CHARACTERISTICS OF THREONINE, VALINE AND METHIONINE ABSORPTION IN THE JEJUNUM AND ILEUM OF SHEEP

by

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INTRODUCTION

Protein units are generally used to express the nitrogen intake of an animal, but proteins are altered once they reach the gastrointestinal tract by chemical and enzymatic hydrolysis to yield amino acids and peptides. These amino acids are still considered outside the body of the animal until they cross the mucosal epithelium which stands as a barrier between the intestinal lumen and the circulatory system.

The majority of the information relating to the absorption of amino acids has been derived by *in vitro* procedures using monogastrics, usually laboratory animals. Results from this research suggest that different transport systems are present in the small intestine and that amino acids interact with each other for these systems. Amino acids differ in the affinity they possess for the transport systems which in turn dictates the ability of an amino acid to inhibit the absorption of other amino acids. The interrelationships among amino acids for absorption by the small intestine of monogastric animals has been researched quite extensively.

Ruminants have a uniquely different digestive system in comparison to monogastric animals. The microbial population of the rumen is able to synthesize all amino acids, utilizing carbohydrate sources such as callulose and various non-protein nitrogen sources as substrates. This has delayed, until recently, the development of interest in amino acid nutrition of the ruminant. Little is known about amino acid

transport systems or the interactions among amino acids for these systems in the ruminant small intestine. In order to increase protein utilization and in turn increase growth, efficiency and production, all aspects of amino acid nutrition of the ruminant should be explored. Experiments were undertaken to determine the amino acid absorption characteristics of three structurally different amino acids at two different intestinal sites. Double re-entrant cannulae were employed to ascertain these characteristics *in vivo*.

REVIEW OF LITERATURE

Proteins are macromolecules which are composed of the basic component amino acids. Proteins are ingested, hydrolyzed to amino acids, absorbed, transported to the site of utilization and reconstructed into proteins. Thus, the protein unit ingested results in animal protein in the body, but the transition from the intestinal lumen to the utilization site requires that the amino acids be absorbed across the intestinal epithelium. The functional characteristics of the mucosal epithelium thus determines which amino acid will be available later for protein synthesis.

Procedures Used to Study Absorption

In Vitro Procedures. Many in vitro procedures have been used to ascertain amino acid absorption characteristics. Each procedure allowed the researcher to control the environment within the closed system. Because the system was closed, the amino acids could be studied without interference from other digestive products and endogenous amino acid sources.

These procedures require that the intestinal section to be studied be removed from the animal as quickly as possible. The greatest concern has been whether the tissue remained alive and viable within each system. Agar et al. (1954) developed the use of intestinal rings as a means of studying amino acid absorption. Once the

intestinal segment to be studied had been removed and flushed of all digesta, it was everted and cut into segments 0.5 cm in length. This resulted in rings with edges curled inward and with the mucosa located on the outside of the ring. These rings could then be incubated in a warm, oxygenated, buffered medium containing amino acids. The removal of amino acids from the media by the tissue could be measured and expressed as amounts per gram of dry tissue to standardize the units. The circulatory system, normally present to remove the amino acids after absorption, is not present. Thus, this procedure would only yield data relating to the ability of tissue to retain or concentrate amino acids. It was found that the movement of amino acids between the tissue and incubation media was bidirectional. The tissue maintained a certain ratio between tissue and media. By expressing the amino acid content of the tissue as a concentration based on the amount of tissue water, it was shown that a concentration gradient had developed. The concentration in the tissue was higher than in the incubation media. Tissue in intestinal rings would reach a maximum amino acid concentration after 20 minutes of incubation.

The procedure used by Wilson and Wiseman (1954) was reported at the same time as the ring procedure, but it added another dimension to the collection of data. The intestinal section was removed in a similar manner, but longer segments were cut, 2 to 3 cm, after the segment was everted. By tying one end of the section to form a sac, filling the sac with test solution or media, and tying the remaining end, another compartment had been added. The sac could now be incubated

in buffered media containing amino acids. The amino acids would be absorbed from the mucosal fluid by the mucosa on the exterior of the sac and transported into the tissue. Once the concentration of amino acids in the tissue had reached saturation, the amino acid could diffuse or be transported into the fluid inside the sac. The fluid inside the sac was called serosal fluid. The serosal fluid would be similar to the circulatory system of the animal. Thus, a more natural path for amino acid absorption had been developed. This procedure could provide information on tissue absorption rates since the amino acids were being removed from the tissue. For the first time, researchers could look at the concentration gradients between the three compartments, the intestinal lumen, tissue and serosal fluid. Information derived from the use of this procedure showed that amino acids are concentrated in the tissue at levels above the incubation media and serosal fluid.

