TRANSPORT AND EXCHANGE OF AMINO ACIDS FROM PLASMA, ERYTHROCYTES, PEPTIDES AND SERUM PROTEINS ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS

by

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Chapter I

INTRODUCTION

Amino acids are the building blocks of proteins in all forms of life. They are involved in the intermediary metabolism of carbohydrates and fats and function as integral components in many other body processes. As such, protein nutrition and amino acid metabolism are of utmost concern to biological scientists as they attempt to solve problems associated with inadequate or altered nutritional and physiological states, optimization of growth rates and studies of 'steady-state' metabolism.

Studies dealing with amino acid transport and metabolism in aminals are conducted using many different techniques or approaches. Many of the underlying issues in this area stem from research on the amino acid requirements and metabolism in microbes. In addition, in vitro experiments using tissue or cell cultures prove valuable for the understanding of the more fundamental concepts involved. However, for an accurate integration of the basic concepts of nitrogen metabolism, it becomes necessary to perform in vivo studies within the animal body.

Blood plasma has been the common media used for assaying the movement and levels of amino acids in the body. The

bulk of research in the past has used only plasma, however, recent evidence has implicated additional amino acid pools within the blood that function in the transport of amino acids. Quantitatively, the role of erythrocytes, plasma peptides and serum proteins are unknown at this point. As research techniques become more refined the relative importance of these additional pools will become evident. It is therefore important in the overall interpretation of amino acid transport and metabolism that these additional sources of amino acids to the different tissues be considered.

Chapter II

LITERATURE REVIEW

The changes of free amino acids in blood in relation to different nutritional and physiological conditions have been widely studied. Many different analysis techniques, experimental designs and reporting styles appear in the literature. Therefore, interpretation of the many different pools of amino acids in blood may result in some confusion. For the sake of clarity, discussions referring to plasma free amino acids will be described simply as amino acids in blood and reference to other pools will be specifically indentified as they are presented.

INTERORGAN TRANSPORT OF AMINO ACIDS

Muscle

The effects of fasting on the metabolism of amino acids have been intensively examined by researchers in several different species of animals. One of the earliest studies conducted examining the effects of fasting on plasma amino

acids was that of Wu (1950) on rats fasted for 9 days. He showed that this type of fast resulted in a reduction of alanine, glutamate, glutamine, glycine, serine and cysteine and elevated levels of threonine, leucine and isoleucine. The classification of these amino acids into essential (E) and nonessential (NE) groupings yield a substantial widening of the E/NE ratio. Tuttle et al. (1962) noted a similar pattern of amino acid changes in men fasted for several days. When the fast was extended to 4 weeks, however, the essential amino acids previously elevated were depressed below prefasting levels. Speculation at that time centered around adaptation mechanisms affecting the animal's ability to maintain homeostasis and basic metabolic functions.

Observations of fluctuations of amino acid patterns in the blood of fasted (short-term protein-calorie deprivation) and starved (long-term protein-calorie deprivation) subjects led to the inception of studies on different tissue beds in order to isolate the location of these responses. Prior to the early 1960's, it was believed that the liver was the primary site of protein and amino acid metabolism. Information is now accumulating which shows the skeletal muscle to be intimately associated in the metabolism of certain amino acids.

An amino acid infusion study (McMenamy et al., 1962) on perfused dog livers indicated that the liver was responsible for the very rapid uptake of most amino acids absorbed from the gut after a meal. Of notable exception was the failure of the liver to remove valine, isoleucine and leucine (the branched-chain amino acids - BCAA). This large uptake of amino acids by the liver results in the increased synthesis of liver and blood proteins. The supposition was made that the liver acts as a temporary storage reservoir for amino acids in the form of proteins and subsequently makes them available to other organs and peripheral tissues of the body.

The work of Miller (1961) showing that the BCAA's are catabolized at similar rates in normal and hepatectomized animals and that of Mimura et al. (1968) indicating that muscle and kidney tissues of rats contain greater levels of the BCAA transaminases than liver tissues, aided in the estabilshment of a new definition for the role of muscle tissue in amino acid catabolism. The synthesis of amino acids (notably, alanine and glutamine) by muscle tissues has also been documented by several researchers (Pozefsky et al., 1969; Marlis et al., 1971; Ruderman et al., 1974; Odessey et al., 1974) and further indicates that at least for certain specified amino acids, the muscle functions as an important site of amino acid metabolism.

Studies on amino acid metabolism in the muscle using C-labeled amino acids and the determination of oxidation potentials within the rat diaphragm (Goldberg and Odessey, 1972) have shown that only leucine, isoleucine, valine, alanine, glutamic acid and aspartic acid could be oxidized in the muscle. Other amino acids (glycine, serine, proline threonine, lysine, methionine, phenylalanine, histidine, tyrosine and tryptophan) were degraded very little or not at all. These relationships are important in the evaluation of metabolic changes that occur in the muscle in steady-state metabolism or when animals are subjected to fasting or starvation situations.

The flux of amino acids across muscle tissue (arteriovenous differences) have been used as a gross indication of metabolic changes occurring in the muscle when the body is subjected to nutritional stress. Early workers studied the post-absorptive state (approximately 12 to 14 hr post-feeding) vs sampling immediately after feeding to determine changes of amino acids in the blood. Numerous reports document a net output of amino acids from muscle when subjects are in the post-absorptive state (Pozefsky et al., 1969; Felig et al., 1970). By far the most significant increase is that of alanine and glutamine which account for greater than 50% of the total free amino-N release from the muscle

(Felig et al., 1970; Marliss et al., 1971; Ruderman and Berger, 1974). These facts are of interest in that patterns exhibited are uncharacteristic of normal protein turnover. For example, glutamine is an amide that is present in very low amounts in muscle protein while the output of it from muscle can account for 20% or more of the total release. Similarly, the alanine content of muscle protein is less than 10%, yet it accounts for a much greater proportion (approximately 30%) of the total amino acid release from post-absorptive muscle (Felig, 1975). Uptakes of certain amino acids (i.e., serine, cysteine and glutamate) by the muscle have also been noted under similar conditions (Felig et al., 1970; Marliss et al., 1971).

As the length of the fast progresses, further changes in amino acid patterns are noted. As previously discussed, there occurs a widening of the E/NE ratio (Wu, 1950, Tuttle et al., 1962). Felig et al. (1969) reported elevated plasma levels of valine, leucine, isoleucine and methionine during the first week of starvation by obese human subjects and delayed progressive increases in glycine, serine and threonine after the fifth day without food. Fasting for even longer periods resulted in decreased concentrations of most plasma amino acids, of which alanine was the most affected.

The changes in amino acid patterns in the blood and the differential action of muscle in response to fasting and starvation are important in terms of homeostatic mechanisms within the body that function in periods of food deprivation. Young and Scrimshaw (1971) outlined the various adaptations that occur during this type of nutritional stress. In the post-absorptive period, there is a depletion of blood glucose which functions as the primary energy source for most organs and tissues under normal conditions. The initial body response is the maintenance of blood glucose levels by increasing the output of hepatic glucose through increased gluconeogenesis in the liver (Adibi, 1968; Felig et al., 1969).

Several amino acids are classified as glucogenic, meaning very few biochemical steps are needed for conversion into pyruvate or intermediates of the Krebs-Henseleit cycle (Krebs, 1970). These amino acids are alanine, glutamine, glutamate, serine and glycine. The increased output of alanine and glutamine by muscle represents attempts by the body to increase the flow of gluconeogenesis precursors to the liver. Ruderman and Berger (1974) indicated that alanine and glutamine were being formed de novo in the muscle by the action of glutamate-pyruvate transaminase and glutamine synthetase, respectively.

As previously discussed (Goldberg and Odessey, 1972) only certain amino acids can be degraded by muscle. Further analysis (Odessey et al., 1974) revealed that the BCAA were the only amino acids in the muscle capable of increasing the de novo synthesis of alanine. Marliss et al. (1971) reported that the muscle was the major site of glutamine synthesis in man. Even though uptake of glutamate is often observed, the output of glutamine is far in excess of that which would describe the simple addition of ammonia to glutamate. In the post-absorptive state, the precursors for alanine and glutamine appear to be free amino acids already present throughout the system and the breakdown products of labile proteins.

Further fasting results in the continued demand for glucose, of which the brain accounts for greater than 60% of this requirement, resulting in a depletion of the immediate gluconeogenic precursors in the blood and liver (Young and Scrimshaw, 1971). Thus we see a depression of the nonessential amino acids in peripheral plasma indicative of increased hepatic utilization for gluconeogenesis. Furthermore, plasma levels of essential amino acids become elevated. These changes are significant in terms of the adaptation response to starvation. With the initial depletion of the nonessential "labile" free amino acids, the elevation

in the essential amino acids indicate that muscle protein is being degraded for the purpose of providing additional precursors for the synthesis of glucose in the liver (Felig, 1975).

These occurrences led to increased interest concerning the actual changes occurring within the muscle. An in vitro study on the rat diaphragm (Odessey et al., 1974) found that protein breakdown in the muscle was a first-order linear process. However, recoveries of alanine and glutamine were greater, tyrosine and phenylalanine approximately the same as, and the BCAA lower than originally present in muscle protein. This information is of interest when studied in relation to that of the high level of activity of BCAA transaminases in the muscle relative to the liver (Mimura et al., 1968). In addition, it is also known that during certain physiological states in which protein catabolism occurs, the ability of the muscle to degrade the BCAA increases three to five fold (Meikel and Klein, 1972; Goldberg and Odessey, 1972; Buse et al., 1973; Adibi et al., 1975). This enzyme activity response has not been observed for other amino acids in the muscle.

The catabolism of the BCAA in the muscle pose a particularly important problem in that skeletal muscle is unable to synthesize urea from the excess ammonia produced from

amino acid catabolism (Munro, 1969). Therefore, alternative means for the removal of excess ammonia are necessary. An additional function of alanine and glutamine now becomes apparent in regard to their role in the transport of endogenous ammonia from the muscle to the liver where conversion to urea for excretion does occur (Elwyn, 1972).

It was originally hypothesized that alanine functioned primarily as a shuttle for gluconeogenic precursors to the liver in a proposed 'alanine-glucose' cycle operating between skeletal muscle and the liver (Felig et al., 1970). A modification of this system has recently been proposed that attempts to account for the role of the BCAA's as they function in the energy and nitrogen metabolism of the muscle. The basis of this proposal stems from the work of Odessey et al. (1974) which showed that the production of alanine is not the result of protein degradation but in fact appears to be synthesized de novo from exogenous glucose and amino groups liberated by BCAA catabolism within the muscle cell. The addition of BCAA to an isolated muscle culture resulted in elevated glutamic acid levels within the cell as a result of transamination, primarily with a-keto glutarate and subsequent increases in the production of alanine and glutamine (Chang and Goldberg, 1978a). Thus, the inclusion of the BCAA in the definition seemingly aids in broadening the

understanding of the mechanisms that are functioning in the muscle.

Relative levels of alanine and glutamine production appear to be dependent upon levels of intracellular free ammonia. This arises from exogenous sources or the degradation of purines and amino acids. It has been shown that increased amounts of ammonia enhance glutamine and depress alanine synthesis (Goldberg and Chang, 1978). Chang and Goldberg (1978b) provided additional information as to the source of the carbon skeleton of glutamine by studying the utilization of ^{12}C-labeled amino acids in in vitro rat diaphragm preparations. They showed that the carbon skeletons of several amino acids (aspartate, asparagine, glutamate, isoleucine and valine) were converted to glutamine via TCA cycle intermediates.

An alternative hypothesis has been proposed for the description of the synthesis of the carbon skeleton of alanine (Garber et al., 1976). They reported that most of the amino acids act as precursors for the synthesis of alanine and glutamine in skeletal muscle and, therefore, alanine results principally from degradation of all amino acids in the muscle. This proposal is based upon in vitro analysis on rat skeletal muscle preparations where alanine and glutamine release into the medium were monitored following seper-

ate addition of physiological levels (.1 to .5 mM) of 21 individual amino acids. The difference between this study and that of Chang and Goldberg (1978b) was the inclusion of glucose (5 mM) in the incubation medium. The exogenous glucose could be acting as a precursor for the synthesis of the carbon skeleton of alanine. Upon addition of amino acids a net synthesis of alanine is possible regardless of which amino acid is added. Unfortunately, glucose disappearance from the medium was not examined by Garber.

Goldstein and Newsholme (1976) reported that isoleucine and glutamate were converted to pyruvate which was then transaminated to alanine and ultimately released from the muscle. They concluded that the BCAA may be responsible for approximately 40% of the carbon skeleton formation of alanine. Snell and Duff (1977) evaluated the effects of glutamate, valine, leucine and glucose on the formation of alanine in muscle and were in basic agreement with the previous concept of primary alanine synthesis from glucose derived pyruvate. However, they also stated that in situations of glucose depletion, several amino acids function to fill this evacuated role.

The continued loss of muscle protein in prolonged starvation would soon result in death, however, a series of adaptive changes occur which allow for increased survival.

After a period of several days without food, the brain acquires the ability to utilize alternative energy sources, i.e., acetoacetic acid, B-hydroxybutyrate and acetone (Owens et al., 1967b) which arise from the increased catabolism of fats. This results in decreases in the need for glucose and a concommitant depression of gluconeogenosis at the liver (Felig et al., 1969). Felig et al. (1969) recorded the plasma amino acid alterations under these conditions. Most amino acids (including the essential amino acids) clearly show decreased concentrations in blood plasma indicating a reduction in protein catabolism by the muscle. Of special interest was the very large decrease in plasma alanine levels, which was primarily due to a reduction in the release of alanine by the muscle. Also noted in this study was the progressive increase of three amino acids (glycine, serine and threonine). This increase began after approximately 5 days of fasting and transiently increased up to 21 days and then remained elevated for the duration of the fast. Apparently, these three amino acids act in direct opposition to the BCAA as it was noted that at 5 days the BCAA were at their greatest concentration followed by a progressive decrease to 21 days at which point a steady concentration in plasma was maintained. Their explanation involved the observation that renal glycine extraction increased four to

six fold in starvation, indicating a conservation of glycine by the kidney. The biochemical relationships of serine and threonine explain the complementary increases observed for these amino acids. These authors concluded that increases of these amino acids were due to increased peripheral release and strict conservation by the kidney.

Most of the previous studies deal with human experiments, however, results of a similar nature in different species have been observed. For example, studies using rabbits (Block and Hubbard, 1962) pigs (Chavez and Bailey, 1976) dogs (Elwyn et al., 1968) and rats (Adibi, 1971) show similar patterns of plasma amino acids when fasting is imposed. Adibi (1976) proposed the rat as a particularly appropriate model for studying the effects of starvation and protein deprivation relative to man because of the close similarities observed. In addition, most of the in vitro muscle work has been performed using different muscles isolated from the rat. Studies utilizing the chick (Hill and Olsen, 1963; Stephens and Evans, 1971a) present results that are divergent from those observed in mammals in several respects.

Fasting and starvation cause significant changes in basic intermediary metabolism as the body attempts to adapt to severely altered nutritional states. Protein and amino

acids in muscle tissue function as a 'buffer' against extreme adaptation measures by providing precursors for the glucose that is needed by other organs in short-term situations. Several metabolic processes have been identified that allow these changs to transcribe. In more severe situations, further changes are noted that serve to protect the body from extreme protein depletion.

Other Organs

A discussion pertaining to the fluxes of amino acids across the other main metabolic regions of the body (gut, liver, kidney and brain) allows for a more complete evaluation and understanding of the changes observed across the muscle. One of the earliest reports dealing with the inter-organ transport of amino acids is that of Elwyn et al. (1968). These authors reported that whole blood amino acid composition of the portal drained viscera (PDV) of dogs generally reflect the amino acid composition of the ingested meal. The absence of aspartate and glutamate output was observed and could be accounted for by conversion to alanine, ammonia, glutamine and glutathione. Felig et al. (1973) also reported a net ouput of alanine by the gut.

Windmueller and Spaeth (1974) showed that in rats, glutamine uptake by gut tissue was very extensive and accounted for 25-35% of total plasma glutamine extraction for each passage through the gut tissue bed. The ^{15}N-label of glutamine became incorporated in the following manner; alanine (33%), citrulline (32%), proline (10%), and ammonia (23%). They concluded that glutamine acts as an important respiratory substrate for the mucosal cells of the small intestine. The large conversion of glutamine to alanine at the gut has also been confirmed in sheep (Tagari and Bergman, 1978). Marliss et al. (1971) described the splanchic area, in particular the gut, as the major site of glutamine catabolism for the body under normal conditions. In fasting, the basic patterns of amino acid interconversions remained unchanged. The most notable difference is a greater influx of amino acids from the periphery relative to the lack of input from the intestinal region (Heitmann et al., 1978).

The overall metabolism of amino acids by gut tissue in sheep differ in several respects from that of non-ruminants. Release of ammonia into portal blood accounts for approximately 65% of the total nitrogen (Wolff et al., 1972b). This is in contrast to the 5% level observed in the dog (Elwyn et al., 1968). However, the patterns of the remaining amino acids basically reflect the composition of rumen

microbial protein. Again, glutamine was utilized by gut tissues and the lack of output of asparate or glutamate by the gut was noted. Apparently, glutamine from the peripheral areas of the body is being used for energy and the synthesis of alanine and other amino acids by the gut tissue in the sheep (Bergman and Heitmann, 1978).

Portal blood enters the liver where the blood amino acid composition is greatly altered giving credence to the description of the liver as the primary homeostatic control region in regard to amino acid and protein metabolism (Elwyn, 1972). In general, the liver is responsible for the removal of essentially all amino acids absorbed by the gut in fed animals (Elwyn, 1972; Felig, 1975; Bergman and Heitmann, 1978).

The 'branched-chain' amino acids, however, are not removed totally by hepatic tissue and thus pass through with minimal change between portal and hepatic venous blood to the periphery where they are utilized (Elwyn, 1972). This has been disputed in the case of sheep (Wolff et al., 1972; Heitmann and Bergman, 1978). It appears there may be a difference of interpretation involved in this matter. Results from the Bergman lab indicate an uptake of the BCAA by the liver which does indicate utilization within the liver. However, they also show a net splanchnic output of the BCAA

into the blood. This output from the liver was not apparent for any of the other essential amino acids. Based upon previous results in non-ruminants the ability of peripheral tissue to metabolize the BCAA still applies. Certainly, there are numerous other arguments for these observed differences including an inter-species difference. But other factors also may be influencing the observations, for example, steady-state metabolism vs post-feeding, post-absorptive or starvation sampling, the length of time after surgery before sampling, lack of large numbers of observations and also simply the presence of large sampling errors which are common in this type of research.

As previously noted, the liver is responsible for the near total removal of ammonia from blood added by the gut in the dog and sheep (Elwyn et al., 1968; Wolff et al., 1972). Arginine uptake and citrulline and ornithine release from the liver have been measured in man and sheep (Felig et al., 1973; Heitmann and Bergman, 1980a). This is additional evidence that the liver is responsible for the synthesis of urea.

Observations of differential fluxes of other amino acids have been noted. The kidney appears to be the major source of circulating serine in both man and sheep (Felig et al., 1969; Bergman et al., 1974). A substantial output of

serine has been reported and is apparently balanced by an almost equal uptake of threonine (Felig et al., 1969). In sheep several other fluxes have been reported by Bergman et al. (1974) including a near equal uptake of citrulline and release of arginine which occurred in both the fed and fasted state. It also appears that the kidney assumes additional importance under varying physiological conditions by changing from a state of glutamine uptake under normal conditions to that of glutamine removal in cases of fasting or acidosis (Bergman et al., 1974). This change functions to neutralize large amounts of acidic urine produced in the kidney with the ammonia liberated upon glutamine deamidation. A net uptake of alanine by the renal tissues of fed sheep has also been reported by these authors. This uptake is in accord with the knowledge that the kidney (and liver) are the only tissues in the body capable of synthesizing glucose via gluconeogenesis (Krebs, 1964). The kidney, as the organ responsible for the filtration of blood and excretion of metabolic end-products, is much less involved in the overall metabolsim of amino acids relative to the liver and peripheral muscle. This is mainly due to quantitative factors. However, the efficiency with which the kidney reabsorbs amino acids under normal conditions is phenomenal (Smith, 1960).

The large requirement for glucose by the brain has previously been discussed and is probably one of the most noted transfers associated with this organ. Felig et al. (1973) demonstrated that most amino acids are taken up by the brain. The BCAA and especially valine demonstrate the most pronounced uptake. Felig (1975) speculated that the brain serves an important role in the uptake of the BCAA, particularly in times of fasting when release rates of the BCAA from muscle are elevated.

It is evident that several groups of amino acids are transported throughout the body performing many different functions. The study of the synthesis, breakdown and transformations of amino acids as they traverse various organs and tissue beds aid in the interpretation of basic metabolic functions as they occur in the whole body.

Ruminant Studies

The study of the fasting metabolism and transport of amino acids in ruminants, and in particular cattle, are noticably lacking when the literature prior to the 1970's is examined. The massive amount of pregastric digestion and manipulation of nutrients, in particular proteins and car-

bohydrates, that occurs in the rumen has led to the belief by many workers in this field that the ruminant is uniquely different from monogastrics in many more ways than actually may be (Purser, 1970).

One of the first reports on amino acid patterns in fasted beef cattle is that of Brown et al. (1962). They studied the effects of a 72-hr fast on dwarf and normal yearling heifers and found that both groups responded with elevated levels of the essential amino acids plus glycine and cysteine and decreased amounts of serine, alanine, glutamate and citrulline. This widening of the E/NE ratio basically describes the classic response observed in fasting non-ruminants.

Certainly the anatomical and functional aspects of the ruminant digestive and absorptive systems are unparalleled. In regard to basic metabolism within the body, variations in terms of nutrient assimilation and utilization do exist. Specific areas of interest include the continual and more pronounced occurrence of gluconeogenesis (Bergman et al., 1966) and greater emphasis upon nitrogen recycling and excretion (Weston and Hogan, 1967). However, many similarities are also apparent. More specifically, the same amino acids that are essential for monogastrics are also essential metabolically in the ruminant (Purser, 1968). In addition,

the amino acids involved in the transport of carbons and nitrogen between the periphery and liver (alanine and glutamine) appear to be playing similar roles in ruminants and non-ruminants (Bergman and Heitmann, 1978).

The use of the ruminant in the study of amino acid metabolism during various physiogical or nutritional situations present several distinct advantages over that of non-ruminants. The larger physical size of the animal relative to the rat, dog or pig allow for easy placement of cannulas for the sampling of various organs and tissue beds (Kaufman and Bergman, 1971; McGilliard, 1971a, 1971b, 1972). Perhaps most importantly, however, is the ability to establish a 'steady-state' metabolism through regimes of frequent feeding that result in stabilization of diurnal fluctuations known to occur following meal eating (Wolff and Bergman, 1972a; Wolff et al., 1972).

Much of the information on ruminants concerning fasting and steady-state metabolism, gluconeogenesis and amino acid metabolism and interorgan transport has been generated from sheep in the laboratory of Bergman and associates at Cornell University. Their research effort over the past decade has provided much information related to gluconeogenesis and nitrogen metabolism during various stages of different metabolic states (Bergman and Heitmann, 1978). Bergman et al.

(1970). determined that in steady-state metabolism of mature sheep, 75 to 85% of the glucose turnover is derived from the liver while the kidneys contributed 5-15% of the body glucose turnover (Kaufman and Bergman, 1971). The plasma amino acid contribution to the synthesized glucose in the liver accounts for approximately 30% of the total with the remainder coming from propionate (50%), glycerol (5%) (Wolff and Bergman, 1972a) and lactate (15%) (Annison et al., 1963).

The infusion of ^{14}C-labeled amino acids by Bergman et al. (1970) indicated that alanine and glutamate were by far the most important contributors from the gloconeogenic amino acid pool. Glutamate, rather than alanine, was observed to be the most important amino acid precursor for glucose synthesis in the lactating dairy cow indicating possible differences due to physiological function, species or sampling procedure (Egan and Black, 1968). The gluconeogenic role of glutamine in these studies is not available because the infused amino acid was ^{14}C-glutamate in both studies. Undoubtedly, glutamate is being converted to glutamine to a large extent by the muscle and then back to glutamate by the gut (Elwyn et al., 1968; Heitmann and Bergman, 1978). Therefore, the direct relationship of glutamate as a primary gluconeogenic precursor must be considered as it inter-converts with glutamine.

Additional information on the origin and fate of the gluconeogenic amino acids in sheep (Wolff and Bergman, 1972b; Wolff et al., 1972) indicate that to a large extent, alanine, glycine and serine are produced from peripheral areas and the gut and utilized or removed by the liver. In addition, other relationships appear in the ruminant that are not as pronounced in the monogastric. For instance, rather large amounts of ammonia are added to the portal drained viscera and removed by the liver. Also the role of the urea cycle amino acids (arginine,. ornthine and citrulline) appear more pronounced in fluxes between the liver, kidney and peripheral tissues.

Of special interest are the observations of Wolff et al. (1972b) indicating greater liver and decreased muscle utilization of the branched-chain amino acids in 'steady-state' sheep. Bergman and Heitmann (1978) proposed that BCAA are utilized differently by the muscle and liver of sheep as opposed to observations in other species. They propose that the liver is more functionally involved in the catabolism of the branched-chain amino acids. This is in direct contrast to previous observations in rats Mimura et al., Chang and Goldberg, 1978b), dogs (Elwyn et al., 1968) and humans (Felig et al., 1969). Elwyn et al. (1968) also showed uptake of the BCAA in the dog liver and determined

that the bulk of the disappearance of the plasma BCAA from the liver could be accounted for by liver protein syntehsis and breakdown. However, the catabolism of the BCAA in the liver was less than the other essential amino acids.

Ballard et al. (1976) studied the flux of amino acids in whole blood across the hindlimb (muscle) of mature sheep. In fed animals, there was a significant release of alanine, glutamine and tyrosine and an uptake of serine, glutamate and lysine. Fasting for 72-hr resulted in the release of most amino acids by the hindlimb. Levels of alanine release were much lower than reported for humans and the release of the BCAA was greater than the net release of alanine. They also suggested that sheep muscle may not be as effective in the degradation of the BCAA as other species.

