake of ten amino acids was reported in man following a 10- to 14-hr fast (Felig et al., 1973).

TABLE 4. NET SPLANCHNIC RESPONSE OF PLASMA FREE AMINO ACIDS

	Fed[a]		Fasted[a]			
Amino acid	uM/dl	P[d]	uM/dl	P[d]	P[b]	EMS[c]
ASP	-.36	(.01)	.07	(.28)	.01	.02
THR	-.91	(.01)	1.03	(.02)	.01	.28
SER	-1.26	(.01)	.89	(.05)	.01	.45
ASN	-1.55	(.01)	-.09[e]	(.73)	.02	.18
GLU	-2.51	(.01)	.54	(.33)	.01	1.03
GLN	.99	(.14)	1.19	(.13)	.82	1.73
GLY	-1.59	(.12)	2.63	(.05)	.03	3.76
ALA	-3.42	(.01)	3.29	(.01)	.01	1.55
CIT	-.35	(.01)	-.37	(.02)	.88	.04
VAL	-1.86	(.01)	.12	(.75)	.01	.52
CYS	-.04	(.31)	.08	(.11)	.08	.01
MET	-.18	(.01)	.19	(.01)	.01	.01
ILE	-1.78	(.01)	.34	(.42)	.01	.62
LEU	-2.64	(.01)	.40	(.50)	.01	1.32
TYR	-.65	(.04)	.45	(.16)	.03	.29
PHE	-.74	(.01)	.37	(.13)	.01	.16
ORN	-.89	(.49)	-.42	(.78)	.80	8.27
LYS	-2.01	(.01)	-.16	(.60)	.01	.35
HIS	-1.02	(.05)	.68	(.18)	.04	.75
ARG	-.49	(.59)	-.56	(.60)	.96	4.09

[a] Negative values represent a net output from the splanchnic bed. Values represent means of six observations for fed and five observations for fasted.

[b] Probability that fed and fasted mean differences this large or larger could occur by chance.

[c] Error mean square.

[d] Values in () indicate the probability that the net splanchnic response is different from zero.

[e] Least square mean based on four observations.

The large splanchnic output of branched chain amino acids in fed animals was reversed by fasting reflecting increased hepatic uptake of these amino acids. The removal of these amino acids by the liver is correlated with protein synthesis in the liver of dogs (Elwyn et al., 1968). Research has established that skeletal muscle is very active in the catabolism of branched chain amino acids in nonruminants and muscle has been suggested to be the chief site of branched chain amino acid metabolism (Goldberg and Chang, 1978). The hepatic removal of these amino acids from plasma observed in the present study with calves and by Heitmann and Bergman (1980b) with sheep would indicate that the liver does have a role in branched chain amino acid metabolism in the ruminant. These amino acids may be degraded or used for protein synthesis. Fasting appears to enhance the hepatic response to these amino acids resulting in no net splanchnic output. It has generally been the belief that the liver removes most amino acids added to portal blood resulting in no net splanchnic output (Elwyn et al., 1968; Felig and Wahren, 1971; Heitmann and Bergman, 1980b). Net outputs of amino acids in this study with fed calves may reflect the nutritional status of the animal. In mature sheep fed a maintenance diet, amino acids were removed by the liver at a rate similar to their addition to portal blood (Heitmann and Bergman, 1980b). In the present study animals were fed to gain .9 kg/d resulting in a net output of amino acids to peripheral circulation in the plasma pool. Outputs of branched chain amino acids in plasma of fed animals accounted for 26% of the net output. These amino acids may be

transported to extrahepatic tissues for catabolism (Elwyn, 1972).

Erythrocyte free amino acid A/V differences. Amino acids were generally removed from the erythrocyte pool by GIT tissues in fed and fasted calves (table 5). This response of the erythrocyte free amino acid pool is opposite that observed for plasma in terms of uptake and release of free amino acids. Variability among animals tended to be greater for concentration changes in the erythrocyte pool than those of the plasma or peptide pools. Although the magnitude of response was often as large as that observed for the plasma pool, animal variability may have precluded statistical significance. Missing observations are a result of the calculation of a negative erythrocyte free amino acid concentration for one or more sampling sites. In fed animals a positive A/V difference was recorded for all amino acids as blood traversed the GIT with concentration changes ranging from .10 to 5.32 uM/dl blood for ASP and ALA, respectively. The red blood cell carries large quantities of ALA in man accounting for 22 to 32% of net ALA movement (Felig et al., 1973). Arterial blood contained greater concentrations of VAL, LEU, TYR, PHE and ORN than portal venous blood of fed calves ($P<.10$). Fasting resulted in a large uptake of GLY from the erythrocyte ($P<.01$), accounting for over 50% of the total amino acid uptake from the erythrocyte pool. This response is opposite that of GLY in the plasma pool. These observations indicate that blood cells may provide amino acids to gut tissue and that this response is diminished with fasting.

In fed calves, all erythrocyte free amino acids except GLU and

TABLE 5. ERYTHROCYTE FREE AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Amino acid	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
ASP	.10	(.80)	.09	(.80)	-.66	(.09)	-.84	(.06)	.04	.81	.80
THR	.82	(.20)	-.22	(.72)	-.57	(.37)	-.28	(.69)	.27	.56	2.31
SER	1.15[f]	(.13)	.25	(.69)	-.58[g]	(.48)	-.45	(.52)	.10	.61	2.26
ASN											
GLU	1.95[h]	(.15)	-.22	(.77)	.82[h]	(.52)	.74[g]	(.49)	.93	.31	3.12
GLN											
GLY[j]	1.82	(.19)	6.42	(.01)	-1.26	(.36)	-1.58	(.31)	.01	.14	10.64
ALA	5.32[h]	(.25)	2.01[i]	(.79)	-2.95[h]	(.46)	1.95[i]	(.79)	.46	.90	32.55
CIT											
VAL	.92[f]	(.06)	.92	(.04)	-.26[f]	(.58)	-.23	(.61)	.02	.98	.93
CYS											
MET	.12	(.39)	.05	(.72)	-.33	(.02)	-.12	(.43)	.04	.61	.10
ILE	.58[g]	(.13)	.32	(.25)	-.12[h]	(.78)	-.06	(.85)	.12	.79	.42
LEU	1.20	(.01)	.67	(.13)	-.57	(.20)	-.16	(.74)	.01	.89	1.06
TYR	.52	(.01)	.29	(.13)	-.48	(.02)	-.38	(.08)	.01	.73	.19
PHE	.32[h]	(.04)	.22[g]	(.03)	-.29[h]	(.06)	-.38[h]	(.01)	.01	.43	.04
ORN	1.81[h]	(.07)	1.11[g]	(.15)	.34[h]	(.69)	-1.20[h]	(.19)	.05	.22	1.80
LYS[j]	1.55[g]	(.12)	-.65[f]	(.40)	-1.32[h]	(.25)	.70[h]	(.52)	.42	.93	2.35
HIS	.60	(.64)	-.86	(.50)	-.63	(.62)	1.70	(.24)	.61	.74	9.18
ARG											

[a] Values represent the concentration difference between arterial and venous samples expressed as uM/dl whole blood.
[b] Negative values indicate greater concentrations in venous blood.
Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in ( ) indicate the probability that the A/V difference does not differ from zero.
[f,g,h,i] Least square mean based on five, four, three or one observations, respectively.
[j] Tissue x state interaction (P<.10).

ORN tended to be higher in hepatic venous blood than in blood entering the liver but responses were generally small and nonsignificant. Fasting had no effect on A/V differences across the tissues examined ($P>.10$) but a tissue x state interaction was evident for GLY and LYS ($P<.10$) due to differences in uptake and release and magnitude of response across the two tissues. A net movement of amino acids in the erythrocyte pool (table 6) was not observed ($P>.10$). Exceptions were a net output of ASP ($P<.09$) in fed and fasted calves and a net uptake of GLY ($P<.01$) in fasted animals. The erythrocyte free amino acid pool supplies a greater quantity of GLY to the splanchnic bed than does the plasma pool. Free amino acids in the plasma and erythrocyte pools responded differently indicating separate and distinct roles for each pool. Support is given to this concept by observations of opposing roles for plasma and erythrocytes across hepatic tissues of the dog (Elwyn, 1972) and hindlimb tissues in calves (Danilson et al., 1983). However, plasma and blood cell free amino acids have been reported to respond similarly in ewes (Heitmann and Bergman 1980a). Elwyn (1966) found that uptake of labeled GLY was much faster by dog erythrocytes than by human red cells and concluded that the dog may have a more active transport system for some amino acids allowing the dog erythrocyte to concentrate amino acids to a greater extent than human erythrocytes. Perhaps these differences occur for sheep and cattle as well. It will be difficult to ascertain the role of transport by the red blood cell and species differences until the mechanisms of membrane transport are elucidated.

TABLE 6. NET SPLANCHNIC RESPONSE OF ERYTHROCYTE FREE AMINO ACIDS

	Fed[a]		Fasted[a]			
Amino acid	uM/dl	P[d]	uM/dl	P[d]	P[b]	EMS[c]
ASP	-.57	(.09)	-.70	(.08)	.75	.39
THR	.39	(.38)	-.52	(.33)	.21	.94
SER	.72[f]	(.52)	-.14	(.87)	.57	2.41
ASN						
GLU	2.19[g]	(.24)	-.06[f]	(.96)	.31	4.20
GLN						
GLY	.39	(.77)	6.30	(.01)	.04	9.32
ALA	2.37[g]		3.97[i]			
CIT						
VAL	1.22[e]	(.17)	.68	(.39)	.63	1.97
CYS						
MET	-.22	(.20)	-.02	(.90)	.43	.12
ILE	.23[g]	(.56)	.46	(.24)	.65	.13
LEU	.63	(.20)	.62	(.27)	.99	1.03
TYR	.04	(.83)	-.05	(.81)	.75	.18
PHE	.19[g]	(.26)	.01	(.98)	.32	.03
ORN	2.33[g]	(.24)	-.64[g]	(.61)	.27	1.86
LYS	-1.38[g]		.43[h]			
HIS	-.03	(.99)	2.10	(.39)	.49	20.06
ARG						

[a] Negative values represent a net output from the splanchnic bed. Values represent means of six observations for fed and five observations for fasted.

[b] Probability that fed and fasted mean differences this large or larger could occur by chance.

[c] Error mean square.

[d] Values in () indicate the probability that the net splanchnic response is different from zero.

[e,f,g,h,i] Least square mean based on five, four, three, two or one observations, respectively.

Peptide amino acid A/V differences. All plasma peptide amino acids increased as blood traversed the GIT in fed and fasted calves (table 7). A/V differences were different from zero for more than one-half of the amino acids ($P<.10$). The magnitude of the A/V difference was affected only slightly by fasting. Responses of amino acids in the peptide pool paralleled those of plasma free amino acids in direction but magnitude approached threefold greater for peptide amino acids. The large A/V differences observed for all amino acids in the peptide fraction indicate a substantial release of amino acids in the form of peptides into portal circulation. The peptide pool appeared to serve as a source of amino acids for liver as indicated by the large positive A/V values, however, no amino acid showed a significant response in either fed or fasted calves. Statistical significance may have been precluded by the large animal variability and small sample size.

While the liver removes a portion of the amino acids from this pool, a considerable net splanchnic output of all amino acids in the form of peptides was observed in fed and fasted calves (table 8). The peptide pool provided 50% more total amino acids to peripheral circulation than did the plasma pool. McCormick and Webb (1982) reported that the peptide fraction appeared to be an important source of ALA, ASP, GLU, PHE and branched chain amino acids for the muscle of growing calves. Danilson (1981) found that large quantities of GLU, LYS, HIS, VAL and GLY were removed from the peptide amino acid pool as blood crossed the hindlimb of calves fed soy protein. In the present study these and other amino acids were supplied to peripheral tissues

TABLE 7. PEPTIDE AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Amino acid	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
ASP	-6.44	(.04)	-4.52	(.14)	1.84	(.53)	3.02	(.37)	.02	.61	49.80
THR	-4.86	(.07)	-4.07	(.12)	1.75	(.49)	2.14	(.45)	.03	.82	36.72
SER	-6.42	(.06)	-5.58	(.09)	1.46	(.64)	2.75	(.44)	.02	.74	56.58
ASN											
GLU	-5.82	(.10)	-2.53	(.46)	1.56	(.65)	2.17	(.57)	.10	.58	66.89
GLN											
GLY	-8.05	(.05)	-11.56	(.01)	.89	(.81)	-1.14	(.79)	.02	.48	82.36
ALA	-3.55	(.22)	-2.80	(.33)	.78	(.78)	1.62	(.61)	.15	.78	45.55
CIT											
VAL	-3.63	(.06)	-2.42	(.20)	1.12	(.54)	1.04	(.61)	.04	.76	19.14
CYS											
MET	-.39	(.06)	-.24	(.20)	.10	(.61)	-.21[g]	(.39)	.22	.68	.20
ILE	-2.93	(.01)	-1.42	(.16)	1.10	(.28)	.03	(.98)	.02	.83	5.60
LEU	-6.47	(.06)	-3.93	(.24)	2.46	(.45)	2.06	(.58)	.04	.75	61.00
TYR	-1.70	(.07)	-1.73	(.07)	.42	(.64)	.37	(.71)	.03	.96	4.60
PHE	-3.74	(.05)	-2.76	(.13)	1.56	(.38)	1.14	(.56)	.02	.88	17.78
ORN											
LYS	-6.97	(.03)	-2.57	(.38)	2.21	(.45)	1.64	(.62)	.04	.53	48.85
HIS	-.18	(.86)	-1.75	(.09)	-.57	(.57)	1.17	(.30)	.22	.94	5.58
ARG	-2.94	(.16)	-1.83	(.36)	.48	(.81)	.20	(.93)	.20	.84	22.92

[a] Values represent the concentration difference between arterial and venous samples expressed as uM/dl whole blood.
[b] Negative values indicate greater concentrations in venous blood.
[c] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[d] Probability that fed and fasted mean differences this large or larger could occur by chance.
[e] Error mean square.
[e] Values in () indicate the probability that the A/V difference does not differ from zero.
[f,g] Least square mean based on five or four observations, respectively.