Procedures were developed whereby intestinal sections were removed and connected at both ends to a perfusion apparatus (Crane and Wilson, 1957; Fisher and Parsons, 1949). Test solutions could then be circulated through the lumen while the exterior, serosal side, was being bathed with media. The amino acids which were diffused from the tissue were picked up by the flowing media on the outside. This procedure was quite similar to the everted sac but added movement to simulate digesta flow and the circulatory system.

The three previously described procedures can be classified as whole tissue preparations. These types of preparations resulted in

viable tissue (Fisher and Parsons, 1949), but the heterogenous composition of the intestine has resulted in criticism that the non-epithelial tissue formed a barrier to amino acid movement thus biasing the results (Munck, 1972). Dickens and Weil-Malherbe (1941) developed a procedure which used only the mucosal cells. These cells were obtained by scraping the intestinal segment once it had been removed from the animal and cleaned. The cells could then be used to study amino acid absorption over short periods of time without the presence of muscular tissue. Munck (1972) compared data obtained by this method to previous work using whole tissue preparation. The results were contradictory, which he attributed to the system used.

Each procedure has problems which limit its use and data interpretation. But each provides a means by which the intestinal environment can be controlled and closed in order to ascertain the location of transport systems, requirements of each to function and characteristics as well as the movement of amino acids from the intestinal lumen to the serosal side of the intestine or circulatory system. An understanding of the procedures will be useful in comparing data from several sources with different procedures being used.

In Situ Procedures. Other procedures were developed to study characteristics, but these procedures left the intestine intact in the test animal (Gibson and Wiseman, 1951; Williams, 1969). The animal would be anesthetized and the intestine exposed by a midline incision so that the section to be studied could be located and loops

or small sacs formed by ligating the intestine, after it had been cleaned. Test solutions could be introduced into the sacs and the sacs pushed back into the cavity so that they could be kept warm and moist. Absorption would be measured by the removal of the test substance or amino acid from the loop. This type of procedure moves the researcher one step closer to actual absorption conditions of the intestine. The blood system was available to remove amino acids as they were absorbed by the mucosa as well as provide energy and remove metabolic products formed. The changes in environment would be minimized, but it has not been established how much the anesthesia would affect the absorption rate of the small intestine. This type of procedure did attack the problem with a more physiological approach.

Other researchers, such as Jacobs and Lang (1965), used a similar procedure. The intestine remained in its normal environment with its blood supply intact, as the loop described in the previous paragraph. Instead of forming loops or sacs, the segment was left complete. They attached the intestine to a perfusion apparatus and perfused solutions through the intestine during the absorption period. This added movement was similar to digesta flowing through the lumen. Removal of the test substance from the perfusion solution could be monitored as a function of time. Also, the concentration of the test substance in the perfusion solution could be maintained at a more constant level by allowing it to pass through the intestine only one time. But once again the effects of the anesthesia on absorption were not known.

Intubation Procedures. In the 1920's, Cori (1925) studied

absorption of sugars by feeding the test substance. Rats were fasted for 48 hours with access to water, then the test solution was administered by a stomach tube. After one hour, the animal was sacrificed and the contents of the tract removed and analyzed. The amount of test ingredient absorbed could be determined by difference. Endogenous sources of the test ingredient could be accounted for by using blanks or controls treated with water. But Badway et al. (1957) reported that the means by which an animal is sacrified would affect the amount of epithelium or mucosal cells that are shed. Although the Cori method is not very sensitive and only one observation can be obtained from each animal used, it did remove many of the criticisms of the in vitro and in situ procedures.