This work when taken at face value, would tend to support the earlier premise proposed by Bergman and Heitmann (1978). The Ballard study determined whole blood amino acids while most of the previous work was with plasma free amino acids. However, the data of McCormick (1980) on fed and fasted Holstein calves give indication of an interesting phenomenon that may be occurring. The decreased role of alanine in sheep relative to human studies may be a function of the pool of measurement, rather than a true physiological difference between ruminants and non-ruminants. This is

indicated by the work of McCormick (1980) which showed the hindlimb release of alanine and glutamine into plasma during fasting accounted for 30.7% and 18.9% of the total free amino acid release from muscle, respectively. This is in complete agreement with results on humans (Felig, 1975). However, when whole blood free amino acids were compared, the relative amount of alanine release from the muscle decreased by 20% which is in close agreement with Ballard as to the contribution of alanine in fasted animals.

Heitmann and Bergman (1980a) integrated the study of amino acid metabolism by studying combined effects over several organs and tissues concurrently. Again, steady-state and fasted mature sheep were compared. They identified the functional importance of several groups of amino acids that serve as carriers of nitrogen and carbons to and from peripheral regions of the body, gut, liver and kidney. The urea cycle amino acids (arginine, ornithine and citrulline) appear to be functioning as an entire body nitrogen cycle similar to the 'alanine-glucose' cycle with the final step, i.e., the catalyzed removal of urea from arginine occurring largely in the liver. The basis of this cycle lies in the observation of ornithine and citrulline release by the liver and the uptake of ornithine and citrulline by the kidney and hindquarter. These two tissues then release arginine which

is taken up by the liver for the final removal of urea and re-initiation of the cycle (Heitmann and Bergman, 1980a). This occurred in both fed and fasted states. Studies of growing steers (McCormick, 1980) are basically in agreement to the observations made in sheep. In the whole blood of fed steers, ornithine and citrulline were taken up by the hindlimb, however, arginine flux was near zero. In the fasted state, results were identical to the above discussion.

Other amino acid couplets also appear to be functioning in the interorgan transport of amino acids. If emphasis given to a topic in the literature is interpreted as implying the relative importance of a particular event, the glycine-serine amino acid couplet as it flucuates in various metabolic states is of little significance. Only cursory discussion of the metabolic changes and functions of glycine under these conditions are approached in the majority of studies.

Glycine and serine are both glucose precursers in the sense that the conversion of glycine to serine followed by dehydration and deamination to form pyruvate is a metablic pathway that does occur in mammals (Lehninger, 1975). Even though the glycine-serine couplet is often referred to in a similar sense as alanine, glutamate and glutamine, the meta-

bolic significance of these two amino acids appear to be of a different nature. As previously discussed, (Felig et al., 1969) glycine levels in plasma of man exhibit a delayed but prolonged elevation as the length of a fast progresses. Of interest is the near exact opposite response illicited by the branched-chain amino acids. In early fasting, when the BCAA are at their highest level in peripheral blood, plasma glycine is at its lowest level. Progessive decreases in BCAA levels in blood plasma are surprisingly consistent with progressive increases in the glycine level. Very little attention has been given to the role of glycine and serine in nutritional stress situations.

The effects of fasting on the metabolism and transport of amino acids by peripheral regions of the body have been discussed. Many of the same relationships between specific amino acids and their metabolic function exist between ruminants and non-ruminants. In the fed or 'steady-state' amino acid fluxes across muscle tissue generally reflect the dynamic nature of protein metabolism which consists of constant assimilation and breakdown of protein in the muscle. Some amino acids (alanine, glutamine and arginine) appear to act as 'scavengers' of certain catabolic processes within the cells.

In ruminants, the role of the urea cycle amino acids (citrulline, ornithine and arginine) are elevated and appear to be functioning as a 'whole-body cycle' in the transport of nitrogen. Furthermore, glutamine relative to alanine has been shown to contribute more to the gluconeogenic amino acid pool of ruminants. Both of these differences are perhaps simply a reflection of the greater ammonia concentration of ruminant blood due to their unique digestive system.

The other major difference that has been proposed is the decreased ability of the muscle in ruminants to catabolize the branched-chain amino acids. Evidence supporting this premise is sketchy and indirect and in need of further clarification.

The ruminant is much more dependent upon continous glucose production in the fed state than non-ruminants due to the lack of glucose absorbed from the gut. The primary precursors for glucose in ruminants are proprionate, amino acids, glycerol and lactate. Evidence illustrating a greater contribution of the gluconeogenic amino acids to glucose production in ruminants is indicated by the lower levels of circulating alanine and glutamine in the plasma of sheep as compared to dogs and humans. This is suggestive of increased utilization of these amino acids for the production of glucose in the 'steady-state'.

RELATIONSHIP OF DIETARY PROTEIN TO BLOOD AMINO ACID PATTERNS

Much of the information on interorgan transfer and muscle metabolism of amino acids is based on animals in fed or 'steady-state' conditions or under varying degrees of fasting and starvation. The analysis of amino acid tissue fluxes in relation to dietary protein or protein deficiency have not been extensively studied. However, a large volume of research has been generated as to the effects of various protein levels, protein sources or specific amino acids in a variety of animal species as they relate to animal growth and production, nitrogen balance, blood amino acid patterns and other general blood biochemical variables.

Non-protein nitrogen and amino acid metabolism

It is suggested that plasma amino acids are reflective of the nutritional adequacy of ingested dietary protein in monogastrics. Numerous studies have shown depressed levels of specific amino acids in response to consumption of a diet in which a respective amino acid is limiting (Longnecker and Hause, 1959; Hill et al., 1963; Swendseid et al., 1966).

This response is not observed in ruminants, however, due to the extreme changes effected on dietary proteins by ruminal microorganisms. However, similarities between the physiological systems apparently do exist. The work of Leibholz (1966) showed that plasma free amino acid patterns of very young calves with non-functional rumens are generally reflective of their dietary amino acid intake and as rumen function develops these similarities disappear. The breakdown of dietary protein in the rumen and re-synthesis into microbial protein result in the presentation to the intestine for absorption, amino acid concentrations which vary due to varying amounts of non-digested bypass dietary protein relative to a rather constant composition and supply of microbial protein (Weller, 1957; Weller et al., 1958). Leibholz (1969) studied the relationship of various dietary proteins fed at different levels with the plasma amino acid patterns of peripheral blood in mature wethers. Although there was an overall increase in plasma free amino acids following feeding, there was no apparent relationship between dietary amino acid intake and plasma free amino acid levels. Research by Wolff et al. (1972) on mature 'steady-state' sheep fed alfalfa pellet diets indicate that changes in the plasma free essential amino acids in portal blood were similar to the essential amino acid composition

of microbial protein. These alterations of dietary proteins by rumen microorganisms have hindered the ability of ruminant nutritionists to conduct traditional studies related to the evaluation of limiting amino acids of specific feedstuffs or the amino acids requirements of the different ruminant species.

The study of amino acid metabolism in ruminants was greatly influenced by the work of Loosli et al. (1949) which showed that the microorganisms within the rumen were capable of synthesizing all of the known essential amino acids required by mammals. This information, when taken in context with the function and anatomy of the ruminant digestive system, resulted in a general lack of research concerning protein and amino acid metabolism in the ruminant for the next decade.

Oltjen et al. (1962) utilizing purified diets with soy protein or urea as the sole source of nitrogen in the diets found that performance of sheep fed urea was reduced by 30 percent. Oltjen (1969) reviewed the literature pertaining to the feeding of nonprotein nitrogen (NPN) to ruminants and summarized the effects. As already stated, growth rate and feed efficiency are consistently reduced by approximately 35% in animals fed urea vs purified soy protein. Milk production is similarly depressed. Nitrogen retention is

reduced to about 60-70% of soy-fed animals. Improvement in nitrogen retention is often noted with increased length of time on feed. This improved retention is apparently due to decreased urinary nitrogen loss indicating an adaptive response within the animal body that is achieving a greater utilization and conservation of nitrogen by the system. This has been noted as a gradual response that reaches normal values in a 30 to 50 day period (Ludwick et al., 1971).

Studies on plasma amino acid patterns in relation to dietary NPN and purified diets were first presented by Virtanen (1966) in dairy cattle and Oltjen and Putnam (1966) in beef cattle. Virtanen (1966) noted that the concentrations of most of the free amino acids in both plasma and whole blood were lower in long-term studies on dairy cows fed urea as the sole source of dietary nitrogen. The depression was most evident in the essential amino acids, however, he stressed the variation of histidine and possibly methionine as being the most important. Very low histidine levels were measured in urea-fed cows after they calved and were maintained at low levels for several months. This was related to the stress of high protein synthesis needs in relation to lactation. As the amount of milk production decreased, histidine levels in blood increased indicating a decreased histidine need within the body relative to productive output.

Glycine was the only amino acid that was found in greater concentrations in the blood of cows fed urea. He speculated that histidine may be the limiting amino acid in dairy cattle fed purified diets in which ammonium salts supply the sole source of dietary nitrogen by forming a "bottleneck in protein synthesis".

Results on growing beef steers (Oltjen and Putnam, 1966) in a series of metabolism trials also showed a marked depression in the essential amino acids in plasma. However, total amino acid levels were nearly the same as animals fed purified soy protein. This effect was mainly due to a substantial increase in glycine and serine in relation to either no change or a slight decrease in the other nonessential amino acids. The most significant response observed in the depressed essential amino acids was that of the BCAA and phenylalanine. No difference in histidine response was noted in this study.

Similarities between the two studies do exist in that the total essential amino acids were always depressed and glycine was elevated when NPN was the sole nitrogen source. There were several differences in the experimental procedures between the two studies that could have significantly affected the different observations. Ammonium salts were the NPN source in Virtanen's study and these dairy cows were

consuming their diets ad libitum for monthly or yearly periods. The Oltjen work utilized restricted feeding (1.25% body weight) of urea purified diets to beef steers in metabolism studies for short periods of time (<40 days). The fact that animals in these studies had different physiological functions is a most obvious factor related to the observed differences in specific essential amino acids. In addition, different sources of NPN and more importantly, the length of time and level of consumption are also probably related to differential animal response.

In comparison to studies on rats (Swendseid et al., 1963a) and humans (Young and Scrimshaw, 1968), it has been shown that short-term protein deprivation results in depressed levels of essential amino acids while the total amino acid concentration in the blood is unaffected. Prolonged protein deficiency, however, results in the depression of all amino acids in the blood except glycine and possibly serine (Holt, 1963). If it could be assumed at this point that NPN purified diets fed to ruminants result in a protein deficiency then results from certain monogastric species seemingly correspond to some of the discrepancies observed between these two studies.

Research on the feeding of urea to ruminants have raised several questions pertaining to the dietary and meta-

bolic aspects of protein and amino acid nutrition in ruminants. The ability of NPN to supply the total exogenous nitrogen for rumen microorganism protein synthesis has led researchers to pursue several divergent theorys in attempts to explain both the productive and biochemical differences that occur with NPN feeding. These areas include microbial protein quality, microbial protein quantity and the 'urea' adaptation phenomenon.

Microbial protein quality and quantity

Purser (1970) reviewed the literature on the amino acid composition of rumen microorganisms. The literature confirms that dietary factors do not appear to affect the amino acid composition of rumen bacteria and protozoa. Therefore, the effect of NPN on the composition of microbial protein is minimal. However, research conducted on rats fed harvested microbial protein (Bergen and Purser, 1968) indicate that histidine and tryptophan are growth limiting in the rat.

Analysis of microbial populations in the rumen of animals fed primarily NPN as the nitrogen source indicate that protozoal numbers decrease significantly while bacterial concentrations increase (Virtanen, 1966; Schelling et al.,

1967). In addition, it has been shown that rumen protozoa protein contains slightly greater concentrations of leucine, isoleucine, phenyalanine and lysine (Hungate, 1966). This is of interest when compared to the results of Oltjen and Putnam (1966) where depressed levels of the BCAA in plasma were observed. The reduction of protozoa in the rumen microflora by high urea diets could possibly account for these observations.

The BCAA are of further interest in that the dietary source of these compounds is plant protein. Slyter et al. (1971) found that the branched-chain fatty acids were much lower in the ruminal fluid of NPN supplemented steers as compared to steers fed natural dietary protein sources. The lack of natural plant protein in the diet, therefore, results in a large reduction in the carbon skeleton precursors or actual BCAA that are needed for maximal protozoal protein synthesis.

Branched-chain volatile fatty acids (B-methylubutyrate, isovalerate and isobutyrate) and phenylacetate have been shown to be the carbon skeleton precursors in the rumen for the synthesis of the BCAA and phenyalanine by the rumen microbes (Allison, 1969). Studies have been conducted on the dietary supplementation of these precursors in urea or soy purified diets in attempts to explain the various phe-

nomena previously observed (Oltjen et al., 1971; Hume et al., 1970). The results obtained were discouraging due to failure to alter previously observed plasma amino acid relationships and lack of consistent improvements in growth and performance. However, Oltjen et al. (1970) reported that abomasal infusion of the BCAA and phenylalanine in urea-fed steers resulted in elevated plasma levels of these amino acids and improved nitrogen retention. As levels of BCAA increased there was also a large corresponding decrease in glycine concentrations in the blood. These findings are interesting in that they imply a metabolic relationship in the body between glycine/serine and the BCAA regardless of dietary nitrogen source. The improvements noted with supplemental amino acids provide additional evidence that amino acid deficiencies are in part responsible for the deleterious effects associated with urea in ruminants.

There have been many studies attempting to define limiting amino acids or amino acid imbalances present in microbial protein for several different amino acids. Results are variable and cases have been made by separate scientists for the potential of every single essential amino acid (histidine, arginine, lysine, isoleucine, leucine, valine, phenylalanine, methionine, threonine and tryptophan) to be limiting in urea-fed animals (Chalupa, 1972).

This information thus leads to the supposition that there may simply be a lack of total amino acids available for absorption or stated differentially, the quantity of microbial protein is insufficient to promote high levels of growth or other performance parameters by ruminants. That this may be the case is indicated by the results of several studies showing a reduction (10 to 30%) in the amount of post-ruminal protein available to urea- vs soy-fed animals (Potter et al., 1969; Tucker and Fontenot, 1970; Oltjen et al., 1971).

A growth and metabolism study on young steers fed varying combinations of purified soy protein and urea to account for crude protein dietary equivalencies from 9.2 to 23% showed linear decreases in plasma serine and glycine as the level of nitrogen in the diet increased. No response, however, was noted in the essential amino acids in regard to nitrogen level although the essential amino acids were generally slightly higher for animals fed some proportion of soy protein (Oltjen et al., 1972). Similar results were obtained (Boling et al., 1972; Young et al., 1973) on young growing steers fed ground ear corn and corn silage based diets supplemented with soy protein, urea or no additional nitrogen source. Performance of steers supplemented with urea was slightly greater than those without any additional

nitrogen while both were inferior to the soy protein supplemented steers.

Plasma amino acid patterns were also reflective of a particular nitrogen source. Soybean meal fed steers had significantly greater levels of valine, isoleucine, leucine and lysine than the urea or non-supplemented steers. Urea vs no supplemental nitrogen showed greater levels of the BCAA at 56 days but no differences at 112 days. The only difference between these two diets at the termination of the trial was the greater level of glycine in the low-nitrogen diet. These authors concluded that young growing steers need a performed protein supplement in the early stages of growth primarily because the high protein needs in early stages of development cannot be met solely by microbial protein synthesis from urea.

From the previous discussion, it is apparent that several different factors are interrelated in the interpretation of urea supplementation to ruminants. Not only is the balance of absorbed amino acids important but also the absolute quantity of absorbed amino acids is of concern when evaluating the dietary nitrogen adequacy for maximal tissue growth.

Many similarities exist between ruminants and monogastrics in terms of blood chemistry flucuations associated

with protein deprivation. Examples have already been discussed as to the general responses observed in short-term vs long-term protein deficiency. However, it is interesting to note that descriptions of amino acid profiles of children afflicted with kwashiorkor are as follows: (a) depressed levels of all amino acids in the blood, (b) the E/NE ratio is decreased due mainly to large decreases in the essential amino acids of which the BCAA are most effected, and (c) relatively smaller decreases in the non-essential amino acids with glycine being notably elevated (Snyderman et al., 1963; Arroyave et al., 1962). This pattern fits very closely to the description of plasma amino acid profiles of ruminants fed urea purified diets.

Urea Adaptation

The improvement in nitrogen utilization over time by ruminants fed urea has been well documented. The mechanisms responsible for this adaptation, however, are not clearly defined. Many changes are noted to occur in the rumen, in particular shifts in microbial populations (Schelling et al., 1967) and changes in the activity of certain microbial enzymes (Caffery et al., 1967; Chalupa et al., 1970) in response to high levels of dietary non-protein nitrogen.

Efforts have also focused on metabolic changes occurring within the body as the principal site of the adaptation process. The elevation of urinary nitrogen in ruminants fed high levels of NPN is commonly observed (Oltjen, 1968). Improved nitrogen retention and thus improved growth rate as length of time on feed increases is commonly observed and usually achieves equivalence to soy-fed animals by 30 to 50 days (McLaren et al., 1965; Clifford and Tillman, 1968; Ludwick et al., 1971). Changes in plasma amino acid patterns of animals fed urea as the total nitrogen source have previously been outlined. Reversion of plasma amino acid patterns to pretreatment levels as the animal 'adapts' have generally been shown not to occur (Virtanen, 1966; Ludwick et al., 1972; Prior et al., 1972). It has been shown, however, that amino acid differences do disappear in animals fed urea supplemented diets that contain some preformed protein (Bergen et al., 1973) after a period of 30 days.

The earlier discussed work of Oltjen et al. (1970) showed improved nitrogen retention and elevation of the BCAA and depression of glycine and serine upon abomasal infusion of valine, isoleucine, leucine and phenylalanine to steers maintained on urea diets. Opposite responses were noted upon glycine and serine infusion with soy diets. These stu-

dies were conducted on steers previously adapted to their respective diets 42 days prior to beginning of the infusion studies. A follow-up study, however, failed to show the previously observed positive responses obtained by BCAA infusion or the antagonistic response associated with serine and glycine infusion (Chalupa, personal communication). The results, however, are suggestive of some type of metabolic relationship between these specific amino acids.

Several studies have been conducted in rats that have implicated high levels of dietary glycine as growth depressants. Hepburn and Bradley (1968) reported a linear reduction in weight gains associated with concommitant increases in glycine and serine intake. Greater levels of amino acids in the urine were observed in high glycine diets. Waterhouse et al. (1979) indicated that infusion of glycine in humans resulted in extremely high levels of glycine in circulating blood which could not be accounted for simply by levels of infused glycine. Pui and Fisher (1979) found that diets supplemented with glycine resulted in depressed nitrogen retention and growth in rats. Inclusion of arginine in the diet eliminated the effects observed with glycine alone.

Glycine is unique from the standpoint of its classification as a nonessential amino acid and its association with growth depression and nitrogen retention. Harper (1970)

indicated that the tolerance to high levels of dietary glycine is much less than that of the other nonessential amino acids with the exception of serine. The metabolic similarities of glycine and serine may account for many of the parallel functions that have been observed. Sauberlick (1961) initially commented on the uniqueness of glycine relative to other amino acids in toxicity studies in rats. He found that the growth depression caused by glycine was not due to depressed feed consumption as is the case shown by most other growth depressing amino acid imbalances. These studies were later confirmed by Sanahuja and Harper (1963) which demonstrated that appetite controlling centers in the rat brain were unaffected by glycine. These findings suggest that metabolic changes are occurring somewhere in the body in response to elevated levels of glycine which thus far have not been totally delinated.

Many studies have been conducted in attempts to isolate metabolic changes occurring in monogastrics in response to dietary protein deprivation. However there is much less information available on the metabolic changes in ruminants fed urea diets and more specifically, on the adaptive response that is so often noted. Chalupa et al. (1964) fed varying levels of urea to steers and reported a depression in nitrogen utilization in congruence with high NPN diets.

Urine analysis indicated increasing levels of creatinine as dietary urea increased suggesting increased rates of muscle catabolism. In addition, plasma amino acids are usually lower in urea- vs soy-fed animals (Ludwick et al., 1972). Particularly striking is the extreme depression of the essential amino acids. A decreased supply of amino acids from dietary sources could be the cause of the depressed plasma amino acids and thus result in a lack of all amino acids or possibly particularly limiting amino acids at the tissue level which are necessary for normal protein synthesis (Munro, 1969).

It has been shown that the accumulation of protein and normal protein turnover in the muscle is regulated not only by the rate of protein synthesis but also to a lesser extent, by the rate of degradation (Millward and Waterlow, 1980). It was pointed out by these authors that when growth is suppressed due to protein or energy deficiencies there is an immediate and sustained decrease in the rate of protein synthesis in the muscle. Long-term maintenance of these depleted states result in decreases in the RNA:protein ratios in muscles which indicate that protein synthesis may be suppressed due to the lack of translational material in muscle cells (Millward et al., 1975). Furthermore, they noted that differences between fasted and protein-deficient mus-

cles in terms of rate and extent of muscle RNA depletion were difficult to differentiate. Cabak et al. (1963) also expressed difficulties in differentiating responses in fed and fasted muscles.

Increased concentrations and activities of the BCAA transaminases of in vitro muscle specimens of both fasted and protein-deficient rats are further indications that fasting and protein deprivation ellicit similar responses in muscle tissue (Mimura et al., 1968). In addition, they further indicated that the BCAA transaminase in the muscle influences the rate of leucine deamination to a much greater extent than either isoleucine or valine.

These factors are interesting when considered in context with recent evidence presented by Goldberg and Chang (1978) that the BCAA-leucine is the only amino acid capable of promoting protein synthesis and inhibiting protein catabolism in skeletal and cardiac muscle. This action may be direct or through the action of increased levels of leucyl-tRNA. Further research from this laboratory (Tischler and Goldberg, 1980) indicate that intermediates of leucine catabolism actually inhibit protein synthesis.

The accumulation of evidence may be offering an explanation to the short-term adaptive mechanisms occuring in the muscle. Under regular nutritional conditions protein syn-

thesis occurs, possibly stimulated by normal levels of intracellular leucine. Nutritional stress leads to the increased activity of BCAA aminotransferase due to some as yet unknown mechanism. Deamination of the BCAA proceed leading to an accumulation of leucine degradative by-products which effect the "shut-down" of protein synthesis in muscle. This theory is unsubstanitated by in vivo studies and based upon conclusions drawn from inter-species observations and differential experimental designs.

Analysis of this phenomenon in the live animal is very limited. Bergen et al. (1973) measured the free amino acids in muscle of lambs fed five diets of varying protein level. The muscle from lambs fed the basal diet (6.5% crude protein, corn-base) had significantly reduced levels of total essential amino acids and in particular the BCAA's and phenyalanine. Nonessential amino acids were unaffected. The basal diet supplemented with 3.5% urea showed no differences relative to other more conventional diets. Comparisons to other urea studies are difficult to make from these results because preformed protein from the basal diet contributed largely to the total nitrogen in the urea treatment. However, the observations from the low protein diet are in accord with previous disscussions. Observations on protein synthesis and amino acid metabolism by muscle tissue in rum-

inants fed urea diets are not available for direct comparisons with the work done on protein-deficient and fasted nonruminants. It awaits to be determined whether similar factors are responsible for many of the observed phenomenon.

Most of the research concerning metabolic changes in urea adapation have dealt with the liver. Many liver enzymes have been shown to be altered by the feeding of urea. Several studies by Prior et al. (1969; 1970; 1972) have shown that several enzymes involved in intermediate carbohydrate metabolism are depressed in urea-fed lambs resulting in altered carbohydrate metabolism. Ludwick et al. (1972) fed soy and urea purified diets and found only glutamic-pyruvate transaminase to be significantly lower in urea-fed lambs. Trends were noted in the increasing activity of several other enzymes over time that could indicate tissue adaptation to urea feeding. Chalupa et al. (1970a) examined liver urea cycle enzymes and noted reduced activity of carbamyl phosphate synthetase, ornithine transcarbamylase and arginase and increased activity of glutamate dehydrogenase and glutamate-oxaloacetate and glutamate-pyruvate transaminase in urea-fed lambs. They also observed large increases in liver ornithine concentration. Even though the liver was very efficient in the removal on ammonia from portal blood, these authors concluded that the accumulation of

ornithine rather than increased arginine synthesis in the liver was responsible for handling the increased demand on liver capacity for ammonia detoxification.

Accumulation of ornithine in the liver may not simply be the result of urea cycle enzyme disfunction as was postulated by these authors. The following biochemical pathway represents the first step in the synthesis of creatine: (Lehninger, 1975):

arginine + glycine ------> ornithine + guanidinoacetic acid

High levels of glycine noted in urea-fed animals may indicate an interaction thus far not examined. Furthermore, this could have a relationship ship to the increased levels of urinary creatinine in a previously discussed report since quanidinoacetate is a direct precursor of creatine.

These findings could be of importance in short-term diurnal adaptation to high nitrogen loads. Conclusive studies on ruminants have not been made that show enzymatic adaptation responses that are able to account for long-term improvements in nitrogen retention.

Many investigations have been made in the rat on metabolic changes in liver arginase activity in response to dietary protein changes. It has been shown that arginase

activity decreases with very low protein diets and increases with high protein diets or in fasting (Ashida and Harper, 1961; Schimke, 1962). Schimke (1964) reported that changes in arginase activity were related to turnover/synthesis mechanisms which allow for short-term changes in enzyme replacement. Das and Waterlow (1974) showed that following low protein intake and repletion studies several urea cycle enzymes (arginase, arginosuccinate lyase, arginosuccinate synthetase) and glutamate dehydrogenase reach new levels of acitvity 30-hr after feeding. Their results also suggest that the activity of the urea cycle enzymes depend in part on the amount of nitrogen remaining after specific demands for protein synthesis are met. In low protein diets, enzymes functioning in nitrogen utilization appear to be in excess and are not usually stressed in this situation.

Ruminants, however, are unique in this aspect in that they may be subject to low protein, deficiency type diets but the nitrogen load presented to the liver is very high due to ruminal ammonia production. These are actually conflicting metabolic phenomena not normally observed in the non-ruminant.

Indications are that liver enzymes can undergo adaptive responses in activity rates and concentration changes due to differing dietary regimes. The significance of these find-

ings relative to short or longterm adjustments to nitrogen utilization are not clearly known and their relevance to the urea adaptation phenomenon is yet to be determined.