TABLE 8. NET SPLANCHNIC RESPONSE OF PEPTIDE AMINO ACIDS

Amino acid	Fed[a] uM/dl	Fed[a] P[d]	Fasted[a] uM/dl	Fasted[a] P[d]	P[b]	EMS[c]
ASP	-4.60	(.01)	-3.58	(.01)	.27	1.62
THR	-3.12	(.01)	-3.70	(.01)	.49	1.45
SER	-4.96	(.02)	-5.38	(.03)	.85	11.56
ASN						
GLU	-4.26	(.01)	-3.48	(.01)	.39	1.70
GLN						
GLY	-7.16	(.17)	-11.37	(.09)	.56	110.36
ALA	-2.77	(.05)	-2.87	(.07)	.95	5.82
CIT						
VAL	-2.51	(.05)	-1.80	(.17)	.64	4.98
CYS						
MET	-.29	(.09)	-.70[e]	(.03)	.15	.09
ILE	-1.83	(.08)	-1.85	(.12)	.99	3.81
LEU	-4.01	(.02)	-4.18	(.03)	.92	7.48
TYR	-1.28	(.09)	-2.06	(.04)	.43	1.97
PHE	-2.19	(.04)	-2.92	(.03)	.55	3.23
ORN						
LYS	-4.76	(.03)	-2.73	(.20)	.43	13.48
HIS	-.76	(.35)	-.97	(.32)	.86	3.12
ARG	-2.45	(.06)	-1.58	(.23)	.59	5.46

[a] Negative values represent a net output from the splanchnic bed. Values represent means of six observations for fed and five observations for fasted.

[b] Probability that fed and fasted mean differences this large or larger could occur by chance.

[c] Error mean square.

[d] Values in () indicate the probability that the net splanchnic response is different from zero.

[e] Least square mean based on four observations.

by the splanchnic bed. Peptides appear to be the principal form in which amino acids are absorbed and presented to the liver. The number of amino acid residues present in these peptides has not been determined. It has been reported that deproteinization with sulfosalicylic acid will leave traces of small proteins in plasma filtrates (Dickensen et al., 1965). If the peptides that appear in portal plasma were actually absorbed from the intestinal lumen it is likely that they have low molecular weights and consist of a small number of amino acid residues. It has been suggested from in vitro research that only di- and tripeptides enter the mucosal cells where they are hydrolyzed to free amino acids for absorption into the portal system (Adibi and Kim, 1981). Infusion studies with rats (Boullin et al., 1973) and guinea pigs (Sleisenger et al., 1977) have indicated absorption of peptides into the portal circulation. The present study is to our knowledge the first to indicate that peptides may escape hydrolysis and appear in portal plasma of animals under "steady state" conditions. Vasoactive intestinal peptide (VIP) present in intestinal musoca of several species is removed from portal circulation by rat liver and VIP degradation products are rapidly released (Misbin et al., 1982). Another gastrointestinal peptide of similar size, GIP, is not taken up by liver cells. Whether gastrointestinal peptides, peptides absorbed from the lumen or a combination of these are contributing to the peptide amino acid pool monitored in this study remains unclear. The large concentration changes observed for this pool may indicate that both sources are responsible for transporting amino acids to the liver.

Concentration changes in blood glutathione were also monitored in this study but were unaffected by transport across tissues and nutritional state.

The findings of this study support the concept that the monitoring of only plasma free amino acids may not reflect the total movement of amino acids across the GIT and liver. Plasma and erythrocyte free amino acid fluxes across these tissues responded differently for most amino acids monitored indicating an independent role for these two free amino acid pools. The plasma peptide pool was quantitatively more important in amino acid transport than the free amino acid pools. The results indicate that plasma, erythrocytes and plasma peptides perform a function in transporting amino acids in calves. The extent and direction of transport is influenced by tissue bed, blood pool and nutritional state of the animal. The large arteriovenous differences in peptide amino acids across the GIT offer new evidence for peptide absorption into portal blood and for peptide amino acid transport in calves. Further research is needed to define the roles of each blood pool in amino acid transport and to determine the factors influencing peptide transport and hydrolysis in the gut wall.

SERUM PROTEIN AMINO ACID MOVEMENT ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF CALVES

Summary

Amino acid concentration arteriovenous differences (A/V) in four serum protein fractions were quantified across the gastrointestinal tract (GIT) and liver of calves in a "steady state" metabolism and following a 72-hr fast. Six growing Holstein steer calves (136 kg) surgically equipped with cannula in the abdominal aorta, portal vein and hepatic vein were fed a natural diet as 24 hourly meals per day. Serum samples were simultaneously obtained from all cannulae at 9 to 12 d postsurgery and again following a 72-hr fast. Serum was desalted (Sephadex G-25) then separated into four fractions (F-I to F-IV) on a DEAE Sepharose CL-6B column. Proteins were hydrolyzed with 4 N methanesulfonic acid and analyzed for amino acid concentrations. Generally amino acids were added to F-I, -II and -IV of fed animals by the GIT while the albumin fraction supplied amino acids to the GIT. Fasting reversed the direction of response for amino acids in all fractions. A/V differences for amino acids in F-II and -III were opposite in direction across both tissues suggesting a transport role for unidentified proteins in F-II. A variable response of amino acids in the gamma-globulin fraction was observed across the liver of fed calves. Generally the albumin fractions picked up amino acids from the liver while the mixed protein pool supplied amino acids to the liver. Fasting resulted in a release of amino acids from the liver into all fractions. Net splanchnic output or uptake appeared to depend on individual amino

acids in both nutritional states. The magnitude of response of amino acids in the four fractions and the affects of nutritional state indicate a potential role of these serum proteins in interorgan amino acid movement.

Introduction

The animal body maintains a dynamic state of protein metabolism through counterbalancing protein synthesis with degradation. During the life of a cell, essentially all proteins are replaced (Schimke, 1977). Estimates of fractional catabolic and synthetic rates of 3%/d and 3.2%/d, respectively, for muscle protein of growing calves illustrates this extensive and dynamic metabolism (McCarthy, 1981). With rapid growth, muscle protein synthesis greatly exceeds net protein deposition due to turnover (Pencharz et al., 1977). Thus in growing animals the rate of protein synthesis must exceed the rate of protein degradation. Maintenance of this protein turnover requires a continuous supply of amino acids. The blood amino acids available for protein synthesis arise from intestinal absorption, synthesis and tissue degradation (Kawai, 1973). The liver has a fundamental role in the regulation of amino acids entering peripheral circulation. Generally the liver has been reported to remove most free amino acids added to portal blood resulting in no net splanchnic amino acid output (Elwyn et al., 1968; Felig and Wahren, 1971; Wolff et al., 1972; Heitmann and Bergman, 1980b). Thus the question arises as to how amino acids reach the peripheral tissues. Elwyn (1970) suggested that the plasma proteins

may supply amino acids to these tissues. These proteins are synthesized primarily in the liver but degraded elsewhere thus transporting amino acids to these sites. The plasma proteins also have an important role in maintenance of the blood amino acid pool (Kawai, 1973) and are continuously being lost and replaced. Serum albumin is the major protein synthesized by the liver and may account for 52 to 68% of serum total protein (Peters, 1970). Albumin functions in transporting many compounds throughout the body including the amino acid tryptophan (McMenamy and Onely, 1958). Others have also suggested that albumin serves as a "circulating reservoir" of amino acids for use by tissues in times of depletion (Peters, 1970). Dietary protein restriction and short term fasting results in decreases in both albumin synthesis (Yap and Hafkenscheid, 1981) and catabolism (Hoffenberg et al., 1966; Kawai, 1973). Under these conditions, there is also an increased degradation of tissue protein in liver and muscle (Gan and Jeffay, 1967; Millward, 1970; Rothschild et al., 1980). Generally conditions predisposing tissue protein catabolism result in an increased catabolism of plasma proteins (Kawai, 1973) and may affect availability of amino acids to peripheral tissues.

McCormick (1980) recently demonstrated an uptake of amino acids from a highly albumin serum fraction by the hindlimb of growing calves. Following a 72-hr fast, amino acids were released into the albumin and a non-specific globulin fraction. Danilson (1981) reported these two protein fractions to be of considerable importance in supplying amino acids to the hindlimb of urea fed calves. The effects of the splanchnic

bed on the amino acids of these fractions have not been investigated.

The objective of this study was to quantify the movement of amino acids from serum proteins across the gastrointestinal tract and liver of calves in a "steady state" and fasting metabolism.

Experimental Procedure

Animals, feeding, surgical preparation, sampling, calculations and statistical analyses were described in the previous chapter. Serum was harvested from whole blood following centrifugation and was stored frozen (-20 C). Four distinct protein fractions were separated from serum by ion exchange chromatography. Two milliliters of serum were desalted on a 1.5 x 15 cm Sephadex G-25M column[9] by gel filtration. Protein was eluted with 3.5 ml of a pH 5.0 sodium acetate buffer (25 mM/l). Three milliliters of this protein solution were applied to a 2.6 x 9.5 cm DEAE-Sepharose CL-6B[10] ion exchange column for separation with sodium acetate buffers (pH 5.0, I = .025; pH 4.65, I = .025; pH 4.0, I = .1) into three distinct peaks (Curling et al., 1977). Buffer transitions at 95% transmittance resulted in optimum peak resolution (McCormick, 1980). Approximately 3 hr (separation time = 1.5 hr and regeneration time = 1.5 hr) were required for each separation at a maximum pump efficiency of 160 ml/hr. The column eluent was scanned

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[9]Column PD-10, Pharmacia Fine Chemicals, Uppsala, Sweden.
[10]Anion Exchange, Pharmacia Fine Chemicals, Uppsala, Sweden.
[11]Model UA-5 Absorbance Monitor, Instrument Specialities Co., Lincoln, NE.

by a UV-monitor[11] at 280 nm and collected as 7-min fractions into 20 ml graduated tubes via a fraction collector[12]. A typical fractionation of the serum proteins illustrates the three distinct protein peaks (figure 1). The volume of eluent in each of the four tubes containing the fractions being monitored was recorded and each fraction was analyzed for protein content by the method of Lowry et al. (1951) as modified by Bergmeyer (1974). Approximately 500 ug of protein from each fraction was dried under vacuum [13] then hydrolyzed with 100 ul of 4 N methanesulfonic acid for 22 hr at 115 C. Hydrolyzed samples were adjusted to pH 2 with 1 ml of .35 N NaOH, filtered through glass wool and immediantly applied to sample cartridges for amino acid analysis [14]. Arterial and venous samples for each animal within a protein fraction were hydrolyzed simultaneously and analyzed successively. A qualitative assessment of the types of proteins present in each fraction was made by McCormick (1980).

Results and Discussion

Fractions. The fractionation technique employed resulted in the separation of serum proteins into four fractions. A qualitative evaluation of the types of proteins present in each fraction was performed by McCormick (1980) using disc gel electrophoresis. With

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[12]Model 328 Fraction Collector, Instrument Specialities, Co., Lincoln, NE.
[13]Speed Vac-Concentrator, Savant Instruments, Long Island, NY.
[14]Model TSM, Amino acid analyzer, Technicon Instruments, Tarrytown, NY.

