# *IN VITRO* ABSORPTION OF VALINE, THREONINE AND METHIONINE BY THE SMALL INTESTINE OF SHEEP

by

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# INTRODUCTION

Amino acids are the basic components of the macromolecule proteins. These proteins are essential in creating supramolecules which develop into organelles that sustain life. Amino acid nutrition of monogastric species has been studied extensively for several decades. As a result of this research, amino acids have been isolated and their structures derived. The daily requirements have been determined for some species and the metabolic importance and pathways have been studied in detail.

*In vitro* procedures developed to study absorption characteristics of amino acids have centered around such laboratory animals as rats an hamsters. Amino acid absorption by dogs and chickens has been studied recently using these procedures. To date, only one published report is available where such procedures have been used in ruminants.

Ruminants are unique in their digestive characteristics, differing distinctively from the monogastrics. The microbial population of the rumen enables the ruminant to utilize carbohydrate sources such as cellulose and various non-protein nitrogen sources. The knowledge that the microbial population of the rumen is capable of synthesizing all amino acids delayed the development of interest in amino acid nutrition of ruminants. In order to increase growth, efficiency and production as these relate to protein needs, more must be known concerning the amino acid nutrition of ruminants.

Before the animal can utilize amino acids metabolically, these amino acids must be absorbed. Thus, the intestinal level interactions and requirements should be explored. Experiments were undertaken to develop an *in vitro* procedure to study amino acid absorption characteristics of ruminant intestine. Three amino acids were selected to be studied by this procedure.

## REVIEW OF LITERATURE

The nitrogen intake of an animal is generally expressed in protein units, but once proteins reach the gastrointestinal tract they are hydrolyzed by enzymatic and chemical processes to yield amino acids and peptides. The mucosal epithelium stands between the digestive products and the circulatory system as a barrier that must be crossed before the animal can benefit from these nutrients.

### Procedures Used in Transport Studies

In order to isolate the intestinal tract and to study specific reactions of the tissue to various amino acids or combinations of amino acids, the early researchers strived to develop reliable *in vitro* procedures. These procedures would allow studies to be conducted on specific portions of the tract with various parameters. Parsons (1968) reported that attempts were made in the 1700's to ascertain intestinal absorption of food components. The basic fact learned from these early ventures was that the tissue loses its viable functions once the animal dies. It was not until the 1900's that renewed interests in *in vitro* procedures were revived.

Four basic types of *in vitro* procedures emerged from this activity. They were the use of rings, everted sacs, intestinal segments and isolated mucosa. Although modifications have been made by many scientists the basic concepts remain. Each procedure is applicable and sound,

but the one selected provides the necessary characteristics that appeal to the scientists involved. Opinions vary as to the best approach to the amino acid absorption studies, but one main factor is common among all procedures. Each procedure, to some extent, simulates the actual processes that are involved in *in vivo* absorption.

The concept of removal of the intestinal section from the animal, but keeping it alive led Agar, Hird, and Sidhu (1954) to develop the intestinal ring procedure. Rats were anesthetized and the intestinal tract exposed by a midline incision. The intestinal section to be used was flushed of its contents while the blood system remained intact. The removal was as quick as possible in order to minimize the time from removal to incubation. The segment was dipped in buffer to remove all remaining particles before tissue preparation. It was then everted by passing a glass rod through the lumen and cut into segments approximately 0.5 cm in length. These narrow segments, which could be prepared quickly, formed rings as the edges of the tissue curled inward. Rings could be allotted to treatments and the incubation begun. The villi of the mucosa were adequately exposed to the oxygenated medium containing the test substance, but absorption of the amino acids could be measured only for short periods. The vascular system that would normally remove the amino acids absorbed from the mucosa are not present, thus a build up of amino acids can occur at the mucosa level or the amino acids can move through the tissue and reenter the medium. As a result the ring procedure would yield only tissue retention, not the overall uptake capacity, during long experiments. Agar *et al.* (1954) reported amino acid uptake with this procedure, expressed as μmoles per gram of dry tissue. These

measurements revealed the amount of tissue absorption from the mucosal fluid. The amount found in the tissue was expressed as a concentration in the tissue water. They also noted that the amino acid in the tissue would rapidly move from the epithelial tissue into the mucosal medium if there was no amino acid present in the fluid and an equilibrium would be reached.

During the same period of time that the ring procedure was being developed, Wilson and Wiseman (1954) developed the everted sac technique. The removal of the intestine was the same as Agar _et al_. (1954) had reported, but the tissue preparation varied in order to achieve another approach to the problem. The intestinal section was everted and cut into 2 to 3 cm segments. One end of a segment was tied, the sac was filled with medium, then the other end was tied. The sac now had the exposed lumen that Agar _et al_. (1954) achieved, which is necessary for absorption. The amino acids that are absorbed by the mucosal epithelium can be transferred across the cells to the serosal side, but instead of being released into the medium they are concentrated in the serosal fluid. This type of movement can be determined by the increase in amino acid content of the serosal fluid above the initial level. The increase in the serosal fluid will account for part of that which was missing from the mucosal fluid and the remainder will be present in the tissue. Wilson and Wiseman (1954) calculated the concentrations of both the serosal and mucosal fluid and found that a concentration gradient had developed on the serosal side of the sacs. Agar _et al_. (1954) reported a concentration gradient had developed within the tissue water of the rings, which was higher than the incubation medium. The concentration in the mucosal tissue could be assumed

to be higher than the serosal and mucosal fluids. It could then be concluded that the movement from mucosal fluid to tissue is active transport while the movement from tissue to serosal fluid is diffusion. The everted sacs can also be used to determine the amount of amino acid that can be absorbed per unit of time.

Perfusion of an intestinal section which has the blood system intact has been used in *in vivo* studies. This principle can be employed in an *in vitro* system also. Removing an intestinal segment from the animal and connecting it to a perfusion apparatus allows the medium to flow through the intestinal lumen continuously in a circular path. At the same time the outside tissue of the intestine is bathed with medium to retain the amino acids that are absorbed (Crane and Wilson, 1957; Darlington and Quastel, 1952; Fisher and Parsons, 1949). Fisher and Parsons (1949) and Darlington and Quastel (1952) perfused fluids through and around the intestine continuously by using reservoirs of fluid and gas pressure as the propelling force. Samples of either fluid can then be taken from the reservoir. The movement of medium through the lumen moves the villi. Crane and Wilson (1957) employed an intestinal segment in a similar fashion, but they did not perfuse continuously with the medium. Instead, the segment was everted and closed at one end, allowing the other end to be attached to a cannula and filled with fluid. The segment was then emersed in a test medium that was oxygenated. Sampling of the serosal and mucosal fluids was relatively easy, which allowed the use of the procedure to study changes in absorption with time. The methods developed by Darlington and Quastel (1952) and Fisher and Parsons (1949) employ the advantage described for the everted sac. These methods

afford the opportunity to study the accumulation in the tissue as well as the subsequent movement into the serosal fluid. The volume of the serosal fluid is much larger for these methods and results in a slower change of the serosal concentration during incubation.

The three previously described procedures can be classified as whole tissue preparations. This type of preparation results in viable tissue as indicated by the histological studies performed by Fisher and Parsons (1949), which showed no difference between fresh tissues and tissue that had been incubated for one hour. Adequate oxygenation and temperatures simulated the natural environment and prevented structural damage. These whole tissue preparations have been criticized for not being representative of the actual processes that occur (Munck, 1972). Munck (1972) felt that the non-epithelial tissue formed a barrier to amino acids. This barrier forced the tissue to accumulate amino acids, which would not normally happen. He suggested that another type of preparation would be more suitable. This type uses only the mucosal cells and was developed by Dickens and Weil-Malherbe (1941). The procedure was formerly used in studies of the large intestine, but Dickens and Weil-Malherbe (1941) felt that it could also be applied to small intestinal studies. The intestinal section is removed in the same manner as ring and everted sac methods. Once the contents have been flushed out, the lumen is opened and the mucosal cells scraped off. This system has some problems also. Removing the mucosal cells can be damaging to the cells. Bacterial contamination of the isolated cells is another problem. The disintegration of tissue or loss of dry matter varied both among and within the experiments, and did not develop a particular pattern

(Dickens and Weil-Malherbe, 1941). Munck (1972) studied the effect of leucine on the steady state accumulation of lysine. He compared results obtained by the isolated mucosa method and the intestinal ring method. The results obtained by these two methods showed opposite effects with the addition of leucine. Munck (1972) explained the differences as a result of the different procedures.

Problems exist with each procedure described. The isolated mucosa method pinpoints the specific active site of absorption, the mucosal cell, and allows experiments to be performed at the site of absorption. Whole tissue preparations can be used to study tissue accumulation and uptake rates of individual amino acids as well as mixtures. Kinetic studies of amino acids are usually done in short intervals and involve the active sites of the lumen. The isolated mucosa method would be more appropriate for this type of study.

## Absorption of Amino Acids from the Lumen of the Small Intestine

Active transport has been defined as the process of moving molecules against a concentration gradient. This movement could be against electrical or pressure differences. The movement of any substances by active transport requires energy and a carrier. The carrier transports the substance through the membrane, picking it up at one side and releasing it on the other. Energy is required to operate the carrier. Facilitated diffusion also uses a carrier, but no energy is used to operate the carrier (Guyton, 1971).