Feeding of high urea diets have also resulted in depressed levels of serum proteins (Oltjen, 1968) of which albumin appears to be the most affected (Ludwick et al., 1972). The depression of serum proteins and specifically albumin are also classic clinical symptoms of protein deficiency (kwashiorkor) in children (Pimstone et al., 1966). It is theorized that synthesis of liver proteins are depressed due possibly to low levels of circulating plasma free amino acids (Elwyn, 1972). Virtanen (1966) observed that blood proteins were lower in cows not yet adapted to high urea levels in the diet relative to cows subjected to long-term urea feeding and cows fed 'natural' diets. Amino acid composition analysis of these blood proteins showed no differences due to dietary treatment. This implies that changes are due to quantitative aspects of protein synthesis rather than qualitative changes in the protein composition. Ludwick et al. (1972) showed continued decreases in both total serum proteins and albumin for sheep fed both soy and urea purified diets as the length of the trial progressed. Similar results were also reported by Singh and Sawhney (1967) with lambs fed varying levels of urea.

The many conflicting reports demonstrating changes or lack of changes of various metabolites and enzymes occurring in response to urea feeding lead to possible conclusions that the adaptation mechanism is either so complex that specifics are difficult to eluciadate or the possibility that no such changes attributed to urea per se actually occur. Indications of this are also found in the literature. Some studies have shown no significant changes in nitrogen balance (Johnson and McClure, 1964; Caffrey et al., 1967) over long periods of urea feeding. Even with the tremendous amount of research done in this area it is still unclear as to what exactly is happening in ruminants fed high levels of dietary non-protein nitrogen.

BLOOD POOLS INVOLVED IN AMINO ACID TRANSPORT

The measurement of plasma free amino acids has traditionally been the medium of choice in nutritional studies when amino acid concentrations, tissue exchange and protein turnover rates have been examined. Winter and Christensen (1964) determined from in vitro studies on human erythrocytes (RBC) that the equilibration time for amino acid transport across the RBC membrane was very slow. This evi-

dence contributed to the belief that the role of the RBC in amino acid transfer across tissues was minimal.

Research efforts in the past 15 years by a growing number of scientists have cast doubt on the non-functional role of the RBC in terms of evaluating amino acid metabolism in the animal body. It is now obvious that the RBC is some way involved in the transport of amino acids although precise definitions regarding inter-species variations, characterization of the role of the RBC relative to altered physiological or nutritional states and actual mechanisms responsible for RBC/plasma differentials and similarities are as yet unclear.

Recently other amino acid pools within the blood have been implicated as playing a role in the transfer of amino acids to and from peripheral regions of the body. These include peptides that are known to be present in circulating blood and different blood proteins commonly classified as serum proteins. Very little research has been conducted on the peptides and blood proteins in relation to their amino acid transport role.

A report by Elwyn (1969) on the fate of free amino acids absorbed into portal blood and passed on to the liver in dogs fed a high protein meal indicated that 57% of the absorbed amino-N is converted to urea in the liver while 23%

enters the general circulation as free amino acids. Plasma proteins accounted for 6% of the absorbed amino-N leaving the liver and the remaining 14% was unaccounted for. He speculated that this latter amount was retained in the liver as hepatic protein although measurement of peptides or other nitrogenous compounds were not made. These findings are interesting in that they implicate other factors in the blood that may be of functional significance in total amino acid transport and as yet have received very little attention in terms of quantitative and qualitative verification.

Erythrocytes.

Elwyn (1966) demonstrated in dogs that concentration of free amino acids in the RBC were almost always greater than that of plasma. In addition, he noted concentration changes between these pools of some amino acids and glutathione as blood passed through the gut and liver. In several instances, these changes in the RBC were seemingly independent or of different magnitudes than changes noted in plasma. In addition, glutamate exhibited concentration changes in opposite directions between plasma and RBC in passage through the gut and liver. Overall conclusions from

this study, however, still refer to plasma as being quantitatively more important in the transport of free amino acids and generally more reflective of changes occurring in whole blood.

Subsequent work on the role of the RBC in dog blood (Elwyn et al., 1968) showed that a marked difference occurs between RBC and plasma concentrations as different tissues or organs are traversed. For example, the plasma amino acids of portal blood were generally more concentrated than the RBC but in blood leaving the liver, free amino acids in the RBC were more concentrated than plasma. The action of these two pools were similar for peripheral arterial and hepatic venous blood. Elwyn (1972) summarized much of his work in dogs and stated that the gut releases amino acids primarily into plasma with little contribution by the RBC except for near total transport of glutathione. The RBC, however, appears to be much more involved in the transport of amino acids from the liver to peripheral areas of the body due possibly to direct membrane transfer of amino acids between erythrocytes and hepatic cells (Elwyn et al., 1972c).

Several studies have been conducted on humans where both plasma and RBC amino acid contributions have been analyzed. Snyderman et al. (1970) reported that free amino

acid content of the RBC reflected the changes occurring in plasma when measured in 4 to 17 day old premature infants fed different protein intakes. They concluded that the RBC and plasma reacted in a parallel manner in terms of free amino acid concentrations and thus there was no apparent advantage in the measurement of the RBC free amino acids in protein studies. They did note, however, a much greater decrease in plasma lysine concentration relative to the RBC on low protein diets. Levy and Barkin (1971) noted that the human RBC in peripheral venous blood contained high levels of aspartate and glutathione. These substances were not detectable in plasma. McCormick (1980) observed similar concentration differences between the plasma and RBC of young calves.

A dynamic role played by the RBC in glutamate transfer has been shown in studies on the effect of insulin on muscle glutamate uptake by the human forearm. Research by Pozefsky et al. (1969) failed to detect an uptake of glutamate from plasma across insulin treated forearm muscle. Aoki et al. (1972) also noted the same effect for the plasma pool. However, whole blood analysis revealed significant glutamate uptake in response to infused insulin. These authors theorized that insulin was acting in some manner to sensitize the RBC membrane thus allowing for a very rapid efflux of gluta-

mate from the cell into the plasma or possibly directly into the muscle.

Felig et al. (1973) also contributed to the evaluation of the RBC in interorgan amino acid transport in humans by studying plasma and blood cell amino acid fluxes across the splanchnic bed and peripheral muscle. They showed that 30% of the alanine output from the leg and 22% of its uptake by the splanchnic area was quantitatively performed by the erythrocyte. In addition, plasma alone could not account for the total flux of several other amino acids (threonine, serine, glutamine, methionine, leucine, isoleucine, tyrosine and citrulline). They concluded that the RBC is important in the total evaluation of net amino acid uptake and release by muscle and splanchnic organs.

Heitmann and Bergman (1980b) recently commented on their observations of amino acid differences between whole blood and plasma in 'steady-state' sheep. Their conclusion was that amino acid transport by the RBC was always reflective of plasma transport across all tissues measured. However, the sole use of plasma amino acids in the evaluation of net tissue flux resulted in an underestimate of total amino acid tissue contribution by at least the amount of the packed cell volume (PCV). They also described specific amino acid patterns that were not entirely related. For

example, glutamate in the sheep RBC was more concentrated than in plasma while glutamine was not detectable in the erythrocyte. The RBC also does not contain arginine (Heitmann and Bergman, 1980a; McCormick 1980).

This lack of arginine is interesting when considered in context with the known presence of arginase in the RBC (Eyob et al., 1968). Reports in the literature are not available as to whether this lack of arginine is due to immediate catalytic conversion of arginine to urea and ornithine or the inability of arginine to pass through the RBC membrane.

Examination of RBC function in amino acid transport under different physiological and nutritional conditions have been reported. Snyderman et al. (1970) considered RBC amino acids of limited importance relative to plasma in the diagnosis of protein deficiency in humans. Similar conclusions were drawn by Mikhail et al. (1973) in studies on children afflicted with severe protein-calorie malnutrition. In contrast, the RBC in chickens fed protein-free diets (Stephens and Evans, 1971) appeared to carry a substantial proportion of the free amino acids and depletion patterns between plasma and the RBC were not symmetrical.

A series of experiments in rats (Coleman, 1980) and humans (Coleman et al., 1980) subjected to different dietary protein regimes suggest that the RBC is more sensitive to

changes in levels of the essential amino acids. Thus, the RBC in these cases proved to be a better indicator of protein nutritional status than the traditional evaluation of plasma amino acids.

McCormick (1980) compared RBC and plasma free amino acid arteriovenous differences across the hindlimb of fed and fasted calves. This work also indicated a differential role for the RBC under these conditions. During fasting the RBC increased its total carrying capacity of amino acids by 25 percent. Furthermore, failure to monitor RBC free amino acids in fed animals would have resulted in an underestimation of amino acid flux by approximately 30 percent. This agrees closely with the PCV underestimation expressed by Heitmann. However, failure to account for RBC amino acids during the 72-hr fast would have given an underestimation of nearly 50% of the total tissue amino acid flux.

It is thus evident from these results that studies of protein turnover or amino acid tissue and organ fluxes that fail to account for the amino acid concentrations of cellular components in the blood likely result in underestimations of total free amino acid contributions. Furthermore, even greater differences become apparent when animals are exposed to physiological or nutritional stress.

The mechanisms involved in the trans-membrane movement of amino acids in the RBC are poorly understood. Reiser (1962) indicated that the entry rate of amino acids into the RBC was greatly affected by the structure of the amino acid. Oxender and Christensen (1963) determined that entry rates of neutral amino acids into the RBC were closely related to the size of the hydrocarbon side chain. In addition, they also identified three different transport mediating systems in the RBC membrane that were involved in the transport of neutral amino acids. The systems were defined as follows: (a) L-system (leucine preferring); (b) A-system (alanine preferring); and (c) imino acid and glycine system. However, there is considerable overlap and non-specifity associated with these systems with in vitro analysis techniques.

Several other transport systems associated with the RBC have also been reported (Christensen, 1973). The γ-glutamyl cycle has been proposed as one of the systems that functions in the transport of amino acids across cell membranes (Meister and Tate, 1976). The presence of glutathione (γ-glutamylcysteinylglycine) in the RBC and not plasma is indicative of red cell membrane glutathione synthesis. Furthermore, γ-glutamyl cycle enzymes have been found in rabbit reticulocytes and human erythrocytes. The γ-glutamyl cycle has several features that link it with other amino acid transport

systems. These include cell membrane amino acid binding sites, carrier-mediated translocation. carrier release of amino acids into intracellular spaces and energy dependency for reactivation of the carrier (Meister and Tate, 1976).

It appears that the significance of amino acid transport and function within the RBC may be related to aging factors involved in the maturation of the erythrocyte. Several studies have noted severely curtailed activity of transport systems, increased length of time of amino acid uptake from in vitro mediums and lowered capacity to concentrate amino acids against chemical gradients as the cells change from reticulocytes to mature erythrocytes (Levy and Barkin, 1971).

The cellular nature of the mature RBC is quite different from that of the reticulocyte. Mature mammilian RBC's do not contain nuclei, mitochondria, ribosomes or m-RNA. Therefore, specific amino acid requirements for cellular function are unneeded since protein synthesis does not occur (Young and Ellory, 1977). However, the synthesis of glutathione proceeds throughout the life of the RBC and specific requirements for glycine, glutamate and cysteine are required for the continued biosynthesis of glutathione (Grimes, 1980). Elwyn et al. (1968) described the gut and liver as the principal sites of glutathione synthesis. In

these studies the RBC functioned exclusively in the transport of glutathione to peripheral regions. This indicates that a transport system functioning in the uptake and removal of glutathione from the RBC is needed. Srivastava (1977) has confirmed the presence of a glutathione transport system in the RBC membrane that requires energy and apparently is active rather than passive in nature.

Research on mechanisms of RBC amino acid transport are basically confined to the above discussed areas. Attempts to directly determine the causes of the observed differential roles of RBC amino acid transport relative to different nutritional states are not availabe in the literature. It is thus difficult to either refute or confirm many of the phenomenon that have been observed.

Peptides

Studies on the biological and nutritional roles of peptides are currently in vogue. Many different and varied functions in various tissues and organs have been proposed. Some of these include neurotransmittor activity in the brain (Margolis, 1974), the many functions of glutathione related to maintenance of cellular integrity (Meister and Tate,

1976) and quantitative and qualitative absorption of amino acids in the gut (Matthews, 1977).

Currently interest is also being generated in assessing the contribution of circulating peptides in concert with free amino acids in the interorgan transport of amino acids and nutriture of muscle tissues.

Generally, peptides are present in blood plasma in very low amounts (Mathews and Payne, 1975). However, Elwyn et al. (1968) identified two peptides (glutathione and carnosine) that were readily apparent in blood and appeared to be functioning in the interorgan movement of amino acids. For example, glutathione was synthesized primarily in the gut and liver and transported to peripheral areas by the erythrocyte. They considered the significance of this glutathione transport as a possible mechanism for conserving cysteine in a relatively nonreactive form as it is being transported to peripheral areas. Upon reaching target tissues the glutathione is catabolized thus yielding cysteine to the tissue. Simarly, carnosine (B-alanylhistidine, a dipeptide often associated with muscle protein) was released by the liver and taken up by peripheral tissues perhaps signifying a means of interorgan histidine protection and transfer.

McCormick (1980) monitored amino acid arteriovenous differences of hydrolyzed plasma peptides across the hindlimb of fed and fasted calves. He noted a substantial uptake of peptide amino acids in fed animals and a large release of peptide amino acids in fasted animals. Specific peptides involved were not identified in this study. However, the exchanges noted for the hydrolyzed amino acids of the peptide fraction implicate this pool as having some as yet undefined significance in the transport of amino acids between various organs and tissues.

Matthews (1977) provides a complete discussion on intestinal absorption of peptides. Several peptide transport systems have been identified in the mucosal cells of the small intestine which facilitate large uptakes of peptides by gut tissues. However, it has been shown that peptides in the blood are not of dietary origin. It appears that peptide hydrolases found in the cytosol of the enterocyte are responsible for the very rapid hydrolysis of peptides after they have been absorbed (Matthews and Payne, 1975). These factors likely account for the failure to note accumulations of peptides within the cells and the failure to detect increased peptide levels in portal blood (Bergen et al., 1978).

The importance of peptides isolated from blood thus far are only beginning to be realized. Many functions have been attributed to glutathione, however, the role of carnosine is unknown. Carnosine has been found in significant levels in muscle and distributed in other areas of the body including the brain (McGilvery, 1979).

These and other peptides exhibit a unique property that distinguish them from other protein-like substances. Glutathione and carnosine, the posterior pituitary hormones (oxytocin and vasopressin), the hypothalamic factor (TRF) and bradykinin are examples of peptides or polypeptides that are synthesized enzymatically rather than by translation (Lehninger, 1975). Substances of this nature are usually synthesized for specific purposes and contain bonding properties unlike those of normal proteins and peptides which are essential for preventing premature degradation by non-target tissues. These properties are indicative of very specific metabolic and regulatory roles and the importance of these compounds in terms of contribution to the amino acid nutrition of tissues is unknown.

There are indications of additional sources of peptides, polypeptides, or oligopeptides that are not of dietary or enzymatic origin. Coulsen and Herbert (1974) reported that the interruption of protein syntheses results

in incomplete protein chains. In nutritional stress situations where amino acid levels are low or imbalances may be present, the synthesis of protein chains may be halted due to the lack of a specific limiting amino acid resulting in peptides, polypeptides or incomplete proteins. This report suggests several interesting connotations: (a) greater levels of circulating peptides may be playing a larger role in periods of nutritional inadequacy, and; (b) this may help to explain the often observed phenomena of low plasma levels of limiting amino acids in unbalanced diets (Harper, 1970).

The significance of blood peptides as vehicles for interorgan amino acid transport are currently unknown. Reports showing a general lack of small peptides in the blood make it difficult to perceive a large role being played by circulating peptides in the transfer of amino acids across tissue beds. The results presented by McCormick (1980), however, implicate peptides as playing a major role in the transfer of amino acids across the hindlimb under imposed 'steady-state' and fasting conditions. Similar studies have not been conducted examining these particular factors. Therefore, firm conclusions are unable to be drawn relative to this topic in the absence of further supportive material.

Blood Proteins

The role of blood proteins in the transport of amino acids between organs was alluded to by Katz et al. (1961) and Peters (1970) who determined that over 50% of the albumin produced in the liver was catabolized by peripheral tissues. Elwyn (1972) stated that plasma proteins are synthesized primarily by the liver and degraded largely in peripheral tissues. Proteins in the blood are of many different types (globulins, albumin, lipoproteins, glycoproteins, etc.), however, albumin comprises as much as 50% or more of the total protein in blood (Rothschild et al., 1972).

Analysis of changes in blood proteins is a common measurement taken to monitor or assess differing nutritional or physiological states. This appears to be a quantitative change rather than qualitative as as indicated by Virtanen (1966). As previously stated, he showed that the amino acid composition of various whole blood protein fractions were unaffected by dietary treatment. Elwyn (1966) speculated that the contribution of these proteins to the amino acid nutrition of peripheral areas could be equal to or much greater than that of circulating free amino acids in normal meat-eating dogs. Changes in the levels of these blood proteins due to altered nutritional states could have profound

effects upon the total amino acid nutriture of peripheral tissues.

Adibi (1976) summarized results from several experiments and showed that after 8 days of starvation, 25% of muscle protein and 65% of liver protein is depleted indicating that amino acids from all tissues are being mobilized to provide energy. Eight days of protein deprivation, however, resulted in minimal losses in muscle protein content but liver protein was decreased by 30 percent. This could indicate that proteins produced in the liver are acting as amino acid supplements for maintenance of protein synthesis in the muscle. It is thus generally believed that blood proteins are very important in amino acid transport although very few studies have been conducted on different tissue and organ sites to more accurately assess this additional source of circulating amino acids.

McCormick (1980) monitored amino acid changes across the hindlimb of several grossly separated serum proteins in 'steady-state' and 72-hr fasted steers. Results are interesting from the standpoint that similar studies on specific amino acid fluxes of blood proteins across any tissues have not been presented. The amino acid composition of a mixed albumin fraction appeared to be affected by fasting. Total amino acid concentration for this albumin fraction in fasted

animals was depressed. In addition, a differential response to fasting was noted between a non-specific globulin fraction and the mixed albumin fraction in terms of flux across the hindlimb. In the fed state, most of the amino acids in the globulin fraction were at greater levels in venous blood vs arterial blood indicating an output of amino acids from the hindlimb into this particular fraction. Conversely, the albumin fraction generally demonsrated an uptake of amino acids by the hindlimb. Upon fasting both fractions demonstrated a pronounced release of amino acids from the hindlimb.

Large outputs of amino acids during fasting are consistent with previous data indicating general protein catabolism during fasting. The interpretation of these findings result in several observations that can not be substantiated from previous research endeavors but are unique and possibly significant owing to the fact that no other research of this type is available for comparison. These interpretations are as follows: (a) by some mechanism the actual composition of the blood proteins are altered as they pass through a tissue bed; (b) various free amino acids in rather large amounts are being tightly bound or adsorbed to the proteins.

There are reports that blood proteins have the ability to exogenously bind tryptophan (McMenamy and Olney, 1958)

and polypeptide chains (Tritsch et al., 1968). Binding of other amino acids by blood proteins have as yet not been shown.

It is obvious from this discussion that plasma accounts for only a portion of the amino acids that are transported in the blood. The RBC is better defined than other pools that contribute to amino acid transport. However, specific relationships between these different pools as they function relative to varying nutritional status are not clearly defined. In addition, information regarding muscle metabolism and amino acid transport in ruminants in varying nutritional status are limited.

Chapter III

OBJECTIVES

The objectives of this study were to evaluate the relationship and importance of plasma, erythrocye, peptide and serum protein amino acid pools in the transport and hindlimb exchange of amino acids in young calves fed soy or urea purified diets.

Chapter IV

TRANSPORT AND HINDLIMB EXCHANGE OF PLASMA AND ERYTHROCYTE AMINO ACIDS IN CALVES FED SOY AND UREA PURIFIED DIETS

INTRODUCTION

Feeding of high levels of urea to ruminants frequently results in growth depression and decreased nitrogen retention. Improvement of these factors with increased length of time on feed describe the so-called 'urea adapation' phenomenon (Oltjen, 1968).

Changes in plasma free amino acids are noted in response to dietary nonprotein nitrogen (NPN) supplements. Total levels of essential amino acids (EAA) are depressed and nonessential amino acids (NEAA) have been shown to decrease (Virtanen, 1966), remain unchanged (Oltjen and Putnam, 1966) or increase (Bergen et al., 1973) resulting in various degress of narrowing of the essential/nonessential (E/NE) ratio. Generally, improvements or changes of amino acid patterns have not been demonstrated for animals fed urea for extended periods of time (Ludwick et al., 1972). Efforts to determine the causes of reduced performance and site(s) of metabolic adaptation have focused primarily on

the rumen (Chalupa, 1972) and liver (Chalupa et al., 1970; Ludwick et al., 1972; Prior et al., 1972). Very little attention has been applied to the muscle tissue response in the feeding of urea to ruminants. Skeletal muscle is known to be very important in the maintenance of homeostasis and adaptation to nutritional inadequacies in protein- deficient monogastrics (Munro, 1969a) and in fasted monogastrics and ruminants (Felig, 1975; Bergman and Heitmann, 1978; McCormick, 1980). A depressed E/NE ratio in the muscle of sheep fed low protein diets has been observed (Bergen et al., 1972). This is due mainly to decreased levels of the branched-chain amino acids which indicates that changes in muscle tissue may be occurring.

The evaluation of amino acid transport and metabolism has traditionally been accomplished by measuring plasma free amino acids. However, recent evidence indicates that erythrocyte (RBC) free amino acids are functioning in both qualitative and quantitative aspects in the interorgan transport of amino acids (Elwyn, 1966; Elwyn et al., 1972; Aoki et al., 1974; Heitmann and Bergman, 1980b; McCormick, 1980). Controversy exists over the relative contribution of the RBC in terms of evaluating amino acid nutrition in ruminants (Heitmann and Bergman, 1980b). However, it has recently been shown that the RBC functions differently in 72-hr

fasted steers and failure to account for RBC contributions result in large misrepresentations of amino acid fluxes across skeletal muscle (McCormick, 1980).

Since little information is available on amino acid muscle metabolism in growing ruminants fed high urea levels this study was designed to monitor free amino acid fluxes across the hindlimb (muscle) in young Holstein steer calves fed soy and urea based purified diets for 30-day periods. Particular emphasis is given to the role of the RBC relative to plasma in the transport and exchange of free amino acids across the hindlimb.

EXPERIMENTAL PROCEDURE

Animals and Feeding

Holstein steer calves ranging in weight from 110 to 177 kg and age from 3 to 6 months were used in this study. Three to 6 weeks prior to the beginning of the experiment animals were castrated, vaccinated for blackleg, injected with vitamins A and D[1] and Vitamin E and the mineral

[1]Administered intramuscularly: Vitamin A, 1.5 million IU; Vitamin D, 225,000 IU.

selenium[2] and treated for internal parasites.

Animals were housed in confinement stalls under constant lighting conditions on slatted floors and provided with fresh water from an automatic nipple watering system. Feeding was done by an automatic feeder which provided 24 hourly meals per day. This feeding technique appears to achieve near 'steady-state' conditions of ruminal fermentation, gastrointestinal passage, rates of absorption and concentrations of blood metabolites (Minson and Cooper, 1966, Wolff and Bergman, 1972).

Dietary treatments imposed in this study were composed of purified ingredients with either soy protein or urea constituting the total nitrogen intake (Oltjen and Putnam, 1966). The composition of the experimental diets are listed in table 1. The purified ingredients were divided into three subgroups; wood pulp, mineral mix and a concentrate mix including all other ingredients (see Appendix A). The mineral mix was periodically premixed in large batches (14 kg) and stored in airtight containers. Thorough mixing was effected in a bowl-type food mixer. The concentrate mix was prepared in 70 kg batches in a horizontal mixer and stored in an airtight container. This provided for sufficient amounts of all dietary components for one animal during an

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[2]Administered intramuscularly: Vitamin E, 680IU; Selenium, 10 mg.

entire test period. The mixing of daily feed allotments was performed in a bowl-type mixer and preceded actual feeding by 1 to 5 days. The daily feed allotment was then subdivided into 24 equal portions. Each diet was fed to four animals at a level of 2% body weight for 30 days.

Following surgery animals were reacclimated to the pretest diet[3] over a 3 to 5 day period. When a 'normal eating level' was reached, a 5-day transition to the purified diet was initiated. A 10% incremental change between the two diets occurred at 12-hr intervals as the animals were fed the hourly feed allotments until only the purified diet was offered.

Surgical Preparation

Abdominal aorta and caudal vena cava cannulae were surgically implanted by techniques described by McGilliard (1972) and modified by McCormick (1980). A detailed description on cannulae preparation is given in Appendix B. Prior to surgery animals were subjected to a 24-hr fast. Animals were induced into a state of anesthesia[4] and the

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[3]Diet composition (%): ground corn, 50%; chopped orchard grass hay, 30%; soybean meal, 13.3%; molasses, 5%; limestone .78%; deflourinated rock phosphate, .42%; trace mineral salt, .5%.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

Item	Diet	
	Soy	Urea
	%	%
Cornstarch	23.3	28.4
Glucose [1]	23.3	28.4
Wood Pulp [2]	30.0	30.0
Isolated Soy Protein [3]	14.9	0
Urea [4]	0	4.7
Refined Corn Oil [5]	2.0	2.0
Choline Chloride	0.1	0.1
Mineral Mix [6]	6.4	6.4
Vitamins	+	+

[1] Cerelose, Corn Products Co., Argo, IL.

[2] Solka-floc, BW-20, Brown Co., Berlin, NH.

[3] Assay protein, RP-100, Ralston Purina Co., St. Louis, MO.

[4] Reagent grade urea, Fisher Scientific Co., Fair Lawn, NJ.

[5] Mazola Oil, Corn Products Refining Co., New York, NY.

[6] Oltjen et al., 1966, (see Appendix A).

cannulae were inserted into the aorta and vena cava via the fermoral artery and vein and/or the circumflex illiac artery and vein. The venous 'sampling' cannulae and arterial 'infusion' cannulae were positioned posterior to the renal vein and artery. Arterial 'sampling' cannulae were placed anterior to the arterial infusion cannulae.

Following recovery from surgery, the cannulae were flushed with 10 ml of an anticoagulant/antibacterial solution.[5] This flushing procedure was rigorously adhered to at 12-hr intervals throughout the entire experiment. Animal recovery following surgery was monitored by observation of rectal body temperature, hematocrit stabilization and animal eating habits. Intramuscular antibiotic[6] injections of 6 to 10 ml/day were given until rectal temperatures had stabilized at 38 to 39 C and signs of external swelling had diminished. Strict asepsis was maintained in all phases of these procedures with the result of minimal infection and near complete recovery within 3 to 5 days.

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[4] 1 to 3% Fluothane (Bromochlorotrifluorethane), Ayerst Laboratories, New York, NY.