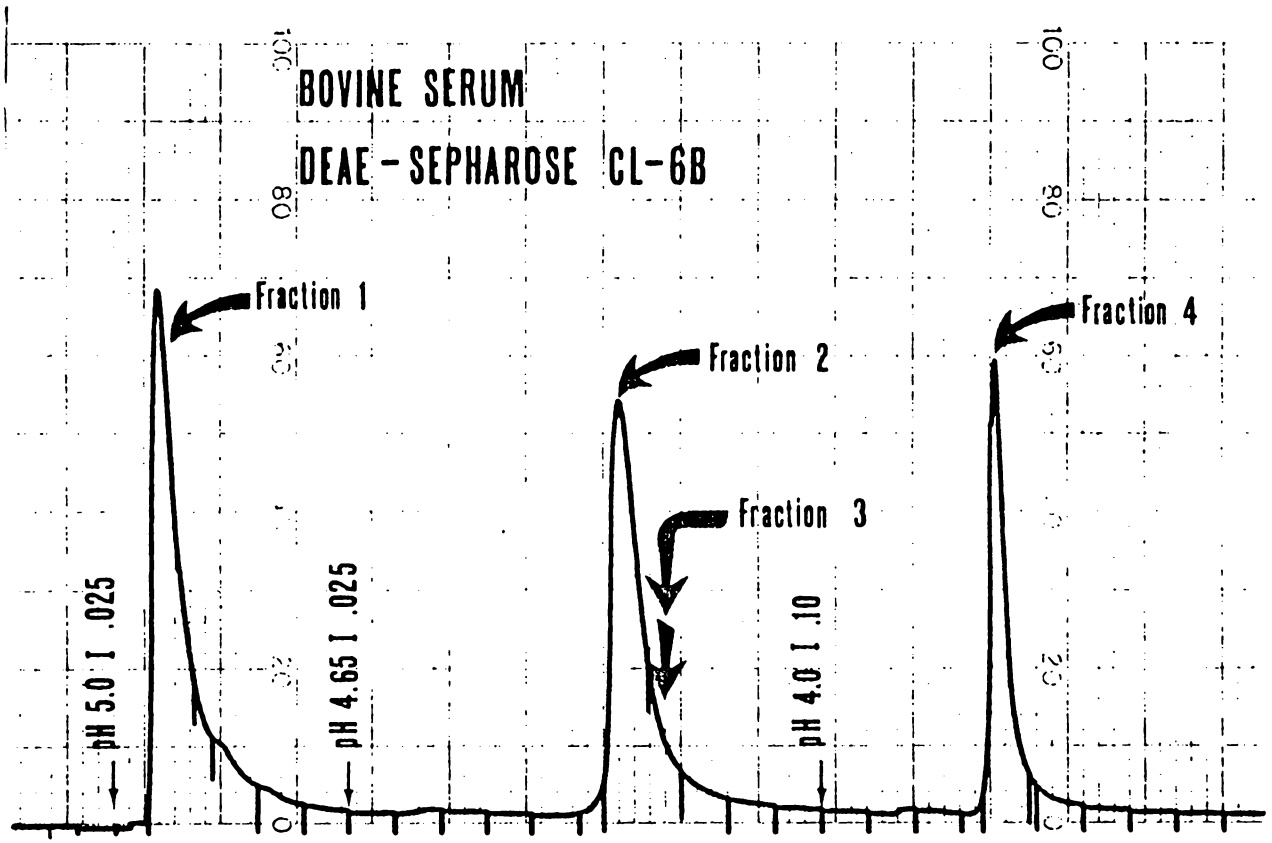

Figure 1. Typical fractionation of serum proteins on DEAE-Sepharose CL-6B with acetate buffers.

albumin, alpha-, beta- and gamma-globulin as standards, fraction I (F-I) was found to contain a large amount of gamma-globulin with some albumin and mixed globulins. Fraction II (F-II) was largely but not totally albumin and fraction III (F-III) was essentially pure albumin. Fraction IV (F-IV) contained a mixture of unidentified proteins.

Arterial concentrations. Concentrations of amino acids in F-I ranged from 13.7 and 16.6 for MET to 155.6 and 184.6 mg/dl serum for GLU in fed and fasted calves, respectively (table 9). Fasting resulted in increases ($P<.10$) in concentrations of ASP, SER, GLU, PRO, GLY ALA, LEU and PHE. Concentrations of others, except ILE tended to increase causing increases ($P<.01$) in total and nonessential amino acids (NEAA) and thus a widening ($P<.02$) of the essential/nonessential (E/NE) ratio.

A similar response occurred for amino acid concentrations in F-II (table 10). Arterial concentrations ranged from 8.4 and 11.8 for MET to 258.0 and 298.7 mg/dl serum for GLU in fed and fasted calves, respectively. Fasting tended to result in an increased concentration of all amino acids ($P<.01$) except ILE and VAL which appeared to be unaffected. An increase in NEAA ($P<.07$) caused a narrowing of the E/NE ratio ($P<.01$).

Arterial concentrations of all amino acids in F-III increased following the 72-hr fast (table 11). As a result, essential (EAA) ($P<.10$), NEAA ($P<.08$) and total ($P<.09$) amino acid concentrations were all increased. The E/NE ratio was also altered ($P<.08$) from 1.01 in fed animals to .99 following fasting.

TABLE 9. PROTEIN FRACTION I AMINO ACID CONCENTRATIONS IN ARTERIAL BLOOD OF FED AND FASTED CALVES

Amino acid	Fed[a]	Fasted[a]	P[b]	EMS[c]
ASP	134.64	153.42	.01	79.7
THR	119.41	121.11	.82	156.2
SER	138.20	164.32	.01	121.6
GLU	155.59	184.64	.01	114.5
PRO	84.62	105.88	.01	15.0
GLY	56.99	62.52	.06	15.5
ALA	56.35	66.97	.01	10.2
VAL	91.53	94.64	.39	32.8
CYS	19.17	20.46	.54	11.6
MET	13.67	16.60	.22	13.0
ILE	30.15	29.61	.62	3.2
LEU	107.24	119.18	.03	48.6
TYR	78.16	82.02	.31	34.6
PHE	45.49	52.54	.01	9.3
LYS	98.12	107.88	.17	112.8
OH-LYS				
HIS	26.06	29.74	.26	25.1
ARG	65.83	71.80	.24	60.7
BCAA	228.92	243.43	.14	202.1
EAA	597.52	643.09	.12	1730.8
NEAA	723.72	840.24	.01	1211.4
TOTAL	1321.24	1483.33	.01	5392.6
E/NE	.82	.77	.02	.001

[a] Mean concentrations are expressed as mg/dl serum and represent six observations each.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.

TABLE 10. PROTEIN FRACTION II AMINO ACID CONCENTRATIONS IN ARTERIAL BLOOD OF FED AND FASTED CALVES

Amino acid	Fed[a]	Fasted[a]	P[b]	EMS[c]
ASP	163.29	184.77	.07	267.7
THR	94.80	99.16	.44	82.8
SER	64.43	78.82	.02	47.6
GLU	257.98	298.72	.03	592.7
PRO	80.19	89.08	.22	121.8
GLY	27.57	30.25	.18	9.0
ALA	79.78	89.73	.11	79.3
VAL	79.19	78.92	.96	83.9
CYS	35.74	37.52	.49	16.9
MET	8.37	11.83	.03	4.1
ILE	34.20	33.68	.80	11.0
LEU	169.97	185.64	.19	321.0
TYR	81.66	87.63	.38	114.8
PHE	94.68	101.81	.30	115.0
LYS	194.60	216.87	.17	574.0
OH-LYS				
HIS	57.54	60.16	.48	34.8
ARG	87.22	96.40	.23	135.5
BCAA	283.36	298.25	.43	917.4
EAA	820.58	884.47	.26	7770.4
NEAA	790.48	896.52	.07	6393.0
TOTAL	1611.24	1780.99	.14	2798.2
E/NE	1.04	.99	.01	.001

[a] Mean concentrations are expressed as mg/dl serum and represent six observations each.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.

TABLE 11. PROTEIN FRACTION III AMINO ACID CONCENTRATIONS IN ARTERIAL BLOOD OF FED AND FASTED CALVES

Amino acid	Fed[a]	Fasted[a]	P[b]	EMS[c]
ASP	23.71	32.77	.08	52.0
THR	13.09	18.12	.06	13.0
SER	10.57	13.95	.13	10.2
GLU	36.63	49.94	.10	126.8
PRO	11.50	16.27	.06	11.6
GLY	4.44	5.91	.07	1.3
ALA	11.78	16.23	.08	12.6
VAL	11.22	14.54	.16	12.5
CYS	4.74	6.86	.01	.6
MET	1.22	1.91	.05	.2
ILE	4.90	6.13	.17	1.7
LEU	24.26	32.96	.08	48.4
TYR	11.68	15.67	.15	16.5
PHE	13.11	18.02	.12	21.3
LYS	28.10	36.49	.14	66.7
OH-LYS				
HIS	7.58	11.29	.05	6.4
ARG	13.22	17.65	.16	21.6
BCAA	40.38	53.63	.11	139.1
EAA	116.70	157.12	.10	1254.2
NEAA	115.05	157.61	.08	1174.2
TOTAL	231.75	314.74	.09	4841.2
E/NE	1.01	.99	.08	.0002

[a] Mean concentrations are expressed as mg/dl serum and represent six observations each.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.

Amino acid concentrations associated with F-IV responded in a manner similar to that observed in the previous three fractions (table 12). Fasting resulted in an increased ($P<.10$) concentration of most amino acids. Consequently, all categories were increased ($P<.01$) including branched chain (BCAA), EAA, NEAA and total amino acids. There was, however, no change in the E/NE ratio.

The increased concentration of amino acids assoicated with the protein of these four fractions as a result of fasting was rather consistent. While changes in total protein as determined by the method of Lowry et al. (1951) were directionally the same as observed with the amino acids, it appears that the changes observed are not simply the result of uniform changes in protein concentration. The relatively different response of some amino acids compared to others (i.e. EAA vs. NEAA) negate this conclusion. Alternately, it might be speculated that the proportions of different proteins composing a fraction might be changing or these proteins are binding and carrying free amino acids or peptide fragments as has been shown to accur with TRP (McMenamy and Onely, 1958).

Arteriovenous differences. Observed fluxes of amino acids from F-I across the GIT in fed animals were about equally divided between addition and removal (table 13). Those differing from zero ($P<.13$) included addition of PRO and removal of THR, VAL, ILE and TYR. Following the fast all amino acids appeared to be removed from the F-I pool except HIS. Particularly large ($P<.05$) uptakes of THR, PRO and the branched chain amino acids were recorded. A variable response of

TABLE 12. PROTEIN FRACTION IV AMINO ACID CONCENTRATIONS IN ARTERIAL BLOOD OF FED AND FASTED CALVES

Amino acid	Fed[a]	Fasted[a]	P[b]	EMS[c]
ASP	63.67	75.89	.01	8.1
THR	38.56	44.58	.01	4.4
SER	37.88	45.34	.01	2.5
GLU	82.11	97.18	.01	16.1
PRO[d]	34.25	41.44	.03	7.0
GLY	18.69	21.77	.01	.9
ALA	26.98	32.30	.01	1.8
VAL	29.32	31.46	.04	1.7
CYS	5.36	6.88	.12	2.1
MET	1.42	1.04	.76	4.2
ILE	18.38	19.09	.22	.8
LEU	61.22	71.15	.01	8.3
TYR	36.90	46.04	.01	3.9
PHE	30.73	37.44	.01	2.0
LYS	61.97	76.18	.17	231.9
OH-LYS	18.24	20.46	.39	16.6
HIS	25.02	35.01	.09	65.4
ARG	32.93	37.28	.02	4.6
BCAA	108.92	121.69	.01	24.5
EAA	299.35	353.22	.01	531.6
NEAA	319.47	380.66	.01	501.1
TOTAL	618.82	733.88	.01	721.4
E/NE	.92	.93	.88	.01

[a] Mean concentrations are expressed as mg/dl serum and represent six observations each.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.
[d] Least square means based on five observations.

TABLE 13. PROTEIN FRACTION I AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Amino acid	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
ASP[g]	-2.67	(.50)	4.96	(.22)	1.58	(.68)	-7.41	(.11)	.32	.86	88.3
THR	7.28	(.13)	9.78	(.05)	-1.23	(.79)	-14.09	(.01)	.01	.28	121.6
SER	-3.50	(.48)	1.30	(.79)	.56	(.91)	-.01	(.99)	.78	.68	137.1
GLU	-1.79	(.84)	9.57	(.28)	-4.20	(.63)	-4.17	(.67)	.37	.52	430.7
PRO[g]	-7.95	(.08)	11.65	(.01)	4.72	(.28)	1.00	(.83)	.82	.09	105.6
GLY	.33	(.83)	1.48	(.35)	1.07	(.50)	-2.16	(.23)	.37	.52	14.1
ALA[g]	-1.88	(.23)	2.56	(.11)	.54	(.72)	-2.49	(.16)	.40	.65	13.3
VAL[g]	4.86	(.06)	9.88	(.01)	1.65	(.50)	-6.88	(.02)	.01	.49	33.9
CYS	.77	(.56)	.84	(.52)	2.14	(.12)	-2.11	(.16)	.55	.14	9.8
MET	-.22	(.87)	1.86	(.25)	1.34	(.40)	-.69	(.70)	.76	.99	14.3
ILE[g]	3.22	(.01)	3.85	(.01)	-.22	(.79)	-2.86	(.01)	.01	.24	3.8
LEU[g]	-.59	(.80)	5.54	(.03)	1.65	(.49)	-5.82	(.04)	.08	.78	32.5
TYR	4.66	(.13)	1.14	(.70)	1.08	(.72)	-2.58	(.45)	.25	.25	51.3
PHE	-.39	(.84)	3.21	(.12)	-2.22	(.27)	-2.63	(.25)	.08	.44	22.9
LYS	-.07	(.99)	8.98	(.18)	-4.39	(.50)	-8.14	(.27)	.12	.69	238.3
OH-LYS											
HIS	-.92	(.81)	-5.24	(.18)	2.23	(.56)	2.82	(.51)	.16	.63	81.6
ARG	2.11	(.50)	1.06	(.73)	-1.38	(.66)	-3.18	(.37)	.24	.65	55.0

[a] Values are means of six observations and represent the concentration difference between arterial and venous samples expressed as mg/dl serum.
Negative values indicate greater concentrations in venous blood.
[b] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in () indicate the probability that the A/V difference does not differ from zero.
[f] Least square mean based on five observations.
[g] Tissue x state interaction (P<.10).

amino acids in F-I was observed across the liver of fed animals. Both removals and additions were observed with the total value for one essentially equalling the value for the other. A/V values different from zero were not detected. Fasting resulted in a release of essentially all amino acids into F-I. THR alone accounted for 22% of the total amino acid release while the branched chain amino acids accounted for another 24%.

Concentration changes of amino acids in F-II (table 14) were similar to those in F-I, however, the direction of response appeared to be less dependent on individual amino acid. Amino acids were generally accumulated by this highly albumin fraction in fed animals as blood crossed the GIT and were removed from this fraction following the fast. GLU and LYS accounted for 20 and 11%, respectively, of the total gut amino acid output in F-II of fed calves while GLU was 16% and LYS 12.5% of the total gut uptake in fasted steers. ASP and LEU were also highly involved in movement across the gut accounting for approximately 20% of the gut output and uptake in fed and fasted calves, respectively. Under both nutritional conditions, amino acids tended to appear in higher quantities in hepatic venous blood than in blood presented to the liver. Exchanges across the liver were generally of smaller magnitude than those across the GIT, however, GLU and LYS continued to show a large but less significant exchange. GLU, LYS and ASP are found in large amounts in albumin (Kawai, 1973).