The intestinal tract is not homogenous tissue. The epithelial tissue lines the lumen of the tract and is in constant contact with the

digestive fluids. The entire tissue is not epithelium, thus, it reacts differently to substances transported through it. Newey, Sanford and Smyth (1970) proposed a model of the intestinal tract. They considered the tissue as four compartments; the mucosa and serosa served as the first and last compartments with the intestinal tissue as the middle two. The first tissue compartment was the mucosal epithelium and the second compartment was the submucosal cells. An amino acid would have to be transported across these two compartments to reach the serosal side from the mucosal. To study these types of movements, Akedo and Christensen (1962) employed model amino acids, cycloleucine and aminoisobutyric acid. These models were used to prevent loss by metabolic action. The models were concentrated across the intestinal wall 2.7 times the concentration in the mucosal fluid. These models were actively transported across the small intestine. Similar results for tryptophan were reported by Cohen and Huang (1964). Concentrations of the serosal fluid, tissue and mucosal fluid changed during the incubation. The mucosal fluid dropped from its initial concentration while the serosal fluid and tissue concentrations increased. Although both increased above the mucosal level, the tissue concentration was greater than the serosal fluid. Additional work with labeled tryptophan illustrated that the movement was from the mucosal fluid to serosal fluid, but the process was not reversible. The pathway for amino acid absorption appeared to be an active unidirectional flow from the mucosa to the serosa with the highest concentration of amino acids being found in the tissue. They theorized that the absorption from the lumen by the epithelial cells was active transport, but movement from these cells to the serosal fluid was by

diffusion. Two experiments conducted by Agar, Hird, and Sidhu (1953, 1954) demonstrated that the first step of uptake by intestinal rings and segments was in the epithelial cells. They also concluded that the amino acid not accounted for in the serosal or mucosal fluids was in the tissue, which reached saturation first. Additional work with intestinal rings resulted in tissue accumulation of amino acids ten times the medium concentration (Agar, Hird and Sidhu, 1956). In vivo, the blood supply would constantly be renewed, thus maintaining a lower concentration than the tissue (Agar et al., 1956). This would enable the amino acids to move out of the tissue by diffusion. Using 1-aminocyclopentane-carboxylic acid and aminoisobutyric acid as models, Christensen (1963) studied the relationship between intestinal amino acid concentration and plasma level. He found that the plasma level was 20 to 90 times more concentrated than the lumen contents. Movement of amino acids from the lumen to the plasma is against a concentration gradient. Amino acids appear to be actively absorbed and concentrated in the tissue so that movement from the tissue to the serosa will be diffusion.

Any given biological pump has a carrier and a system to provide energy for the carrier. These two components are intimately related but the nature of the relationship is still obscure. The carrier recognizes the specific substance to be transported, but this specificity is not necessary for the energy system. A general inhibitor can reduce the energy available, but it takes a highly specific inhibitor to act as the carrier (Csaky, 1961). The carrier which transports amino acids across the epithelium has not been identified but some characteristics

have been described. Cohen and Huang (1964) reported a linear response in uptake of tryptophan by everted sacs with increased concentrations in the mucosal fluid. The linear increase peaked at a mucosal concentration of 3 mM and decreased with additional increases in concentration. The decrease would be due to carrier saturation or damage to tissue at high concentrations of amino acids. Histidine showed a similar response with irregular uptake patterns resulting at concentrations above 30 mM.

For amino acids to be actively transported, energy for the carrier must be provided. Wilson and Wiseman (1954) compared transfer rates of everted sacs under two different gaseous environments. Absorption of methionine was reversed by replacing the 95% oxygen gas environment with 95% nitrogen. Negative values were obtained for transference under the nitrogen atmosphere, indicating a movement of methionine from the serosa to the mucosa. Water movement for both environments followed the trend of amino acid transport. Water moving from the serosal to the mucosal fluid under the nitrogen environment did create an increase in methionine concentration, but net flux to the serosal side was negative. Thus the anaerobic environment could not support amino acid transport. Addition of cyanide or 2,4-dinitrophenol inhibited amino acid transport in _in vitro_ environments (Agar _et al._, 1954, 1956; Finch and Hird, 1960a). The ability of these compounds to stop respiration and phosphorylation indicates the energy needs of the system.

The dependence of amino acid transport on the presence of sodium has been demonstrated. Cohen and Huang (1964) removed sodium from the medium of the serosal or mucosal fluids. They found that amino acid uptake was reduced when sodium was absent from the mucosal side, but no

effect was evident on the serosal side. The transport system must then be located on the mucosal side in the brush border cells (Alvarado, et al., 1970). Alvarado (1966) theorized that the sodium ion was necessary in amino acid absorption in order to form a ternary complex with the carrier.

Ehrlich ascites tumor cells were used to study the role of potassium in amino acid absorption (Christensen and Oxender, 1960). Potassium imbalance was established between the two fluids separated by the tumor cells. Glycine was added to the non-potassium side only so its movements could be monitored. Glycine was absorbed into the cells and exchanged for potassium. This allowed the movement of potassium toward the glycine side to relieve the imbalance. Thus, potassium movement could drive the carrier for amino acid absorption. Riggs, Walker and Christensen (1958) reported decreased Ehrlich ascites cell potassium concentrations with increased glycine uptake. They concluded that either potassium is being exchanged for the amino acid or another ion is involved. The absorption of amino acids could be driven by alkali metal distribution.

Removing sodium from the medium and adding potassium or lithium, results in inhibition of absorption (Cohen and Huang, 1964; Reiser and Christiansen, 1967; Schultz, Curran, Chez and Fuisz, 1967). Reiser and Christiansen (1967) removed the sodium ion in fractions by replacing it with potassium. They found that little effect was noted until a sodium concentration of 30 mM was reached. Measurements of intracellular sodium were also made after a normal incubation. The concentration was 3 times the initial level, but this was still below the sodium concentration

in the medium. Schultz *et al.* (1967) found that alanine influx was strongly dependent upon sodium in the mucosal solution, but it was not influenced by a marked reduction of intracellular sodium concentration. These results follow the theory of Riggs *et al.* (1958), that potassium leaves the cell as a result of sodium entering it.

*In vivo* pH of the intestinal contents is relatively constant. Amino acid transport systems have been shown to be affected by pH variations in the medium of *in vitro* systems (Reiser and Christiansen, 1967). Valine transport was optimum from pH 5 to 8. Decreases in absorption were noted on either side of this range with the greatest decrease in transport on the acid side being at pH 3 and 4, and on the alkaline side at pH 8.5 and 9.

Alvarado (1970) explains two approaches to the studies of amino acid absorption, analytic and synthetic. He describes analytic absorption as the intestine possessing many separate exclusive transport systems. The synthetic approach looks at the transport systems as having common links between each other. The carrier that is responsible for amino acid active transport by these systems has not been identified, but the site of absorption and limiting factors have, to some extent, been ascertained. Membrane structure, by its very nature, has some effect. Munck and Schultz (1969b) viewed the intestine as one compartment separating the lumen fluid and the vascular system. They concluded that the rate limiting step of absorption was crossing the mucosal cellular layers. The amino acid could influx into the mucosa cell from both the serosal and mucosal sides, and the amino acid in the cell could efflux out of the cell to both sides. The amount retained by the cell was the net result of these four fluxes. The inhibition by the lack of energy

or ionic changes would affect the flux into the cell. The lower cellular concentration would then have its effect on the movement to the serosal side.

Another similar model was suggested by Newey _et al_. (1970). They suggest that the one compartment presented by Munck and Schultz (1969b) is divided into mucosal and submucosal cells. These two cellular compartments could interact to control absorption rates. The amino acid would be absorbed from mucosal fluid and concentrated in the epithelial area. This would be the active transport which required energy. The movement of the amino acid through the submucosal cells would be diffusion with no requirement for energy. The permeability of the submucosal barrier to the amino acid would dictate the amount finally absorbed. If permeability was high, the diffusion rate would proceed no faster than the accumulation of amino acids in the mucosal layer, due to the diffusion method of transport. The rate limiting step would be the active transport. Assuming that the permeability was low, the reverse would take place. The amino acids could not penetrate the submucosa as fast as the active transport system accumulated the amino acid in the mucosal cells. The concentration would increase in the submucosal cells until it backed up into the mucosa and blocked the active accumulation of more amino acid (Newey _et al_., 1970). Schultz, Curran, and Fuisz (1967) reported a similar conclusion about structural effects. They concluded that amino acids had to cross two barriers, the brush border cells and the combined serosal and lateral membranes. They also pointed out that the two barriers differed structurally and functionally.

Munck (1965) added additional strength to the ion theory that

the mucosal epithelium was the site of concentration. He preloaded the mucosa with proline and inhibited the absorption of glycine. These two amino acids are inhibitory, thus, the proline blocked the absorption of glycine at the carrier site or the epithelial cells. This agreed with the results reported by Alvarado _et al_. (1970) that the carrier system is located in the brush border cells. This carrier is specific for amino acids and can also distinguish different amino acids of the same general group (Baker and George, 1971). The carrier amino acid complex can be studied in the same manner as enzymes. Christensen (1966) theorized that enzymatic principles of substrate and enzymes can be applied to the amino acid carrier complex and that dissociation constants can be derived.

Christensen (1963) and Jacobs (1965) reported that the amino acid transport was not unidirectional. Munck and Schultz (1969b) defined their theory of fluxes in and out of the tissue in two directions. Jacobs (1965) infused the intestinal segment of rats _in situ_ to ascertain the amino acid flux from the tissue into the lumen. By using a model amino acid, aminoisobutyric acid, or perfusions of the single amino acid glycine, he found that amino acids were released into the lumen by the tissue. Intravenous injections of aminoisobutyric acid resulted in the appearance of this amino acid in the lumen of the small intestine. The presence of alanine alone in the lumen elicited the release of other amino acids into the luminal fluid. Jacobs (1965) concluded that the maginitude of the bidirectional flow was specific for an amino acid. The ability of an amino acid to elicit the release of others into the lumen would depend on the individual amino acid.

Intestinal absorption of amino acids depends on a balance between the diet, the lumen contents, and endogenous amino acids pools. These results confuse the value of plasma amino acids levels as an indicator of the amino acid status (Christensen, 1963).

Amino acid structure has been shown to be a determining factor in their absorption from the small intestine. Amino acids can be grouped into classes according to the nature of the side chain, which is the variable in the amino acid structure. Generally, amino acids are classified into three groups: basic, acid, and neutral.