[5] 40 units heparin and 2.4 mg penicillin/ml in .9% sterile saline.

[6] Combiotic, Pfizer Agricultural Div., New York, NY.

Sampling and Analysis Procedure

Simultaneous arterial and venous blood samples were obtained at day 10 and 30 within each respective dietary treatment. Samples were collected on any given sampling day between 0900 and 1200 hours. Four successive samples of heparinized and non-heparinized blood were taken at 15-min intervals over a 1-hr period between successive feedings. Approximately 350 ml were withdrawn for each sampling period.

Immediately following blood withdrawal protein-free filtrates of plasma were prepared by adding 1 ml of 20% sulfosalicylic acid to 4 ml of plasma, shaking vigorously, cooling, centrifuging (174 g) and filtering through glass wool. Filtrates from the four samples obtained were composited on an equal volume basis, flushed with nitrogen and stored at -10 C for later analysis of plasma free amino acids.

Erythrocyte free amino acids were determined by precipitating the proteins from 5 ml whole blood with 5 ml 10% sulfosalicylic acid (Joo et al., 1975). Filtrates from the four samples were composited on an equal volume basis and 3 ml aliquots were then freeze-dried and stored at -10 centigrade.

Analysis of the composited plasma and whole blood filtrates of arterial and venous samples were performed on an automatic amino acid analyzer[7] equipped with a peak integrator.[8] Amino acid analyses were performed in duplicate and plasma and whole blood samples from a common treatment were analyzed concurrently. Plasma filtrates were applied to the amino acid analyzer requiring no further preparatory steps. Prior to analysis of whole blood samples the freeze-dried filtrate was reconstituted with 1.5 ml .01 N HCL in order to produce an appropriate pH for sample application. Hematocrits were used to determine the volume of erythrocytes in whole blood and it was assumed that 20% of the packed cell volume was plasma (Elwyn, 1966).

Microhematocrits were determined on all whole blood arterial and venous samples using microcapillary tubes. Serum total protein (Oser, 1965) and serum albumin (Rodkey, 1965) were determined from blood serum samples. Blood ammonia nitrogen was determined by microdiffusion analysis (Conway, 1958) from whole blood within 2-hr of sampling time. Determinations of blood urea nitrogen (BUN) were derived from protein-free filtrates of the arterial and venous whole blood samples (Coulombe and Favreau, 1963). Reduced blood

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[7]Model TSM, Technicon Instruments, Tarrytown, NY.

[8]Systems I Computing Integrator, Spectra Physics, Santa Clara, CA.

glutathione was determined on all whole blood samples in procedures described by Buetler et al. (1963). The purity of the glutathione standard[9] was determined iodometrically (Woodward and Fey, 1932) and the appropriate corrections were made.

Statistical Analysis

Differences between soy and urea treatments were analyzed according to a split-plot design using least square procedures (SAS, 1978). Diet and animal within diet (main plot error) were the main plot effects while time, diet by time and residual (sub-plot error) were the sub-plot effects. Arteriovenous differences between paired samples were evaluated by the Student's t-test. A sample analysis of variance is presented in Appendix D.

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[9]Glutathione, reduced, Sigma Chemical Co., St. Louis, MO.

RESULTS AND DISCUSSION

Animal response to surgery, dietary transition and immediate and continued consumption of the hourly feed allotments at the 2% body weight level were very acceptable for all animals used in this study. Weight gains averaged .5 to .6 kg/day throughout the 30 day trials. Amino acid concentrations for plasma and erythrocytes are expressed as um amino acid/dl blood. For discussion purposes, the three letter international symbol for specific amino acids will be used. A complete listing of amino acid groups discussed below is available in Appendix C.

Plasma Free Amino Acid Concentrations

Presented in table 2 The plasma free amino acid concentrations in venous blood from calves fed soy or urea purified diets are presented in table 2. Total concentrations of plasma free essential amino acids (P-EAA): nonessential amino acids (P-NEAA) and the essential/nonessential ratio (P-E/NE) of calves fed the soy-protein based purified diet agree very closely to levels observed under similar experimental conditions in previously conducted studies in this laboratory on calves fed 'normal' (hay/grain) diets (McCormick, 1980).

TABLE 2. PLASMA FREE AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

Amino acid	Diet		P[b]	SE[c]
	Soy	Urea		
	um/dl	um/dl		
Aspartic acid [e,f]	1.68	1.71	.85	.14
Threonine [e]	7.85	4.42	.02	1.01
Serine [e]	10.68	7.93	.08	1.32
Asparagine	4.27	2.10	.02	.66
Glutamic acid	14.57	17.02 [d]	.56	3.53
Glutamine	14.20	15.04 [d]	.72	2.08
Proline	4.12	2.75	.07	.61
Glycine	40.43	32.73	.09	5.87
Alanine	13.79	10.16	.20	2.53
Citrulline	4.90	4.57	.59	.12
Valine	21.32	13.40	.05	3.29
Cysteine [e]	1.57	1.18	.34	.38
Methionine	1.37	1.10	.17	.17
Isoleucine	10.26	6.57	.09	1.81
Leucine	12.57	7.10	.05	.45
Tyrosine	3.61	2.03	.01	.34
Phenylalanine	4.86	2.96	.02	.59
Ornithine	8.00	4.98 [d]	.05	.97
Lysine	8.44	5.63	.02	.88
Histidine	4.41	3.67	.24	.57
Arginine	9.34	6.14	.03	1.14
BCAA	44.15	27.08	.04	7.25
EAA	80.42	50.98	.02	9.74
NEAA	121.83	102.68 [d]	.08	9.51
Total	202.26	152.74 [d]	.04	16.61
E/NE	.66	.49 [d]	.11	.07

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.
[b] Probability of a chance difference between diet means.
[c] Standard error of mean.
[d] Least square mean based on seven rather than eight observations. Standard error is underestimated.
[e] Time effect was significant, see Appendix table 17.
[f] Diet x time interaction, see Appendix table 17.

The feeding of urea resulted in a 24.5% reduction ($P<.05$) of total-free amino acids in plasma (P-Total). This was caused by lower levels of P-EAA ($P<.05$) and P-NEAA ($P<.10$) although the magnitude of depression was more than double for the former (36.6% and 15.7%, respectively). Thus the P-E/NE ratio was lower for urea-fed animals. These results generally agree with gross observations made by previous workers (Virtanen, 1966; Oltjen and Putnam, 1966). However, some specific differences are apparent. Plasma levels of GLY and SER were lower ($P<.10$) in the urea treatment. Generally, these two amino acids are elevated in animals fed urea vs soy purified diets (Oltjen and Putnam, 1966) although Ludwick et al. (1972) found no such differences. It is of interest that GLY was greater in both treatments relative to calves fed 'normal' diets (McCormick, 1980). Also, in contrast to Virtanen (1966) who found MET and HIS limiting in lactating dairy cows, in this study these two amino acids were the only P-EAA that did not show a significant response. However, a trend towards decreased levels in animals fed urea is apparent. Depressions in the other essential amino acids were consistent and statistically significant, ranging from 36.3% to 49.9% below that of animals fed soy protein. The consistency of this response is more indicative of a protein or amino acid deficiency as

it does not fit descriptions associated with specific limiting amino acids or dietary amino acid inbalances (Harper, 1970; Nimrick, 1970; Richardson and Hatfield, 1978).

In no case was any amino acid higher in urea-fed animals. Time effects were noted for three amino acids (Appendix table 17). ASP ($P<.05$) and SER ($P<.10$) concentrations were higher for the 30-day sampling period. This was due mainly to increases observed when soy was fed. Conversely, CYS decreased equally ($P<.05$) from 10 to 30 days in both dietary treatments. A nonsignificant trend toward increased ARG at 30 days was noted for the urea treatment. Explanations of these differences are difficult to make although speculations in association with the roles of ARG and ASP in the urea cycle may be justified. The lack of changes in amino acid patterns over time have been documented (Ludwick, 1972) and these results are in general agreement.

Erythrocyte Free Amino Acid Concentrations

Concentration changes of erythrocyte (RBC) free amino acids in venous blood are presented in table 3. Erythrocyte essential amino acids (R-EAA) were decreased by 30% in the animals fed urea. Erythrocyte-NEAA were unaffected while a

slight decrease in R-TOTAL was noted. Accordingly, the RBC-E/NE ratio was narrowed. Statistical analysis is precluded for these variables due to the compiling of several missing observations. Reductions of specific amino acids relative to urea vs soy feeding are as follows: ILE, 61.7% ($P<.10$); VAL, 61.0% ($P<.05$); MET, 40.9% ($P<.05$); PHE, 34.0% ($P<.10$) and; THR, 23.4% ($P<.10$). Large percentage decreases were also noted for LEU and the nonessentials, ASP and PRO while GLY and CIT increased. These latter responses, however, were nonsignificant. The only NEAA to be significantly affected by treatment was ALA ($P<.05$) which was substantially lower in the urea treatment. The responsiveness of the EAA due to dietary treatment support the concept that the RBC may be more sensitive than plasma to changes in dietary EAA (Coleman, 1978).

Time effects were observed for GLU ($P<.10$) and VAL ($P<.05$) and a diet x time interaction ($P<.10$) for THR was also noted (Appendix table 18). The increase in GLU was substantial and consistent over time for both soy and urea treatments. Similar long-term studies are not available for comparison of the results obtained from the erythrocyte pool, especially in reference to the time effects observed.

Direct comparison of plasma and RBC amino acid concentrations within each diet are shown in table 4. Concentra-

TABLE 3. ERYTHROCYTE FREE AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

	Diet			
Amino acid	Soy	Urea	P [b]	SE [c]
	um/dl	um/dl		
Aspartic acid	6.32	4.47	.26	1.48
Threonine [h]	4.49	3.44	.10	.54
Serine	4.09	3.52	.50	.79
Asparagine	2.72 [d]	2.35	.71	.89
Glutamic acid [g]	8.86	7.84	.76	3.20
Glutamine	--	--	--	--
Proline	4.03	3.22 [d]	.60	1.57
Glycine	36.45	43.94	.60	13.28
Alanine	2.61	.18	.03	.89
Citrulline	.83	1.23 [e]	.72	.63
Valine	4.28	1.67	.05	1.04
Cysteine	--	--	--	--
Methionine	1.86	1.10	.02	.24
Isoleucine	1.93	.74	.07	.55
Leucine	4.85	3.16	.13	.96
Tyrosine	2.10	2.11	.98	.46
Phenylalanine	1.38	.91 [d]	.08	.21
Ornithine	3.04	2.68 [d]	.58	.51
Lysine	4.09	3.68 [d]	.63	.76
Histidine	6.25	5.65	.74	1.72
Arginine	--	--	--	--
BCAA	11.07	5.56 [e]	.06	2.38
EAA [f]	29.13	20.35	.18	4.73
NEAA [f]	71.18	72.23	--	--
Total [f]	100.31	92.58	--	--
E/NE [f]	.41	.28	--	--

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.
[b] Probability of a chance difference between diet means.
[c] Standard error of mean.
[d] Least square mean based on seven rather than eight observations. Standard error is underestimated.
[e] Least square mean based on six rather than eight observations. Standard error is underestimated.
[f] Least square means non-estimable due to missing observations. Values represent column sums.
[g] Time effect was significant, see Appendix table 18.
[h] Diet x time interaction, see Appendix table 18.

tions of EAA, NEAA and TOTAL amino acids were always greater in plasma. Several differences are apparent between the plasma and RBC pools in terms of specific amino acids. ASP ($P<.001$) and HIS ($P<.005$) were more concentrated in the erythrocyte. In contrast, GLN, CYS and ARG were not detected in the erythrocyte. Concentrations of ALA ($P<.001$), ILE ($P<.001$) and VAL ($P<.001$) were much lower in the RBC than plasma when expressed equally on a whole blood basis. GLY was present in the largest amounts and was equal to concentrations in plasma. There were also no differences for PRO and MET between the RBC and plasma pools. These responses were consistent across dietary treatment. Concentrations of the remaining amino acids were usually lower in the RBC although some amino acids (ALA, VAL, ISO) appeared to be depressed to a greater extent in the urea treatment.

Coleman et al. (1980) reported that high protein diets effected a depression in RBC essential amino acids. The results of this experiment show that the urea diet had a similar effect. This may in fact signify a response to high dietary nitrogen rather than protein level.

Comparison of these results to previous findings in cattle (McCormick 1980) indicate that levels of the branched-chain amino acids (VAL, ILE, LEU), CIT and ALA are much lower in calves fed purified diets while GLY is higher.

TABLE 4. COMPARISON OF PLASMA AND ERYTHROCYTE FREE AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

	Soy		Urea			
Amino acid	Plasma	RBC	Plasma	RBC	P [b]	SE [c]
	----- um/dl -----		----- um/dl -----			
Aspartic acid [g]	1.68	6.32	1.71	4.47	.001	.61
Threonine [f]	7.85	4.49	4.42	3.44	.001	.43
Serine	10.68	4.09	7.93	3.52	.001	.64
Asparagine [d,g]	4.27	2.72	2.10	2.35	.05	.53
Glutamic acid	14.84	8.86	17.02	7.84	.001	1.68
Glutamine	14.20	--	15.04	--	--	2.08
Proline [d]	4.12	4.03	2.75	3.22	.77	.75
Glycine	40.43	36.45	32.73	43.94	.81	5.61
Alanine	13.79	2.61	10.16	.18	.001	.74
Citrulline [d]	4.90	.83	4.57	1.23	.001	.30
Valine [g]	21.32	4.28	13.40	1.67	.001	1.42
Cysteine	1.57	--	1.18	--	--	.38
Methionine [h]	1.47	1.86	1.10	1.10	.096	.14
Isoleucine [g]	10.26	1.93	6.57	.74	.001	.77
Leucine [g]	12.57	4.85	7.10	3.16	.001	1.02
Tyrosine [g]	3.61	2.10	2.03	2.11	.025	.29
Phenylalanine [d,f]	4.86	1.38	2.96	.91	.001	.31
Ornithine [d,g]	8.00	3.04	4.98	2.68	.001	.54
Lysine [d]	8.44	4.09	5.63	3.68	.001	.65
Histidine	4.41	6.25	3.67	5.65	.003	.55
Arginine	9.34	--	6.14	--	--	1.14
BCAA [e]	44.15	11.07	27.07	5.56	.001	3.15
EAA [e]	80.42	29.13	50.98	20.35	--	--
NEAA [e]	121.83	71.18	102.68	72.23	--	--
Total [e]	202.26	100.31	152.74	92.58	--	--
E/NE [e]	.66	.41	.49	.28	--	--

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.
[b] Probability of a chance difference between plasma and RBC means.
[c] Standard error of mean.
[d] Standard errors are underestimated due to missing observations.
[e] Least square means non-estimable due to missing observations.
[f] Values represent column sums.
[g] Diet x pool interaction ($P<.01$).
[h] Diet x pool interaction ($P<.05$).
Diet x pool interaction ($P<.10$).

These differences are more accentuated in the urea vs soy treatment.

The absence of certain amino acids in the RBC are unexplained. CYS is a functional component of the γ-glutamyl cycle (Meister and Tate, 1976) and may represent a control or limit on the synthesis of gluthathione by the RBC membrane. The presence of arginase in the RBC (Eyob et al., 1968) may explain the lack of ARG due to immediate conversion to urea and ornithine following passage into the cell. The possibility of arginine synthesis within the RBC is also plausable as evidenced by the plasma/RBC differentials in ASP and CIT which function as intermediates in this pathway. Information is not available to verify this hypothesis.

Arteriovenous differences in plasma amino acids

Plasma free amino acid arteriovenous (A/V) differences across the hindlimb are reported in table 5. Generally, in animals fed soy protein, the hindlimb exibited an uptake (removal) of most amino acids. Uptake ordered according to quantity was noted for GLU, ORN, LEU, ILE, CIT, PRO, ASN, and MET ($P<.10$). VAL ($P<.13$) and ASP ($P<.12$) approached statistically significant uptakes. The branched-chain amino

acids (BCAA) accounted for 75% of the total EAA uptake. Fluxes of the remaining EAA were small and nonsignificant. Output (release) of amino acids from the hindlimb was slight, however, a small release of CYS ($P<.13$) approached significance. Large quantities of GLY and small amounts of GLN release were also noted, however, large standard errors preclude definitive statements.

Comparison of calves fed soy purified diets with those fed 'normal' diets (McCormick, 1980) show similar patterns of uptake and release although the magnitude of flux was much less in the present study. The difference was the failure to detect a large, significant uptake of LYS and release of GLN in soy-fed animals. Heitmann and Bergman (1980a) reported consistent outputs of three gluconeogenic amino acids (GLN, ALA, GLY) and ARG in 'fed', fasted and acidotic sheep and uptakes of GLU, SER, CIT, ORN and LYS. Ballard et al. (1976) demonstrated the release of GLN, ALA and TYR and uptakes of GLU, SER and LYS in 'fed' sheep. The study of McCormick (1980) on 'fed' steers indicated GLN was the only amino acid released in significant quantities while ALA, GLU, LEU, ASN, LYS, VAL and MET were removed from arterial blood. In this study GLY was the only amino acid to exhibit substantial release in animals fed soy protein. In contrast, a large but nonsignificant uptake of ALA by the hindlimb was noted.

TABLE 5. PLASMA FREE AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

Amino acid	Soy A/V [b]		Urea A/V [b]		P [c]	SE [d]
	um/dl	P [e]	um/dl	P [e]		
Aspartic acid	.13	(.12)	-.02	(.76)	.18	.10
Threonine	.07	(.77)	-.45	(.11)	.19	.36
Serine	.17	(.55)	.10	(.72)	.91	.58
Asparagine	.31	(.01)	-.05	(.59)	.01	.10
Glutamic acid	.99	(.03)	.92	(.04)	.90	.53
Glutamine [h]	-.06	(.86)	-.88 [f]	(.06)	.23	.57
Proline	.41	(.03)	.17	(.30)	.36	.24
Glycine	-.63	(.41)	-.43	(.58)	.83	.91
Alanine	.63	(.21)	-.60	(.13)	.21	.87
Citrulline	.45	(.01)	.38	(.02)	.69	.18
Valine	.47	(.13)	-.16	(.57)	.09	.31
Cysteine [g]	-.06	(.13)	.01	(.74)	.42	.08
Methionine [h]	.10	(.10)	.03	(.29)	.30	.07
Isoleucine	.52	(.02)	.05	(.76)	.01	.13
Leucine	.59	(.06)	.01	(.99)	.01	.16
Tyrosine	.03	(.73)	-.04	(.60)	.46	.08
Phenylalanine	.05	(.56)	-.03	(.66)	.12	.04
Ornithine	.68	(.01)	.14 [f]	(.43)	.57	.74
Lysine	.19	(.61)	-.14	(.71)	.40	.35
Histidine	.09	(.48)	.05	(.71)	.87	.26
Arginine	.04	(.93)	-.17	(.55)	.55	.35
BCAA	1.58	(.05)	-.10	(.88)	.01	.49

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[c] Probability of a chance difference between diet means.
[d] Standard error of mean.
[e] Values in ( ) depict probability that A/V difference does not differ from 0.
[f] Least square mean based on seven rather than eight observations. Standard error is underestimated.
[g] Time effect was significant, see Appendix table 20.
[h] Diet x time interaction, see Appendix table 20.

Feeding of urea resulted in the overall depression of amino acid uptake by the hindlimb. Thus a change from a net positive balance in animals fed soy was shifted to near zero of a slightly negative balance of amino acids in animals fed urea. Large decreases in uptake were noted for LEU ($P<.01$), ILE ($P<.01$), VAL ($P<.10$) and ASN ($P<.01$) which changed from large uptakes to essentially a zero flux in the respective soy and urea treatments. PHE changed from a small uptake to that of a small output which approached significance ($P<.12$). ALA and GLN displayed perhaps the most dramatic changes between soy and urea feeding although these responses were not significant.

The bulk of amino acid release by the hindlimb into the plasma pool for calves fed urea was accounted for by GLN ($P<.10$), ALA (NS), GLY (NS) and THR ($P<.12$). Large uptakes of GLU ($P<.05$) and CIT ($P<.05$) were apparent while PRO, ORN and SER showed smalller, nonsignificant positive balances. The EAA accounted for less than 30% of the total release of amino acids into plasma whereas GLN, ALA and GLY account for more than 60% of total output.

A time effect was noted for CYS ($P<.10$) and diet x time interactions ($P<.10$) for GLN and MET (Appendix table 20). The presence of GLN uptake for the 30-day soy treatment explains the failure to observe overall GLN release in ani-

mals fed soy as previously discussed. Gross observation of table 20 indicates posssible changes of amino acid flux over time. Uptake of most amino acids were increased for the 30-day samples from animals fed soy protein while the release of amino acids at 30 days with urea feeding were much larger than those observed at 10 days. These data imply that animals fed soy protein were becomming adapted to their dietary regime via increased uptake and utilization of amino acids for protein synthesis at the tissue level. Animals fed urea, on the other hand, show a more severe negative tissue amino acid balance at the 30-day observation implying possibly continued and accelerated muscle protein catabolism. Previous studies on muscle metabolism and amino acid flux of ruminants fed urea are not available for comparison. However, results reported by Chalupa et al. (1964) associated urea diets with muscle protein catabolism by observing elevated urine creatinine. The magnitude of amino acid output across the hindlimb in calves fed urea is much less than that of calves fasted for 72-hr (McCormick, 1980) indicating a less severe metabolic stress. However, the hindlimb fluxes of plasma free amino acids over a 30 day trial can not account for the adaptation response commonly observed in urea feeding.

Arteriovenous differences in erythrocyte free amino acids

The mechanism of amino acid transfer between the RBC and tissue beds are unknown. For this reason discussion of amino acid fluxes in this section refer to uptake and output from the RBC as it traverses the hindlimb rather than direct movement between the RBC and the tissue bed. Erythrocyte free amino acid A/V differences are presented in table 6.

For soy-fed animals only ILE and MET demonstrated significant releases from the RBC across the hindlimb. All other amino acids were non-significant in their response. Gross observation indicates a general negative balance for most RBC amino acids. This is opposite to the flux observed in plasma and may simply indicate the movement of amino acids from the RBC into plasma in response to the depletion of plasma free amino acids removed across the hindlimb. In animals fed urea, release of MET and ASN were the only amino acids that even approached significance ($P<.14$). The consistent response of MET and the biochemical association of MET and CYS indicate involvement in the -glutamyl cycle and glutathione synthesis which is a proposed transport system of the RBC membrane (Meister and Tate, 1976). Large outputs of ILE ($P<.05$), LEU ($P<.11$) and VAL (NS) were observed in animals fed soy protein but not in those fed urea. GLY also demonstrated substantial dietary flucuations. Extreme ani-

TABLE 6. ERYTHROCYTE FREE AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

Amino acid	Soy A/V [b]		Urea A/V [b]		P [c]	SE [d]
	um/dl	P [e]	um/dl	P [e]		
Aspartic acid	.02	(.93)	-.16	(.46)	.72	.46
Threonine [h]	-.12	(.66)	.17	(.54)	.67	.64
Serine	-.04	(.89)	-.16	(.58)	.77	.38
Asparagine	-.11	(.42)	-.20	(.14)	.76	.25
Glutamic acid	-.48	(.66)	.54	(.62)	.45	1.27
Glutamine	--	--	--	--	--	--
Proline	-.22	(.32)	-.28[f]	(.27)	.69	.14
Glycine	1.54	(.40)	-1.59	(.39)	.11	1.67
Alanine	.02	(.97)	.01	(.99)	.99	.96
Citrulline	-.09	(.68)	-.29	(.18)	.41	.19
Valine	-.43	(.19)	.11	(.71)	.41	.61
Cysteine	--	--	--	--	--	--
Methionine	-.25	(.01)	-.12	(.14)	.36	.13
Isoleucine	-.51	(.07)	.08	(.73)	.05	.24
Leucine	-.55	(.19)	.04	(.93)	.11	.32
Tyrosine	.03	(.76)	.09	(.37)	.67	.14
Phenylalanine	-.09	(.44)	.07[g]	(.60)	.44	.18
Ornithine	-.17	(.55)	.09[g]	(.78)	.49	.10
Lysine [h,i]	.30	(.33)	-.02[f]	(.98)	.40	.84
Histidine	-.17	(.49)	-.32	(.22)	.35	.48
Arginine	--	--	--	--	--	--
BCAA	-1.49	(.11)	.23	(.78)	.15	1.03

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[c] Probability of a chance difference between diet means.
[d] Standard error of mean.
[e] Values in ( ) depict probability that A/V difference does not differ from 0.
[f] Least square mean based on seven rather than eight observations. Standard error is underestimated.
[g] Least square mean based on six rather than eight observations. Standard error is underestimated.
[h] Time effect was significant, see Appendix table 21.
[i] Diet x time interaction, see Appendix table 21.

mal variability associated with this amino acid make interpretations difficult. It is interesting to note, however, that these four amino acids have been linked together in neutral transport systems possibly functioning in the RBC membrane (Young and Ellory, 1977).

Plasma and RBC A/V differences are directly compared in table 7. Amino acid fluxes of plasma and RBC across the hindlimb were in opposite directions for ASN ($P<.05$), PRO ($P<.01$), CIT ($P<.01$) and MET ($P<.10$). A diet x pool interaction was observed for ILE ($P<.01$) and LEU ($P<.05$). In both cases there was substantial uptake from the plasma pool and release from the RBC pool in animals fed soy protein. Conversely, there was only a slight uptake from both pools when urea was fed. Exchange of ALA by the RBC was not apparent. This is in contrast to previous reports which show the RBC to be highly involved in transport and exchange of alanine (Felig et al., 1973; McCormick, 1980). Hindlimb exchange of HIS occurred primarily via the erythrocyte. GLY also exhibited very large RBC exchanges. It is interesting to note the opposite but equally large fluxes of the BCAA for plasma and RBC in animals fed soy protein while exchanges from these pools in urea-fed animals were depressed to very low levels.