The direction of response of amino acids across the GIT in F-III

TABLE 14. PROTEIN FRACTION II AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Amino acid[g]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
ASP	-19.26	(.05)	22.05	(.03)	-1.57	(.86)	-6.11	(.55)	.58	.07	475.4
THR	-9.81	(.09)	13.17	(.03)	.40	(.94)	-9.12	(.15)	.29	.24	169.7
SER	-9.44	(.03)	10.44	(.02)	-2.20	(.59)	-1.10	(.81)	.61	.02	95.9
GLU	-31.08	(.04)	35.72	(.02)	-10.46	(.45)	-8.80	(.57)	.41	.03	1102.7
PRO	-6.21	(.41)	8.44	(.27)	.25	(.97)	-1.71	(.84)	.81	.42	321.2
GLY	-2.14	(.18)	3.82	(.02)	-.11	(.94)	-1.74	(.32)	.27	.18	13.5
ALA	-7.54	(.12)	10.48	(.04)	-.88	(.85)	-3.49	(.50)	.45	.12	123.1
VAL	-.15	(.97)	13.74	(.01)	-2.07	(.63)	-4.75	(.33)	.03	.21	103.8
CYS	-2.36	(.37)	5.12	(.07)	-.45	(.86)	-2.75	(.36)	.28	.34	39.8
MET	-1.92	(.25)	-.04	(.98)	-1.16	(.48)	-1.51	(.42)	.83	.65	15.6
ILE	-.75	(.66)	5.50	(.01)	-1.14	(.51)	-2.34	(.23)	.03	.17	16.8
LEU	-15.69	(.10)	24.16	(.02)	-3.27	(.72)	-7.13	(.49)	.32	.07	470.2
TYR	-6.52	(.20)	12.91	(.02)	-1.45	(.77)	-5.08	(.37)	.22	.14	142.5
PHE	-7.56	(.19)	13.77	(.02)	-1.09	(.84)	-5.09	(.42)	.29	.15	179.9
LYS	-17.66	(.10)	28.31	(.01)	-7.37	(.48)	-2.69	(.82)	.34	.03	618.1
OH-LYS											
HIS	-5.90	(.19)	5.66	(.21)	1.23	(.78)	-4.19	(.40)	.77	.50	112.5
ARG	-10.52	(.10)	12.90	(.05)	-.68	(.91)	-6.19	(.37)	.46	.16	208.8

[a] Values are means of six observations and represent the concentration difference between arterial and venous samples expressed as mg/dl serum.
Negative values indicate greater concentrations in venous blood.
[b] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in () indicate the probability that the A/V difference does not differ from zero.
[f] Least square mean based on five observations.
[g] Tissue x state interaction (P<.10) occurred for all amino acids except PRO and MET.

was generally opposite that of those in F-II (table 15) even though both fractions contain large proportions of albumin. All amino acids in F-III were found in higher concentrations in the aorta than in portal venous serum of fed calves. GLU, LYS, ASP and LEU continued to account for a large portion of the amino acid movement. GLU and LYS were responsible for 30% of the gut exchange under both nutritional states while ASP and LEU accounted for an additional 20%. A significant response was recorded for 70% of the amino acids in F-III of fed calves. F-II and F-III amino acids responded similarly across the liver. Hepatic exchanges were generally of less magnitude than those across the GIT and were not different form zero. GLU and LYS A/V differences were large but not statistically different from zero. Previous research illustrates that fasting of rats results in decreased albumin synthesis and degradation rates (Rothschild et al., 1972). These researchers suggested this as a mechanism for conservation of the albumin pool. Plasma albumin levels appear to require several days to weeks to show a decrease which may result from extravascular albumin redistribution. These observations support the lack of decrease in serum albumin levels noted in the present study. A large portion of albumin degradation occurs in gastrointestinal tissue (Kawai, 1973). In this study, the removal of amino acids from the F-III albumin pool ceased following a 72-hr fast which suggests that whole protein catabolism was occurring under fed conditions. The tendancy for continued hepatic output of amino acids into this albumin pool after fasting may support observations of albumin synthesis from degradation of liver proteins in

TABLE 15. PROTEIN FRACTION III AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Amino acid	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
ASP	4.70	(.09)	-4.57	(.10)	-.96	(.72)	-2.13	(.48)	.56	.07	39.9
THR	2.86	(.07)	-1.35	(.36)	-1.02	(.49)	-1.39	(.41)	.21	.15	12.5
SER	1.75	(.12)	-2.58	(.03)	-.12	(.91)	-.88	(.47)	.94	.04	6.7
GLU	7.70	(.08)	-7.60	(.08)	-1.48	(.72)	-3.92	(.41)	.52	.05	99.9
PRO	2.73	(.10)	-.99	(.53)	-.92	(.56)	-2.38	(.19)	.13	.13	14.2
GLY	.86	(.04)	-.62	(.14)	-.22	(.59)	..39	(.40)	.32	.06	.9
ALA	2.48	(.07)	-2.31	(.09)	-.50	(.70)	-1.21	(.41)	.48	.05	9.5
VAL	2.83	(.05)	-.65	(.62)	-.62	(.64)	-1.22	(.42)	.16	.15	10.0
CYS	.80	(.20)	-.84	(.17)	-.31	(.60)	-.02	(.98)	.82	.29	2.1
MET	.08	(.67)	-.29	(.16)	-.32	(.12)	-.23	(.32)	.40	.50	.2
ILE	1.28	(.02)	-.42	(.40)	-.33	(.51)	-.67	(.24)	.08	.06	1.4
LEU	5.17	(.06)	-3.81	(.14)	-1.24	(.62)	-3.04	(.29)	.29	.05	36.7
TYR	2.84	(.08)	-1.16	(.45)	-.72	(.63)	-2.79	(.12)	.11	.07	13.2
PHE	3.09	(.08)	-2.52	(.15)	-.75	(.66)	-2.06	(.29)	.34	.06	16.5
LYS	6.90	(.09)	-4.32	(.27)	-1.80	(.64)	-5.70	(.20)	.21	.07	83.6
OH-LYS											
HIS	1.22	(.48)	-1.24	(.48)	-.60	(.73)	-.68[g]	(.76)	.73	.50	17.0
ARG	2.82	(.14)	-2.74	(.15)	-.55	(.76)	-1.13	(.59)	.64	.12	19.6

[a] Values are means of six observations and represent the concentration difference between arterial and venous samples expressed as mg/dl serum.
Negative values indicate greater concentrations in venous blood.
[b] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in () indicate the probability that the A/V difference does not differ from zero.
[f,g] Least square mean based on five or four observations, respectively.

rats during early starvation (Rothschild et al., 1972).

Amino acids were generally released into F-IV by the gut (table 16). Fasting reversed the direction of response of most amino acids with greater quantities of many amino acids being removed by the GIT than were added in fed calves. The basic amino acids were responsible for 40% of the total uptake by the GIT tissues while the branched chain amino acids accounted for another 17% in fasted calves. The liver tended to remove amino acids presented in the portal blood. Fasting reversed the direction of the response resulting in a tendancy for hepatic output of amino acids. An additional amino acid corresponding to a standard of hydroxylysine was observed in F-IV of all animals. Hydroxylysine, the 5-hydroxy derivative of LYS, is found in collagen and as a structural component of some bacterial cell walls (Lehninger, 1975). Hydroxylysine (OH-LYS) resulting from collagen degradation appears to be totally excreted in the urine by rabbits (Robins, 1977). However, during collagen synthesis terminal polypeptides must be cleaved and may represent the hydroxylysine appearing in F-IV proteins. The concentration of this amino acid in F-IV did not appear to be influenced by either tissue bed or nutritional state.

Considering the four protein fractions in terms of direction of amino acid movement reveals several interesting changes. Generally amino acids were added to F-I, -II and -IV of fed calves while they were removed from F-III as blood traversed the GIT and this response was reversed by fasting. Response across the liver was similar for amino acids in F-I and F-IV and for those in F-II and F-III. Fasting

TABLE 16. PROTEIN FRACTION IV AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Amino acid[j]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
ASP	-4.86	(.17)	4.10	(.24)	3.02	(.38)	-2.81	(.47)	.89	.66	66.4
THR	-.79	(.72)	5.61	(.02)	1.46	(.51)	-2.29	(.36)	.23	.56	28.0
SER	-3.98	(.12)	1.39	(.57)	2.01	(.42)	-2.48	(.38)	.68	.86	35.0
GLU	-5.28	(.23)	5.86	(.18)	4.31	(.32)	-4.04	(.41)	.97	.75	106.0
PRO	-.30[g]	(.92)	2.76[f]	(.31)	-1.40[f]	(.60)	-1.10[g]	(.72)	.37	.58	30.2
GLY	-1.18	(.25)	1.49	(.15)	.94	(.36)	-1.30	(.26)	.74	.83	5.8
ALA	-2.19	(.15)	1.70	(.25)	1.91	(.20)	-1.10	(.51)	.67	.77	12.4
VAL	-.05	(.97)	4.06	(.01)	.94	(.53)	-2.73	(.12)	.07	.88	12.5
CYS	.23	(.75)	1.07	(.15)	-.06	(.94)	-.32	(.69)	.27	.70	3.0
MET	-.76[h]		.65[h]		2 79[i]		.60[i]				
ILE	.24	(.80)	2.55	(.02)	.45	(.65)	-1.59	(.16)	.07	.89	5.5
LEU	-3.44	(.29)	5.12	(.12)	3 62	(.26)	-3.12	(.39)	.86	.78	57.9
TYR	-4.92	(.07)	2.74	(.30)	2.85	(.28)	-2.05	(.48)	.58	.61	38.7
PHE	-2.52	(.25)	2.40	(.28)	2.07	(.34)	-1.53	(.53)	.88	.77	26.8
LYS	5.27	(.64)	12.80	(.27)	.52	(.96)	.43	(.97)	.47	.75	744.9
OH-LYS	-1.75	(.34)	.83	(.65)	-.71	(.70)	-1.75	(.40)	.68	.68	19.2
HIS	1.33	(.79)	10.76	(.05)	1.65	(.74)	-.59	(.92)	.30	.50	148.6
ARG	.03	(.98)	2.32	(.12)	3.49	(.03)	-2.78	(.10)	.58	.20	12.2

[a] Values are means of six observations and represent the concentration difference between arterial and venous samples expressed as mg/dl serum.
Negative values indicate greater concentrations in venous blood.
[b] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in ( ) indicate the probability that the A/V difference does not differ from zero.
[f,g,h,i] Least square mean based on five, four, two or one observations, respectively.
[j] Tissue x state interaction (P<.10) occurred for all amino acids except CYS, MET, LYS, OH-LYS and HIS.

resulted in a hepatic outpouring of amino acids into all fractions. Adibi et al. (1973) reported a 25% reduction in skeletal muscle protein and a 66% decrease in liver protein of rats following an 8-d fast. A slight increase in catabolic rate of liver proteins following a 3-d fast was also observed in rats (Millward, 1970).

Protein exhange. The exchange of total protein across the tissues may aid in understanding amino acid changes (table 17). Protein concentration of F-I was higher in portal venous serum than in arterial serum of fed calves ($P<.06$). This fraction contains large quantities of gamma-globulins (McCormick, 1980) which are synthesized largely in the spleen and gut mucosa (Kawai, 1973). Since the portal vein drains these tissues, the increase in portal concentration of F-I protein may reflect globulin synthesis and explain the output of several amino acids into this pool. The synthesis appears to cease after a 72-hr fast. The presence of other proteins in this fraction may be reflected in the large output of serum protein by the liver after fasting ($P<.01$) indicating hepatic protein synthesis. Although protein changes across the two tissue beds for F-II and F-III were small and nonsignificant, they do illustrate the opposing directions of protein exchange across these tissues. F-II is known to be largely albumin but also contains other proteins while F-III is almost purely albumin. It is apparent from the contradictions that exist in amino acid concentrations, A/V differences and protein exchange between these two pools, that the unidentified proteins in F-II may have a very functional role in amino acid movement. McCormick (1980) also reported opposing responses of

TABLE 17. ARTERIOVENOUS DIFFERENCES FOR LOWRY PROTEIN IN SERUM PROTEIN FRACTIONS ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Fraction	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a]	P[e]	A/V[a,f]	P[e]	P[b]	P[c]	EMS[d]
I[g]	-.88	(.06)	.64	(.16)	.48	(.28)	-1.30	(.01)	.52	.78	1.11
II	-.47	(.54)	.30	(.70)	.10	(.90)	-.37	(.66)	.95	.85	3.34
III	.54	(.30)	-.10	(.84)	-.09	(.87)	-.38	(.51)	.40	.38	1.54
IV[g]	-.27	(.60)	.43	(.42)	1.23	(.03)	-.34	(.57)	.51	.43	1.63

[a] Values are means of six observations and represent the concentration difference between arterial and venous samples expressed as mg/ml serum.
Negative values indicate greater concentrations in venous blood.
[b] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in () indicate the probability that the A/V difference does not differ from zero.
[f] Least square mean based on five observations.
[g] Tissue x state interaction (P<.10).

certain amino acids in F-II and F-III as blood traversed the hindlimb of calves under fed and fasted conditions. It will be necessary to identify these proteins more completely before their role and relative importance in transport can be determined. The direction of total protein response of F-IV paralleled that of amino acid exchange in this fraction. The large positive uptake of protein by the liver of fed calves would indicate a hepatic degradation of proteins in this fraction. The relative importance of a protein fraction in the movement of individual amino acids varied with the individual animals. This difference among animals may explain the lack of significant response observed in many cases. In terms of total amino acid exchange, F-II was quantitatively more important than the other three fractions.