Researchers began to explore new methods of studying absorption in the small intestine so that more observations from the same individual could be obtained as well as approach a more normal environment for the intestine. Monogastrics, especially man, were anatomically able to be intubated to study absorption in the intact intestine (Cummins, 1952; Fleshler et al., 1966). Double or triple lumen tubes were fed through the nasal cavity down the esophagus and into the stomach. The tubes were then moved down the tract with the peristaltic action of the intestine until the test section had been reached. Each lumen of the tube was connected to the outside by holes. Two of the lumens were used to administer and collect the test solution which contained a marker to calculate volume. The third lumen, if present, was used to inflate a balloon or some other rubber membrane to seal off that portion of the tract. This gave the researcher a tool to

study absorption in the intact animal, but his control over the conditions within the lumen were limited.

Cannulation of Small Intestine. In order to add more control and easy access to the intestinal segment to be studied, Newman and Taylor (1958) modified an old technique from the 1800's referred to as the Thiry-Vella fistula. This preparation isolates a segment of intestine by exteriorizing both ends of the segment once the remaining ends of the tract have been rejoined to reestablish its continuity. The procedure allowed easy access to the intestine for repeated measurements while keeping the blood supply intact with the segment lying free in the abdominal cavity. Johnston (1932) reported that enzymatic activity was still present in the Thiry-Vella loop after functioning for 7 months. Raudin et al. (1933) felt that their Thiry-Vella loops were still sound after 6 months because the absorption rate of glucose remained constant during this period of time. Histological studies of the mucosa of the chicken small intestine after fistulation showed no changes except for the area around the fistula (Newman and Taylor, 1958). Huan and Hung (1972) reported that atrophy occurred in rats after 6 months, but no digestive problems or loss of weight were noted. The rats lived for as long as 2.5 years without any major problems. Intestinal segments of this type were usually flushed daily with a glucose solution to remove debris from the lumen, but Keren and Elliot (1973) felt that some factor present in the chyme was needed to prevent the mucosal changes they noted in their Thiry-Vella loops. This type of preparation has been used to study the absorption of amino acids (Annegers,

1969), drugs (Sample et al., 1968), glucose and electrolytes (Bywater, 1970) as well as enzymatic activity (Cajori, 1933) of the small intestine.

Thus, more modification of the Thirry-Vella loop was required to allow passage of digesta through the isolated loop, but still maintain the capabilities to isolate this segment for absorption studies. Earlier researchers used intestinal cannulas to study digesta composition and flow rate (Goodall and Kay, 1965; Harrison and Hill, 1962; Phillipson, 1952). These procedures described cannulae which were installed in the ends of the intestine after it had been transected. This provided a means of interrupting the intestine to collect digesta and return it. Such procedures are referred to as re-entrant cannulae. From this previous work many important techniques and characteristics of intestinal cannulation were developed. Ash (1962) reported that the composition of the diet would influence the number of times blockage would occur in the cannulae. He found that hay fed in either the long or chopped form produced fewer numbers of blocked cannulae than rations with concentrates added. Leakage between the cannula and the intestine could be reduced by inverting the mucosa around the cannula with purse string sutures so that none was exposed (Streeten and Williams, 1951). Exposed mucosa would continue to grow and enlarge the opening. Massive adhesions of intestine to the abdominal wall after cannulation was a problem, until Brown et al. (1968) reported that placing the omentum around the cannula would prevent this. Effects of fluid losses from cannula leaks were detrimental to the animal, but treatments were developed to return the

animal to a normal electrolyte balance (Horney et al., 1972).

Re-entrant cannula were successfully employed in different species and different sections of the intestine of these species for absorption and digesta studies (Easter and Tanksley, 1973; Holmes et al., 1973; Horney et al., 1972, 1973; Phillipson, 1952). This type of cannulation was durable. Ash (1962) reported animals remaining functional for as long as 18 months. With improvements in cannula construction and more expertise in their inplacement, double re-entrant cannula could be employed (Horney et al., 1972, 1973). With two sets of re-entrant cannula, the intestine can be interrupted at two points. The section between the two pairs of cannulae could then be used for absorption studies because each end contains a cannula. With this procedure, the intestine functions normally, maintaining its absorption and secretory functions, but it is still available to the researcher for absorption studies. It can be isolated for periods of time to study its absorption characteristics by the infusion of different solutions containing the test ingredient. Absorption of these ingredients, as measured by disappearance from the lumen, can be determined but their fate after absorption cannot be followed.