Wiseman (1953) reported there was no active transport of glutamic and aspartic acids, the dicarboxylic amino acids. The concentration of these amino acids decreased during the incubation, but none was recovered in the tissue. Other amino acids studied using the same system were actively transported. Matthews and Wiseman (1953), studying the same amino acids, found alanine in both the serosal and mucosal fluids, but was more concentrated in the serosal fluid. Tissue incubated under the same conditions, but with alanine, phenylalanine or histidine resulted in the appearance of only the original amino acid in the serosal fluid. This would indicate that aspartic and glutamic acids are involved in a transamination reaction to form alanine. In later studies Neame and Wiseman (1957) reported the transport of glutamic and aspartic acids in dogs. The system was an _in situ_ perfusion of the small intestine. The appearance of the two amino acids was monitored by collecting the blood that drained that intestinal segment. Perfusion of glutamic acid at various levels influenced both the glutamic and alanine veinous concentrations. Two percent glutamic acid resulted in less glutamic acid and

more alanine in the blood than at the higher level, 10% glutamic acid. Aspartic acid showed the opposite effect. At 0.15%, aspartic acid appeared in the veinous blood as well as alanine and glutamic acid. Increasing aspartic acid to 0.5% increased the amount of aspartic and glutamic acid in the veinous blood but the amount of alanine remained the same. At lower concentrations aspartic and glutamic acids are apparently transaminated more than at higher concentrations.

The neutral amino acids comprise a larger group, and use more than one system. Baker and George (1971) studied seven different neutral amino acids. They theorized two systems, $N_1$ and $N_2$. The first system is more favorable for the longer side chains and the affinity for this system increases with chain length. The second system prefers the substituted forms such as aminoisobutyric acid and betaine. Hagihira, Wilson and Lin (1962) reported that betaine and sarcosine used the same transport system. They also found that proline and hydroxyproline use the $N_1$ transport system as well as the $N_2$ system which Baker and George (1971) described. Other systems may exist within the neutral amino acid class. Newey and Smyth (1964) used inhibition to study this possibility. Methionine appeared to be transported by a different system than glycine or proline, but some overlapping did exist. Hagihira _et al_. (1960) used the same principle to study valine, leucine, and isoleucine. The inhibitory relationship of these three amino acids indicated that they may also be transported by a different system than methionine and histidine.

## Factors Affecting the Rate of Amino Acid Absorption

The specificity of the carrier has been demonstrated to select

the amino acids to be absorbed. One of the most documented factors is the selectivity of transport systems for the natural forms of amino acids. Wiseman (1951) reported that the L-forms of amino acids were concentrated in the serosal fluid of everted sacs at twice the level present in the mucosal fluid. The D-form studied in the same manner showed no movement. Lin, Hagihira and Wilson (1962) reported the movement of D-methionine. Other experimenters, using different procedures, have reported the same effect of stereochemical selectivity (Agar _et al._, 1954).

The major structural difference in amino acids is the side chain and this can affect absorption. Methionine and sarcosine are transported by two transport systems that react differently to chain length. The methionine system is specific for straight chained aliphatic amino acids and responds to the short chain amino acids first (Daniels _et al._, 1969). Adibi, Gray and Menden (1967) studied branched chain amino acids in amino acid mixtures and discovered that they are absorbed more rapidly than straight chains. They also compared essential and nonessential amino acid absorption by humans from amino acid mixtures. The essential amino acids were absorbed more rapidly from the mixture than were the nonessential ones.

Altering the position of the amine group can produce reductions in in absorption similar to the effect of chain length. These two factors are also additive (Daniels _et al._, 1969). Cohen and Huang (1964) concluded that the amine group was essential for proper recognition by the carrier. Lin _et al._ (1962) proposed that three positions are needed for carrier contact: the alpha amine group, alpha hydrogen and a carboxyl

group. N-methyl substituted amino acids, including sarcosine and betaine are transported less than the neutral amino acids which do not have altered amine groups (Larsen, Ross and Tapley, 1964). Replacing the alpha hydrogen with a methyl group reduced the absorption of that amino acid. This could be due to a charge or molecule size effect (Lin et al., 1962).

Dipeptides, as well as amino acids, can be absorbed by the small intestine. A dipeptide, glycine-glycine, was absorbed by the intestine but appeared on the serosal side as glycine and the dipeptide. Apparently hydrolysis occurred within the mucosal cells. Additional work with a tripeptide of glycine units demonstrated the hydrolytic capabilities of the mucosal cell (Agar et al., 1953). Both glycine and the dipeptide were found in the serosal fluid, but no tripeptide was found. Similar results were obtained when Agar et al. (1953) used leucine-glycine as a dipeptide. The dipeptide was hydrolyzed to yield leucine and glycine in the serosal fluid. Traces of the dipeptide were also detected. Cheng and Matthews (1970) reported that the same amount of methionine was absorbed by intestinal rings incubated in methionine or the dipeptide, methionine-methionine. They also reported that the presence of both the dipeptide and the individual amino acid increased total uptake. Advantage has been taken of the ability of humans to absorb dipeptides in preventing malnutrition in humans suffering from cystinuria. In cystinuria, the ability to absorb dibasic amino acids is not present. Administering lysine in the form of a dipeptide with glycine allows lysine to be absorbed (Hellier, Perrett and Holdworth, 1970). The peptidase activity of the mucosa did hydrolyze the dipeptide and lysine leaked back into the lumen.

Dickens and Weil-Malherbe (1941) noted the thickness of the mucosa is greatest at the upper end of the intestinal tract and diminishes steadily toward the rectum. Alvarez, Goldner, and Curran (1969) determined that the luminal area per serosal area was the greatest in the jejunum. This was due to taller and more numerous villus. Serosal surface area decreases down the tract. Many experiments have dealt with site of absorption. Larsen et al. (1964) found maximum uptake of neutral, dibasic and N-methyl substituted amino acids to be in the mid-intestinal region. The duodenum was reported to be the slowest section for neutral amino acid absorption (Matthews and Laster, 1965). Studies with individual amino acids have indicated that maximum absorption occurs in the lower jejunum and upper ileum (Cohen and Huang, 1964; Samily and Spencer, 1961). Baker and George (1971) divided the small intestine of the rat into eight parts to study each separately. They found that the pattern of absorption of each amino acid was different and the relative magnitude of absorption of individual amino acids varied with segments. Newey et al. (1970) discarded the lower ileum as not being representative of the intestine. Glucose, as well as amino acids, is maximally absorbed by the mid-intestine (Spencer and Samily, 1961).

Johns and Bergen (1973) were the first to report information on the in vitro absorption of amino acids by sheep. They reported that, for the amino acids studied, the site of maximum absorption was the ileum. They felt this displayed a species difference in absorption. Alvarado (1968) used hamsters for his studies. He attributed the differences between his data and those obtained in other laboratories with rats, rabbits, and fish to be most likely the result of species differences.

Substances that are diffused through the intestinal tissue are dependent on the concentration present. Jervis and Smyth (1959) used urea as a diffusion material to compare it to amino acid response to concentration. Urea diffusion increased as the amount present, or as concentration, increased. The rates of absorption for the amino acids studied, methionine and histidine, were not proportional to the concentration, and the concentration could reach a saturation point. Methionine saturated the system at 50 mM while histidine saturated the system at about 100 mM concentration. Uptake of histidine at three concentrations, 5, 10, and 15, mM were studied as a function of time by Agar _et al_. (1954). They reported linear uptakes at the three concentrations for the first 20 minutes. After 20 minutes the amount transported was much smaller and the magnitude of uptake was positively related to the concentrations. The amount absorbed is dependent on the interaction of the concentration of the amino acids and affinity for the system (Finch and Hird, 1960b). Seventeen different amino acids were studied by Finch and Hird (1960a) to ascertain the effects of two concentrations, 1 mM and 10 mM, on uptake by the isolated intestine of the rat. Variation among amino acids was evident in the increase in uptake as concentration increased. Matthews and Laster (1965a) use different initial concentrations presented to the tissue. The lower the initial concentration the higher the serosal to mucosal concentration ratio. Glycine uptake increased with increased concentration, but the percent of the initial mucosal concentration presented to the gut segment decreased four units when the concentration was increased from 1 mM to 10 mM (Spencer _et al_. 1962).

Finch and Hird (1960a) reported that some amino acids are metabolized by the small intestinal tissue. The amino acids listed by these

workers were ornithine, arginine, aspartic acid and glutamic acid. Less than 60% of the amino acid that disappeared from the medium was located in the tissue. The percentage recovered in the tissue was below 10 for aspartic and glutamic acid, 20 for glutamine, and 40 for arginine and and ornithine. Alanine was not present in the medium but was found in the tissue. Alanine could account for 29% to 47% of the amino acid losses. The amino acids not found in the tissue were probably involved in transamination and metabolism. McLead and Tyor (1967) found that 9% of the ornithine absorbed was changed to citrulline. They also used radioactive arginine to determine the presence of arginase. The more distal parts of the small intestine of the hamster had the greatest arginase activity. Baker and George (1971) used labeled alanine to determine its susceptibility to metabolic action. They found that 20 to 25% of the radioactivity added to the medium as labeled alanine was found in an unidentified metabolit which did not give a positive reaction to ninhydrin. They also reported that methionine, leucine, proline, glycine, aminoisobutyric acid and betaine were stable during the incubation. Sugawa, Akedo and Suda (1960) approached the problem of absorbed amino acids being metabolized by *in vivo* and *in vitro* procedures. They found little of the amino acid absorbed incorporated into nucleic acid. Methionine, which was absorbed both *in vitro* and *in vivo*, was oxidized to methionine-sulfoxide. This accounted for approximately 20% of the amount absorbed *in vitro*, but this was much larger *in vivo*. Analysis of the blood *in vivo* revealed that, although the methionine was oxidized in the tissue, it was reduced in the blood, creating a large methionine to methionine-sulfoxide ratio.