TABLE 7. PLASMA AND ERYTHROCYTE AMINO ACID A/V DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

Amino acid	Soy[b]		Urea[b]		P[c]	SE[d]
	Plasma	RBC	Plasma	RBC		
	----- um/dl -----		----- um/dl -----			
Aspartic acid	.13	.02	-.02	-.16	.53	.17
Threonine	.07	-.12	-.45	.17	.54	.35
Serine	.17	-.04	.10	-.16	.46	.31
Asparagine [h]	.31	-.11	-.05	-.20	.05	.12
Glutamic acid	.99	-.48	.92	.54	.23	.74
Glutamine	-.06	--	-.88	--	--	.15
Proline	.41	-.22	.17[e]	-.28	.01	.20
Glycine [g]	-.63	1.54	-.43	-1.59	.70	1.29
Alanine	.63	.02	-.60	.01	.99	.51
Citrulline	.45	-.11[e]	.38	-.29	.01	.11
Valine	.47	-.43	-.16	.11	.38	.35
Cysteine	-.06	--	.01	--	--	.08
Methionine	.10	-.25	.03	-.12	.01	.07
Isoleucine [i]	.52	-.51	.05	.08	.02	.19
Leucine [j]	.59	-.55	.01	.04	.07	.29
Tyrosine	.03	.03	-.04	.09	.45	.09
Phenylalanine	.05	-.09	-.03	.01[f]	.63	.12
Ornithine	.68	-.17	.14[e]	.09	.28	.43
Lysine	.19	.30	-.14	-.07[e]	.85	.49
Histidine	.09	-.17	.05	-.32	.59	.25
Arginine	.04	--	-.17	--	--	.35
BCAA	1.58	-1.49	-.10	.23	.08	.74

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.

[b] Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.

[c] Probability of a chance difference between plasma and RBC A/V differences.

[d] Standard error of mean.

[e] Least square mean based on seven rather than eight observations. Standard error is underestimated.

[f] Least square mean based on six rather than eight observations. Standard error is underestimated.

[g] Soy and urea means differ ($P<.05$).

[h] Soy and urea means differ ($P<.10$).

[i] Diet x pool interaction ($P<.01$).

[j] Diet x pool interaction ($P<.05$).

Across both diets, amino acids were generally released from the RBC. In contrast, plasma free amino acids were taken up by the hindlimb with soy feeding and released with urea feeding. Similar results were shown by McCormick (1980).

Whole blood amino acid exchanges (plasma + RBC) are shown in table 8. Heitmann and Bergman (1980b) have concluded that in sheep RBC and plasma exchanges are similar. Thus the use of only plasma reflects general patterns of amino acid fluxes but underestimates exchange rates and total transport by approximately the extent of the packed-cell volume. This was not the case in the present study. In animals fed soy protein, plasma actually overestimated the total amino acid exchange across the hindlimb by approximately 20 percent. Conversely, plasma underestimated total amino acid exchange by 62% in animals fed urea. These observations are not due to large changes in RBC function. Rather, the greatest effect was noted in the plasma free amino acid pool. Elwyn et al. (1968) reported differential relationships between plasma and RBC pools across the gut and liver in dogs but observed no such differences in peripheral blood.

The use of plasma only would also result in misinterpretations of exchanges of several individual amino

TABLE 8. TOTAL FREE AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

Amino acid	Soy A/V [b]		Urea A/V [b]		P [c]	SE [d]
	um/dl	P [e]	um/dl	P [e]		
Aspartic acid	.15	(.41)	-.18	(.32)	.50	.45
Threonine	-.05	(.87)	-.28	(.35)	.56	.38
Serine	.13	(.59)	-.06	(.82)	.76	.60
Asparagine	.20	(.17)	-.25	(.16)	.06	.22
Glutamic acid	.51	(.62)	1.50	(.19)	.52	1.40
Glutamine	-.06	(.47)	-.88 [f]	(.24)	.04	.44
Proline	.19	(.31)	-.15	(.42)	.29	.29
Glycine	.91	(.50)	-2.01	(.17)	.03	1.00
Alanine	.65	(.25)	-.59	(.29)	.39	1.34
Citrulline [h]	.38	(.01)	.14	(.18)	.14	.14
Valine	.04	(.91)	-.05	(.89)	.82	.35
Cysteine [h]	-.06	(.35)	.01	(.44)	.47	.08
Methionine [h]	-.15	(.05)	-.09	(.17)	.65	.11
Isoleucine	.02	(.93)	.14	(.48)	.56	.19
Leucine	.04	(.90)	.05	(.90)	.99	.26
Tyrosine	.00	(.99)	.06	(.92)	.99	.12
Phenylalanine	-.04	(.72)	-.05	(.71)	.99	.17
Ornithine	.51	(.12)	.14 [g]	(.49)	.65	.49
Lysine	.49	(.05)	-.16	(.78)	.15	.33
Histidine	.26	(.18)	-.27	(.16)	.12	.96
Arginine	.04	(.93)	-.17	(.70)	.34	.66
BCAA	.09	(.91)	.13	(.87)	.96	.66

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[c] Probability of a chance difference between diet means.
[d] Standard error of mean.
[e] Values in ( ) depict probability that A/V difference does not differ from 0.
[f] Least square mean based on seven rather than eight observations. Standard error is underestimated.
[g] Least square mean based on six rather than eight observations. Standard error is underestimated.
[h] Time effect was significant, see Appendix table 22.

acids. ASP and HIS are foremost because of their greater concentration in the RBC relative to plasma. Several other amino acids (ASN, GLY, VAL, MET, ILE, LEU and LYS) also show variable responses in comparisons of plasma, RBC and whole blood amino acid exchange.

Statistical analysis of hindlimb exchange for total free amino acids in whole blood resulted in fewer statistically significant treatment and A/V flux differences.

Other Blood Parameters

Results obtained from the measurement of several other nitrogenous compounds found in blood are presented in table 9. Blood urea nitrogen (BUN) was greater for animals fed urea ($P<.05$). This is a common observation in urea feeding studies (Oltjen, 1968). Decreases in BUN values over time have been associated with the urea adaptation phenomenon (McLaren et al., 1965; Ludwick et al., 1972). In this study no time effects were observed for any of these variables. However, a trend for increased BUN from 10 to 30 days with urea feeding was noted (Appendix table 25). This information coupled with the increased amino acid release observed from the 30-day samples when urea was fed imply that certain

aspects of nitrogen metabolism within the body are becoming further impeded rather than improved.

There were no differences for blood ammonia nitrogen between soy or urea treatments. This has also been shown in previous work (Ludwick et al., 1972). This information adds to concepts of the efficiency of the liver in the detoxification of ammonia in blood (Wolff et al., 1972). Differences in serum total protein and albumin in venous blood were not observed to be influenced by the source of dietary nitrogen. An uptake ($P<.10$) of protein from serum in animals fed soy protein was observed.

Glutathione (GSH) showed significant dietary differences. Urea feeding resulted in decreased GSH ($P<.05$) concentration in venous blood and a large A/V GSH release ($P<.10$) across the hindlimb. Low levels of GSH may possibly be the result of decreased availability of its constituent amino acids (GLU, CYS, GLY). Elwyn (1972) speculated that GSH may function in the interorgan transport of CYS by protecting it in a nonreactive form until target-tissue degradation.

TABLE 9. VENOUS CONCENTRATION AND A/V DIFFERENCE ACROSS THE HINDLIMB OF OTHER BLOOD PARAMETERS FOR CALVES FED PURIFIED DIETS [a]

Item	Soy	Urea	P [b]	SE [c]
Blood urea nitrogen, mg/dl				
Venous	12.47	19.81	.02	2.24
A/V [d]	.22 (.23)	-.09 (.61)	.38	.33
Blood-NH_3, mg/dl				
Venous	2.62	2.66	.92	.44
A/V [d]	-.06 (.45)	.02 (.74)	.34	.08
Serum protein, g/dl				
Venous	5.94	6.10	.86	.78
A/V [d]	.35 (.08)	.13 (.47)	.45	.27
Serum albumin, g/dl				
Venous	3.83	3.93	.77	.36
A/V [d]	.25 (.21)	.09 (.63)	.70	.40
Glutathione, mg/dl RBC				
Venous	79.12	59.03	.05	8.43
A/V [d]	-.07 (.96)	-2.74 (.07)	.13	1.54
Hematocrit, %				
Venous	30.44	27.01	.37	3.57

[a] Main diet effects presented with eight observations per treatment.
[b] Probability of a chance difference between diet means.
[c] Standard error of mean.
[d] Values in ( ) depict probability that A/V differences do not differ from 0.

SUMMARY

Plasma and erythrocyte (RBC) free amino acid concentrations and arteriovenous (A/V) differences across the hindlimb via abdominal aorta and caudal vena cava cannulae were determined on growing Holstein steer calves (130 kg) fed purified diets containing soy protein or urea as the sole source of dietary nitrogen. Calves were fed 24 hourly feedings/day to promote 'steady-state' metabolism. Initial blood samples were obtained at least 15 days post-surgery and each trial lasted 30 days and consisted of four animals per treatment. Urea feeding resulted in a reduction of plasma free amino acid levels caused by a 30% decrease in EAA ($P<.05$) and 16% decrease in NEAA ($P<.10$). All EAA except MET and HIS were significantly affected. The NEAA of the RBC were not affected by urea feeding with the exception of decreased ALA ($P<.05$). However, the erythrocyte EAA were deoressed due to significant decreases in THR, VAL, MET, ILE and PHE. Concentrations of amino acids in the RBC were either equal to or lower than concentrations in plasma. Exceptions were HIS and ASP which were always more concentrated in the red cell. A net positive amino acid balance across the hindlimb for soy fed animals was noted for most plasma free amino acids. GLY release accounted for nearly all of the observed output. Urea feeding resulted in a

small negative amino acid balance due mainly to output of NEAA by the hindlimb. The BCAA ($P<.01$) changed from a very large uptake in animals fed soy protein to essentially a zero flux in animals fed urea. Releases of most of the other EAA were observed but were nonsignificant. Time differences were generally not significant although a tendancy toward greater amino acid output by the hindlimb was apparent at 30 vs 10 day in animals fed urea. The RBC total amino acid flux across the hindlimb showed no treatment differences although specific amino acids behaved differentially. The BCAA changed from large outputs from the RBC in animals fed soy protein to essentially a zero flux in animals fed urea. This study reports data on amino acid transport and muscle metabolism of urea-fed ruminants previously not recorded. In addition, further supportive evidence of both quantitative and qualitative aspects of RBC amino acid transport relative to different dietary regimes are also presented.

Chapter V

TRANSPORT AND HINDLIMB EXCHANGE OF PEPTIDE AND SERUM PROTEIN AMINO ACIDS IN CALVES FED SOY AND UREA PURIFIED DIETS

INTRODUCTION

The exchange of free amino acids in blood has been the traditional method for in vivo evaluation of tissue metabolism and protein turnover. There is increasing evidence, however, indicating additional amino-N pools in circulating blood that contribute to the amino acid nutriture of tissues and organs. Absorption of dietary amino acids by the gut and near complete removal of these amino acids by the liver led Elwyn (1972) to postulate that certain peptides and proteins synthesized by the gut and liver are functional in the transport of amino acids to peripheral tissues.

The nutritional significance of peptides is unclear. Intestinal uptake of peptides of dietary origin is believed to play an important role in amino acid absorption by the gut (Matthews and Payne, 1975). However, peptides of dietary origin are generally not found in portal blood (Bergen et al., 1978). Thus, circulating peptides and possibly small proteins likely result from enzymatic formation as is

the case with glutathione (Meister and Tate, 1975) or possibly through interruption of protein synthesis which may yield incomplete protein fragments (Coulsen and Herbert, 1974). McCormick (1980) demonstrated large uptakes across the hindlimb in 'steady-state' calves and large outputs in fasted calves of amino acids from a non-descript peptide and/or small protein pool in plasma which was not associated with free amino acids. These findings clearly indicate tissue exchange of amino acids from pools other than free amino acid pools.

The amino acids of serum proteins constitute a very large proportion of total amino acids found in blood, yet the nutritional significance of these proteins is unknown. Schimke (1977) postulated that a lysosomal based "intracellular autophagy" system becomes activated under conditions of massive protein degradation. These conditions exist in fasting and to a lesser extent in protein deficiency.

Results by McCormick (1980) on fed and fasted steers have shown differing hindlimb exchanges of amino acids from gel-filtrate separated protein fractions of serum proteins in fed animals. Upon fasting, amino acid concentrations of these fractions in venous blood became greatly elevated. This indicates a conceptual involvement of serum proteins in the mass mobilization of amino acids from peripheral areas in response to starvation.

Depression of serum proteins and specifically albumin are classically associated with severe protein deficiency (Arroyave et al., 1962). Singh and Sawhney (1967) reported that serum protein levels decreased as urea intake in sheep increased. Ludwick et al. (1972) showed decreasing serum protein and albumin levels over time in lambs fed soy and urea purified diets and levels of both were progressively lower for lambs fed urea as to the sole nitrogen source.

Information on the tissue flux of blood protein amino acids in response to these nutritional conditions are not available. Therefore, the purpose of this experiment was to evaluate the hindlimb flux of amino acids associated with peptides and blood proteins in young calves fed soy protein and urea purified diets.

EXPERIMENTAL PROCEDURES

Animals, feeding regime, surgical techniques, sampling procedures, experimental design and statistical analyses are as described in Chapter IV. The peptide amino acid fraction was generated from plasma free amino acid filtrates described in the previous chapter. Duplicate aliquots (500 ul) of the plasma filtrate were placed in 2 ml ampules and dried

under vacuum.[10] One hundred ul of 4N methanesulfonic acid was then added to each ampule which was hermetically sealed and subjected to hydrolysis for 22 hr at 115 C (Simpson et al., 1976). This procedure results in the release of amino acids from peptides and small proteins remaining in sulfosalicylic acid filtrates of plasma. The pH of the resulting hydrolysate was adjusted to 2.0 by the addition of 500 ul of .7N LiOH and filtered through glass wool. Amino acid analyses were performed (chapter IV) and the values observed were corrected for contributions of the free amino acids present prior to hydrolysis.

Serum protein amino acids were determined on two distinct protein fractions by ion-exchange chromatography. Two milliliters of serum were first desalted by gel-filtration chromatography in a 1.5 x 15 cm Sephadex G-25M desalting column[11] and the protein eluted with 3.5 ml of acetate buffer (I=25mm/1, pH=5.0). Three milliliters of the desalted protein solution were then applied to a 2.6 x 9.5 cm DEAE-Sepharose CL-6B[12] ion exchange column (Curling et al., 1977). Separation with acetate buffers (pH 5.0, I=25; pH

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[10]Speed Vac-Concentrator, Savant Instruments, Long Island, NY.

[11]Column PD-10, Pharamacia Fine Chemicals, Uppsala, Sweden.

[12]Anion Exchanger, Pharamacia Fine Chemicals, Uppsala, Sweden.

4.65; I=25; pH 4.0, I=100) resulted in the resolution of three distinct peaks. Optimum peak resolution was achieved with buffer transitions at 95% transmittance. The eluted protein peaks were scanned by a UV-Monitor[13] at 280 mu and separated into 20 ml fractions by a fraction collector.[14] The effluent volumes of these four fractions were recorded and the corresponding protein contents were determined (Lowry et al., 1951). Amino acid analysis was performed only on the first two fractions (I & II) eluted from the column. This is due to results from McCormick (1980) that indicate fraction I (primarily globulins and small quantities of albumin and mixed globulins) and fraction II (primarily albumin) were most consistently involved in amino acid transport.

Protein fraction hydrolysates of fraction I and II were prepared in a similar manner as described for plasma peptide amino acids with the following exceptions. Approximately 500 ug of protein from each fraction were hydrolyzed with 4N methanesulfonic acid. The pH was adjusted to 2.0 with 1000 ul of .35N LiOH. This solution was filtered through glass wool and 100 to 200 ul were then analyzed for amino acid

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[13]Model UA-5 Absorbance Monitor, Instrument Specialties Co, Lincoln, NE.

[14]Model 328 Fraction Collector, Instrument Specialties Co., Lincoln, NE.

concentration. Complementary arterial and venous protein fractions were hydrolyzed simultaneously and arteriovenous differences across the hindlimb were determined.

RESULTS AND DISCUSSION

Peptides

Peptide fraction (peptide) amino acid concentrations in venous blood of calves fed soy or urea purified diets are presented in table 10. The total concentration of amino acids in peptides was approximately 30% greater than levels of free amino acids in whole blood (chapter IV). No significant differences were noted due to dietary treatment. A tendency was observed for levels of most amino acids and especially the EAA to be lower when urea was fed. MET and TYR were higher ($P<.05$) for both treatments at 30 days than at 10 days.

A diet x time interaction ($P<.10$) was observed for MET due to a lesser increase from free amino acid pools. Care must be taken in interpreting these data as representing purely 'peptides' in the blood. It is known that deprotein-

TABLE 10. PLASMA PEPTIDE AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE [b]
	10-day	30-day	10-day	30-day	
	----- um/dl -----		----- um/dl -----		
Aspartic acid	42.65	43.76	33.40	37.22	2.10
Threonine	27.09	27.16	20.81	22.45	1.27
Serine	33.85	36.33	29.16	30.69	2.21
Glutamic acid	66.64	69.40	60.90	61.40	4.15
Proline [c]	15.21	27.97	21.62	33.28	4.88
Glycine	58.84	49.49	48.69	49.83	9.87
Alanine	29.75	28.95	21.83	23.36	1.36
Valine	17.92	18.25	12.18	13.66	1.59
Methionine [e,f]	1.31	2.76	1.76	2.05	.30
Isoleucine	15.77	15.92	9.60	11.38	1.06
Leucine	37.39	37.97	28.75	31.98	1.79
Tyrosine [e]	7.48	9.64	6.66	7.16	.49
Phenylalanine	20.54	20.52	14.82	16.62	1.27
Lysine	33.14	30.91	22.76	26.69	2.46
Histidine	14.93	9.76	7.49	8.83	2.39
Arginine	16.22	16.14	13.39	14.85	1.40
EAA [d]	184.31	179.39	131.56	148.51	9.84
NEAA [d]	254.42	265.54	222.26	242.94	--
Total [d]	438.73	444.93	353.82	391.45	--
E/NE [d]	.72	.68	.59	.61	--

[a] Mean concentration of four animals per treatment. Expressed as um/dl blood.
[b] Standard error of mean.
[c] Least square means based on several missing values per observation. Standard error is underestimated.
[d] Least square means non-estimable due to several missing observations. Values represent column sums.
[e] Means for 10- and 30-day samples differ ($P<.05$).
[f] Diet x time interaction ($P<.10$).

ization with sulfosalicylic acid leaves traces of protein in plasma filtrates (Dickensen et al., 1965).

Hindlimb exchanges of peptide amino acids are presented in table 11. Several amino acids were removed from this fraction across the hindlimb of animals fed soy protein. A very large uptake of GLU ($P<.10$) and smaller uptakes of LYS ($P<.05$), HIS ($P<.01$) and VAL ($P<.10$) were observed. GLY was also taken up in large amounts, however, large variation associated with this amino acid precluded statistical response. GLY and GLU accounted for nearly 80% of the total amino acid uptake across the hindlimb in animals fed the soy diet.

Arteriovenous differences were generally of a lesser magnitude when urea was fed. The tendency was for all amino acids to be removed by the hindlimb except for ASP, SER and GLU. The uptake of ILE ($P<.09$) was the only difference observed to be significant.

McCormick (1980) found much larger uptakes of most amino acids in this peptide fraction in 'steady-state' calves of which ASP, SER, GLU, ALA and PHE made the greatest contribution. However, the variability associated with his measurements make interpretations difficult. Variability of measurements in the present study were much less and the magnitude and extent of arteriovenous differences were also

TABLE 11. PLASMA PEPTIDE AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS [a]

Amino acid	Soy A/V [b]		Urea A/V [b]		P [c]	SE [d]
	um/dl	P [e]	um/dl	P [e]		
Aspartic acid	.41	(.44)	-.38	(.47)	.36	.80
Threonine	-.14	(.70)	.39	(.31)	.50	.75
Serine	-.32	(.52)	-.40	(.44)	.95	1.21
Glutamic acid	2.17	(.07)	-1.24	(.26)	.01	.77
Proline [g]	-.13	(.89)	.44	(.59)	.54	.45
Glycine	2.28	(.52)	.36[f]	(.86)	.11	1.60
Alanine	.13	(.77)	.17	(.71)	.96	.65
Valine [h,i]	.27	(.08)	.15	(.29)	.85	.66
Methionine	.05	(.78)	.00	(.99)	.81	.20
Isoleucine [h]	.08	(.49)	.22	(.09)	.83	.63
Leucine	-.20	(.57)	.02	(.96)	.81	.88
Tyrosine	-.39	(.14)	.08	(.69)	.33	.41
Phenylalanine	.00	(.99)	.13	(.52)	.73	.35
Lysine	.72	(.04)	.39	(.21)	.60	.60
Histidine	.70	(.01)	.14	(.44)	.31	.51
Arginine [h]	.01	(.98)	.39	(.20)	.51	.55

[a] Main effects of diet across time presented with eight observations per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[c] Probability of a chance difference between diet means.
[d] Standard error of mean.
[e] Values in ( ) depict probability that A/V difference does not differ from 0.
[f] Least square means based on seven rather than eight observations. Standard error is underestimated.
[g] Least square means based on several missing values per treatment. Standard error is underestimated.
[h] Time effect was significant, see Appendix table 26.
[i] Diet x time interaction, see Appendix table 26.

lower. The large fluxes of GLU observed in both studies, however, suggest that the peptide fraction may be very actively involved in the transport of GLU to peripheral tissues.

Comparison of soy and urea treatments reveal very few differences due to diet. Exceptions included GLU ($P<.01$) which was removed from this fraction in large quantities when soy protein was fed and was released into this fraction when urea was fed. Additionally, the uptake by the hindlimb of GLY ($P<.11$) was much less pronounced when urea was fed. Time and diet x time interactions are presented in Appendix table 26. VAL ($P<.15$), ILE ($P<.10$) and ARG ($P<.10$) exchanges were positive and of greater magnitude in 30-day vs 10-day samples. VAL also exhibited a diet x time interaction ($P<.10$) resulting from a relatively greater uptake at 30 vs 10 days when the soy diet was fed compared to the urea diet. Consistent uptakes of LYS and HIS were noted for all sampling times. These responses may indicate additional means for tissue accumulation of these two amino acids.

McCormick (1980) reported the peptide fraction to be highly involved in the transport of amino acids away from the hindlimb in fasted steers. The data from the present study are not as conclusive although it does appear as if this peptide fraction is playing some as yet undefined role in amino acid tissue exchange.

Serum Proteins

Amino acid concentrations of serum protein fraction I (primarily globulins) and II (primarily albumin) are presented in tables 12 and 13, respectively. MET in fraction I was the only amino acid in either fraction that responded to dietary treatment. It was observed to be present in greater ($P<.10$) concentrations when soy protein was fed. When urea was fed (table 12) there was a tendency for the concentrations of all amino acids associated with fraction I to be higher in the serum at 30 compared to 10 days.

Conversely, there was a tendency for the concentrations of all amino acids associated with fraction II (table 13) to be lower in serum for both dietary treatments at 30 compared to 10 days. These declines in fraction II amino acids were significant for ASP ($P<.05$), THR ($P<.10$), SER ($P<.10$), GLU ($P<.10$), GLY ($P<.10$) and ALA ($P<.10$)

Total concentrations of amino acids in fraction II are nearly double those found in fraction I. This is consistent with the types of proteins associated with each fraction and the fact that albumin is the most prevalent protein found in blood.

Results of the hindlimb exchange of fraction I are listed in table 14. MET ($P<.14$) and GLY ($P<.13$) uptakes were substantially elevated in animals fed urea. Large ani-

TABLE 12. PROTEIN FRACTION I AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE [b]
	10-day	30-day	10-day	30-day	
	-----	/dl -----	-----	/dl -----	
Aspartic acid	130.03	124.80	124.74	125.94	4.17
Threonine	104.07	99.73	100.53	105.75	5.09
Serine	145.14	141.44	129.05	136.04	5.74
Glutamic acid	149.48	142.52	139.88	144.27	5.55
Proline	77.06	76.68	72.93	77.96	3.68
Glycine	68.78	66.57	64.52	67.03	2.80
Alanine	61.53	58.61	56.57	58.00	1.91
Valine	92.12	90.05	90.52	101.67	5.19
Cysteine	16.65	15.52	15.46	16.76	.68
Methionine [c,d]	9.34	11.41	8.03	6.71	.94
Isoleucine	30.45	29.03	29.96	33.44	1.63
Leucine	105.01	100.10	97.90	105.50	3.82
Tyrosine	75.79	72.86	71.99	79.20	3.68
Phenylalanine	51.17	48.26	48.64	51.77	2.18
Lysine	99.71	110.66	90.50	101.44	7.11
Histidine	25.25	47.50	20.30	43.88	6.66
Arginine	70.37	65.71	61.46	71.32	1.80
EAA	587.47	602.45	547.82	621.47	22.25
NEAA	724.46	699.00	675.14	705.20	27.77
Total	1311.57	1301.45	1222.96	1326.67	39.94
E/NE	.81	.86	.81	.88	.05

[a] Mean concentration of four animals per observation. Expressed as mg/dl serum.

[b] Standard error of mean.

[c] Soy and urea means are different ($P<.10$).

[d] Diet x time interaction ($P<.10$).

TABLE 13. PROTEIN FRACTION II AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE[b]
	10-day	30-day	10-day	30-day	
	----- mg/dl -----		----- mg/dl -----		
Aspartic acid [c]	240.65	215.59	240.95	217.44	10.42
Threonine [d]	131.93	120.10	132.10	118.91	6.42
Serine [d]	115.91	103.23	103.15	94.36	5.57
Glutamic acid [d]	365.53	334.74	365.68	332.16	16.77
Proline	120.93	117.34	131.71	107.11	6.00
Glycine [d]	51.67	47.60	48.89	44.01	2.35
Alanine [d]	135.45	124.72	136.99	123.04	6.42
Valine	120.67	116.07	124.68	119.35	7.82
Cysteine	50.11	46.50	50.85	47.79	2.98
Methionine	13.20	12.19	11.85	11.80	1.77
Isoleucine	50.23	47.00	51.90	50.29	3.61
Leucine	245.33	230.93	249.52	231.75	12.12
Tyrosine	118.64	112.30	120.78	113.26	6.31
Phenylalanine	135.80	127.40	137.41	128.64	6.81
Lysine	268.89	240.44	262.42	258.31	13.15
Histidine	74.68	67.20	71.86	74.66	3.98
Arginine	130.32	118.75	127.18	124.80	9.19
EAA	1171.04	1080.07	1168.90	1118.50	56.15
NEAA	1198.89	1100.02	1199.00	1079.19	60.50
Total	2369.94	2182.10	2367.92	2197.67	122.81
E/NE	.98	.98	.97	1.04	.02

[a] Mean concentration of four animals per treatment. Expressed as mg/dl serum.
[b] Standard error of mean.
[c] Means for 10- and 30-day samples differ ($P<.05$).
[d] Means for 10- and 30-day samples differ ($P<.10$).

mal-to-animal variability prevented statistical significance for other amino acids, however, gross observation indicates a very interesting phenomenon. Exchange in animals fed soy protein was variable with no distinct patterns. In contrast, when urea was fed consistent and large uptakes of most amino acids were observed of which over half were significant. The magnitude of these differences and the statistical significance would lead one to conclude that the proteins of fraction I are an important source of amino acids to the hindlimb when urea is fed.