Serum total protein, determined by the method of (Oser, 1965) and serum albumin (Rodkey, 1965) arterial concentrations were increased ($P<.02$) by fasting (Appendix E). Concentration changes across tissue beds were not reflected in serum total protein or serum albumin (Appendix F).

Net splanchnic effects. An additional assessment, net splanchnic output, was employed to illustrate the total effect of the portal drained viscera and liver (splanchnic bed) on amino acid movement and output for transport to peripheral tissues. The effect of the splanchnic bed on amino acids in F-I differed with individual amino acid in fed calves (table 18). A total amino acid output of 21.66 mg/dl was counterbalanced by an uptake of 29.85 mg/dl. The branched chain amino acids accounted for 35% of the total net splanchnic uptake. This

TABLE 18. NET SPLANCHNIC RESPONSE OF SERUM PROTEIN FRACTION I AMINO ACIDS

Amino acid	Fed[a] mg/dl	Fed[a] P[d]	Fasted[a] mg/dl	Fasted[a] P[d]	P[b]	EMS[c]
ASP	-1.08	(.76)	-2.08	(.62)	.85	63.59
THR	6.05	(.39)	-3.39	(.68)	.39	241.95
SER	-2.94	(.28)	.59	(.84)	.39	33.81
GLU	-5.99	(.16)	8.17	(.11)	.06	70.49
PRO	-3.23	(.60)	9.20	(.24)	.23	192.11
GLY	1.40	(.43)	-.29	(.89)	.53	15.37
ALA	-1.34	(.15)	.36	(.70)	.22	3.40
VAL	6.52	(.05)	2.99	(.34)	.39	33.26
CYS	2.91	(.11)	-2.79	(.17)	.06	11.84
MET	1.12	(.65)	1.09	(.71)	.99	32.15
ILE	3.00	(.01)	1.29	(.10)	.10	1.63
LEU	1.06	(.68)	-.21	(.94)	.74	33.43
TYR	5.74	(.30)	-1.47	(.81)	.39	142.78
PHE	-2.62	(.36)	1.68	(.60)	.33	38.06
LYS	-4.46	(.34)	4.33	(.42)	.24	100.69
OH-LYS						
HIS	1.32	(.47)	-3.91	(.12)	.11	16.30
ARG	.73	(.88)	-1.60	(.79)	.77	136.29

[a] Negative values represent a net output from the splanchnic bed. Values represent means of six observations for fed and five observations for fasted.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.
[d] Values in () indicate the probability that the net splanchnic response is different from zero.

uptake reflects the large quantity of amino acids entering the splanchnic region from peripheral tissues. Fasting tended to alter the direction and/or magnitude of exchange of several amino acids especially ILE ($P<.10$), GLU and CYS ($P<.06$). Approximately 60% of the splanchnic uptake following the fast could be attributed to GLU and PRO.

A net splanchnic output of F-II amino acids in fed calves reflected the GIT and hepatic release into this pool (table 19). In this largely albumin fraction, GLU and LYS were responsible for 36% of the net output and ASP and LEU accounted for an additional 21%. A large output of SER ($P<.02$) was also observed. Fasting resulted in a more variable response dependent upon individual amino acids, however, GLU and LYS continued to account for a large portion (53%) of the net uptake reflecting the nonhepatic uptake of these amino acids.

F-III amino acids responded differently than those in F-II (table 20). This albumin pool generally supplied amino acids to the splanchnic region of fed steers. This is in agreement with a large amount of albumin degradation occurring in the GIT (Kawai, 1973). Fasting reversed this response yielding a net splanchnic output of amino acids into F-III. GLU and LYS accounted for approximately 30% of the net splanchnic amino acid exchange in calves in both nutritional states.

Net splanchnic output of amino acids in F-IV of fed calves was generally small and variable (table 21). ARG was the only amino acid showing a net splanchnic response different form zero ($P<.05$). A splanchnic uptake of amino acids from F-IV reflected the large

TABLE 19. NET SPLANCHNIC RESPONSE OF SERUM PROTEIN FRACTION II AMINO ACIDS

Amino acid	Fed[a] mg/dl	Fed[a] P[d]	Fasted[a] mg/dl	Fasted[a] P[d]	P[b]	EMS[c]
ASP	-20.83	(.05)	-1.08	(.91)	.17	353.68
THR	-9.40	(.14)	-3.68	(.57)	.50	154.06
SER	-11.63	(.02)	1.44	(.71)	.05	56.86
GLU	-41.56	(.04)	3.08	(.85)	.10	1084.62
PRO	-5.96	(.46)	.17	(.99)	.62	316.23
GLY	-2.24	(.19)	-.73	(.69)	.53	12.28
ALA	-8.42	(.18)	-1.01	(.88)	.41	162.14
VAL	-2.22	(.74)	2.37	(.77)	.66	237.41
CYS	-2.81	(.54)	-.54	(.92)	.74	106.68
MET	-3.08	(.21)	-1.60	(.55)	.67	25.50
ILE	-1.89	(.40)	.82	(.75)	.44	24.81
LEU	-18.96	(.14)	.72	(.96)	.29	656.84
TYR	-7.97	(.36)	.83	(.93)	.51	364.32
PHE	-8.64	(.38)	1.60	(.88)	.49	465.43
LYS	-25.03	(.13)	6.04	(.72)	.20	1057.83
OH-LYS						
HIS	-4.67	(.16)	-4.37	(.25)	.95	44.49
ARG	-11.20	(.20)	-3.08	(.74)	.52	328.68

[a] Negative values represent a net output from the splanchnic bed. Values represent means of six observations for fed and five observations for fasted.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.
[d] Values in () indicate the probability that the net splanchnic response is different from zero.

TABLE 20. NET SPLANCHNIC RESPONSE OF SERUM PROTEIN FRACTION III AMINO ACIDS

Amino acid	Fed[a]		Fasted[a]			
	mg/dl	P[d]	mg/dl	P[d]	P[b]	EMS[c]
ASP	3.73	(.45)	-7.41	(.23)	.18	119.44
THR	1.84	(.46)	-2.80	(.36)	.26	31.08
SER	1.63	(.46)	-3.90	(.18)	.15	24.17
GLU	6.22	(.44)	-12.68	(.22)	.17	321.93
PRO	1.81	(.41)	-3.17	(.25)	.18	23.53
GLY	.65	(.37)	-1.02	(.25)	.17	2.52
ALA	1.98	(.42)	-3.77	(.22)	.16	28.62
VAL	2.22	(.36)	-1.82	(.51)	.29	27.66
CYS	.48	(.38)	-1.10	(.13)	.11	1.44
MET	-.24	(.01)	-.51	(.01)	.03	.02
ILE	.95	(.32)	-1.14	(.31)	.18	4.19
LEU	3.94	(.41)	-7.27	(.23)	.17	111.02
TYR	2.12	(.46)	-4.07	(.25)	.20	40.23
PHE	2.34	(.47)	-4.96	(.23)	.18	51.67
LYS	5.10	(.29)	-10.44	(.10)	.07	104.62
OH-LYS						
HIS	.62	(.77)	-2.11	(.42)	.43	24.09
ARG	2.27	(.46)	-3.73	(.32)	.24	46.71

[a] Negative values represent a net output from the splanchnic bed. Values represent means of six observations for fed and five observations for fasted.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.
[d] Values in () indicate the probability that the net splanchnic response is different from zero.

TABLE 21. NET SPLANCHNIC RESPONSE OF SERUM PROTEIN FRACTION IV AMINO ACIDS

Amino acid	Fed[a] mg/dl	Fed[a] P[d]	Fasted[a] mg/dl	Fasted[a] P[d]	P[b]	EMS[c]
ASP	-1.84	(.68)	3.51	(.52)	.46	105.47
THR	.67	(.84)	4.70	(.27)	.45	58.18
SER	-1.98	(.45)	.25	(.93)	.57	32.83
GLU	-.96	(.87)	4.18	(.55)	.57	174.04
PRO	-.94	(.74)	3.09	(.38)	.45	41.38
GLY	-.24	(.86)	.86	(.61)	.61	10.23
ALA	-.29	(.88)	1.42	(.52)	.56	18.13
VAL	.89	(.68)	2.40	(.37)	.65	23.99
CYS	.18	(.84)	.61	(.56)	.75	3.97
MET	.31	(.16)	.73	(.03)	.04	.20
ILE	.69	(.57)	1.73	(.26)	.58	7.58
LEU	.18	(.96)	4.20	(.38)	.52	79.37
TYR	-2.07	(.50)	2.11	(.56)	.39	46.39
PHE	-.45	(.86)	2.05	(.50)	.52	32.40
LYS	5.79	(.67)	14.44	(.39)	.68	979.91
OH-LYS	-2.47	(.52)	-1.24	(.78)	.83	72.06
HIS	2.98	(.66)	8.34	(.33)	.62	245.63
ARG	3.52	(.05)	.85	(.60)	.24	9.52

[a] Negative values represent a net output from the splanchnic bed. Values represent means of six observations for fed and five observations for fasted.
[b] Probability that fed and fasted mean differences this large or larger could occur by chance.
[c] Error mean square.
[d] Values in () indicate the probability that the net splanchnic response is different from zero.

nonhepatic uptake as blood traversed the splanchnic bed in fasted calves.

The two albumin fractions monitored in this study appear to be quantitatively most involved in movement of amino acids across the splanchnic region of the calf. The difference observed in direction and magnitude of response of amino acids in these fractions suggests the presence of other proteins in F-II which influence amino acid movement. Although statistical significance was often precluded by animal differences and small number of animals, exchange of amino acids in these four protein fractions was large and often responded differently to nutritional stress indicating a potential role in amino acid movement across tissue beds. The observed changes in the amino acid composition of the protein in these four fractions monitored in the present study of the GIT and liver and in previous studies of the hindlimbs from this laboratory (McCormick, 1980; Danilson, 1981) are supportive of a hypothesis that serum preoteins do contribute to the interorgan movement of amino acids. Whether amino acid exchange in these fractions represented total protein exchange or the attachment and release of amino acids to and from pre-existing serum proteins cannot be determined from the present study. Further isolation and identification of specific proteins in these fractions is essential to our understanding and interpretation of their role in transport of individual amino acids.

EPILOGUE

The concentrations and arteriovenous differences of amino acids in four blood pools have been discussed in terms of transport across the GIT and liver. It is the objective of the following discussion to relate other aspects of this study that are perhaps less significant than those previously discussed but may be of interest in terms of an overall look at amino acid transport.

Blood flow data obtained from a limited number of animals will be related to mean A/V differences in an attempt to gain some knowledge of net amino acid exchange. The data obtained in this study will be related to those measured across the hindlimb by McCormick (1980) in order to integrate transport across the three tissues. Finally, other blood parameters evaluated in the present study will be presented.

NET FREE AMINO ACID EXCHANGE

Movement Across Tissues. Although attempts were made to quantify blood flow across the tissues monitored in this study using the dye-dilution technique, these data were not utilized due to considerable animal variation, lack of repeatable measurements and failure to acquire this information for all animals due to cannula malfunction. Problems associated with this technique are discussed in appendix B. Idealy, one would calculate the net exchange of these amino acids in terms of grams transported per day across these tissues by multiplying the concentration difference across the tissue by the blood flow rate through that tissue. This should most accurately be accomplished within each animal, i.e. using the blood flow rate for each animal

paired with concentration differences recorded for that animal. Due to the problems encountered in this study, a mean blood flow rate may be applied to mean A/V differences to gain an indication of the net exchange of amino acids per day across these tissues. It is necessary to remember that these are not exact calculations but are based on mean values. The net exchange of plasma and erythrocyte free amino acids and the total free amino acid exchange has been calculated for both tissue beds (table 22). The blood flow rates used to compute these figures were 6.99, 4.70 and 9.74 liters/min for fed calves across the GIT, fasted calves across the GIT and across the liver, respectively. These estimates are based on three observations for the GIT of fed calves, five observations for the GIT of fasted calves and six observations for the liver. Values obtained through these calculations reflect the trends in A/V differences already discussed. A large absorption of amino acids into portal blood was observed in fed calves. This absorption provided 353 g/d via the plasma free amino acid pool. ALA was responsible for a large proportion of this uptake. These absorbed amino acids may result from microbial protein, endogenous proteins and dietary bypass protein. GLU and GLN were the only amino acids removed from the plasma pool as blood traversed the GIT.