Other nutrient components are found in the lumen of the intestine

during absorption. Alvarado (1966) detected inhibition of amino acid absorption by sugars. He reported that transport of neutral amino acids can be inhibited by galactose, glucose and methyl glucoside. He also ranked the three sugars studied in the order of their ability to inhibit the absorption of amino acids. Galactose had the greatest inhibitory effect and glucose the least. Methyl glucoside was intermediate. He also concluded that the inhibition could be direct or a competition for energy. Later Alvarado (1968) tested the ability of galactose to inhibit internally or externally in relation to the membrane. Using cycloleucine as the amino acid to be inhibited, he concluded that the inhibition was an external interaction. Two years later, Alvarado (1970) divided the theories into an allosteric effect and an internal effect. He had previously indicated (Alvarado, 1968) that inhibition was allosteric, but the sugars could have internal effects by disrupting the carrier energy system responsible for the accumulation of amino acids or sugars may cause an ionic imbalance involving potassium and sodium. The question of how sugars and amino acids interacted was also probably confounded with species differences. Glucose did decrease the rates of absorption of 18 amino acids infused into human intestine but did not alter the pattern of absorption (Orten, 1963).

The most likely inhibitory element for the absorption of a given amino acid is another amino acid. Larsen et al. (1964) studied three different amino acid transport systems. The systems studied were the neutral, dibasic and methyl substituted systems. The most inhibitory element for one particular group would be a member of that group. A neutral amino acid is inhibitory toward other neutral amino acids because it is able to attach itself to that particular carrier more readily.

Inhibition is not limited within a group. Neutral amino acids can inhibit basic amino acids (Reiser and Christiansen, 1969). This inhibition is partially competitive and is best explained as competition for independent carriers or multifunctional carrier sites. Arginine was the most powerful inhibitor of lysine while the others, in order of potency, were leucine, valine, glycine and proline.

Baker and George (1971) could achieve a 44% inhibition of proline absorption with methionine. The uptake pattern of proline resembled betaine. The addition of betaine did not inhibit proline until the ratio of betaine to proline was 10 to 1. Betaine inhibition was also dependent on the position of the tract. The inhibition was exerted most strongly at the area of greatest betaine uptake. From these results they concluded that methionine shifted the uptake of proline from one neutral amino acid system to one of the other which would not transport methionine. Methionine has been demonstrated not only to inhibit proline, but to inhibit glycine as well (Newey and Smyth, 1964). Glycine and proline had no effect on methionine transport, although individually, methionine is absorbed slower than proline or glycine. Newey and Smyth (1964) theorized that methionine uses one neutral amino acid system and glycine and proline another. They also added leucine to the methionine system. The smaller water soluble neutral amino acids are more inhibitory toward the sarcosine system; whereas the larger more lipid-soluble ones are more inhibitory toward the methionine system (Daniels *et al.*, 1969). Daniels *et al.* (1969) blocked proline from one system with methionine, then added sarcosine as a test inhibitor. Sarcosine had no effect on proline absorption, but the D-form had more affinity for the system than the L-form. It was inhibitory toward L-proline as indicated by the $K_M$

12.2 to 10.2 respectively.

Hagihira et al. (1962) reported that methionine inhibited valine absorption, but had no effect on betaine or sarcosine; proline was inhibitory to both valine and betaine absorption. Thus, proline could be transported by the betaine-sarcosine system as well as the neutral amino acid systems. Munck (1965) preloaded the tissue with proline and inhibited the absorption of glycine and methionine. The proline had built up in the cell and competed for the mediated steps of transport. Methionine also inhibits the absorption of histidine at varying concentrations (Hagihira et al., 1960). Methionine produced 30% inhibition at equal concentrations and 12% when only half as much methionine was present, but histidine had no effect on methionine absorption even at 4 times the methionine concentration. Methionine also decreased valine uptake by 50%, but histidine had no effect on valine absorption. Lin and Wilson (1960) suggested that methionine inhibited the transport of tyrosine as well as glycine by competing for their carrier. Methionine was exposed to equal concentrations of proline, glycine, histidine, lysine, ornithine and glutamic acid. Ornithine and proline were the only two to show a large amount of inhibition toward methionine absorption (Wiseman, 1954).

The inhibitors of valine absorption can be divided into 3 groups according to their proficiency of inhibitions (Reiser and Christiansen, 1965). Leucine, isoleucine, methionine, tryptophan and phenylalanine were very inhibitory, while histidine and alanine were only slightly inhibitory. Serine and theorine were non-inhibitory because the hydroxyl groups decreased the lipophilic properties of the side chain.

Glycine also lacked the ability to inhibit due to its lack of lipophilic properties. Cystine contains a disulfide bond, two amino groups, and two carboxyl groups. This structure may explain its lack of inhibition of valine absorption. Charges that glutamic, lysine and arginine possess prevents their inhibitory effect on valine absorption.

Histidine absorption is inhibited by most amino acids with the greatest effect coming from non-polar side group amino acids such as methionine, leucine and isoleucine. Polar side chain amino acids such as glutamic acid, aspartic acid, arginine and lysine inhibit histidine absorption also, but the effect is minimal (Agar et al., 1956). Finch and Hird (1960a) concluded that the amino acids with non-polar side groups inhibited histidine at the site of absorption, not inside the cell.

Munck (1966a) studied the effects of 7 different amino acids in terms of inhibition of absorption. Betaine had no effect on threonine or glycine absorption, but a glycine concentration of 20 mM can inhibit threonine uptake at a concentration of 2 mM. The absorption of glycine and threonine can also be inhibited by alanine or proline. Matthews and Laster (1965b) reported that glycine absorption can also be inhibited by leucine. Sarcosine absorption is not affected by glycine or leucine at concentrations of 2 mM. Munck (1966a) concluded that glycine can use the neutral amino acid system but also can use the same system as proline, hydroxyproline, sarcosine, alanine and leucine.

Not all experiments have proven inhibition. Reiser and Christiansen (1971) reported a stimulatory effect between neutral and basic amino acids. Lysine uptake was stimulated by leucine, methionine, and alanine. Stimulation resulted in increased accumulation of lysine in the serosal

medium, but a decrease in the tissue. This results in an overall increase in cell flux. The stimulation is best explained on the basis of an intracellular exchange of neutral amino acids for lysine. Similar results of stimulation were reported by Munck (1966b). The uptake of a 10 mM solution of lysine was increased by additional levels of leucine from 0.5 to 5.0 mM. The saturation point of the lysine carrier remained the same but net flux to the serosal side increased. A 15 mM leucine solution did become inhibitory to the absorption of a 2 mM lysine solution. When the concentrations were reversed leucine absorption was decreased 20% and lysine absorption was increased four fold. Leucine was also stimulatory to ornithine and arginine absorption when applied at 2 mM. Munck (1966b) concluded that a counterflow principle applied. The leucine on the serosal side was high enough to prevent the flux of lysine from the serosa to the mucosa. The net flux into the serosa increased with the nullification of a source of loss. Munck and Schultz (1969a) determined that the flux of lysine from serosa to mucosa is kept low but the flux from the mucosa to the serosa is high. Ornithine can employ the counterflow principles and stimulate the uptake of arginine (McLead and Tyor, 1967).

Williams (1969) infused a mixture of 16 amino acids into the isolated intestine of the sheep. Two concentrations were used, 1.5 mM and 3.0 mM. Six sheep were used for each concentration. The nine essential amino acids present were the fastest to be absorbed. Aspartic and glutamic acids were among the bottom third in rate of absorption at 1.5 mM, but an increase in concentration to 3.0 mM improved the rate at which aspartic acid was absorbed, changing its rate to be among the top half. These results agree with Adibi _et al_. (1967) who used 18 amino acids

in vivo with humans. Orten (1963) measured plasma amino acids after infusion of 10 and 18 amino acids. The pattern of absorption of essential amino acids did not change in the presence of the nonessential, but rates did change with concentration.

Proline is able to be transported by three systems: the sarcosine, neutral amino acid, and glycine systems. Uptake depends on the amino acid with which it must compete (Baker and George, 1971; Hagihira et al., 1962; Munck, 1965). Glycine can use two different systems, the methionine and its own system, but the absorption of glycine is easy to inhibit (Newey and Smyth, 1964). Methionine, histidine, alanine, valine, isoleucine, phenylalanine and tryptophan appear to belong to the same system but have different affinities for the carrier (Reiser and Christiansen, 1965). The N-substituted amino acids, sarcosine and betaine, have a system which they share with proline (Daniels et al., 1969a, 1969b). The basic amino acids, with the exception of histidine, have a system in which arginine is the most potent inhibitor. Less reactive amino acids, serine, threonine and cystine, are absorbed at low rates by the neutral system or by some unknown system. The basic amino acids, ornithine, arginine, and lysine, share a transport system which can also be used by cystine. This system is not as active as the neutral amino acid system and requires low concentrations (Wiseman, 1968). Aspartic and glutamic acids are probably transported by a separate system since their charges limit their inhibitory ability (Reiser and Christiansen, 1969).

Fisher and Parsons (1953) plotted the reciprocal of the initial concentration of glucose as the abscissa axis and the reciprocal of the rates of absorption as the ordinate axis. The plot resulted in a straight line which means that the uptake of glucose conformed to a

Lineweaver-Burk plot. From these types of plots the $K_M$ and $V_{max}$ for amino acids and sugars can be determined. Larsen et al., (1964) felt that these were not true values because the velocity of the system and initial rate change during the experiment. A low $K_M$ value indicates a stronger affinity for active sites than a high $K_M$. An amino acid that has a high $K_M$ will probably have a high maximum velocity (Wiseman, 1968). Amino acids with high $K_M$ values can be inhibitory but require a higher concentration (Matthews and Laster, 1965a). The smaller $K_M$ indicates a longer time required to break down the amino acid carrier complex (Wiseman, 1968). Matthews and Laster (1965a) stated that at lower initial concentrations leucine, valine, alanine and glycine developed larger serosal to mucosal ratios. Increasing the concentration results in an increased rate of absorption until a peak is achieved then it decreases, but this peak varies for each amino acid. The uptake rate is dependent on the $K_M$ and the concentration of the amino acid. Matthews and Laster (1965b) used the $K_M$ values determined for glycine, alanine and leucine to estimate inhibition. The estimated and actual values were close. Cohen and Huang (1964) used the Lineweaver-Burk plot to determine the type of inhibition while Daniels et al. (1969b) calculated $K_M$ and $K_I$ to determine the possible inhibitor effects of proline and sarcosine. The affinity indicated by the $K_M$ is inversely related to the rate of uptake (Finch and Hird, 1960b). The $K_M$ was derived for the entire length of the small intestine and was found to be similar throughout its length, so Spencer and Samily (1961) concluded that the only difference in uptake patterns in relation to site was the presence

of greater or lesser amounts of the system. Schedl et al. (1968) did the same thing with methionine. Munck (1972) warns that the use of a Lineweaver-Burk plot is not the best way to determine the $K_M$ and $V_{max}$ because they do not consider the undefined routes in a mixture of epithelial and non-epithelial tissue preparations.