Hindlimb exchanges of amino acids from fraction II proteins are presented in table 15. The arteriovenous differences associated with the feeding of soy protein showed both uptake and release with none of the differences achieving statistical significance. When urea was fed, all amino acids appeared to be removed from the proteins of fraction II as the hindlimb was traversed. Furthermore, very large quantities of amino acids appeared to be removed. Only three amino acids (MET, HIS, ARG) failed to approach statistical significance. The difference between the two diets is manifest in the fact that more than half of the differences observed showed a greater uptake ($P<.12$) of amino acids from these proteins when urea was fed. The patterns of individual amino acid uptake closely resemble the molar ratio con-

TABLE 14. PROTEIN FRACTION I AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS[a]

Amino acid	Soy A/V[b]		Urea A/V[b]		P[c]	SE[d]
	mg/dl	P[e]	mg/dl	P[e]		
Aspartic acid	1.04	(.59)	4.33	(.06)	.34	3.16
Threonine	.72	(.75)	6.61	(.02)	.21	4.20
Serine	-.80	(.65)	4.77	(.03)	.19	3.72
Glutamic acid	.52	(.79)	3.71	(.09)	.45	3.90
Proline	1.38	(.36)	-.57	(.70)	.35	1.90
Glycine	-.89	(.49)	2.46	(.09)	.13	1.93
Alanine	-.66	(.55)	1.23	(.28)	.26	1.51
Valine	.74	(.71)	.91	(.66)	.96	3.06
Cysteine	-.10	(.77)	.84	(.05)	.23	.70
Methionine[f]	.12	(.69)	2.22	(.01)	.14	1.24
Isoleucine	-.44	(.47)	.07	(.91)	.76	1.61
Leucine	-.21	(.90)	3.09	(.10)	.39	3.59
Tyrosine	.00	(.99)	1.22	(.42)	.65	2.52
Phenylalanine	.33	(.75)	1.55	(.17)	.54	1.89
Lysine	-.98	(.57)	2.84	(.13)	.27	3.10
Histidine	-.04	(.96)	2.14	(.03)	.18	1.43
Arginine	-.01	(.99)	2.04	(.03)	.59	3.60

[a]Main effects of diet across time presented with eight observations per treatment. Expressed as mg/dl serum.
[b]Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[c]Probability of a chance difference between diet means.
[d]Standard error of mean.
[e]Values in ( ) depict probability that A/V difference does not differ from 0.
[f]Diet x time interaction, see Appendix table 27.

centrations of amino acids in each protein fraction. This is evidence that whole proteins are being takenup across the hindlimb rather than specific amino acid flucuations.

McCormick (1980) found these two protein fractions to be highly involved in the transport of amino acids away from the hindlimb in fasted calves. The data from the present study provide further evidence for the inclusion of serum proteins as transporters of amino acids. When taken in context with reports of decreased blood proteins and albumin in protein-deficient rats and humans (Hoffenberg et al., 1969) and similar effects noted for ruminants fed urea purified diets (Ludwick et al., 1972), the blood proteins appear to be acting as supplemental sources of amino acids to muscle tissue. The in vitro work of Penn (1960) which showed rapid catabolism of albumin following cellular uptake due to mitocondiral lysosomes coupled with Schimke's (1977) theory on increased activity of cellular lysosomes in nutritional stress situations fit nicely with the results observed in the present study for serum protein amino acid tissue exchange.

An area which has not been studied relative to amino acid transport or supply is the extravascular circulation. Serum proteins are present not only in blood vessels, they penetrate the capillaires and become distributed in extra-

TABLE 15. PROTEIN FRACTION II AMINO ACID ARTERIOVENOUS DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED SOY OR UREA PURIFIED DIETS[a]

Amino acid	Soy A/V[b]		Urea A/V[b]		P[c]	SE[d]
	mg/dl	P[e]	mg/dl	P[e]		
Aspartic acid	1.18	(.82)	10.02	(.09)	.01	2.28
Threonine	1.38	(.76)	6.84	(.16)	.03	1.97
Serine	.36	(.92)	5.88	(.12)	.03	1.92
Glutamic acid	3.80	(.69)	20.53	(.06)	.02	6.88
Proline	-.31	(.87)	3.64	(.07)	.08	1.76
Glycine	1.12	(.58)	2.68	(.17)	.34	1.39
Alanine	.34	(.91)	6.55	(.07)	.02	1.96
Valine[f]	-.96	(.80)	5.20	(.20)	.39	6.63
Cysteine	-.18	(.93)	3.92	(.08)	.05	1.62
Methionine	.01	(.99)	.39	(.53)	.65	.81
Isoleucine	-1.27	(.43)	2.14	(.20)	.32	3.15
Leucine	-.51	(.94)	11.83	(.11)	.12	6.82
Tyrosine	.21	(.96)	7.19	(.14)	.19	4.75
Phenylalanine	-.30	(.94)	8.66	(.07)	.07	4.16
Lysine	9.47	(.34)	16.29	(.13)	.12	3.75
Histidine	2.27	(.46)	3.68	(.25)	.52	2.09
Arginine	1.30	(.72)	3.50	(.36)	.69	5.31

[a] Main effects presented as eight observations per treatment.
[b] Expressed as mg/dl serum. Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[c] Probability of a chance difference between diet means.
[d] Standard error of mean.
[e] Values in ( ) depict probability that A/V difference does not differ from 0.
[f] Time effect was significant, see Appendix table 28.

vascular fluids (Kawai, 1973). Moreover, he suggested that the extravascular portion of serum proteins are not stationary but are actively recycled both in and out of the blood vessels and through the lymphatic vascular system. The substantial involvement of serum proteins noted in the present study as well as that reported by McCormick (1980) would appear to offer strong evidence that these play an important function in amino acid transport. The recycling of plasma proteins by the lymphatic system must, however, be further evaluated for a clear understanding of this process.

SUMMARY

Holstein steer calves (130 kg) were used to monitor the transport and exchange of peptides and serum proteins via changes in the amino acid concentrations of hydrolyzed fractions obtained from arterial and venous blood samples. Calves were fed purified diets containing soy protein or urea as the sole source of dietary nitrogen. Twenty-four hourly feedings per day were imposed to promote 'steady-state' metabolism. Initial blood samples were obtained at least 15 days post-surgery and each trial lasted 30 days and consisted of four animals per treatment.

The peptide fraction exhibited amino acid concentrations in venous blood approximately 30% greater than whole blood free amino acids. HIS was the only amino acid depressed ($P<.13$) by urea although trends for lower levels of all amino acids in urea-fed animals were noted. Uptakes of GLU ($P<.10$), VAL ($P<.10$), LYS ($P<.05$) and HIS ($P<.01$) were observed in soy-fed animals and ILE ($P<.10$) in urea-fed animals from the peptide fraction. In urea vs soy-fed animals the uptake of GLU ($P<.01$) and GLY ($P<.11$) changed to output and near zero flux, respectively. A large role of peptides in amino acid transport is not apparent in this data although it appears that it is possibly functioning in GLU metabolism and as a supplemental source of certain EAA (LYS, HIS, VAL, ILE) under varying conditions.

Two serum protein fractions were evaluated in this study: fraction I (primarily globulins) and fraction II (primarily albumin). Reduced MET ($P<.10$) in fraction I of urea-fed animals was the only amino acid in either fraction to respond significantly to dietary treatment. In fraction II there was a tendency towards lower amino acid levels for the 30-day observations in both soy and urea treatments. This response was significant ($P<.10$) for THR, SER, GLU, GLY, ALA and ASP ($P<.05$). Hindlimb amino acid exchanges for soy-fed animals were inconsistent and non-significant for

both serum protein fractions. Conversely, hindlimb uptakes in urea-fed animals for both protein fractions were large and statistically significant for the majority of amino acids. Fraction II contributed much greater quantities of amino acids to the hindlimb than fraction I. Soy and urea treatment differences were also much more pronounced in fraction II being statistically significant for over half of the amino acids.

These data indicate large tissue fluxes of serum protein amino acids that react differently to varied nutritional regimes. The large uptakes of protein amino acids in urea-fed animals may represent compensation by the tissue in response to depressed free amino acid levels observed under the same dietary conditions.

Chapter VI

GENERAL DISCUSSION

Integration of free amino acid and blood protein transport

Results presented in chapter IV represent the first direct evidence showing amino acid mobilization and possible muscle protein catobilsm by ruminants fed urea diets. This action was alluded to by Chalupa et al. (1964) who showed elevation of urine creatinine with increasing dietary urea. Calves fed soy diets showed a small but consistent uptake of amino acids while calves fed normal hay/grain diets (McCormick, 1980) exhibit much larger uptakes of most amino acids across the hindlimb. In addition, amino acid mobilization during fasting and starvation is very pronounced. The amino acid outputs shown in the urea treatment are small in comparison. These data give possible insights into growth and performance differences often observed between these types of diets.

It can be seen, however, that prolonged negative fluxes of this nature in urea-fed animals could soon result in rather severe depletion of muscle protein. In vitro analyses of rat muscles have shown that starvation results in

increased protein catabolism of both muscle and liver proteins. In protein deficiency, however, muscle protein appears to be conserved while liver proteins are catabolized (Adibi, 1976). Traditional evaluation of this problem using free amino acid pools in blood fail to show compensations made by muscle tissue in response to severely restricted plasma free amino acids due to protein deficiency. Millward and Waterlow (1978) reported that in growth suppressed, protein-deficient rats there is a decrease in the rate of protein synthesis. They surmised that the rate of protein breakdown was also decreased due to their failure to observe marked loss of proteins from the muscle.

The data in chapter V present striking in vivo evidence of increased hindlimb uptake of liver-synthesized blood proteins in urea-fed animals. This information in association with the low concentrations and uptakes of free amino acids under these same conditions implicate additional mechanisms which may be functioning to protect muscle protein from sever depletion in marginal nutritional states. If the theory of increased lysomal autophagy in nutritional stress is valid (Schimke, 1977) then we have a situation where degradation of these blood proteins are functioning to supplement muscle tissues with additional sources of amino acids for the maintenance of muscle protein integrity.

Short-term response to deprived nutritional states show remarkable attempts by the organism to maintain homeostasis. Studies on the intact animal vs in vitro cellular studies allow a more complete analysis of additional factors that may be functioning for this purpose.

There is also other evidence that supports the observations made in this experiment. Blood proteins and especially albumin are noted to decrease in protein deficiency (Hoffenberg et al., 1969) and in the feeding of urea purified diets to ruminants (Ludwick et al., 1972). Additionally, blood proteins are known to pass from vascular fluids through the capillaries into extravascular spaces (Kawai, 1973). This evidence implies that blood proteins are being taken up and utilized resulting in the eventual depletion noted under these conditions.

Previous observations of this phenomenon in the intact animal have not been made. This is likely due to the low level of sensitivity associated with the measurement of whole proteins vs the amino acid determinations employed in this study.

Analysis of Appendix table 28 indicates that this response may be important in the early stages of protein deficiency. Uptakes are much greater in the 10-day vs 30-day urea-fed animals. The lower uptakes observed at 30 days may

be the result of decreased muscle protein synthesis in manners similar to those previously described (Millward and Waterlow, 1978).

Adaptive responses to starvation show parallel mechanisms in that the short-term response results in maintenance of important physiological functions and the shut-down of certain nonessential functions (Young and Scrimshaw, 1971). It is, thus, reasonable to assume that simalar responses may also occur due to protein deprivation. For example, attempts to maintain normal cellular growth and protein synthesis are followed by the elimination of these processes when nutrients remain depleted.

Further conjecture on this topic is limited until more definitive evidence on extracellular fluids and the role of the lymphatics are better understood. Additionally, more refined techniques that allow determination of actual protein exchanges in in vivo experiments are needed.

Urea Adaptation

Data collected in this experiment provide little evidence to implicate extensive muscle involvement in the 'urea adaptation' phenomenon. Free amino acid levels in urea-fed

calves remained low throughout the entire 30-day experiment. In addition, elevated blood urea nitrogen (BUN) in urea-fed calves did not decrease with time. Decreasing BUN, improved nitrogen retention and enhanced growth are parameters often associated with urea adaptation (Oltjen, 1968). Failure to observe improvements in plasma free amino acid concentrations were not unexpected (Ludwick et al., 1972).

The lack of improvement in BUN status may be due to several factors. The length of these trials was only 30 days. Many of the improvements noted in urea adaptation are usually noted after 30 to 50 days. In addition, the calves used in this study were very young (<6 months). Supplementation of higher protein levels in the diet are much more critical in terms of optimum growth for calves of this size than for larger or more mature animals (NRC, 1976). Thus, it is likely that calves of this size are subject to a more severe protein deficiency when fed urea purified diets. Hungate (1966) stated that microbial protein production in rumen fermentation yields less than 10% dietary crude protein equivalent. Therefore, the calves in this study fed urea purified diets were probably receiving below-maintenance crude prtoein levels from the microbial protein.

There are also reports in the literature that changes observed with urea diets are not due to urea per se but

rather to adjustments to different nutritional regimes (Caffery et al., 1967). Examination of plasma free amino acid A/V differences tend to support this theory (Appendix table 21) in the following manner. The 10-day samples from both dietary treatments showed essentially zero or slightly negative fluxes across the hindlimb. At the 30-day sample taken from animals fed soy protein uptakes were generally positive and of larger magnitude. This indicates some type of adjustment occurring for this particular diet and time. In contrast, 30-day samples taken from animals fed urea showed increased amino acid output from the hindlimb relative to the 10-day sample. This is further indication of the nutritional severity of the urea diet in this particular experiment.

Horn and Beeson (1969) theorized that low levels of amino acids observed in urea diets were the result of more efficient utilization of amino acids in protein synthesis. The hindlimb flux data of this study indicate that even though amino acids may be utilized more efficiently, there is still an overall deficiency and muscle protein synthesis is likely impaired. This is certainly in agreement with observations of reduced protein storage in ruminants fed high urea diets (Clifford and Tillman, 1969).

Microbial Protein Quality and Quantity

The significant reduction of most EAA (except MET and HIS) in urea-fed animals is an indication that amino acids in general were limiting in this study. When expressed as um/dl blood the EAA decreased 33 to 44% for the urea vs soy treatments. Schelling and Hatfield (1968) reported that MET was the first limiting amino acid in sheep fed urea purified diets. Plasma free MET was not significantly depressed by urea in this experiment although a slight trend was noted. However, blood glutathione was 20% lower for urea-fed animals. This is possibly due to a lack of precursors for glutathione synthesis and implicates a possible metabolic deficiency of the sulphur containing amino acids. This is further supported by lower MET levels in protein fraction I ($P<.10$) and a nonsignificant decrease observed for MET in protein fraction II (chapter V).

Conclusions from this study would indicate that there is a general lack of amino acids available to calves fed the urea purified diets. This does not preclude the fact, however, that any specific essential amino acid (for exapmle, MET) could be first limiting. It is beyond the scope of the data collected in this experiment to isolate specific limiting amino acids.

Plasma and Erythrocyte Free Amino Acids

When amino acid concentrations are expressed as um/dl RBC and as um/dl plasma the concentrations of ASP, THR, SER, ASN, GLU, PRO, GLY, MET, TYR, LYS and HIS were greater in the RBC while the remaining amino acids showed greater levels in plasma. In contrast, when expressed on a whole blood basis only ASP and HIS were more highly concentrated in the RBC. Observation of greater amino acid concentration in the RBC of dogs (Elwyn, 1972) and chickens (Stephens and Evans, 1971) or the failure to observe such differences as found by Heitmann and Bergman (1980b) in sheep are likely due to differences in the way data are interpreted. However, the fact that the RBC is associated with a significant portion of the free amino acids in blood cannot be disputed.

Presented in table 16 is yet another method for expressing amino acids in blood. Molar proportions of free amino acids in plasma and erythrocytes from fed and fasted calves (McCormick, 1980) and calves fed soy and urea purified diets give indications of amino acid changes relative to each other and also to the differing diets.

It can be seen that ASP, THR, PRO, GLY, MET and HIS are consistently more concentrated in the RBC. This is a slightly different profile than previously observed. These figures indicate that the RBC is able to concentrate spe-

TABLE 16. MOLAR PROPORTIONS OF PLASMA AND ERYTHROCYTE FREE AMINO ACIDS IN VENOUS BLOOD OF CALVES UNDER DIFFERING NUTRITIONAL REGIMES

Amino acid	Plasma [a]		Plasma		RBC [a]		RBC	
	Fed	Fast	Soy	Urea	Fed	Fast	Soy	Urea
	moles/100 moles							
ASP	.6	.4	.8	1.1	8.3	11.3	6.3	4.8
THR	3.7	3.6	3.9	2.9	4.3	4.2	4.5	3.7
SER	3.7	2.8	5.3	5.2	4.1	4.2	4.9	3.8
ASN	3.9	2.3	2.1	1.4	---	---	2.7	2.5
GLU	3.1	1.6	7.2	11.1	9.8	5.7	8.8	8.5
GLN	11.7	9.5	7.0	9.9	---	---	---	---
PRO	3.6	3.3	2.0	1.8	6.2	4.2	4.0	3.5
GLY	14.2	19.7	20.0	21.4	19.5	22.3	36.3	47.5
ALA	9.7	6.7	6.8	6.7	6.5	4.9	2.6	.2
CIT	2.4	1.8	2.4	3.0	3.0	1.0	.8	1.3
VAL	9.5	12.7	10.5	8.8	8.5	5.6	4.3	1.8
CYS	1.9	2.0	.8	.8	---	---	---	---
MET	1.1	.9	.7	.7	1.8	2.1	1.9	1.2
ILE	5.4	6.1	5.1	4.3	3.9	4.9	1.9	.8
LEU	8.1	8.2	6.2	4.7	7.8	11.8	4.8	3.4
TYR	2.2	1.4	1.8	1.3	2.9	1.8	2.1	2.3
PHE	2.5	2.2	2.4	1.9	2.2	1.5	1.4	1.0
ORN	2.6	2.6	4.0	3.3	1.5	2.7	3.0	2.9
LYS	2.9	3.9	4.2	3.7	3.9	2.9	4.1	4.0
HIS	2.6	2.9	2.2	2.4	5.7	8.9	6.2	6.1
ARG	4.4	3.1	4.6	4.0	---	---	---	---

[a] Adapted from McCormick (1980).

cific amino acids against a concentration gradient. Mechanism are not available to describe this particular action observed in the RBC.

The RBC has been described as being particularly important in the transport of ALA. This was not observed in this study. In fact ALA percent in the RBC was much lower in soy vs 'fed' animals and severely depressed in urea-fed animals. Similar responses were also noted for several other amino acids (ASP, PRO, VAL, ILE, LEU, MET, PHE).

In contrast, GLY% increased 86% and 144% in respective soy and urea treatments relative to 'fed' animals. The very high levels of GLY may be inhibiting transport of other amino acids across the RBC membrane. Similar occurances relative to amino acid transport systems have been shown in intestinal absorption studies with amino acid imbalances (Matthews and Payne, 1975). Of particular interest is the in vitro association of GLY, ALA, PRO, VAL, MET, ILE and LEU in neutral amino acid membrane transport systems (Christensen, 1973). These data represent possible in vivo evidence of functioning neutral amino acid transport systems in the RBC membrane that have been described in in vitro systems by Young and Ellory (1977).

The extreme changes noted in GLY and the relationship to its possible effect on transport systems parallel actions

observed in the small intestine and may indicate a metabolic imbalance of this amino acid. This is unique from several standpoints. An imbalance of this nature would have been created within the body rather than from imposed dietary imbalanced conditions. Furthermore, GLY and SER are the only nonessential amino acids that have been observed to ellicit toxic responses when high levels are fed to rats and chickens (Harper, 1970). The toxic response in rats is primarily presented as a general depression in growth and nitrogen retention (Pui and Fisher, 1979). This response is graded relative to incremental amounts of GLY in low-protein diets (Benevenga and Harper, 1966). Furthermore, adaptation to high dietary GLY is shown to occur in 1 to 2 weeks in rats (Harper, 1970). Metabolic sites of GLY adaptation are unknown at this time.

The causes of GLY elevation in blood observed in differing nutritional states are also not clear. Increased curculating GLY due to muscle prtoein breakdown cannot account for the large elevations observed in starvation, protein deficiency or urea-fed ruminants unless GLY degradation is inhibited in some manner. This has not been shown. Elwyn et al (1962) using perfused dog livers, found that high levels of GLY in plasma were positively correlated with increased efflux of GLY from the liver. The liver of ver-

tebrates have the ability to convert carbon dioxide and ammonia GLY via catalysis with glycine synthetase in the following manner (Lehninger, 1975):

$$CO_2 + NH_3 + 5,10\text{-}CH_2\text{-}H_4 \text{ folate} \longleftrightarrow GLY + H_4\text{folate}$$

Under normal conditions this reaction is the major pathway for GLY and SER catabolism, however, it is freely reversible. GLY may thus serve an important function in ammonia detoxification when nonphysiological levels of ammonia are present. This situation occurs in starvation, protein deficiency and in high urea feeding of ruminants.

From the above discussion the following theory is proposed in an attempt to interrelate the metabolic activity of GLY and the BCAA as they respond to differing nutritional states. Excess ammonia due to muscle protein breakdown or high urea diets is presented to the liver. Normal mechanisms for ammonia detoxification are overloaded (i.e., the urea cycle and ammonia excretion by the kidney) and GLY is synthesized as an additional buffer against ammonia toxicity. Continued ammonia influx results in long-term elevation of GLY and SER. This occurs because the major catabolic route of GLY and SER has been reversed due to the high levels of ammonia. These high levels of GLY exert inhibi-

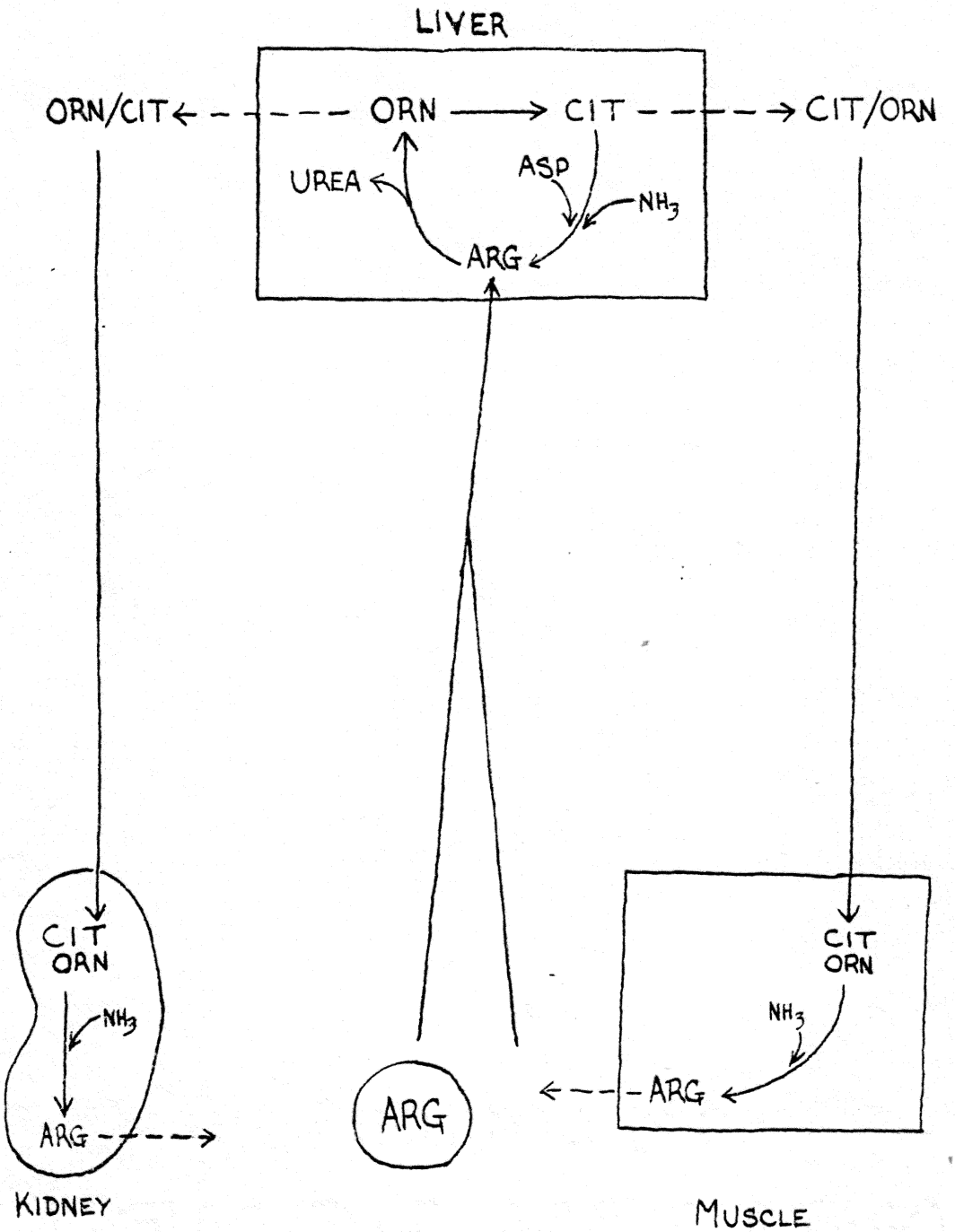
tory effects on neutral amino acid transport systems. Under these conditions muscle protein is alos being mobilized, however, the BCAA and other neutral amino acids are unable to pass through the tissue membrane resulting in failure of amino acids to pass in or out the muscle cells. A resulting build-up of BCAA within the cell may, therefore, occur. The work done in the laboratories of Goldberg (Goldberg and Chang, 1978) indicate that catabolic products of LEU degradation exhibit inhibitory effects upon cellular protein synthesis. Slowing of protein synthesis due to inhibition by LEU degrative products may account for the growth depressions observed under these conditions. Excessive tissue levles of the BCAA are not observed due to high activity levels of the BCAA transaminases in skeletal muscle (Mimura et al., 1968).