The erythrocyte free amino acid pool served as a source of amino acids for the gut supplying 220 g/d. Even though the erythrocyte contributed amino acids to the GIT while the plasma carried absorbed amino acids to the liver, the net free amino acid exchange in blood favored a net transport of most amino acids to the liver. A net of 189

TABLE 22. NET 24-HR FREE AMINO ACID EXCHANGE ACROSS TISSUE BEDS OF CALVES

Amino acid	Gastrointestinal tract						Liver[c]					
	Fed[a]			Fasted[b]			Fed			Fasted		
	Plasma	RBC	Whole blood[d]	Plasma	RBC	Whole blood[d]	Plasma	RBC	Whole blood[d]	Plasma	RBC	Whole blood[d]
	g/d[e]											
ASP	-7.0	1.3	-5.7	-2.5	.8	-1.7	2.8	-12.3	-9.5	8.0	-15.7	-7.7
THR	-15.1	9.8	-5.3	-1.7	-1.8	-3.5	2.2	-9.5	-7.3	19.7	-4.7	15.0
SER	-20.6	12.2	-8.4	-4.7	1.8	-2.9	10.2	-8.5	1.7	27.7	-6.6	21.1
ASN	-24.6		-24.6	-4.8		-4.8	5.7		5.7	18.9		18.9
GLU	9.9	28.8	38.7	10.8	-2.2	8.6	-65.6	16.9	-48.7	-19.6	15.2	-4.4
GLN	16.2		16.2	17.6		17.6	-2.5		-2.5	-1.6		-1.6
GLY	-27.9	13.7	-14.2	-9.5	32.6	23.1	22.1	-13.2	8.9	55.1	-16.6	38.5
ALA	-48.4	47.6	-.8	-8.0	12.1	4.1	24.7	-36.8	-12.1	64.7	24.3	89.0
CIT	-7.8		-7.8	-3.6		-3.6	2.2		2.2	.5		.5
VAL	-23.1	10.8	-12.3	-2.8	7.3	4.5	1.6	-4.3	-2.7	13.1	-3.8	9.3
CYS	-1.7		-1.7	-.3		-.3	1.7		1.7	2.5		2.5
MET	-4.4	1.8	-2.6	-1.0	.5	-.5	2.1	-6.9	-4.8	7.3	-2.5	4.8
ILE	-21.1	7.6	-13.5	-2.1	2.8	.7	-3.3	-2.2	-5.5	13.6	-1.1	12.5
LEU	-33.5	15.8	-17.7	-4.4	5.9	1.5	-1.8	-10.5	-12.3	19.1	-2.9	16.2
TYR	-22.2	9.5	-12.7	-3.9	3.6	-.3	14.2	-12.2	2.0	21.3	-9.6	11.7
PHE	-21.9	5.3	-16.6	-4.1	2.4	1.7	13.4	-6.7	6.7	17.8	-8.8	9.0
ORN	-10.1	24.0	13.9	.4	9.9	10.3	-2.6	6.3	3.7	-13.0	-22.2	-35.2
LYS	-30.6	22.8	-7.8	-12.4	-6.4	-18.8	1.4	-27.0	-25.6	31.3	14.3	45.6
HIS	-12.5	9.4	-3.1	9.4	-9.0	.4	-4.8	-13.7	-18.5	-3.9	37.0	33.1
ARG	-20.7		-20.7	-17.4		-17.4	16.8		16.8	30.1		30.1

[a] Based on an average blood flow from three calves of 6.99 liters/min.
[b] Based on an average blood flow from five calves of 4.70 liters/min.
[c] Based on an average blood flow from six sampling of 9.74 liters/min.
[d] Sum of plasma and erythrocyte net exchanges.
[e] Negative values represent greater concentrations in venous blood.

g/d of free amino acids was available when the two pools were combined. The data further illustrate the problems of monitoring amino acid exchange in only one free amino acid pool. Plasma free amino acid exchange overestimated the net exchange of amino acids in this study.

Fasting of animals for 72-hr considerably reduced the magnitude of amino acid uptake from the GIT into the plasma pool. The magnitude of red blood cell amino acid contribution to the GIT was also reduced. Although most amino acids continued to be removed from this fraction, fasting tended to reverse this response for THR, GLU, LYS and HIS. ALA and GLY were supplied in larger quantities by this pool than other amino acids. Total free amino acid exchange in fasted calves was more dependent upon individual amino acids. A net uptake of 72 g/d was counterbalanced by a net output of 53 g/d.

The plasma pool generally supplied amino acids to the liver of fed calves with the exception of a tendancy for outputs of GLN, ILE, LEU, ORN, HIS and an extremely large output of GLU (65 g/d). The liver tended to release amino acids into the erythrocyte pool with the exceptions of GLU and ORN. ALA was transported in large quantities by the red blood cells. The total free amino acid exchange favored a release of amino acids from the liver. Fasting had little effect on the direction of amino acid response across the liver, however, the magnitude of the net exchange was increased. The liver supplied 406 g/d of free amino acids.

These data may also be expressed as net splanchnic output or uptake to indicate the availability of amino acids to peripheral tissues

(table 23). The splanchnic region supplied 430 g/d of plasma amino acids to peripheral circulation. No net splanchnic output of these amino acids has generally been observed in other species. GLN was the only amino acid taken up by the splanchnic bed. The red blood cell supplied amino acids to the splanchnic region with the exception of ASP, MET, LYS and HIS. Total net amino acid exchange favored a net splanchnic output of 313 g/d. Twenty percent of this exchange was in the form of LYS due to an output into both free amino acid pools. Fasting reversed this response resulting in a net uptake of free amino acids from blood. Extremely large uptakes of GLY and ALA may reflect the increased use of these amino acids for energy.

Quantitatively, the plasma free amino acid A/V differences were larger than those for the erythrocyte free amino acids. However, the direction of response of amino acids in these two pools was frequently opposite and in some cases resulted in a difference in direction for plasma and whole blood amino acid exchange. These results clearly indicate a role in amino acid transport for these two pools which cannot be accounted for by monitoring either alone. Although this discussion reflects the A/V differences proviously reported, these data give an estimate of quantities transported per day.

Cycles of free amino acid exchange. Two of the major researchers in the area of interorgan amino acid transport, Bergman and Cahill, have offered diagramatic representations of amino acid exchange across tissues of sheep and man (Bergman and Heitmann, 1980; Cahill et al., 1981). Such cycles have not been described for

TABLE 23. NET 24-HR FREE AMINO ACID EXCHANGE ACROSS THE SPLANCHNIC BED OF CALVES

Amino acid	Fed[a]			Fasted[a]		
	Plasma	RBC	Whole blood[b]	Plasma	RBC	Whole blood[b]
	g/d[c]					
ASP	-6.7	-10.6	-17.3	1.3	-13.0	-11.8
THR	-15.2	6.5	-8.7	17.2	-8.7	8.5
SER	-18.6	10.6	8.0	13.1	-2.1	11.0
ASN	-28.7		-28.7	-1.7		-1.7
GLU	-51.8	45.1	-6.7	11.1	-1.2	9.9
GLN	20.3		20.3	24.4		24.4
GLY	-16.7	4.1	-12.6	27.7	66.2	94.0
ALA	-42.7	29.6	-13.1	41.1	49.6	90.6
CIT	-8.6		-8.6	-9.1		-9.1
VAL	-30.5	20.0	-10.5	2.0	11.2	13.2
CYS	-.7		-.7	1.4		1.4
MET	-3.8	-4.6	-8.4	4.0	-.4	3.6
ILE	-32.7	4.2	-28.5	6.2	8.4	14.6
LEU	-48.5	11.6	-36.9	7.3	11.4	18.7
TYR	-16.5	1.0	-15.5	11.4	-1.3	10.1
PHE	-17.1	4.4	-12.7	8.6	.2	8.8
ORN	-16.5	43.1	26.6	-7.8	-11.8	-19.6
LYS	-41.2	-28.2	-69.4	-3.3	8.8	5.5
HIS	-22.2	-.6	-22.8	14.8	45.6	60.4
ARG	-12.0		-12.0	13.7		13.7

[a] Based on an average blood flow from three calves of 6.99 liters/min
[b] Sum of plasma and erythrocyte net exchanges.
[c] Negative values represent a net output from the splanchnic bed.

cattle or any growing ruminants other than those eludited to by McCormick (1980) and Danilson (1981).

The results of this study along with the data obtained by McCormick (1980) using similar animals and a similar dietary regiment may give an indication of amino acid movements among three major tissues (muscle, GIT and liver). The movement of amino acids across the kidney has not been evaluated in cattle. A diagramatic representation of plasma free amino acid cycles indicates the amino acids which responded differently than the majority or to a greater magnitude (figure 2). Under conditions of continuous feeding, amino acids are absorbed from the GIT and presented to the portal blood for transport to the liver. ALA generally constitutes a considerable proportion of this uptake. GLU and GLN are the only free amino acids removed from plasma. The liver removes a portion (approximately 30%) of the amino acids presented to it while the remainder represents a net splanchnic output to peripheral tissues. A large portion of the output is attributable to GLU, ALA and the branched chain amino acids. Exceptions include nonsignificant responses for GLN, CYS, ORN and ARG. These amino acids are presented to and removed by the hindlimb (used as an indicator of muscle mass). GLN is the only amino acid subsequently released by the hindlimb. The GLU-GLN cycle appears to be extremely functional in these animals. GLU taken up by the muscle may provide a nontoxic transport medium for the transfer of ammonia to the liver for conversion to urea. The enzyme GLN synthetase catalyzes such a reaction in the liver (White et al., 1973) and may be responsible

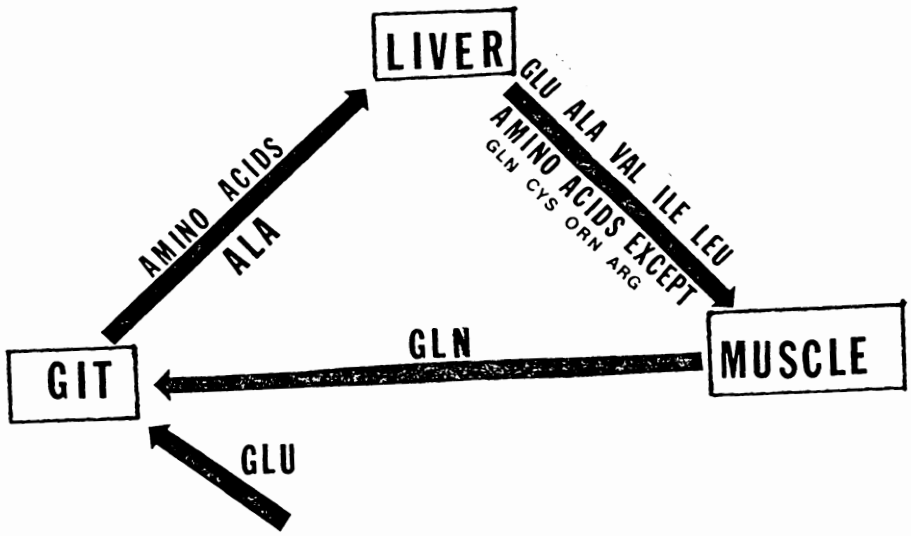

Figure 2. Cycles of plasma free amino acid exchange in calves in a steady state metabolism. Amino acids showing major changes are indicated in bold letters.

for this conversion in the muscle as well. The lack of ALA output by the hindlimb suggests that the ALA cycle is not a major route of ammonia transport. The cycle has been reported to be highly functional as a source of glucose when the supply is limited and demand is high (Pike and Brown, 1975). Perhaps under the feeding conditions of this study, the cycle was less functional because conversion of ALA to pyruvate and finally to glucose was not in demand for energy. Nutritional stress may be expected to reverse this response.

GLU and GLN were also metabolized by the GIT. Both were removed from arterial blood. As previously discussed GLN may be used for energy or the synthesis of other amino acids such as PRO and CIT. GLU may undergo transamination and result in the production of ALA. This may serve to explain the large uptake of this amino acid into portal circulation. Many of the events of this cycle have been eluded to by others studying different species. These were discussed in the first chapter. Differing from previous reports is the net splanchnic output of most amino acids and the large output of GLN and lack of ALA output by muscle. Another interesting aspect is the general lack of response of urea cycle amino acids. An uptake of ARG and release of CIT and ORN across the liver was not observed. Given the cyclic nature of this system and the feeding situation of the present study perhaps the quantities of these amino acids exchanged across the liver were too low to be accurately monitored.

A similar cycle illustrates the response of plasma free amino acids following a 72-hr fast (figure 3). This cycle is similar to that reported

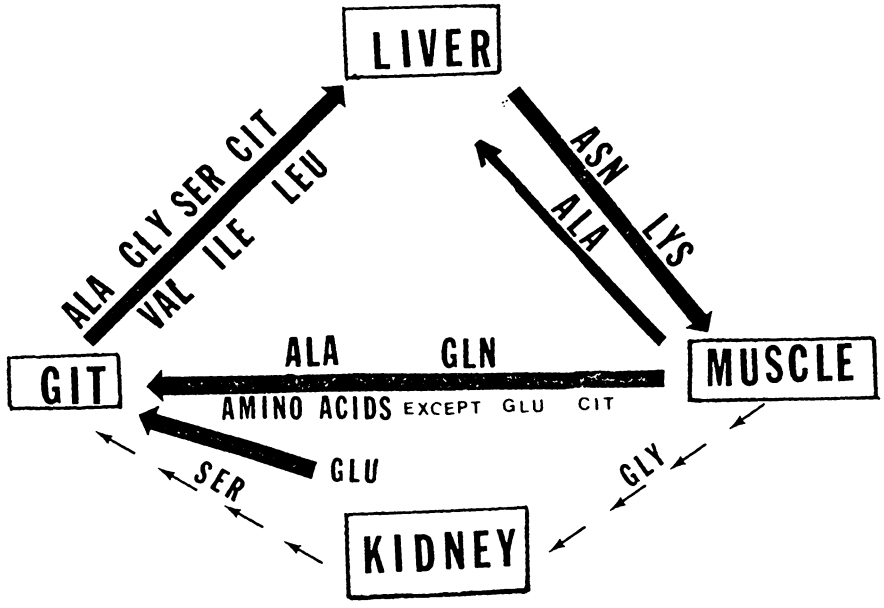

Figure 3. Net plasma free amino acid exchange in calves following a 72-hr fast. Amino acids showing major changes are indicated in bold letters. Pathways indicated by dashed lines are speculative.

by Cahill et al. (1981). Following fasting, amino acids continued to be absorbed into portal plasma. Large quantities of ALA, GLY, SER, CIT and the branched chain amino acids were presented to the liver. A net splanchnic output of amino acids was generally not observed with the exception of ASN and LYS. Cahill et al. (1981) reported a substantial uptake of ALA by the liver and a large net output of GLU in postabsorptive man. In the study by McCormick (1980), the muscle removed GLU and CIT from the plasma while releasing all other amino acids into this pool. This is in agreement with the research in humans. GLU and GLN were removed by the GIT. Cahill et al. (1981) reported that GLY is converted to SER in the kidney. This may have occurred in the present study as well. The GLU-GLN cycle appeared to continue to function following fasting, however, the role of ALA transport of nitrogen and carbon appeared to be enhanced. A considerable release of ALA by the hindlimb and uptake by the liver was observed. These data may reflect increased muscle protein catabolism and increased use of amino acids for glucose production under fasting conditions. The urea cycle amino acids showed little response across the liver. The response of plasma free amino acids did not reflect the entire response of free amino acids in blood since amino acids in the erythrocyte pool generally responded opposingly in terms of A/V differences. It will be difficult to describe such cycles for erythrocyte free amino acids since responses are generally more variable, but generally it can be said that the plasma and erythrocyte have opposing roles in transport of amino acids across the splanchnic bed. The same response was not observed

in the hindlimb research of McCormick (1980) but was reported for hindlimb exchange in calves fed soy protein or urea by Danilson (1981). Factors regulating the response of these two pools appear to be affected by nutritional status and perhaps by species, age of the animal and possibly time after surgery. Once transport mechanisms for amino acid movement into the red cell are elucidated, their role in interorgan transport of amino acids may be better understood.