Nassett, Schwartz and Weiss (1955) suggested that the amino acids available for absorption in the small intestine are constant regardless of the diet. The amount of nitrogen found in the intestine of dogs fed a non-protein diet increased in the distal portions of the tract. The amount of amino acids found in the intestinal contents of different portions of the tract were determined for three diets. One diet contained egg albumin, a high quality protein, another zein, and the third diet was a non-protein diet. Amino acids present in each section were expressed as a ratio to the threonine present. Threonine was used because it occupies an intermediate position. The values obtained for lysine, threonine, valine, tryptophan and methionine from the jejunal content were similar for all diets. The dietary zein contained no lysine, 30% less valine, 90% less tryptophan and 45% less methionine than the egg albumin diet. These differences were not evident in the jejunal contents. The milligrams of lysine, leucine, methionine, threonine, and valine present in the stomach, duodenum, jejunum, upper and lower ileum were measured. All amino acids, except lysine, decreased upon entering the duodenum. Each amino acid increased through the tract to the lower ileum then decreased, except for methionine. Nassett (1964) studied possible sources of endogenous amino acids. Two sources which were identified were enzymes and mucosal cells. The amino acid compositions of pepsin, amylase,

trypsinogen, chymotrypsinogen and carboxypeptidase were determined. Lysine, as well as some other amino acids varied among enzymes but methionine tended to be the lowest of the 10 amino acids listed. Exogenous nitrogen is diluted 7 to 9 fold with endogenous nitrogen. The endogenous nitrogen can be provided by enzymes or by amino acids which are drawn from the mucosal cell. The importance of endogenous nitrogen sources in relation to the amino acid combinations presented to the mucosa is unknown. The inability of some amino acid mixtures to stimulate secretion of enzymes may be a key to their inferiority when compared to natural protein.

## OBJECTIVES

Experiments were conducted to determine the amino acid absorption capability of the duodenum, jejunum and ileum of the sheep.

The specific objectives were:

(1) To study the *in vitro* absorption of three structurally different amino acids, valine, threonine, and methionine.

(2) To determine amino acid uptake, release and tissue accumulation of selected amino acids by the duodenum, jejunum and ileum of sheep.

## EXPERIMENTAL PROCEDURE

The *in vitro* procedure used in this study was an adaptation of the procedure of Wilson and Wiseman (1954). Sections from the small intestine of sheep were obtained to study the absorption of threonine, valine and methionine. Young lambs averaging 26 kg body weight were obtained as donors of intestinal sections. These lambs were housed in an open shed on wire mesh flooring and fed once daily. The ration fed is presented in table 1. The lambs were fed as a group with the amount of feed being adjusted at 2-week intervals so that the average daily gain would approach 113 g. Weights were taken at 2-week intervals to determine the rate of gain and to designate the donors for that period. The largest lambs were selected in order to maintain a continuously growing group of lambs. Parasites were controlled by drenching the lambs twice during the experimental period in order to obtain healthy intestinal sections representative of a normal lamb. One lamb was used for each incubation. Five lambs were used for incubations involving valine and three lambs each for incubations involving threonine and methionine.

The lamb designated as a donor for a given incubation was fasted for 24 to 36 hours before the incubation, but was allowed free access to water. This reduced the volume of rumen ingesta as well as the amount of ingesta in the intestinal tract. Each animal was prepared for tissue collection by clipping the wool from the neck and cannulating the jugular

TABLE 1. COMPOSITION OF RATION FED TO GROWING WETHERS

| Item | Ration |
|---|---|
| Ingredient composition, % | |
| Ground grass hay | 55.40 |
| Ground ear corn | 25.10 |
| Soybean meal | 18.50 |
| Iodized salt | 0.90 |
| Limestone | 0.10 |
| Vitamin A[a] | + |
| Vitamin D[b] | + |
| Chemical composition | |
| dry matter, % | 91.19 |
| Crude protein | 15.08 |
| Ether extract | 1.56 |
| Crude fiber | 26.54 |
| Ash | 5.67 |
| NFE | 51.15 |

[a]Supplied 1300 I. U. per kilogram of ration.

[b]Supplied 220 I. U. per kilogram of ration.

vein. Sodium pentobarbital was administered through the cannula to anesthesize the lamb. Following this, the complete abdominal area plus the flanks were clipped as close as possible with shearing clippers. A midline incision was then made to expose the peritoneal cavity. Moist paper towels were used as drape cloths to surround the incision and protect the intestinal tract from touching the outside area. This working incision was very large in order to locate and remove the desired intestinal portions easily without excessive damage to the intestine.

Intestinal sections of the duodenum, jejunum and ileum were used in these studies. The duodenal section was located and removed first. The duodenal section removed began 6 to 8 cm from the pylorus and was 60 cm in length. The jejunal section was located and removed second. The jejunal tissue used was a 60 cm section which began 1.5 to 2.0 m from the pylorus. The ileal section was located and removed last. This section began 1.5 to 2.0 m proximal to the ileocecal valve and was 60 cm in length.

The mechanical removal of intestinal tissue was similar for all three sections. Care was taken to leave the blood supply to the intestinal segment to be removed intact until immediately before removal from the animal. Silk sutures were placed at appropriate locations on the intestine to minimize spillage of intestinal contents into the peritoneal cavity. The isolated segments were then severed at the sutures and flushed with sufficient 0.9% saline, warmed to 39 C, to remove intestinal contents. The section was then stripped from the

mesentary by blunt dissection and placed in a 250 ml flask containing 100 to 150 ml of Krebs-Ringer bicarbonate buffer. Each section was kept in a different flask until the tissue could be prepared for incubation. These holding flasks were continuously oxygenated with a mixture of 95% $O_2$ and 5% $CO_2$. After all intestinal sections were removed, the lamb was sacrificed.

Each section was further cleaned by changing the buffer in the holding flask before sac preparation. The intestinal sections were everted by a glass rod threaded through the lumen. After eversion, the section was dipped in Krebs-Ringer bicarbonate buffer to remove any remaining feed particles. Sacs were prepared in the same order as the sections were removed from the animal. This was done in an effort to equalize the time for each section from removal to incubation.

Once everted, the section was placed on a clean damp tray and cut into 8 cm sections. Those sections that contained areas of apparent defect, such as a hemorrhaged spot or other damaged tissue, were discarded. Silk sutures were used to close one end of each sac before filling the sacs with the appropriate serosal solution. Sacs were held vertically by two hemostats placed at the very edge of the opening during the filling step. The hemostats were used to minimize the handling of the tissue. A 10 ml syringe was used to fill the sacs until proper distension was noted. The amount placed inside each sac was recorded. The filling procedure was subjective, but proper distension was necessary to expose the villus to the incubation medium. Overdistension was avoided in order to prevent unnecessary pressure on the interior of the sac. As each of the six sacs prepared for each section

was filled, another suture was applied to the remaining open end. The excess suture was clipped off and the sac placed in the designated incubation flask. Of the six prepared for a section, only four were used for absorption determinations, the other two served as blanks. All were prepared at the same time but were filled with different solutions. Both the serosal and mucosal fluids for the absorption flasks contained both the buffer medium and the test amino acid. Blank sacs were employed to detect any endogenous movement of amino acids so both the serosal and mucosal fluids were composed of only the buffer medium.

The incubation containers were 250 ml wide mouth Erlenmeyer flasks fitted with two-hole rubber stoppers. Two pieces of glass tubing were inserted into each stopper, one served as an inlet for the oxygen supply and the other was an outlet. The inlet tube extended to the bottom of the flask by following the contour of the flask wall. At the end of the inlet a crook had been placed to direct the flow of gas away from the side of the flask. The outlet tube extended into the flask 1 to 2 cm. The gas mixture which was 95% $O_2$ and 5% $CO_2$ was bubbled through the buffer and exited to the next flask. Flasks were linked together in groups of six. Eighteen flasks contained sacs prepared from the lamb, two additional flasks contained only the buffered amino acid solution. These two flasks were incubated with the others to determine any effect on the amino acid content as a result of the incubation period.

Krebs-Ringer bicarbonate buffer was prepared from stock solutions for each incubation. The composition of the buffered medium used is presented in table 2. All components, except sodium chloride and

TABLE 2. COMPOSITION OF *IN VITRO* INCUBATION MEDIUM[a]

| Item | g/4liters | Molar concentration |
|---|---|---|
| NaCl | 27.69 | 0.1180 |
| KCl | 1.41 | 0.0050 |
| $CaCl_2 \cdot 2H_2O$ | 1.49 | 0.0025 |
| $KH_2PO_4$ | 0.64 | 0.0012 |
| $MgSO_4 \cdot 7H_2O$ | 1.17 | 0.0012 |
| $NaHCO_3$ | 8.36 | 0.0249 |

[a]Adjusted to pH 7.4.

sodium bicarbonate were prepared in concentrated form to facilitate buffer preparation. The remaining two compounds were weighed and added to the solution at each incubation. The total buffer volume prepared for each incubation was 4 L. The buffer was adjusted to a pH of 7.4 using a Corning Digital 112 Research pH Meter. The amino acid solution was prepared just prior to the incubation. The L-form of each amino acid was dissolved in the Krebs-Ringer bicarbonate buffer to form a 5 mM solution. Burets were used to add exactly 100 ml of fluid (mucosal) to each flask. The blank flasks, two per section, were filled with buffer but no amino acid. The other flasks received the buffered amino acid solution. The blank sacs were filled with buffer solution while those used for amino acid absorption were filled with the buffered 5 mM amino acid solution. Thus, at the beginning of the incubation the amino acid concentration in both the mucosal and serosal fluids was 5 mM.