This discussion seemingly accounts for changes that occur when animals are subject to these types of nutritional states. It does not, however, give insight into metabolic adaptive responses relative to urea-fed ruminants. Further research is needed to more clearly define adaptive change to GLY toxicity. These studies appear to be particularly relevant to the urea adaptation phenemenon observed in ruminants.

The Urea Cycle

Interorgan amino acid transport studies provided evidence of the 'alanine cycle' as it functions in the transport of specific amino acids to and from the liver (Felig et al., 1970). This cycle has become established as an important mechanism in the shuttle of gluconeogenic amino acids and nitrogen between hepatic and non-hepatic tissues. Recent work by Heitmann and Bergman (1980a) on interorgan amino acid transport in sheep have shown similar whole body transport mechanisms for the urea cycle amino acids. The urea cycle has historically been considered to be restricted to the liver. However, Bergman and associates have shown liver, kidney and hindlimb fluxes of ORN, CIT and ARG that appear to be involved in peripheral tissue ammonia detoxification.

Results from the present experiment and from data presented by McCormick (1980) lend further evidence to these observations. Figure 1 illustrates a proposed 'whole-body urea cycle'. Uptake of ARG and release of ORN and CIT from the liver has been documented (Bergman and Heitmann, 1978). Additionally, ORN and CIT uptake by kidney and peripheral tissues and ARG output have also been observed in several studies in sheep and cattle.


LIVER
ORN/CIT
ORN
CIT
CIT/ORN
UREA
ASP
NH_3
ARG
CIT
ORN
NH_3
ARG
KIDNEY
ARG
CIT
ORN
NH_3
ARG
MUSCLE

What appears to be happening is a cycling of ORN and CIT throughout the entire body which aids in nitrogen removal from the various tissues by conversion to ARG. ARG is then transported back to the liver where the urea molecule is removed. This cycle phenomenon has been observed only in sheep (Heitmann and Bergman, 1980a) and cattle (McCormick, 1980; Chapter IV) and not in monogastrics. This perhaps represents another mechanism employed for the detoxification of large amounts of ammonia that are inherent to the peculiar digestive and metabolic systems of ruminants.

Chapter VII

SUMMARY AND CONCLUSIONS

Plasma and erythrocyte (RBC) free amino acids and plasma peptide and serum protein amino acid concentrations and arteriovenous (A/V) differences across the hindlimbs were determined on growing Holstein steer calves (130 kg) fed purified diets containing soy protein or urea as the sole source of dietary nitrogen. Animals were fed at hourly intervals in a constantly lighted enviornment in order to achieve near 'steady-state' metabolic conditions. Experimental trials lasted 30 days with blood samples collected on day 10 and day 30 of each trial. Plasma and RBC free amino acids were determined from filtrates obtained by deproteinization with sulfosalicylic acid. Peptide amino acids were determined from 4 N methanesulfonic acid hydrolyzed plasma filtrates. Serum proteins were grossly separated by ion-exchange chromatography. Specific protein fractions were then hydrolyzed and analyzed for amino acid concentrations.

Urea resulted in the reduction of plasma free amino acid levels due to a 30% decrease in EAA and a 16% decrease in NEAA. All EAA except MET and HIS were significantly depressed. Concentrations of amino acids in blood from the RBC were generally lower than from plasma for all amino

acids, however, HIS and ASP were always more concentrated in the RBC. Urea significantly depressed RBC concentrations of THR, VAL, MET, ILE, LEU and PHE, however, most NEAA in the RBC were unaffected by dietary treatment. Exceptions were a large increase in GLY and decrease of ALA in the RBC of urea-fed animals.

Net A/V difference across the hindlimb of soy-fed animals was positive for most plasma free amino acids. GLU, GLY and CYS accounted for all of the amino acid release. Urea feeding resulted in a small negative amino acid net A/V difference due to decreased uptakes and/or increased outputs by the hindlimb of several amino acids relative to soy-fed animals. The RBC hindlimb fluxes of several amino acids were altered by dietary treatment. The BCAA changed from large outputs in soy-fed animals to essentially a zero flux in urea-fed animals. In contrast, GLY changed from a large uptake in soy treatments to a large output when urea was fed. The free amino acid data from this experiment indicate that the urea-fed calves were subject to diets deficient in total protein and amino acids rather than specific amino acid differences. Negative A/V fluxes in urea-fed calves indicate muscle protein breakdown. Time effects were generally nonsignificant although a trend towards increased amino acid output across the hindlimb in urea-fed calves was

apparent at 30 days. This implies that adaptation under these conditions was not occurring but rather a more severe deficiency state was encountered over time. These data also show further interrelationships between GLY and the neutral BCAA in altered nutritional states.

Plasma peptides exhibited amino acid concentrations approximately 30% greater than whole blood free amino acids. HIS was significantly depressed and a tendency for lower levels of most other amino acids was noted in urea treatments. Peptide hindlimb exchanges were variable and nonsignificant with the exceptions of GLU, LYS, HIS and VAL uptakes in soy-fed animals and ILE in urea-fed animals. Peptides may, thus, be acting as a supplemental source of several amino acids to muscle tissue.

Diet had little effect on amino acid composition of blood protein fraction I (primarily globulins) and fraction II (primarily albumin). Hindlimb amino acid exchanges of both fractions in soy-fed animals were inconsistent and nonsignificant. In contrast, the hindlimb of urea-fed animals removed large quantities of amino acids from both fractions with fraction II making the greatest contribution. Patterns of amino acid uptake closely resemble molar ratios within each fraction indicating whole protein uptake. These data show striking evidence of increased uptake of amino acids

from blood proteins in calves fed urea purified diets. This may represent adaptation by muscle tissue of the animal to protein-deficient diets.

1. The urea diet in this study resulted in an amino acid deficient state as indicated by low levels of free amino acids in blood and zero or negative fluxes of most EAA across the hindlimb tissue bed.

2. When expressed equally on a whole blood basis HIS and ARG were more concentrated in the RBC while the remaining amino acids were equal to or lower than plasma concentrations. The RBC, however, accounted for approximately 40% of the free amino acids in blood.

3. Urea feeding resulted in a substantial reduction of hindlimb amino acid exchange from the RBC. This was due primarily to reduced fluxes of most EAA, especially the BCAA.

4. Peptides or small proteins remaining in plasma filtrates appear to be involved in transport and exchange of amino acids in a non-specific manner. This pool may possibly function as an additional

source of various amino acids to peripheral tissue beds.

5. Diet had little effect on amino acid composition of serum proteins, although total concentrations of amino acids were reduced in urea-fed animals. Time effects were observed in the albumin fraction (II) with 30-day samples showing lower levels of amino acids in both dietary treatments.

6. Serum proteins appear to play a large quantitative role in supplying peripheral tissues with additional amino acids in urea-fed animals. This function was most pronounced in the albumin fraction.

7. Several amino acid pools in blood are involved in the transport of amino acids to and from peripheral regions of the body. Contributions appear to vary relative to nutritional status. In urea-fed animals serum proteins were quantitatively the most important source of amino acids to peripheral tissues.

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Appendix A

PREPARATION OF PURIFIED DIETS

MINERAL PREMIX

The mineral premix was prepared in 14 kg batches in the following manner:

Ingredient	%	Total	% [a]	Total [a]
$CaHPO_4$	49.987	6998.2	55.833	7816.7
K_2CO_3	28.737	4023.2	25.378	3552.9
$MgSO_4$	11.010	1541.4	9.723	1361.2
NaCl	8.478	1186.9	7.487	1048.2
$FeSO_4$	.750	105.0	.662	92.7
$ZnSO_4-7H_2O$	.529	74.06	.467	65.4
$NaB_4O_7-10H_2O$	.361	50.54	.319	44.6
$MnSO_4-H_2O$	.112	15.68	.099	13.86
$CuCO_3$	.030	4.20	.027	3.78
KI	.003	.42	.003	.42
$CoCl_2-6H_2O$	.001	.14	.001	.14
MoO_3	.001	.14	.001	.14
Na_2SeO_3	.001	.14	.001	.14
Total	100	14000	100	14000

[a] $CaHPO_4-2H_2O$ was also used in place of $CaHPO_4$ resulting in the changes listed.

CONCENTRATE PREMIXES

The concentrate premixes were prepared in approximately 70 kg batches in the following manner:

Urea Diet

Ingredient	% of Concentrate Mix	Total (g)
Cornstarch	44.654	30945
Glucose	44.654	30945
Urea	7.390	5121
Refined Corn Oil	3.145	2180
Choline Chloride	.157	109

Soy Diet

Ingredient	% of Concentrate Mix	Total (g)
Cornstarch	36.635	25388
Glucose	36.635	25388
Soy	23.428	16235
Refined Corn Oil	3.145	2180
Choline Chloride	.157	109

VITAMIN PREMIX

Vitamin A (retinyl palmitate)

a) commercial mix contained 650,000 IU/g.
b) diet specification was 8800 IU/kg diet.
c) each kg of diet thus contained .01354 g vitamin A mix.

Vitamin D (D-activated plant sterol)

a) commercial mix contained 200,000 IU/g.
b) diet specification was 1100 IU/kg diet.
c) each kg of diet thus contained .0055 g vitamin D mix.

Vitamin E (dl-α-tocopherol acetate)

a) commercial mix contained 1 IU/mg.
b) diet specification was 28 IU/kg diet.
c) each kg of diet thus contained .028 g vitamin E mix.

Appendix B

CANNULA PREPARATION AND MAINTENANCE

CANNULAE MATERIALS AND ASSEMBLY

The arterial cannulae consisted of teflon[1] tubing within the artery and silastic[2] tubing (.065 ID and .125 OD) fitted 1 inch over the end of the teflon at the point of entry into the artery for the exteriorized portion of the cannula. Teflon was selected for arterial cannula due to the properties of high tensile strength, dimensional stability and reduced implantation time. Venous cannulae were composed entirely of silastic tubing. The properties of inertness to tissue reaction, elasticity and flexibility make the silicon rubber tubing particularly well suited for implantation in small veins (McGilliard, 1972).

The internal end of teflon arterial cannulae were beveled, polished and buffed in order to reduce surface irregularities that could possibly cause damage to the artery resulting in increased fibrosis and cannulae malfunction. A 2 cm^2 piece of polyvinyl sponge[3] was secured around each cannulae at the point where it exited from the blood vessel. The portion of the cannulae protected by the sponge cuff was then sutured to the muscle and the remaining portion of the cannula was exteriorized.

[1]
[2]TFE tubing, Kaufmann Glass Co., Wilmington, DE.
[3]Silastic Medical Grade Tubing, Dow Corning Corp., Midland, MI.
Ivalon sponge. Unipoint Industries, Inc., High Point, NC.

The following guidelines for cannulae lengths were used for calves weighing approximately 150 kilograms:

Femoral	Internal (inches)	External (inches)
a) Arterial infusion	20	32
b) Venous sampling	20	32
c) Arterial sampling	24	32
Iliac		
a) Arterial infusion	14	20
b) Venous sampling	14	20
c) Arterial sampling	18	20

These lengths resulted in placement of cannulae a and b posterior to the renal artery and vein and cannulae c anterior to the renal artery. In addition, cannulae tips are several inches from the point of entry into the aorta and vena cava. Cannulae placement is important not only from a sampling standpoint but also from observations that fibrosis and sheath formation begin near the entry point of the cannula into the vessel, resulting in cannula malfunction (McGilliard, 1972). This appears to be more of a problem with silastic venous cannulae than with teflon arterial cannulae.

PRESURGICAL CANNULA PREPARATION

Several precautions were taken to prevent chemical or bacterial contamination of cannulae. Following assembly, cannulae were washed in a sonic bath containing a non-ionic soap, rinsed with deionized water and autoclaved. Cannulae were subsequently handled only with sterile gurgical gloves.

Appendix C

AMINO ACID TERMINOLOGY

Aspartic acid	ASP
Threonine [1]	THR
Serine	SER
Glutamic acid	GLU
Glutamine	GLN
Proline	PRO
Glycine	GLY
Alanine	ALA
Citrulline	CIT
Valine [1,2]	VAL
Methionine [1]	MET
Isoleucine [1,2]	ILE
Leucine [1,2]	LEU
Tyrosine	TYR
Phenylalanine [1]	PHE
Ornithine	ORN
Lysine [1]	LYS
Histidine [1]	HIS
Arginine [1]	ARG

[1]Essential amino acids

[2]Branched chain amino acids

Appendix D

STATISTICAL ANALYSIS EXAMPLES

EXAMPLE OF STATISTICAL ANALYSIS OF VARIANCE FOR PLASMA AMINO ACID ARTERIOVENOUS DIFFERENCES

GENERAL LINEAR MODELS PROCEDURE

Dependent variable: Valine (um/dl blood)

Source	DF	Sum of squares	Mean square	F value	R-square	CV
Model	9	5.2156	.5794	1.03	.6063	488.7734
Error	6	3.3858	.5643		STD DEV	VAL MEAN
Corrected total	15	8,6004			.7512	.1537

Source	DF	Sum of squares	F value	PR F
Diet	1	1.5681	2.78	.1466
Animal(diet)	6	2.2862	.68	.6722
Time	1	.0855	.15	.7105
Diet x time	1	1.2748	2.26	.1835

Tests of hypothesis using the type IV MS for Animal (diet) as an error term.

Source	DF	Sum of squares	F value	PR F
Diet	1	1.5680	4.12	.0888

EXAMPLE OF STATISTICAL ANALYSIS OF VARIANCE FOR COMPARISON OF PLASMA AND RBC AMINO ACID A/V DIFFERENCES

GENERAL LINEAR MODELS PROCEDURE

Dependent variable: Methionine (um/dl blood)

Source	DF	Sum of squares	Mean square	F value	PR F	R-square	C.V
Model	13	.8282	.0637	1.83	.1160	.5695	311.7793
Error	18	.6260	.0348		STD DEV		VAL MEAN
Corrected total	31	1.4542			.1865		-.0598

Source	DF	Sum of squares	F value	PR F
Diet	1	.0058	.17	.6874
Animal(diet)	6	.1505	.72	.6378
Time	1	.0508	1.46	.2426
Pool	1	.4965	14.28	.0014
Diet x time	1	.0282	.81	.3797
Diet x pool	1	.0870	2.50	.1311
Time x pool	1	.0080	.23	.6377
Diet x time x pool	1	.0014	.04	.8444

Tests of hypothesis using the type IV MS for Animal (diet) as an error term

Source	DF	Sum of squares	F value	PR F
Diet	1	.0058	.23	.6472

MEANS AND STANDARD DEVIATIONS FOR PLASMA AND ERYTHROCYTE VARIABLES

Item	Plasma				Erythrocyte			
	Venous		A/V		Venous		A/V	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Aspartic acid	1.69	.20	.05	.20	5.39	2.00	-.07	.55
Threonine	6.14	.68	-.19	.68	3.97	.87	.03	.74
Serine	9.31	.78	.14	.77	3.80	1.01	-.10	.75
Asparagine	3.19	.66	.13	.26	2.32	1.16	-.14	.32
Glutamic acid	15.93	2.43	.96	1.01	8.35	3.01	.03	2.93
Glutamine	14.92	3.69	-.37	.90	--	--	--	--
Proline	3.43	.82	.29	.42	3.75	2.99	-.28	.55
Glycine	38.76	11.52	-.53	2.03	40.14	9.42	-.02	4.82
Alanine	11.98	1.97	.02	.97	1.31	1.70	.01	1.46
Citrulline	4.74	.83	.41	.34	1.07	.84	.07	.86
Valine	17.36	4.66	.15	.75	2.98	1.65	-.16	.82
Cysteine	1.37	.16	-.02	.09	--	--	--	--
Methionine	1.24	.24	.07	.06	1.48	.37	-.18	.19
Isoleucine	8.42	2.45	.29	.47	1.33	.93	-.21	.64
Leucine	9.84	3.42	.30	.72	4.01	.86	-.25	1.05
Tyrosine	2.82	.68	-.01	.20	2.10	1.00	.06	.28
Phenylalanine	3.91	.90	.01	.21	1.18	.28	-.05	.30
Ornithine	6.71	1.54	.45	.40	2.88	1.24	-.06	.76
Lysine	7.03	1.63	.03	.98	3.79	1.63	.20	.80
Histidine	4.04	.62	.07	.33	5.95	1.56	-.07	.66
Arginine	7.74	1.33	-.02	1.25	--	--	--	--

MEANS AND STANDARD DEVIATIONS FOR PEPTIDES AND SERUM PROTEIN FRACTIONS

	Peptide				Protein fraction I				Protein fraction II			
	Venous		A/V		Venous		A/V		Venous		A/V	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Aspartic acid	39.26	4.19	.02	1.38	126.38	8.34	2.69	5.18	228.66	20.83	5.60	13.98
Threonine	24.38	2.54	.12	.97	102.52	10.18	3.66	6.17	125.76	12.84	4.11	12.10
Serine	32.51	4.43	-.36	1.35	137.92	11.49	1.99	4.78	104.16	11.14	3.12	9.22
Glutamic acid	64.58	8.30	.47	2.81	144.04	11.10	2.21	5.27	349.53	33.55	12.17	25.46
Proline	25.11	8.46	.21	1.78	76.15	7.36	.40	3.96	117.96	12.01	1.73	4.49
Glycine	51.72	19.75	3.19	9.42	66.72	5.60	.78	3.41	48.04	4.70	1.60	4.68
Alanine	25.97	2.72	.15	1.21	58.68	3.82	.29	2.91	130.05	12.84	3.44	8.28
Valine	15.50	3.18	.21	.36	93.59	10.37	.82	5.47	120.19	15.64	2.21	10.30
Cysteine	--	--	--	--	16.10	1.36	.37	.96	48.81	5.96	1.87	5.24
Methionine	1.97	.61	.03	.52	8.87	1.88	1.17	.81	12.26	3.53	.20	1.66
Isoleucine	13.17	2.12	.15	.30	30.72	3.26	-.19	1.62	49.85	7.22	.44	4.24
Leucine	34.02	3.58	-.09	.97	102.13	7.65	1.44	4.46	239.38	24.25	5.65	17.74
Tyrosine	7.81	.98	-.15	.54	74.96	7.35	.61	4.02	116.24	12.63	3.70	11.97
Phenylalanine	18.13	2.53	.06	.53	49.96	4.37	.94	2.83	132.31	13.61	4.18	10.90
Lysine	28.38	4.91	.56	.78	100.57	14.21	.93	4.55	257.51	26.59	12.28	25.95
Histidine	10.26	4.78	.42	.47	34.23	33.33	1.05	2.05	72.10	7.97	2.97	8.17
Arginine	15.15	2.81	.20	.77	67.22	3.60	1.02	3.85	125.26	16.38	2.40	9.93

MEANS AND STANDARD DEVIATIONS FOR OTHER BLOOD VARIABLES

	Venous		A/V	
	Mean	S.D.	Mean	S.D.
Blood urea nitrogen	16.14	4.88	.07	.47
Blood ammonia	2.64	.69	-.02	.20
Serum protein	6.02	.59	.24	.47
Serum albumin	3.88	.46	.17	.50
Glutathione	69.07	13.92	-1.41	3.50
Hematocrit	28.73	1.47	--	--

TABLE 17. PLASMA FREE AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE[b]
	10 day	30 day	10 day	30 day	
	----- um/dl ----		---- um/dl ----		
Aspartic acid [i]	1.53	1.83	1.69	1.72	.06
Threonine [e]	7.93	7.77	4.49	4.35	.34
Serine [f,h]	9.99	11.38	7.18	8.67	.39
Asparagine [e]	4.06	4.49	2.15	2.05	.33
Glutamic acid	14.62	14.52	16.59	17.45	1.21
Glutamine	13.57	14.82	15.13	14.96 [d]	1.84
Proline [f]	4.57	3.49	2.65	2.85	.41
Glycine [f]	39.37	41.50	31.34	34.11	5.76
Alanine	12.55	15.03	10.47	9.86	.99
Citrulline	5.08	4.73	4.29	4.85	.41
Valine [e]	22.99	19.65	13.64	13.16	2.33
Cysteine [g]	1.69	1.44	1.25	1.10	.08
Methionine	1.42	1.32	1.11	1.10	.12
Isoleucine [f]	11.10	9.41	6.48	6.67	1.22
Leucine [e]	13.58	11.57	7.30	6.90	1.71
Tyrosine [d]	3.41	3.81	2.06	2.01	.34
Phenylalanine [e]	4.81	4.92	2.95	2.96	.45
Ornithine [e]	7.96	8.04	4.04 [d]	3.43	.63
Lysine [e]	8.69	8.18	5.61	5.64	.81
Histidine	4.45	4.38	3.61	3.72	.31
Arginine [e]	9.28	9.41	5.08	7.21	.67
EAA [e,f]	84.24	76.61	50.05	51.70	6.59
NEAA [e]	118.58	125.08	100.41	105.27	6.68
Total [e]	202.83	201.69	150.46	156.68	10.16
E/NE	.71	.61	.50	.49	.07

[a] Mean concentration of four animals per treatment. Expressed as um/dl blood.
[b] Standard error of mean.
[c] Least square mean based on three rather than four observations.
[d] Standard error is underestimated.
[e] Soy and urea means differ (P<.01).
[f] Soy and urea means differ (P<.05).
[g] Soy and urea means differ (P<.10).
[h] Means for 10- and 30-day samples differ (P<.05).
[i] Means for 10- and 30-day samples differ (P<.10).
[j] Diet x time interaction (P<.10).

TABLE 18. ERYTHROCYTE FREE AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE[b]
	10 day	30 day	10 day	30 day	
	----- um/dl -----		---- um/dl ----		
Aspartic acid [f,i]	6.67	5.97	5.33	3.61	1.00
Threonine	4.31	4.68	4.29	2.60	.44
Serine	4.07	4.11	3.78	3.26	.50
Asparagine [c]	2.88	2.56	2.64	2.05	.58
Glutamic acid [h]	7.50	10.22	6.04	9.65	1.50
Glutamine	--	--	--	--	--
Proline	5.29	2.76	3.33	3.12 [c]	1.49
Glycine	41.56	31.13	43.94	43.95	4.71
Alanine	2.51	2.71	1.02	.98	.85
Citrulline	.31	1.35	.84 [c]	1.37 [c]	.52
Valine [g]	5.43	3.14	2.63	.70	.83
Cysteine	--	--	--	--	--
Methionine	1.92	1.81	1.08	1.12	.19
Isoleucine	2.45	1.41	.75	.73	.47
Leucine	5.48	4.24	3.30	3.02	.43
Tyrosine	2.35	1.84	2.69	1.53	.50
Phenylalanine	1.51	1.24	.91	.92 [c]	.14
Ornithine	3.16	2.92	2.80 [c]	2.55 [c]	.62
Lysine	3.92	4.27	4.05 [c]	3.32	.81
Histidine	5.71	6.79	4.78	6.52	.78
Arginine	--	--	--	--	--
EAA [d]	30.73	27.57	21.79 [c]	20.40 [c]	1.31
NEAA [d]	76.30	65.56	72.41	72.07	--
Total [d]	107.03	93.14	94.20	91.00	--
E/NE [d]	.41	.42	.30	.28	--

[a] Mean concentration of four animals per treatment.
[b] Expressed as um/dl blood.
[c] Standard error of mean.
[d] Least square mean based on three rather than four observations.
[e] Standard error is underestimated.
[f] Least square means non-estimable due to missing observations.
[g] Values represent column sums.
[h] Soy and urea means differ ($P<.05$).
[i] Soy and urea means differ ($P<.10$).
Means for 10- and 30-day samples differ ($P<.05$).
Means for 10- and 30-day samples differ ($P<.10$).
Diet x time interaction ($P<.10$).

TABLE 19. TOTAL FREE AMINO ACIDS IN VENOUS BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE [b]
	10 day	30 day	10 day	30 day	
	----- um/dl ----		----- um/dl -----		
Aspartic acid [d,g]	8.20	7.79	7.02	5.33	1.00
Threonine [d]	12.24	12.45	8.78	6.95	.47
Serine [d]	14.05	15.48	10.96	11.94	.75
Asparagine [d]	6.94	7.05	4.79	4.10	.66
Glutamic acid	22.12	25.27	22.63	27.10	2.04
Glutamine	13.57	14.82	15.13	14.96 [c]	
Proline	10.04	6.25	5.98	5.97	1.71
Glycine	89.64	72.63	75.27	78.08	9.04
Alanine [g]	15.06	17.38	11.48	10.85	1.13
Citrulline	5.25	6.21	5.13	6.22	.42
Valine [e]	28.42	22.79	16.27	13.86	2.69
Cysteine [f]	1.69	1.44	1.25	1.10	.07
Methionine [d]	3.33	3.13	2.19	2.22	.25
Isoleucine [e]	13.55	10.82	7.23	7.39	1.64
Leucine [e]	19.05	15.80	10.60	9.92	1.94
Tyrosine [e]	5.76	5.64	4.75	3.54	.60
Phenylalanine [d]	6.31	6.16	3.85	3.74	.51
Ornithine [e]	11.12	10.96	8.19 [c]	7.13 [c]	
Lysine	12.61	12.45	9.66	8.95	1.29
Histidine	10.16	11.17	8.38	10.24	.78
Arginine [e]	9.28	9.41	5.08	7.21	.67
EAA [e]	114.95	104.18	72.04	70.48	6.59
NEAA	203.44	190.92	172.58	176.32	----
Total	318.39	295.10	244.62	246.80	----
E/NE	.57	.55	.42	.40	----

[a] Mean concentration of four animals per observation.
[b] Expressed as um/dl blood.
[c] Least square mean based on three rather than four observations.
[d] Standard error is underestimated.
[e] Soy and urea means differ ($P<.01$).
[f] Soy and urea means differ ($P<.05$).
[g] Means for 10- and 30-day samples differ ($P<.05$).
[h] Diet x time interaction ($P<.10$).