RESPONSE OF OTHER BLOOD PARAMETERS

Concentrations of blood urea nitrogen (BUN) and blood ammonia nitrogen in arterial blood were increased ($P<.05$) following a 72-hr fast (table 24). Arterial concentrations of blood glutathione were unaffected by fasting. Expressed as arteriovenous differences, a significant A/V difference for BUN was not observed with the exception of an uptake ($P<.04$) of urea nitrogen from the GIT following fasting (table 25). Blood ammonia nitrogen increased as blood traversed the GIT under both nutritional states and was removed by the liver. Liver removal increased with fasting. Blood glutathione concentrations were not significantly altered by the splanchnic region or nutritional status.

BOUND AMINO ACID EXCHANGE

Peptide amino acid exchange was responsible for a greater addition of amino acids to portal circulation than were the exchanges of free amino acids. Peptide amino acid A/V differences across the GIT of fed calves expressed as net absorption demonstrated an uptake of 813 g/d as peptide amino acids (table 26). The magnitude of this response

TABLE 24. ARTERIAL CONCENTRATIONS OF BLOOD PARAMETERS IN FED AND FASTED CALVES

Item	Fed	Fasted	P[a]	EMS[b]
Blood urea nitrogen, mg/dl[c]	10.36	19.29	.03	18.75
Blood ammonia nitrogen, mg/dl[c]	2.33	2.82	.05	.09
Blood glutathione mg/dl RBC	89.55	83.73	.53	220.84

[a] Probability that fed and fasted mean differences this large or larger could occur by chance.
[b] Error mean square.
[c] Least square means based on five observations.

TABLE 25. ARTERIOVENOUS DIFFERENCES OF BLOOD PARAMETERS MONITORED ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Item	A/V[a]	P[a]	A/V[a]	P[a]	A/V[a]	P[a]	A/V[a,f]	P[a]	P[b]	P[c]	EMS[d]
Blood urea nitrogen, mg/dl[h]	-.26[f]	(.64)	-1.28[f]	(.04)	-.31[f]	(.58)	.78[g]	(.24)	.11	.95	1.51
Blood ammonia nitrogen, mg/dl	-.30[f]	(.05)	-.26[f]	(.08)	.11[f]	(.43)	.45[g]	(.01)	.01	.21	.09
Blood glutathione, mg/dl RBC	-1.71	(.72)	4.30	(.38)	-4.38	(.37)	6.36	(.25)	.95	.11	133.83

[a] Values are means of six observations and represent the concentration difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[b] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[c] Probability that fed and fasted mean differences this large or larger could occur by chance.
[d] Error mean square.
[e] Values in () indicate the probability that the A/V difference does not differ from zero.
[f,g] Least square mean based on five or four observations, respectively.
[h] Tissue x state interaction (P<.10).

TABLE 26. NET 24-HR PEPTIDE AMINO ACID EXCHANGE

Amino acid	Gastrointestinal tract		Liver[c]		Net splanchnic output[c]	
	Fed[a]	Fasted[b]	Fed	Fasted	Fed	Fasted
	g/d[d]					
ASP	-86.2	-40.7	34.3	56.3	-85.8	-66.8
THR	-58.2	-32.8	29.2	35.7	-52.1	-61.8
SER	-67.8	-49.8	21.5	40.5	-73.0	-79.2
GLU	-86.1	-25.2	31.9	44.7	-87.8	-71.7
GLY	-60.8	-58.7	9.4	-12.0	-75.3	-119.6
ALA	-31.8	-16.9	9.7	20.2	-34.5	-35.8
VAL	-42.7	-19.2	18.4	17.1	-41.2	-29.5
MET	-5.8	-2.4	2.1	-4.4	-6.1	-14.6
ILE	-38.6	-12.6	20.2	.6	-33.6	-33.9
LEU	-85.3	-34.8	45.2	37.8	-73.7	-76.8
TYR	-31.0	-21.2	10.7	9.4	-32.5	-52.3
PHE	-62.1	-30.8	36.1	26.4	-50.7	-67.6
LYS	-102.4	-25.4	45.2	33.6	-97.5	-55.9
HIS	-2.8	-18.4	-12.4	25.4	-16.5	-21.1
ARG	-51.5	-21.6	11.7	4.9	-59.8	-38.6

[a] Based on an average blood flow from three calves of 6.99 liters/min.
[b] Based on an average blood flow from five calves of 4.70 liters/min.
[c] Based on an average blood flow from six samplings of 9.74 liters/min.
[d] Negative values represent greater concentrations in venous blood.

was reduced by approximately 50% following a 72-hr fast. The peptide pool served as a source of amino acids for the liver providing 326 g/d in fed calves and 353 g/d in those fasted. Although the liver appeared to remove approximately 40% of peptide amino acids present in portal blood of fed calves, a large portion of these passed through the liver and were released into peripheral circulation. The liver appeared to utilize a greater proportion (approximately 85%) of the peptide amino acids in portal blood following a 72-hr fast. A considerable splanchnic output of these amino acids was observed under both nutritional states. Approximately 825 g/d of peptide amino acids were made available to peripheral tissues. Whether or not these peptides were absorbed intact is not known. The considerable size of this pool may indicate that other sources of these peptides such as synthesis in the gut wall, release as fragments from protein synthesis or active intestinal peptides may be contributing to this pool. Regardless of the source, this pool has a tremendous potential in terms of a circulating pool of amino acids and does not appear to be restricted after fasting in terms of output of amino acids to peripheral tissues. McCormick and Webb (1982) and Danilson (1981) reported that this pool was extremely important in supplying amino acids to the hindlimbs of calves.

It will be interesting to determine the size of these peptides and what proportion if any of those observed in portal blood is actually absorbed intact. This study along with those of McCormick (1980) and Danilson (1981) are the only research, to my knowledge, to report these very large amounts of peptides in circulation. This may be due

to the fact that we have monitored A/V differences as opposed to looking at only concentrations. Further we have hydrolyzed these peptide fractions and reported individual constituent amino acid exchanges. Perhaps this has enabled us to quantify small quantities of peptides which may be present in circulation at any one time.

Changes observed in the serum protein fraction amino acids are more difficult to explain. The large animal variability and lack of knowledge of all of the proteins present in each fraction contribute to this dilemma. It is apparent that amino acids responded differently in each fraction and to nutritional stress. Response of amino acids in the fractions appeared to be more dependent upon individual amino acid than did exchanges in other fractions. These fractions did supply some amino acids to peripheral tissues. This was especially true of the albumin fractions. This is in keeping with the role of albumin as a transport molecule. McCormick (1980) and Danilson (1981) reported that these albumin fractions were an important supplier of amino acids to the hindlimb of growing calves.

SUMMARY AND CONCLUSIONS

Concentrations and arteriovenous (A/V) differences of plasma free, erythrocyte free, plasma peptide and serum protein amino acids were monitored across the gastrointestinal (GIT) tract and liver of growing holstein steer calves fed a natural diet and following a 72-hr fast. Animals were cannulated in the abdominal aorta, portal vein and hepatic vein and were maintained under constant light. A steady state metabolism was maintained by hourly feeding. The concentration of free amino acids in plasma increased as blood traversed the GIT in both fed and fasted calves but the magnitude of release was reduced by fasting. GLU and GLN were the only amino acids removed by the GIT from plasma. ALA accounted for the greatest quantity (19%) of amino acids absorbed. This represented an uptake of 48 g/d of this amino acid. The liver removed a large portion of amino acids presented to it in portal circulation but a net splanchnic output of plasma free amino acids was observed.

The erythrocyte supplied amino acids to the GIT. Although a small hepatic output of amino acids was observed, no net splanchnic output of amino acids in the erythrocyte pool was observed with the exception of ASP in fed calves and an uptake of GLY in fasted calves.

Peptide amino acids increased with both nutritional states as the GIT was crossed in quantities averaging threefold greater than those in the plasma pool. Responses generally paralleled those of plasma free amino acids in direction. A large net output of peptide amino acids

from the splanchnic bed was observed in fed and fasted calves. Quantitatively, peptides appeared to be highly involved in interorgan transport of amino acids supplying as much as 800 g/d to peripheral circulation.

Amino acids were generally added to serum protein fractions I, II and IV as blood traversed the GIT of fed calves and were removed from fraction III. Following a 72-hr fast the direction of A/V response was reversed. The responses of amino acids in fractions I and IV and II and III were similar across the liver. Fasting resulted in an hepatic output of amino acids into these four fractions.

Although these data support the concept of interorgan amino acid transport by each of the pools examined, several factors appear to influence the relative importance of each. Several conclusions may be drawn from this study.

1. Plasma, erythrocytes, plasma peptides and serum proteins perform a function as carriers of amino acids across the gastrointestinal system and liver of growing calves.
2. The extent and direction of this transport varies with tissue, blood pool, nutritional status and individual amino acid.
3. The plasma peptide and plasma free pools appear to be quantitatively more important than the erythrocyte pool.
4. Large arteriovenous differences across the GIT for

the peptide pool suggests that peptide absorption and peptide amino acid transport may occur in the calf.

5. The magnitude of response of amino acids in the four serum protein fractions and the affects of nutritional state indicate a potential role of these serum proteins in interorgan amino acid movement.

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Appendix A

CANNULA MATERIALS AND PREPARATION

MATERIALS

Aorta Cannulae (femoral artery)
45 cm of 2.5 mm Teflon[1] tubing (#15)
80 cm of 1.57 x 3.18 mm Silastic[2] tubing (602-285)

Portal Vein Cannulae
10 cm of 2.8 mm Teflon tubing (#14)
52.5 cm of 1.57 x 3.18 mm Silastic tubing (602-285)
-or-
60 cm of 1.6 x 3.1 mm Tygon[3] tubing

Hepatic Vein Cannulae
45 cm of 2.2 mm Teflon tubing (#17)
60 cm of 1.57 x 3.18 mm Silastic tubing (602-285)
-or-
75 cm of 1.6 x 3.1 mm Tygon tubing

Mesenteric Artery Cannulae
20 cm of 1.8 mm Teflon tubing
60 cm of 1.6 x 3.1 mm Tygon tubing

PREPARATION

Generally all cannulae were composed of Teflon tubing inside the artery or vein attached to a length of silastic tubing for passage under the skin and exteriorization.

STEP 1

All Teflon was evenly cut using a razor or scalpel blade and both ends were beveled, polished and buffed until completely smooth and no

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[1]TFE tubing, Kaufman Glass Co., Wilmington, DE.
[2]Silastic Medical Grade Tubing, Dow Corning Corp., Midland, MI.
[3]TYGON Flexible Plastic Tubing. Norton Plastics and Synthetics Division, Akron, OH.

irregularities could be seen under 5x magnification. This procedure was used in an attempt to reduce damage to the vessel wall which could result in sheath formation around the free end of the cannulae.

STEP 2

Silastic and Tygon tubings were also cut evenly in an attempt to reduce surface irregularities. Leur stub adapters (15 gauge) were placed in the ends of tubing which would be exteriorized.

STEP 3

When Tygon tubing was used as the only cannula material for either the portal or hepatic vein it was necessary to mark the tubing with a waterproof marking pen at the point where the cannulae would exit the vein. It was found, through postmortem examination, that 12.5 cm of tubing could be left inside the liver and still maintain the free end of the cannula in the hepatic vein. Therefore a mark was made 12.5 cm from the end of the tubing. For portal tygon cannulae a mark was made 7.5 cm from the free end.

STEP 4

Cannulae were washed in non-ionic soap in a sonic bath overnight then rinsed in deionized water. Subsequently, cannulae were handled only with gloves. All cannulae materials were then treated in a heparin complex solution {2% (wt/wt) in 1:1 toluene-petroleum ether}[4]. Teflon tubing was refluxed in the complex for 3 hr. Silastic and tygon tubings were soaked, inside and outside, in the complex for 60 sec.

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[4]TDMAC-Heparin Complex Coating, Polysciences, Inc.

All cannulae were then placed in a 50 C oven overnight or until all traces of solvent were removed.