After the addition of the appropriate mucosal fluid to each flask they were placed in a water bath prior to the removal of the tissue from the lamb. Lead weights were affixed to each flask to counter the buoyancy created by the level of water in the bath. The water bath was adjusted to 39 C and maintained at that temperature for the incubation period. All gas lines were connected and oxygenation was begun to insure an adequate oxygen level when sacs were added. The flasks and mucosal solutions were placed in the water bath long enough prior to tissue addition to allow them to reach 39 C.

Incubations were conducted for 45 minutes. Timing began when the last sac for that section (duodenum, jejunum or ileum) was added to

the flask. At the end of the incubation period the gas supply was interrupted and the flasks removed from the water bath. The sacs were removed from the flasks by a pair of long hemostats. The excess fluid on the outside was allowed to drain into the flask. Each sac was then weighed as soon as possible. The serosal fluid from each sac was collected and the sac was reweighed after being blotted to remove excess fluid. The difference between these two weights was used as a measure of the amount of serosal fluid which remained in the sac following incubation. The sutures used to form the sacs were removed and the sac was cut into two approximately equal pieces. Both pieces were weighed and one was dried at 105°C for 36 hours to determine dry matter content. The other tissue section was stored by freezing under an atmosphere of nitrogen for later analysis.

Serosal and mucosal fluids were allowed to equilibrate to room temperature before sampling. Both serosal and mucosal fluids were prepared for amino acid analysis by adding 1 ml of a 20% sufosalicylic acid solution to 4 ml of the fluid. These samples were cooled for at least 6 hours, then centrifuged and filtered through fine glass wool. These samples were then further diluted to 10:1 with a sodium citrate buffer, pH 2.2. The resulting solution was frozen under an atmosphere of nitrogen and stored for later analysis. The remaining mucosal fluid was measured to determine the amount of volume change during the incubation. Initial samples of the amino acid solution were taken and stored as described above.

Tissue samples were prepared for amino acid analysis by hydrolyzing with 6 N HCl at a temperature of 105 C for 24 hours. One-half of

the tissue segment incubated was placed in a 50 ml volumetric flask. The 6 N HCl was then added to volume. The flasks were then placed in a desiccator. The desiccator was flushed with nitrogen, sealed and placed in the oven. After 24 hours the volumetrics were rediluted to volume then the acid hydrolysate was filtered through glass wool. A 2 ml aliquot of this filtrate was taken and evaporated over solid NaOH under vacuum. They were then reconstituted with 2 ml of sodium citrate buffer (pH 2.0) and stored frozen under an atmosphere of nitrogen until later analysis.

Amino acid analyses of all samples were conducted on a Technicon Model TSM Amino Acid Analyzer.

Values which deviated more than 2.5 standard deviations from the mean were excluded from statistical analysis. Each incubation was a replication of one amino acid. Valine was replicated five times and threonine and methionine were replicated three times. Replicates of each amino acid were combined for analysis.

Data were statistically analyzed within amino acids by the least squares analysis of variance procedure of Barr and Goodnight (1971). Differences among means were tested using the multiple range test of Duncan (1955).

## RESULTS AND DISCUSSION

*In Vitro System*. A preliminary experiment was conducted to determine the fluid and amino acid losses due to the *in vitro* system design. Eight flasks were used to form two groups. One group contained six flasks and the other group two flasks. These two groups were representative of the proposed grouping to be used during the experimental incubations. Each flask contained 100 ml of Krebs-Ringer bicarbonate buffer and 455 μmoles of threonine. The two groups were placed in a water bath and heated to 39 C for a two-hour period. The $O_2$ and $CO_2$ gas mixture used during the incubation was attached and bubbled through the mixture during this period. The time interval was established by determining how long the flasks would be exposed to the incubation environment during the actual experiment. Amino acid content of each flask was expressed as μmoles per flask and compared to the initial value of 455 μmoles. Mucosal volumes of each flask were also determined by measuring the remaining fluid in each flask with a graduated cylinder.

Average mucosal fluid recoveries were 98.4 and 98.0 ml from the six-flask group and two-flask group, respectively. From previous experience it had been established that approximately 1.5 to 2.0 ml are retained by the flask and are impossible to recover. The amino acid recovery was expressed as a percentage of the initial amount added. Recovery rates were 97.2% and 103.4% for the six-flask and two-flask

groups, respectively. Standard deviations were also determined for amino acid recovery. The six-flask group had a larger standard deviation, 3.91, than the two-flask group, 0.63. From this preliminary experiment it was concluded that the mucosal volume and amino acid concentration would not be altered substantially as a result of the system.

Serosal to Mucosal Ratio. Final concentrations of each amino acid in the serosal and mucosal fluids of each flask were determined. The concentration of the amino acids are espressed as μmoles per milliliter and a ratio between the serosal and mucosal fluid calculated. These values are presented in table 3. Initial concentrations of amino acids were equal in the serosal and mucosal fluids; therefore, movement of the amino acid from one fluid to the other would be one means of indicating active transport. Values greater than unity indicate a higher concentration of amino acid in the serosal fluid than in the mucosal fluid and *vice versa*.

The serosal to mucosal ratios indicate that the magnitude of valine absorption increased progressively from the proximal to the distal section of the small intestine. Ratios of 0.94, 1.33 and 2.10 for the duodenum, jejunum and ileum, respectively, were significantly different ($P<.01$). Threonine absorption, as indicated by these ratios, was significantly greater ($P<.01$), in the ileal section than in either the duodenum or the jejunum. Likewise, methionine absorption was significantly greater ($P<.01$) in the ileum than in either the duodenum or the jejunum. For both threonine and methionine there was a trend for greater absorption in the jejunum than in the duodenum but these differences were not significant. Serosal to mucosal ratios in the

TABLE 3. RATIO OF SEROSAL TO MUCOSAL AMINO ACID CONCENTRATION AFTER FORTY-FIVE MINUTE INCUBATION

| Site | Amino Acid | | |
|---|---|---|---|
| | Valine | Threonine | Methionine |
| Duodenum | 0.94[a] (0.042) | 0.95[a] (0.053) | 1.00[a] (0.036) |
| Jejunum | 1.33[b] (0.089) | 1.07[a] (0.025) | 1.22[a] (0.131) |
| Ileum | 2.10[c] (0.068) | 2.10[b] (0.320) | 1.61[b] (0.125) |

[a,b,c]Means in the same column with different superscripts are significantly different ($P < .01$).

[d]Values in parentheses are standard errors of the means.

duodenum of 0.94 for valine and 0.95 for threonine indicate no absorption or a negative absorption of these amino acids in this section of the small intestine.

Wiseman (1954) reported an average ratio of 1.18 for methionine obtained by using everted sacs from jejunal and ileal sections of rat intestine incubated in a 20 mM solution. Larsen _et al_. (1964) used a much lower concentration of amino acids, 0.05 mM, with the same segments and reported higher ratios for each amino acid. Their values were 14, 15, and 16 for valine, methionine and threonine, respectively. Serosal to mucosal ratios appear to be somewhat dependent on the initial concentration. The lower the concentration, the higher the ratio will be.

The amino acid concentration in the tissue is higher than the concentration in the mucosal fluid at the end of an incubation (Agar _et al_., 1954). If the movement of amino acid from the tissue to the serosal fluid is by diffusion, then the amino acid concentration in the tissue is higher than the serosal fluid. Baker and George (1971) reported tissue to mucosal ratios of 7.33 and 1.38 for methionine at 1 mM and 10 mM, respectively. Thus the ratios presented in table 3 are much smaller than the tissue to mucosal ratios would be. When a concentration gradient has developed in the serosal fluid this indicates a larger gradient in the tissue.

_Amino Acid Uptake from the Mucosal Fluid_. The μmoles of each amino acid removed from the mucosal fluid are expressed as μmoles per gram of dry tissue per 45 minutes and are presented in table 4.

Valine absorption increased as the distance from the pylorus

TABLE 4. ABSORPTION OF AMINO ACIDS FROM THE MUCOSAL FLUID BY INTESTINAL SECTIONS

| Site | Amino acid[a] | | |
|---|---|---|---|
| | Valine | Threonine | Methionine |
| Duodenum | 83.60[b] (17.700)[d] | 13.05[e] (27.104) | 26.24[e] ( 4.480) |
| Jejunum | 126.53[b] (20.474) | 1.17[e] (8.697) | 177.10[f] (68.460) |
| Ileum | 206.72[c] (16.543) | 140.75[f] (49.871) | 168.76[f] (58.981) |

[a]Expressed as μmoles per gram of dry tissue per 45 minutes.

[b,c]Means in the same column with different superscripts are significantly different ($P<.01$).

[d]Values in parentheses are standard errors of the means.

[e,f]Means in the same column with different superscripts are significantly different ($P<.05$).

increased. Absorption of valine by the duodenal and jejunal sections was not significantly different but the μmoles of valine removed from the mucosal fluid was greater in the jejunal fluid. The ileal section removed almost twice as much valine as the jejunal section, 206.72 and 126.53 μmoles, respectively. The amount absorbed by the ileal section was significantly greater ($P<.01$) than the amounts removed by either the duodenal or jejunal sections.