TABLE 20. PLASMA FREE AMINO ACID A/V DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy				Urea				SE [c]
	10 day [b]		30 day [b]		10 day [b]		30 day [b]		
	um/dl	P [c]	um/dl	P [c]	um/dl	P [c]	um/dl	P [c]	
Aspartic acid	.14	(.22)	.12	(.29)	-.06	(.60)	.01	(.93)	.13
Threonine	-.03	(.93)	.18	(.62)	-.13	(.71)	-.88	(.06)	.34
Serine	-.31	(.45)	.66	(.14)	.09	(.83)	.12	(.77)	.38
Asparagine [f]	.20	(.18)	.43	(.02)	.02	(.91)	-.12	(.39)	.13
Glutamic acid	.81	(.16)	1.18	(.06)	.84	(.15)	1.01	(.09)	.50
Glutamine [j]	-.64	(.22)	.52	(.30)	-.38	(.44)	-1.39[e]	(.06)	.58
Proline	.10	(.65)	.71	(.02)	.09	(.69)	.25	(.28)	.21
Glycine	-.91	(.41)	-.36	(.74)	-.78	(.47)	-.07	(.95)	1.02
Alanine	.82	(.14)	.44	(.41)	-.21	(.68)	-.99	(.09)	.49
Citrulline	.41	(.05)	.49	(.03)	.56	(.02)	.20	(.30)	.17
Valine [g]	.26	(.52)	.68	(.12)	.20	(.62)	-.52	(.22)	.38
Cysteine [h]	-.04	(.45)	-.08	(.14)	-.08	(.13)	-.06	(.25)	.05
Methionine [i]	.09	(.03)	.12	(.01)	.09	(.03)	-.03	(.32)	.03
Isoleucine [f]	.35	(.19)	.70	(.03)	.20	(.44)	-.09	(.72)	.24
Leucine [f]	.32	(.42)	.86	(.05)	.23	(.54)	-.22	(.56)	.36
Tyrosine	.02	(.88)	.07	(.54)	.02	(.87)	-.09	(.38)	.10
Phenylalanine	.00	(.98)	.09	(.40)	.02	(.89)	-.08	(.46)	.10
Ornithine	.39	(.11)	.97	(.01)	.20	(.43)	.08[e]	(.74)	.23
Lysine	-.03	(.95)	.41	(.44)	.17	(.74)	-.44	(.40)	.49
Histidine	-.03	(.85)	.21	(.25)	.02	(.91)	.11	(.53)	.17
Arginine	-.14	(.82)	.22	(.73)	.26	(.68)	-.61	(.36)	.60
BCAA	.92	(.36)	2.24	(.05)	.63	(.53)	-.83	(.41)	.94

[a] Mean concentration of four animals per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples.
Negative values indicate greater concentrations in venous blood.
[c] Standard error of mean.
[d] Values in ( ) depict probability that A/V difference does not differ from 0.
[e] Least square mean based on three rather than four observations. Standard error is underestimated.
[f] Soy and urea means differ (P<.01).
[g] Soy and urea means differ (P<.10).
[h] Means for 10- and 30-day samples differ (P<.10).
[i] Diet x time interaction (P<.10).

TABLE 21. RBC FREE AMINO ACID A/V DIFFERENCES ACROSS THE HINDLIMB OF CALVES FED PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy				Urea				SE [c]
	10 day [b]		30 day [b]		10 day [b]		30 day [b]		
	um/dl	P [d]	um/dl	P [d]	um/dl	P [d]	um/dl	P [d]	
Aspartic acid	-.36	(.24)	.40	(.20)	-.19	(.51)	-.12	(.69)	.28
Threonine [g]	-.15	(.69)	-.09	(.82)	-.54	(.19)	.88	(.05)	.37
Serine	-.02	(.96)	-.06	(.88)	-.57	(.18)	.25	(.53)	.38
Asparagine	-.02[e]	(.72)	-.21	(.25)	-.14	(.41)	-.25	(.17)	.16
Glutamic acid	-.63	(.68)	-.34	(.83)	-.62	(.69)	1.69	(.29)	1.47
Glutamine	--	--	--	--	--	--	--	--	--
Proline	-.05	(.87)	-.38	(.22)	-.33[e]	(.28)	-.23	(.54)	.27
Glycine	2.80	(.29)	.28	(.91)	-.26	(.91)	-2.91	(.27)	2.41
Alanine	.17	(.83)	-.13	(.87)	-.06	(.94)	.07	(.93)	.73
Citrulline	.01	(.97)	-.19	(.42)	-.27	(.26)	-.27	(.20)	.27
Valine	-.59	(.20)	-.27	(.54)	-.39	(.39)	.61	(.19)	.41
Cysteine	--	--	--	--	--	--	--	--	--
Methionine	-.22	(.07)	-.28	(.03)	-.04	(.70)	-.20	(.09)	.10
Isoleucine [f]	-.49	(.18)	-.53	(.15)	.01	(.98)	.16	(.65)	.32
Leucine	-.32	(.57)	-.78	(.19)	-.37	(.50)	.45	(.43)	.53
Tyrosine	.03	(.84)	.03	(.82)	-.05	(.73)	.24	(.14)	.14
Phenylalanine	-.15	(.37)	-.03	(.86)	-.03[e]	(.85)	.17[e]	(.43)	.15
Ornithine	.02	(.97)	-.36	(.39)	-.18[e]	(.70)	.37[e]	(.44)	.38
Lysine [f,g,h]	.22	(.61)	.39	(.37)	-.50[e]	(.53)	.54	(.24)	.40
Histidine	.12	(.74)	.23	(.52)	.17	(.63)	-.80	(.05)	.33
Arginine	--	--	--	--	--	--	--	--	--
BCAA [f]	-1.41	(.26)	-1.57	(.21)	-0.75	(.53)	1.21	(.33)	1.13

[a] Mean concentration of four animals per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples. Negative values indicate greater concentrations in venous blood.
[c] Standard error of mean.
[d] Values in ( ) depict probability that A/V difference does not differ from 0.
[e] Least square mean based on three rather than four observations. Standard error is underestimated.
[f] Soy and urea means differ (P<.05).
[g] Means for 10- and 30-day samples differ (P<.10).
[h] Diet x time interaction (P<.10).

TABLE 22. TOTAL FREE AMINO ACID HINDLIMB A/V DIFFERENCES OF CALVES FED PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy				Urea				SE [c]
	10 day [b]		30 day [b]		10 day [b]		30 day [b]		
	um/dl	P [d]	um/dl	P [d]	um/dl	P [d]	um/dl	P [d]	
Aspartic acid [i]	-.23	(.37)	.52	(.07)	-.25	(.33)	-.11	(.66)	.23
Threonine	-.19	(.66)	.09	(.83)	-.67	(.14)	.01	(.80)	.40
Serine [h]	-.33	(.36)	.60	(.13)	-.48	(.20)	.37	(.31)	.34
Asparagine	.18	(.26)	.22	(.36)	-.13	(.59)	-.37	(.14)	.22
Glutamic acid	.18	(.90)	.84	(.57)	.22	(.88)	2.69	(.10)	1.38
Glutamine	-.64	(.62)	.52	(.60)	-.38	(.74)	-1.39 [e]	(.22)	.77
Proline	.05	(.84)	.33	(.23)	-.24	(.36)	-.03	(.82)	.24
Glycine	1.89	(.35)	-.08	(.97)	-1.04	(.58)	-2.97	(.15)	1.81
Alanine	.98	(.22)	.31	(.68)	-.26	(.73)	-.92	(.25)	.72
Citrulline [i]	.42	(.02)	.35	(.03)	.35	(.03)	-.08	(.57)	.13
Valine	-.34	(.48)	.41	(.40)	-.19	(.69)	.10	(.84)	.45
Cysteine [i]	-.04	(.87)	-.08	(.16)	.09	(.13)	-.03	(.58)	.05
Methionine [i]	-.13	(.18)	-.17	(.09)	.05	(.60)	-.23	(.03)	.08
Isoleucine	-.14	(.59)	.18	(.52)	.20	(.45)	.07	(.80)	.25
Leucine	-.01	(.99)	.09	(.85)	-.14	(.76)	.22	(.63)	.44
Tyrosine	.05	(.90)	.10	(.42)	-.03 [e]	(.78)	.14	(.26)	.11
Phenylalanine	-.15	(.39)	.07	(.70)	-.02 [e]	(.93)	-.08 [e]	(.67)	.16
Ornithine	.41	(.33)	.60	(.18)	.02	(.97)	.45	(.36)	.38
Lysine	.18	(.53)	.80	(.03)	-.33	(.48)	.09	(.75)	.28
Histidine	.08	(.75)	.44	(.12)	.19	(.56)	-.69	(.03)	.24
Arginine	-.14	(.79)	.22	(.74)	.26	(.69)	-.61	(.37)	.62
BCAA	-.48	(.67)	.67	(.55)	-.12	(.91)	.38	(.73)	1.06

[a] Mean concentration of four animals per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples.
Negative values indicate greater concentrations in venous blood.
[c] Standard error of mean.
[d] Values in ( ) depict probability that A/V difference does not differ from 0.
[e] Least square mean based on three rather than four observations. Standard error is underestimated.
[f] Soy and urea means differ (P<.05).
[g] Soy and urea means differ (P<.10).
[h] Means for 10- and 30-day samples differ (P<.05).
[i] Means for 10- and 30-day samples differ (P<.10).

TABLE 23. PLASMA FREE AMINO ACIDS IN ARTERIAL BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE [b]
	10 day	30 day	10 day	30 day	
	----- um/dl ----		---- um/dl ----		
Aspartic acid [h]	1.67	1.95	1.64	1.73	.09
Threonine [d]	7.90	7.95	4.36	3.57	.39
Serine [h]	9.68	11.05	7.27	8.79	.69
Asparagine [d]	4.26	4.92	2.17	1.93	.43
Glutamic acid	15.43	15.70	17.43	18.45	.99
Glutamine	12.94	15.34	14.76	13.57 [c]	1.54
Proline [e]	4.85	4.20	2.75	3.10	.51
Glycine	38.46	41.14	30.56	34.04	4.08
Alanine	13.37	15.40	10.25	8.88	1.18
Citrulline	5.49	5.22	4.86	5.04	.36
Valine [e]	23.24	20.33	13.83	12.65	2.43
Cysteine [e]	1.66	1.37	1.34	1.04	.09
Methionine [e]	1.51	1.43	1.19	1.07	.14
Isoleucine [e]	11.47	10.11	6.68	6.58	1.36
Leucine [e]	13.89	12.44	7.53	6.67	1.90
Tyrosine [d]	3.43	3.87	2.06	1.78	.39
Phenylalanine [e]	4.81	5.01	2.96	2.88	.47
Ornithine [d]	8.35	9.01	5.64	4.71 [c]	.90
Lysine [d]	8.66	8.59	5.79	5.19	1.22
Histidine [e]	4.41	4.59	3.63	3.90	.39
Arginine [e]	9.19	9.63	5.34	6.60	.86
EAA [d,f]	85.08	80.07	51.32	49.10	7.91
NEAA [e]	116.89	129.15	100.72	102.54	6.75
Total [e]	201.97	209.22	152.04	155.20	14.48
E/NE [e]	.73	.61	.51	.51	.05

[a] Mean concentration of four animals per observation. Expressed as um/dl blood.
[b] Standard error of mean.
[c] Least square mean based on three rather than four observations.
[d] Standard error is underestimated.
[e] Soy and urea means differ (P<.01).
[f] Soy and urea means differ (P<.05).
[g] Soy and urea means differ (P<.10).
[h] Means for 10- and 30-day samples differ (P<.05).
Means for 10- and 30-day samples differ (P<.10).

TABLE 24. ERYTHROCYTE FREE AMINO ACIDS IN ARTERIAL BLOOD OF CALVES FED SOY OR UREA PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy		Urea		SE[b]
	10 day	30 day	10 day	30 day	
	----- um/dl -----		---- um/dl ----		
Aspartic acid	6.30	6.36	5.14	3.49	.84
Threonine	4.16	4.59	3.75	3.50	.30
Serine	4.05	4.04	3.22	3.52	.19
Asparagine	2.86[c]	2.36	2.50	1.80	.59
Glutamic acid [j]	6.87	9.88	5.43	11.41	2.62
Glutamine	--	--	--	--	--
Proline	5.24	2.38	3.00	2.88[c]	1.77
Glycine [e]	44.36	31.40	43.17[c]	41.04[c]	4.16
Alanine	2.68	2.42	.96[c]	.41[c]	.53
Citrulline	.32	1.16	.22	1.10	.69
Valine	4.84	2.86	2.24	2.20	.77
Cysteine	--	--	--	--	--
Methionine [f]	1.70	1.53	1.06	.93	.14
Isoleucine	1.96	.89	1.89[c]	.88	.43
Leucine [j]	5.15	3.45	2.60	3.47	.42
Tyrosine [g,i]	2.38	1.87	1.48	1.77	.14
Phenylalanine	1.36	1.21	1.30	.95[c]	.26
Ornithine	3.17	2.56	2.63[c]	2.92[c]	.42
Lysine	4.12	4.67	2.45	3.85[c]	.97
Histidine	5.83	7.02	5.15	5.72	.65
Arginine	--	--	--	--	--
EAA [d]	31.50	26.22	20.44	21.50	1.56
NEAA [d]	75.06	64.43	67.75	70.34	--
Total [d]	109.73	90.65	88.19	91.84	--
E/NE [d]	.40	.41	.30	.31	--

[a] Mean concentration of four animals per treatment. Expressed as um/dl blood.
[b] Standard error of mean.
[c] Least square mean based on three rather than four observations.
[d] Standard error is underestimated.
[e] Least square means non-estimable due to missing observations.
[f] Values represent column sums.
[g] Soy and urea means differ ($P<.01$).
[h] Soy and urea means differ ($P<.05$).
[i] Soy and urea means differ ($P<.10$).
[j] Means for 10- and 30-day samples differ ($P<.10$).
Diet x time interaction ($P<.05$).
Diet x time interaction ($P<.10$).

TABLE 25. CONCENTRATION AND HINDLIMB A/V DIFFERENCE OF OTHER BLOOD PARAMETERS OF CALVES FED PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Item	Soy		Urea		SE [b]
	10 day	30 day	10 day	30 day	
Blood urea nitrogen, mg/dl					
Venous [d]	12.08	12.86	17.46	22.17	2.44
A/V [c]	.38 (.16)	07 (.78)	-.15 (.56)	-.04 (.89)	.23
Blood-NH_3, mg/dl					
Venous	2.64	2.60	2.48	2.85	.35
A/V [c]	-.02 (.86)	-.09 (.38)	-.04 (.68)	-.09 (.39)	.10
Serum protein, g/dl					
Venous [e]	6.48	5.39	6.03	6.17	.30
A/V [c]	.23 (.37)	.47 (.10)	.23 (.37)	.03 (.91)	.24
Serum albumin, g/dl					
Venous	3.93	3.72	4.11	3.76	.23
A/V [c]	.29 (.29)	.21 (.43)	.08 (.76)	.10 (.71)	.25
Glutathione, mg/dl RBC					
Venous [d]	80.56	77.69	57.63	60.42	6.96
A/V [c]	.16 (.93)	-.30 (.87)	-3.53 (.09)	-1.95 (.31)	1.75

[a] Means of four animals per treatment.
[b] Standard error of mean.
[c] Values in ( ) depict probability that A/V differences do not differ from 0.
[d] Soy and urea means differ ($P<.05$).
[e] Diet x time interaction ($P<.10$).

TABLE 26. PLASMA PEPTIDE AMINO ACID HINDLIMB A/V DIFFERENCES OF CALVES FED PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy				Urea				SE[c]
	10 day[b]		30 day[b]		10 day[b]		30 day[b]		
	um/dl	P[d]	um/dl	P[d]	um/dl	P[d]	um/dl	P[d]	
Aspartic acid	.47	(.53)	.35	(.63)	.08	(.91)	-.84	(.27)	.69
Threonine	-.89	(.12)	.61	(.26)	.36	(.50)	.42	(.42)	.50
Serine	-.55	(.45)	-.10	(.89)	-.21	(.77)	-.58	(.42)	.67
Glutamic acid [f]	1.30	(.39)	3.05	(.07)	-.62	(.68)	-1.86	(.23)	1.41
Proline	-.60	(.67)	.34	(.81)	1.57	(.22)	-.69	(.55)	1.26
Glycine	.40	(.88)	5.53	(.07)	.21	(.94)	-.91[e]	(.79)	2.46
Alanine [h]	-.10	(.29)	.97	(.16)	.17	(.79)	.17	(.79)	.60
Valine [h]	-.04	(.82)	.59	(.02)	.13	(.49)	.16	(.40)	.18
Methionine	-.04	(.87)	.15	(.58)	-.09	(.75)	.09	(.74)	.26
Isoleucine [g]	-.14	(.39)	.30	(.10)	.11	(.50)	.33	(.07)	.15
Leucine	-.94	(.10)	.53	(.31)	-.05	(.93)	.08	(.87)	.48
Tyrosine	-.33	(.28)	-.45	(.25)	-.09	(.76)	.25	(.40)	.35
Phenylalanine	-.40	(.31)	.30	(.31)	.24	(.41)	.02	(.95)	.27
Lysine	.26	(.53)	1.19	(.02)	.47	(.27)	.30	(.46)	.39
Histidine	.86	(.01)	.54	(.06)	.15	(.54)	.12	(.62)	.23
Arginine [g]	-.50	(.24)	.51	(.23)	-.09	(.82)	.87	(.06)	.38

[a] Mean concentration of four animals per treatment. Expressed as um/dl blood.
[b] Values represent the difference between arterial and venous samples.
Negative values indicate greater concentrations in venous blood.
[c] Standard error of mean.
[d] Values in ( ) depict probability that A/V difference does not differ from 0.
[e] Least square means based on three rather than four observations. Standard error is underestimated.
[f] Soy and urea means differ (P<.01).
[g] Means for 10- and 30-day samples differ (P<.10).
[h] Diet x time interaction (P<.10).

TABLE 27. PROTEIN FRACTION I HINDLIMB A/V DIFFERENCES OF CALVES FED PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy				Urea				SE [c]
	10 day [b]		30 day [b]		10 day [b]		30 day [b]		
	mg/dl	P [d]	mg/dl	P [d]	mg/dl	P [d]	mg/dl	P [d]	
Aspartic acid	.82	(.76)	1.26	(.65)	3.61	(.21)	5.05	(.10)	2.59
Threonine	.57	(.86)	.86	(.79)	5.33	(.14)	7.89	(.04)	3.59
Serine	-1.19	(.64)	.41	(.87)	5.60	(.06)	3.95	(.15)	2.39
Glutamic acid	-.04	(.99)	1.08	(.70)	3.93	(.19)	3.50	(.23)	2.64
Proline	1.10	(.60)	1.66	(.43)	-1.04	(.62)	-.11	(.96)	1.98
Glycine	-.74	(.68)	-1.05	(.56)	1.75	(.35)	3.17	(.11)	1.70
Alanine	-1.28	(.42)	-.04	(.98)	.64	(.68)	1.82	(.26)	1.46
Valine	.48	(.87)	1.01	(.72)	1.55	(.59)	.26	(.93)	1.74
Cysteine	-.37	(.47)	.17	(.74)	1.04	(.07)	.64	(.23)	.48
Methionine [f]	.68	(.14)	-.44	(.32)	1.87	(.01)	2.58	(.01)	.40
Isoleucine	-.19	(.82)	-.69	(.43)	.62	(.47)	-.49	(.57)	.81
Leucine	-1.58	(.50)	1.17	(.62)	3.55	(.16)	2.63	(.28)	2.23
Tyrosine	-.13	(.95)	.12	(.95)	1.72	(.43)	.72	(.73)	2.01
Phenylalanine	.17	(.91)	.50	(.74)	1.88	(.23)	1.22	(.42)	1.42
Lysine	-1.00	(.68)	-.96	(.69)	4.82	(.08)	.85	(.72)	2.27
Histidine	-1.01	(.36)	.93	(.40)	2.05	(.09)	2.23	(.07)	1.03
Arginine	.11	(.96)	-.13	(.95)	3.58	(.11)	.51	(.80)	1.92

[a] Mean concentration of four animals per observation. Expressed as mg/dl serum.
[b] Values represent the difference between arterial and venous samples.
Negative values indicate greater concentration in venous blood.
[c] Standard error of mean.
[d] Values in ( ) depict probability that A/V difference does not differ from 0.
[e] Diet x time interaction (P<.10).

TABLE 28. PROTEIN FRACTION II HINDLIMB A/V DIFFERENCES OF CALVES FED PURIFIED DIETS AND SAMPLED AT 10 AND 30 DAYS [a]

Amino acid	Soy				Urea				SE [c]
	10 day [b]		30 day [b]		10 day [b]		30 day [b]		
	mg/dl	P [d]	mg/dl	P [d]	mg/dl	P [d]	mg/dl	P [d]	
Aspartic acid [e]	-1.22	(.87)	3.59	(.63)	16.78	(.05)	3.26	(.66)	6.99
Threonine [f]	2.40	(.71)	.36	(.95)	10.17	(.14)	3.52	(.58)	6.05
Serine [f]	-1.48	(.76)	2.19	(.65)	8.66	(.11)	3.10	(.53)	4.61
Glutamic acid [f]	4.21	(.75)	3.40	(.80)	31.50	(.05)	9.57	(.48)	12.73
Proline	-2.63	(.41)	2.01	(.41)	5.07	(.07)	2.22	(.37)	2.90
Glycine	2.93	(.38)	-.68	(.78)	3.82	(.16)	1.55	(.54)	3.02
Alanine [f]	1.25	(.77)	-.58	(.89)	10.31	(.05)	2.79	(.53)	4.14
Valine [h]	5.09	(.36)	-7.02	(.22)	8.90	(.14)	1.50	(.78)	5.15
Cysteine [f]	.07	(.98)	-.43	(.88)	6.47	(.05)	1.37	(.62)	2.62
Methionine	-.47	(.59)	.48	(.59)	.87	(.34)	-.09	(.92)	.83
Isoleucine [f]	1.35	(.55)	-3.89	(.12)	3.24	(.18)	1.05	(.64)	2.12
Leucine	3.73	(.69)	-4.75	(.61)	17.98	(.09)	5.67	(.55)	8.87
Tyrosine	3.61	(.57)	-3.18	(.61)	12.09	(.09)	2.92	(.72)	5.99
Phenylalanine [g]	1.93	(.74)	-2.54	(.66)	13.36	(.05)	3.97	(.49)	5.45
Lysine	4.02	(.77)	14.92	(.29)	29.43	(.06)	3.16	(.82)	12.97
Histidine	3.23	(.42)	1.01	(.81)	7.75	(.11)	-.39	(.93)	4.09
Arginine	1.03	(.84)	1.58	(.76)	7.76	(.17)	-.75	(.89)	4.97

[a] Mean concentration of four animals per observation. Expressed as mg/dl serum.
[b] Values represent the difference between arterial and venous samples.
Negative values indicate greater concentration in venous blood.
[c] Standard error of mean.
[d] Values in ( ) depict probability that A/V difference does not differ from 0.
[e] Soy and urea means differ ($P<.01$).
[f] Soy and urea means differ ($P<.05$).
[g] Soy and urea means differ ($P<.10$).
[h] Means for 10- and 30-day samples differ ($P<.10$).

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TRANSPORT AND EXCHANCE OF AMINO ACIDS FROM PLASMA, ERYTHROCYTES, PEPTIDES AND SERUM PROTEINS ACROSS THE HIND-LIMB OF CALVES FED SOY OR UREA PURIFIED DIETS

by

Dean Alan Danilson

(ABSTRACT)

Plasma and erythrocyte (RBC) free amino acids and plasma peptide and serum protein amino acid concentrations and arteriovenous (A/V) differences across the hindlimbs were determined on growing Holstein steer calves (130 kg) fed purified diets containing soy protein or urea as the sole source of dietary nitrogen. Animals were fed at hourly intervals in a constantly lighted enviornment in order to achieve near 'steady-state' metabolic conditions. Experimental trials lasted 30 days with blood samples collected on day 10 and day 30 of each trial. Plasma and RBC free amino acids were determined from filtrates obtained by deproteinization with sulfosalicylic acid. Peptide amino acids were determined from 4N methanesulfonic acid hydrolyzed plasma filtrates. Serum proteins were grossly separated by ion-ex-

change chromatography. Specific protein fractions were then hydrolyzed and analyzed for amino acid concentrations. Urea resulted in the reduction of plasma free amino acid levels due to a 30% decrease in EAA and a 16% decrease in NEAA. All EAA except MET and HIS were significantly depressed. Concentrations of amino acids in blood from the RBC were generally lower than from plasma for all amino acids, however, HIS and ASP were always more concentrated in the RBC. Urea significantly depressed RBC concentrations of THR, VAL, MET, ILE, LEU and PHE, however, most NEAA in the RBC were unaffected by dietary treatment. Exceptions were a large increase in GLY and decrease of ALA in the RBC of urea-fed animals. Net A/V difference across the hindlimb of soy-fed animals was positive for most plasma free amino acids. GLU, GLY and CYS accounted for all of the amino acid release. Urea feeding resulted in a small negative amino acid net A/V difference due to decreased uptakes and/or increased outputs by the hindlimb of several amino acids relative to soy-fed animals. The RBC hindlimb fluxes of several amino acids were altered by dietary treatment. The BCAA changed from large outputs in soy-fed animals to essentially a zero flux in urea-fed animals. In contrast, GLY changed from a large uptake in soy treatments to a large output when urea was fed. The free amino acid data from this experiment indicate

that the urea-fed calves were subject to diets deficient in total protein and amino acids rather than specific amino acid differences. Negative A/V fluxes in urea-fed calves indicate muscle protein breakdown. Time effects were generally nonsignificant although a trend towards increased amino acid output across the hindlimb in urea-fed calves was apparent at 30 days. This implies that adaptation under these conditions was not occurring but rather a more severe deficiency state was encountered over time. These data also show further interrelationships between GLY and the neutral BCAA in altered nutritional states. Plasma peptides exhibited amino acid concentrations approximately 30% greater than whole blood free amino acids. HIS was significantly depressed and a tendency for lower levels of most other amino acids was noted in urea treatments. Peptide hindlimb exchanges were variable and nonsignificant with the exceptions of GLU, LYS, HIS and VAL uptakes in soy-fed animals and ILE in urea-fed animals. Peptides may, thus, be acting as a supplemental source of several amino acids to muscle tissue. Diet had little effect on amino acid composition of blood protein fraction I (primarily globulins) and fraction II (primarily albumin). Hindlimb amino acid exchanges of both fractions in soy-fed animals were inconsistent and nonsignificant. In contrast, the hindlimb of urea-fed animals

removed large quantities of amino acids from both fractions with fraction II making the greatest contribution. Patterns of amino acid uptake closely resemble molar ratios within each fraction indicating whole protein uptake. These data show striking evidence of increased uptake of amino acids from blood proteins in calves fed urea purified diets. This may represent adaptation by muscle tissue of the animal to protein-deficient diets.