STEP 5

Cannulae were assembled by fitting the silastic over the end of the teflon to a distance of 2.5 cm. The cannulae were tied together with cotton suture at this point. A 2 cm^2 piece of polyvinyl sponge[5] was secured with surgical silk around each cannulae at the point where it exited the vessel. Sponge attached to silastic or tygon tubing was first coated with silastic adhesive[6] then secured carefully with surgical silk to prevent occulsion of the opening. Portal and hepatic cannulae were also fitted with a .5 cm sleeve of 1.57 x 3.18 mm silastic tubing containing a length of single arm surgical silk. This sleeve was placed adjacent to the sponge on the portion of the cannulae remaining outside the vein.

STEP 6

All cannulae were autoclaved prior to use and handled with sterile surgical gloves thereafter.

COMMENTS

Several cannulae preparations were tried throughout the study. Initial portal cannulations were achieved using either a hydrolic needle attached to the vessel or cannulae consisting entirely of silastic tubing. Clotting became a problem with the hydrolic needle since blood tended

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[5]Ivalon sponge. Unipoint Industries, Inc., High Point, NC.
[6]Silastic Medical Adhesive, Silicone Type A. Dow Corning Corp., Midland, MI.

to collect at the sampling port. McGilliard (1972) had reported that silastic was well suited for venous cannulation because of its elasticity, flexibility and inertness, however, in this study it was not rigid enough to maintain patency over several days. It was found that cannulae composed of either teflon or tygon inside the portal vein remained functional longer that other preparations. One cannula of each type was generally implanted in each animal to increase patency of the preparation.

Numerous preparations were tested in the liver as well. A frequent problem was the ability to infuse but not draw blood from this cannulae possibly indicating that the tip of the cannulae was against the vessel wall. Cannulae were prepared with an angled tip and also with several small holes along either side of the tip. These proved to be unsuccessful due to clotting around the tips and fibrosis. Both teflon and tygon appear to be more suitable for hepatic venous cannulation.

Postmortem examination of several implanted calves revealed that all cannulae were properly located with the exception of one hepatic cannulae which had been extended into the vena cava. Samples drawn from this cannulae were not analyzed in this study. Malfunction of several cannulae over the 1-yr sampling period resulted in the loss of several animals from the study. Partial sets of samples were obtained from several calves and were prepared and stored for analysis. Only those animals from which a complete set of samples (all three cannulae in both fed and fasted state) was obtained were used in this study.

APPENDIX B

BLOOD FLOW DETERMINATION

During each sampling period each animal was infused with para-aminohippuric acid (PAH) for determination of blood flow across the GIT and liver. The techniques used was based on the indicator-dilution technique developed for measurement of portal and renal blood flow in dogs and sheep by Katz and Bergman (1969). A 1% solution of PAH in .9% saline was infused into the mesenteric cannula via a screw-driven syringe constant infusion pump[1] at a rate of 6.72 ml/min for 1 hr prior to and during sampling. A priming dose of 10% PAH in .9% saline (8 mg/kg body weight) was injected into the mesenteric artery at the initiation of the infusion. Heparinized blood samples were drawn prior to infusion from the aorta, hepatic vein and portal vein for background determination. These samples along with blood drawn at the midpoint of a 1-hr feeding interval were immediantly deproteinized with 10% trichloroacetic acid (TCA) in a ratio of 3 ml whole blood to 33 ml of TCA. Solutions were filtered through #42 filter paper and placed in a 90 C water bath for 30 min with a marble atop each tube. PAH concentrations were determined by the method of Bratton and Marshall (1939) modified by Smith et al. (1945). The following formulas were used to calculate blood flow:

$$F_{GIT} = \frac{I}{\frac{C_{PV} - C_A}{\text{----------}}}$$

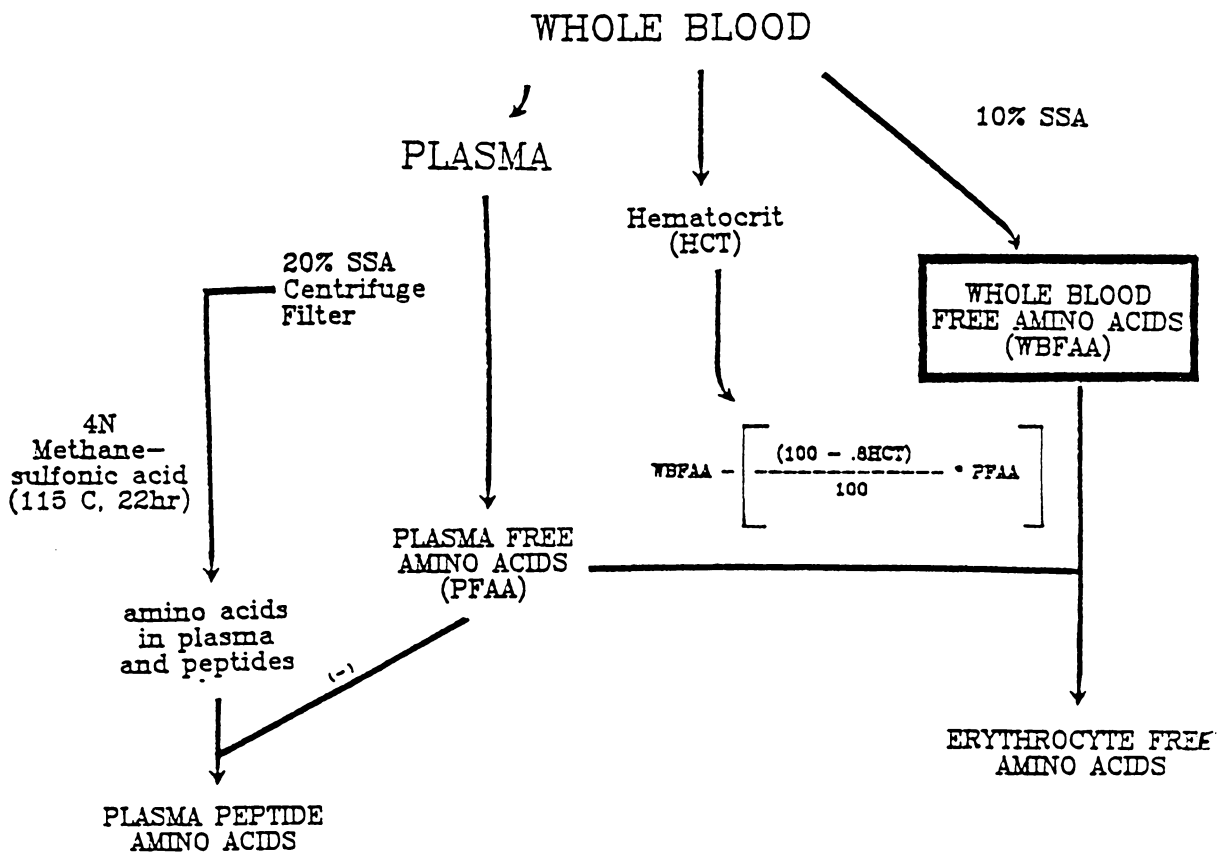
[1]Infusion/Withdrawal Pump. Harvard Apparatus, Co., Millis, MA.

$$F_{LIV} = \frac{I}{C_{HV} - C_A}$$

were F_{GIT} and F_{LIV} represent the rate of blood flow across the gastrointestinal tract and liver, respectively; I is the infusion rate of PAH (mg/min); and C_A, C_{PV} and C_{HV} are the concentrations of PAH (mg/ml) in the aorta, portal vein and hepatic vein, respectively.

Consistent, repeatable measurements of blood flow could not be made using this technique. Standard curves and repeatability among triplicate analyses for each sample were acceptable indicating that the source of the inconsistency may lie in the infusion technique or the preparation of the filtrate. Huntington (unpublished data) has found more consistent results when preparing the filtrate as described by Somogyi (1930). In several animals PAH concentrations in the aorta were greater than those in venous blood resulting in a negative value for blood flow. This may indicate that the mesenteric cannulae were not properly oriented to allow for equilibration of PAH throughout the bloodstream. The rate and concentration of PAH infusion were determined by calculations based on a bovine renal blood flow of 6600 ml/min (Phillips, 1976) and a renal PAH clearance rate of .02 mg/min (Smith et al., 1945). Perhaps these rates did not apply for the animals used in this study. It may be necessary to determine such values for similar animals with the equipment used in this study and under these experimental conditions to accertain the most desirable infusion rate and

concentration. Blood flow values in this study ranged from -12.99 to 15.3 liter/min for the GIT and 3.33 to 47.7 liter/min for the liver.



Appendix C. Diagrammatic representation of laboratory procedures.

APPENDIX D

STATISTICAL ANALYSIS EXAMPLES

EXAMPLE OF STATISTICAL ANALYSIS OF VARIANCE FOR ARTERIOVENOUS DIFFERENCES

General Linear Models Procedure

Dependent variable: GLU

Source	DF	Sum of squares	Mean square	F value	PR>F	R-square	C.V.
Model	8	78.44471899	9.80558987	3.47	0.0203	0.664831	271.2731
Error	14	39.54717667	2.82479833		STD DEV		GLU MEAN
Corrected total	22	117.99189565			1.68071364		-0.61956522

Source	DF	Sum of squares	F value	PR>F
Tissue	1	48.69745562	17.24	0.0010
State	1	9.79110250	3.47	0.0838
Anim	5	10.45811000	0.74	0.6058
TissueXState	1	4.56638062	1.62	0.2243

EXAMPLE OF STATISTICAL ANALYSIS OF VARIANCE FOR CONCENTRATIONS AND NET SPLANCHNIC OUTPUT

General Linear Models Procedure

Dependent variable: GLU

Source	DF	Sum of squares	Mean square	F value	PR>F	R square	C.V.
Model	6	155.25468333	25.87578056	16.54	0.0037	0.952047	13.1954
Error	5	7.81994167	1.56398833		STD DEV		GLU MEAN
Corrected total	11	163.07462500			1.25059519		9.47750000

Source	DF	Sum of squares	F value	PR>F
State	1	85.81400833	54.87	0.0007
Anim	5	69.44067500	8.88	0.0158

APPENDIX E. ARTERIAL CONCENTRATIONS OF SERUM TOTAL PROTEIN AND ALBUMIN IN FED AND FASTED CALVES

Item	Fed	Fasted	P[a]	EMS[b]
Serum total protein, g/dl	6.46	7.14	.02	.12
Serum albumin g/dl	3.57	3.96	.01	.01

[a] Probability that fed and fasted mean differences this large or larger could occur by chance.
[b] Error mean square.

APPENDIX F. ARTERIOVENOUS DIFFERENCES OF SERUM TOTAL PROTEIN AND ALBUMIN MONITORED ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF FED AND FASTED CALVES

	Gastrointestinal tract				Liver						
	Fed		Fasted		Fed		Fasted				
Item	A/V[a]	P[f]	A/V[a]	P[f]	A/V[a]	P[f]	A/V[a,b]	P[f]	P[c]	P[d]	EMS[e]
Serum total protein, g/dl	.10	(.49)	-.01	(.98)	-.19	(.22)	-.16	(.36)	.16	.82	.13
Serum albumin g/dl	.01	(.68)	.01	(.83)	-.02	(.47)	-.01	(.81)	.44	.90	.01

[a] Values are means of six observations and represent the concentration difference between arterial and venous samples. Negative values indicate greator concentrations in venous blood.
[b] Least square mean based on five observations.
[c] Probability that gastrointestinal tract and liver mean differences this large or larger could occur by chance.
[d] Probability that fed and fasted mean differences this large or larger could occur by chance.
[e] Error mean square.
[f] Values in () indicate the probability that the A/V difference does not differ from zero.

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MOVEMENT OF PLASMA FREE, ERYTHROCYTE FREE, PEPTIDE AND SERUM PROTEIN AMINO ACIDS ACROSS THE GASTROINTESTINAL TRACT AND LIVER OF CALVES

by

Linda Lee Koeln

(ABSTRACT)

Peptide, plasma free, erythrocyte free and serum protein amino acid arteriovenous differences were quantified across the gastrointestinal tract (GIT) and liver of growing calves during a "steady state" and fasting metabolism. Six Holstein steer calves (136 kg) surgically equipped with cannulae in the aorta, portal vein and hepatic vein were maintained under constant light and fed a natural diet as 24 hourly meals per day. Blood was obtained simultaneously from all cannulae at 9 to 12 d postsurgery during the midpoint of a 1-hr feeding interval and following a 72-hr fast. Most amino acids were added to plasma by the GIT but fasting reduced the magnitude of this addition. GLN and GLU were the only plasma amino acids removed by the GIT. The liver removed a large portion of amino acids in portal plasma but a net splanchnic plasma free amino acid output occurred. Hepatic removal of amino acids, particularly VAL, ILE and LEU, increased after fasting. The GIT generally removed amino acids from the erythrocyte while the liver contributed amino acids to this pool. Peptide amino acids increased as blood crossed the GIT. Responses paralleled those of plasma free amino acids in direction but were threefold greater for

peptide amino acids. A net output of peptide amino acids from the splanchnic bed was observed. Generally amino acids were added to the primarily globulin, albumin and mixed protein serum fractions while the pure albumin fraction contributed amino acids to the GIT. Fasting reversed the direction of response in the four fractions monitored. Arteriovenous differences for amino acids in two largely albumin fractions responded oppositely indicating a potential transport role of other proteins in these fractions. A more variable response was observed across the liver with direction and magnitude dependent upon individual amino acids. Fasting resulted in a release of amino acids by the liver into all four fractions. Several blood pools appear to be involved in the interorgan transport of amino acids. The extent and direction of transport varies with tissue, blood pool, nutritional status and amino acid. Quantitatively, peptides appear to be highly involved in interorgan movement of amino acids.