Duodenal and jejunal absorption of threonine was significantly lower ($P<.05$) than absorption of threonine by the ileal section. The duodenum and jejunum were not significantly different, but there was a trend for greater absorption in the duodenum. Absorption by the ileal section was more than ten fold greater than by the duodenal or jejunal sections.

Duodenal absorption of methionine was significantly lower ($P<.05$) than absorption in the jejunal and ileal sections. Assorption rates of 177.10 and 168.76 μmoles for jejunal and ileal tissue, respectively, were not significantly different, but were significantly greater ($P<.05$) than the duodenal tissue rate of 26.24 μmoles.

Williams (1969) infused an amino acid mixture into the intestine of sheep to determine uptake rate at different sites in the intestine. He found that maximum absorption of valine was achieved in the lower jejunum and upper ileum. Threonine absorption was maximized in the upper jejunum and methionine absorption was greatest in the upper jejunum and lower ileum. The intestinal sections used in the present study were from the duodenum, upper to middle jejunum and lower ileum.

Absorption sites of valine and methionine by these sections were similar to those reported by Williams (1969).

Larsen et al. (1964) determined $K_M$ values for valine, threonine and methionine in rat intestine. Valine and methionine values were one-half as large as the value reported for threonine, indicating that valine and methionine have a stronger affinity for the neutral amino acid transport system. The absorption of amino acids with strong affinities is not easily inhibited by other amino acids. Thus, the values obtained by Williams (1969) for valine and methionine in a mixture can be compared to the present study. Similar results were obtained for the maximum absorption site of these two amino acids. Threonine $K_M$ values indicate that it could be inhibited in a mixture of amino acids. Williams (1969) reported maximum absorption of threonine in the upper jejunum. Results from this study indicate that when threonine is the only amino acid present, absorption is maximum in the ileal section and minimal in the jejunal section.

Amino Acid Change in the Serosal Fluid. The amount of each amino acid transported into or out of the serosal fluid is presented in table 5. Values are expressed as μmoles of amino acid per gram of dry tissue per 45 minutes present above the initial value. Negative values indicate a loss of amino acid from the serosal fluid.

Valine was transported from the serosal fluid into the tissue by the duodenum and jejunum. This type of movement results in negative values of -16.65 and -4.21 μmoles for the duodenal and jejunal sections, respectively. Values by these sections were not significantly different

TABLE 5. ACCUMULATION OF AMINO ACIDS IN THE SEROSAL FLUID BY INTESTINAL SECTIONS

| Site | Amino acid[a] | | |
|---|---|---|---|
| | Valine | Threonine | Methionine |
| Duodenum | -16.65[b] (4.933)[d] | 10.03[b] (9.327) | 5.51[b] (3.373) |
| Jejunum | - 4.21[b] (11.312) | 5.47[b] (2.384) | 4.35[b] (4.880) |
| Ileum | 82.50[c] (10.130) | 85.93[c] (27.554) | 38.74[c] (6.976) |

[a]Expressed as μmoles per gram of dry tissue per 45 minutes.

[b,c]Means in the same column with different superscripts are significantly different ($P<.01$).

[d]Values in parentheses are standard errors of the means.

from each other, but were significantly lower (P<.01) than that for the ileal section. The ileal section gained 82.50 μmoles in the serosal fluid while the other sections lost valine.

The appearance of threonine in the serosal fluid followed the same pattern as its removal from the mucosal fluid. Accumulation in the serosal fluid of the duodenal section was not significantly greater than in the jejunal section. Tissue from the ileal section accumulated significantly more (P<.01) threonine in the serosal fluid than the more proximal sections.

Accumulation of methionine in the serosal fluid followed a similar pattern as threonine. Duodenal and jejunal section differences were not significant. An appearance of 38.74 μmoles was determined as the ileal rate. This value was significantly greater (P<.01) than the rates of 5.51 and 4.35 μmoles for the duodenal and jejunal sections, respectively.

Valine was the only amino acid that was not accumulated in the serosal fluid by the duodenal and jejunal tissue. The movement of valine into the tissue from both the mucosal and serosal fluids would result in final concentrations lower than the initial concentration. The movement of valine out of the serosal fluid in the duodenal section was not significantly greater than the jejunum, but this movement could be large enough to result in a serosal to mucosal ratio of less than one, which was the case with duodenal absorption of valine.

Threonine serosal to mucosal ratios were low in the duodenal and jejunal sections also, but threonine was accumulated in the serosal fluid of these two sections. For these ratios to become low values,

threonine would have to be released from the tissue into the mucosal fluid. The same situation occurred with methionine.

Tissue Accumulation of Amino Acids. The amino acid content of tissue which was incubated in buffered media containing valine, threonine, methionine or no amino acid was determined. Tissues incubated in buffered media only were used as a base to determine the increase of amino acid in the tissues. Accumulation of an amino acid is expressed as μmoles per gram of dry tissue per 45 minutes above the base established for that section of intestine and the amino acid. These values are presented in table 6.

Accumulation of valine between the duodenal and ileal sections was not significantly different. The jejunal section accumulated significantly less ($P<.01$) valine than either of the other sections. Valine was accumulated in the tissue of the duodenum and the ileum at rates of 51.84 and 58.23 μmoles, respectively. These rates were more than double the rate of 21.90 μmoles accumulated by the jejunum. Tissue accumulation of threonine followed a similar pattern. The ileal tissue accumulated significantly more ($P<.05$) threonine than the duodenal or jejunal sections. The duodenal section accumulated 25.41 μmoles, but this was not significantly different from the jejunal section.

Methionine accumulation by intestinal tissue was unlike the accumulation of either valine or threonine. The three intestinal sections were very similar. Although methionine was accumulated at the same level by all tissue, the amount absorbed from the mucosal fluid (table 4) and released into the serosal fluid (table 5) did vary significantly ($P < .05$) among intestinal sections.

TABLE 6. ACCUMULATION OF AMINO ACIDS BY INTESTINAL TISSUE DURING THE INCUBATION PERIOD

| Site | Amino acid[a] | | |
|---|---|---|---|
| | Valine | Threonine | Methionine |
| Duodenum | 51.84[b]<br>(12.096)[d] | 25.41[e]<br>(5.409) | 18.80<br>(5.412) |
| Jejunum | 21.90[c]<br>(7.200) | 19.95[e]<br>(5.412) | 15.15<br>(1.832) |
| Ileum | 58.23[b]<br>(5.487) | 44.45[f]<br>(6.818) | 16.15<br>(3.529) |

[a]Expressed as μmoles per gram of dry tissue per 45 minutes.

[b,c]Means in the same column with different superscripts are significantly different ($P<.01$).

[d]Values in parentheses are standard errors of the means.

[e,f]Means in the same column with different superscripts are significantly different ($P<.05$).

Baker and George (1971) divided the small intestine of the rat into eight sections. The first one was the duodenum, the next three were the jejunum and the last four were the ileum. Tissue accumulation of methionine increased from the duodenum to the upper ileum at both 1 mM and 10 mM concentrations.

Tissue accumulation in the present study indicates that the duodenum and ileum accumulate similar amounts of valine, or methionine. The jejunum accumulated less of these amino acids with the exception of methionine.

Differences of Intestinal Site. The values presented to this point have been segregated by amino acid. The data from all incubations were combined to evaluate the effect of intestinal site, without regard to amino acid, on all of the parameters observed.

The absorption of amino acid from the mucosal fluid increased as the distances from the pylorus increased (Table 7). The transport of amino acids from the mucosal fluid of the duodenum was 53.67 μmoles of amino acid per gram of dry tissue per 45 minutes. This was one-half the amount transported from the mucosal fluid of the jejunum and one-third of the amount transported by the ileum. This same trend was true when each amino acid was analyzed separately, except for methionine. The jejunum absorbed as much methionine as the ileum and both were significantly greater ($P<.05$) than the duodenum (Table 4).

Appearance of amino acids in the serosal fluid followed the same order as absorption from the mucosal fluid. The duodenum was significantly lower ($P<.01$) than the other two sections in the amount absorbed from the mucosal fluid but there was no significant difference between

TABLE 7. AMINO ACID TRANSPORT AND WATER FLUX BY DIFFERENT INTESTINAL SECTIONS

| Item | Site | | |
|---|---|---|---|
| | Duodenum | Jejunum | Ileum |
| Amino Acid transport, | | | |
| Mucosal fluid[a] | -53.67[b] (12.948)[e] | -101.55[b] (21.511) | -178.63[c] (21.922) |
| Serosal fluid[a] | -3.99[b] (4.138) | 0.37[b] (5.763) | 71.89[c] (9.204) |
| Tissue[a] | 36.34[f] (6.673) | 19.45[g] (3.615) | 43.51[f] (4.339) |
| Change in serosal fluid volume, ml | -2.46[b] (0.370) | -5.00[c] (0.713) | -1.56[b] (0.756) |
| Serosal to mucosal ratios | 0.96[b] (0.026) | 1.24[c] (0.057) | 1.97[d] (0.100) |

[a]Expressed as µmoles per gram of dry tissue per 45 minutes.

[b,c,d]Means in the same row with different superscripts are significantly different ($P<.01$).

[e]Values in parentheses are standard errors of the means.

[f,g]Means in the same row with different superscripts are significantly different ($P<.05$).

the duodenum and jejunum in the amount released into the serosal fluid. The duodenum actually absorbed amino acids from the serosal fluid as indicated by the negative value. The jejunum released a very small amount (0.37 μmoles) of amino acid into the serosal fluid in relation to the amount absorbed from the mucosal fluid (101.55 μmoles). The ileal section absorbed a significantly greater ($P<.01$) amount of amino acid from the mucosal fluid and released a significantly greater ($P<.01$) amount into the serosal fluid. From this study it would appear that amino acids absorbed by the ileum are released to the serosal fluid at a greater rate than by the duodenum or jejunum.

From the two previous values, the mucosal uptake and serosal appearance of amino acids, the amount of amino acid present in the tissue above the initial value can be anticipated. The values presented in table 7 for tissue accumulation are lower than anticipated. The trend of absorption and release of amino acids from the three sections was to increase as the distance from the pylorus increased. The tissue accumulation might be thought to be solely a function of absorption from the mucosal fluid and release into the serosal fluid, however there are other factors involved.

The ileum accumulated significantly more ($P<.05$) amino acid in the tissue than did the jejunum. The duodenum was intermediate and did not differ significantly from the ileum, but was significantly greater ($P<.05$) than the jejunum. The duodenum and jejunum are in the reverse order of the values anticipated. The duodenum did absorb amino acids from both fluids, however, the amount absorbed from the mucosal fluid by the jejunum was twice as large as the amount absorbed

by the duodenum and the jejunum released only a small amount into the serosal fluid. The ileum accumulated amino acids as anticipated.

If it is assumed that the amount of amino acid absorbed from the mucosal fluid and not released into the serosal fluid is in the tissue, then recovery rates calculated from these values are below 100%. Recovery percentages of 63.0, 19.2 and 40.8 were determined for the duodenum, jejunum and ileum, respectively.

Recovery values have been reported by Finch and Hird (1960a) of over 100% for methionine and valine incubated with the small intestine of rats. Agar et al. (1954) used rat intestine in their study, too. They found 100% recovery of histidine, but Finch and Hird (1960a) did report that low recovery of amino acids is possibly due to metabolism and transamination of the amino acids by intestinal tissue. Amino acids can be metabolized after transamination to supply energy. Energy is necessary for the operation of the active transport system which absorbs the amino acids from the mucosal fluid (Guyton, 1971). Before the amino acids can be utilized for energy, accumulated in the tissue or released to the serosal fluid, they must be actively transported into the mucosal cell. Thus, the uptake from the mucosal fluid is indicative of the transport capabilities of the intestine. The amount of amino acid released to the serosal fluid is also relative to the ability of the intestine to release amino acids to the vascular system. Thus, the failure to recover a large percentage of the amino acids absorbed from the mucosal fluid could be due in part to tissue metabolism. Amino acids which were thought to be in the tissue may have been transaminated and some used for energy to power the uptake of more amino acids. The

amount of transamination by the ovine intestine is not known.

No energy source was provided in the incubation media in this study. Glucose has been used in media of this type to provide energy for the intestinal tissue (Agar et al., 1954; Finch and Hird, 1960a). Glucose was not added to this media because Alvarado (1966) indicated that sugars can compete with amino acids for active transport. The results of Agar et al. (1954) and Finch and Hird (1960a), which reported 100% or more recovery of amino acids, used 0.5% glucose in the incubation media.

Recovery of each amino acid also varied. The recovery of valine and methionine were similar to those reported for the sections when the data was combined. Threonine was calculated to have been recovered in excess of 100%. The extremely large values for recovery could have resulted from a low base value being established for the duodenal and jejunal sections for threonine. This would credit the tissue with accumulation when actually there was none or a loss.

The variations in recovery observed for all amino acids could be the result of analytical errors in the analysis of tissue samples, mucosal and serosal fluids. Most likely, however, both tissue metabolism and analytical error are contributing factors.

Fluid volume changes were determined for each sac. The amount lost by each sac was then corrected to one gram of dry tissue per 45 minutes. Intestinal tissue used in this study was determined to be approximately 20% dry matter. The actual dry tissue of the 8 cm sacs used in this study was 0.5929, 0.4549 and 0.5158 per sac for the duodenum, jejunum and ileum, respectively. The values presented in table 7 are

approximately twice that which was actually lost from the sacs of each segment as a result of expressing loss on a dry tissue basis. Fluid movement from the serosal fluid of sacs incubated with threonine or methionine was not significantly different, but sacs incubated with valine were significantly different (P<.01). When the data for all amino acids were combined (Table 7) the jejunum lost significantly more (P<.01) fluid than the duodenum or ileum, which were not significantly different.

The fluid losses presented in table 7 do not follow a particular amino acid movement pattern, but the jejunum lost significantly more (P<.01) fluid than the other two sections. The water movement from the serosal fluid into the tissue may be related to the low tissue accumulation of amino acids by the jejunum.

Finch and Hird (1960a) reported that water movement from the media fluid into the tissue of rat intestinal rings. This was less than one milliliter per gram of dry tissue per 45 minutes. Wilson and Wiseman (1954) reported movement of 4.1 ml and 2.2 ml of fluid from the mucosal into the serosal fluid per gram of dry tissue per hour using rat intestine. Finch and Hird (1960a) found that water exchange between the tissue and the media was essentially complete after 15 minutes of incubation and once tissue is removed from the media the fluid will be released from the tissue. Water losses reported in this study could be a result of removing the sacs from the media at the end of the incubation and fluid moving from the serosal fluid to the mucosal side of the sac and lost.

Wilson and Wiseman (1954), who initially developed the everted sac technique, reported that the mucosal fluid would move into the serosal fluid until a certain level of distension was obtained. The sacs in the present study were filled until adequate distension was obtained, but no sacs were distended to such a point that over-distension was obvious. Thus, the movement of fluid from the serosal fluid could have happened during the initial portion of the incubation to establish the proper fluid balance between the tissue, mucosal and serosal fluids.

The serosal to mucosal ratios are indicative of what happened during the incubation. Significantly greater ($P<.01$) concentration gradients were developed between serosal and mucosal fluids as the sections increased in distance from the pylorus. The duodenum did not develop a gradient, thus its effectiveness as an amino acid absorption site is slight. The jejunum and ileum developed concentration gradients above one, 1.24 and 1.97, respectively. These concentrations indicate the ability of the ileum to release the amino acid absorbed into the serosal fluid.

The duodenum is capable of absorbing amino acids from the mucosal fluid and accumulating these amino acids in the tissue. The release of amino acids into the serosal fluid by the duodenum is small, if any. This section appears not to be very effective as a site of amino acid absorption. The jejunum showed a strong capability of absorbing valine and methionine, but only a slight absorption of threonine. The amino acids it did absorb were neither found in the serosal fluid nor accumulated in the tissue. This may indicate that the jejunum used these amino acids for energy or the structure of the amino acids was altered

by transamination. The ileum maximized movement of all three amino acids. It consistently absorbed the greatest amount from the mucosal fluid and released the greatest amounts into the serosal fluid. The ileal tissue also accumulated as much amino acid as the duodenum, but this was a much smaller portion of the amount transported through the tissue than the duodenum. The ileum appears to be the most active and efficient site of valine, threonine and methionine absorption.

## SUMMARY

Duodenal, jejunal and ileal sections from sheep were used to study *in vitro* absorption of valine, threonine and methionine. Everted sacs were incubated for 45 minutes at 39 C under an atmosphere of 95% $O_2$ - 5% $CO_2$ in Krebs-Ringer bicarbonate buffer (mucosal and serosal fluid) containing 5 µmoles per ml of the test amino acid. The hydrolyzed tissue and mucosal and serosal fluids were analyzed for amino acid content. The duodenum absorbed smaller quantities of amino acids from the mucosal fluid than the jejunum and significantly ($P<.01$) less than the ileum. The release of amino acids from duodenal tissue was small for threonine and methionine and negative for valine. Accumulation of amino acids by duodenal tissue was not significantly different from the ileum, but this represented a larger part of the amount absorbed from the mucosal fluid. The duodenum appears to be of limited importance as a site of amino acid absorption. The jejunum readily absorbed valine and methionine, but absorbed only a small amount of threonine from the mucosal fluid. The release of amino acids into the serosal fluid by the jejunum was very slight in comparison to the ileum ($P<.01$). Tissue accumulation of amino acids was significantly less ($P<.05$) than in either the duodenum or ileum. While the jejunum generally absorbed large amounts of amino acids from the mucosal fluid, it did not release or accumulate amino acids in large amounts. The ileum maximized movement of all amino acids. It absorbed significantly more ($P<.01$) amino

acids from the mucosal fluid and released significantly more (P<.01) amino acids into the serosal fluid. The accumulation of valine and threonine by ileal tissue was significantly greater (P<.05) than the jejunum. The ileum, therefore, appears to be the most active and efficient site of valine, threonine and methionine absorption in the sheep.

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<u>IN VITRO</u> ABSORPTION OF VALINE, THREONINE AND METHIONINE
BY THE SMALL INTESTINE OF SHEEP

by

William Allison Phillips

(ABSTRACT)

Duodenal, jejunal and ileal sections from sheep were used to study *in vitro* absorption of valine, threonine and methionine. Everted sacs were incubated for 45 minutes at 39 C under an atmosphere of 95% $O_2$ - 5% $CO_2$ in Krebs-Ringer bicarbonate buffer (mucosal and serosal fluid) containing 5 μmoles per ml of the test amino acid. The hydrolyzed tissue and mucosal and serosal fluids were analyzed for amino acid content. The duodenum absorbed smaller quantities of amino acids from the mucosal fluid than the jejunum and significantly ($P < .01$) less than the ileum. The release of amino acids from duodenal tissue was small for threonine and methionine and negative for valine. Accumulation of amino acids by duodenal tissue was not significantly different from the ileum, but this represented a larger part of the amount absorbed from the mucosal fluid. The duodenum appears to be of limited importance as a site of amino acid absorption. The jejunum readily absorbed valine and methionine, but absorbed only a small amount of threonine from the mucosal fluid. The release of amino acids into the serosal fluid by the jejunum was very slight in comparison to the

ileum ($P<.01$). Tissue accumulation of amino acids was significantly less ($P<.05$) than in either the duodenum or ileum. While the jejunum generally absorbed large amounts of amino acids from the mucosal fluid, it did not release or accumulate amino acids in large amounts. The ileum maximized movement of all amino acids. It absorbed significantly more ($P<.01$) amino acids from the mucosal fluid and released significantly more ($P<.01$) amino acids into the serosal fluid. The accumulation of valine and threonine by ileal tissue was significantly greater ($P<.05$) than the jejunum. The ileum, therefore, appears to be the most active and efficient site of valine, threonine and methionine absorption in the sheep.