Active Transport of Amino Acids from the Intestinal Lumen (Guyton, 1971)

Movement of molecules across the intestinal mucosa can occur by active transport and diffusion. Molecules that move into the mucosa cells by diffusion, are moving from an area of high concentration to one of low concentration. How much diffuses across the membrane is

dependent on concentration, electrostatic forces and solubility of the molecule in the cell membrane. In comparison, active transport moves molecules against a concentration gradient or from an area of low concentration to one of high concentration. This is accomplished by complexing the molecule with a carrier at one side of the membrane. Energy provides the driving force to move the carrier-molecule complex to the other side of the membrane, where the complex dissociates releasing the molecule into the cell and allowing the carrier to return to form another complex with another molecule. Energy is required to drive the carrier-molecule complex against the electrostatic forces, pressure and concentration gradients. Without energy the molecule would depend on facilitated diffusion which operates on the same principle as active transport, but does not have an energy source to drive it. The carrier is only used to make the molecule more soluble in the membrane, then diffusion begins. Thus, energy and a carrier are necessary components of an active transport system.

Movement Against Concentration Gradient. The movement of amino acids from the lumen is against a concentration gradient. Intestinal rings incubated in a buffered medium with histidine rapidly absorbed histidine for the first 20 minutes of the incubation, at which time the amount present in the tissue peaked and plateaued (Agar et al., 1954). As the initial concentration of histidine increased, the plateau point increased but still occurred after 20 minutes of incubation. Amino acids accumulated in the tissue could obtain levels as high as 10 times that of the incubation media (Agar et al., 1956).

Model amino acids are not common to the body and are resistant to metabolism. Thus, they can be used to follow movement of amino acids in the body without influence from endogenous sources or metabolism. Christensen (1963) used two model amino acids to establish concentration ratios between the intestinal lumen and the blood. Through intravenous injection of labeled model amino acids, equal specific activity was established between the blood and the lumen of the isolated intestinal segment. At steady state, the plasma to lumen ratios were 20 to 1 and 90 to 1 for 1-aminocyclopentane and alpha aminoisobutyric acid, respectively. Without considering the tissue, concentration gradients were established between the intestinal lumen and the blood. Lumen to blood ratios of alpha aminoisobutyric acid reported by Schedl et al. (1969) were similar to those of Christensen (1963), but they also reported tissue accumulation. Tissue to lumen ratios were much higher, 872 to 1, than lumen to blood ratios. From their data, a distribution pattern of amino acids within the three compartments of the intestine with regards to absorption, lumen, tissue and blood, can be established. By looking at the three-way ratio of lumen to tissue to blood, which was 1 to 872 to 93, it can be seen that the amino acids move from the lumen into the tissue against a concentration gradient, then are released to the blood by diffusion.

Energy Requirements. Initial work with everted sacs showed that methionine was removed from the incubation fluid by the mucosa and that this movement required oxygen. Methionine removal from the incubation fluid was reduced from a positive value, under an atmosphere of oxygen, to a negative value when the oxygen was replaced with nitrogen (Wilson

and Wiseman, 1954). The addition of metabolic poisons to the incubation fluid in an *in vitro* system resulted in a decline in amino acid absorption due to the lack of energy (Agar *et al.*, 1956). Agar *et al.* (1954) decreased the uptake of methionine by 66% with the addition of cyanide to the incubation medium.

The amount of glycolytic activity in the intestinal mucosa varies between species, but glucose can provide the energy necessary for transport of amino acids under an anaerobic condition but at a reduced rate (Luisier and Robinson, 1975). Under anaerobic conditions, the addition of glucose increased the uptake of phenylalanine from 9% of control values to 16%. Addition of glucose to a medium containing 2,4-dinitrophenol, a metabolic poison, once again increased uptake of phenylalanine from 12% of control values to 34%. When oxidative phosphorylation has been stopped by the addition of metabolic poisons, amino acid uptake decreases dramatically. Thus, the absorption of amino acids is dependent upon energy. Agar *et al.* (1956) reported that the release of histidine from intestinal rings was not affected by the addition of 2,4-dinitrophenol to the medium. Movement of amino acids out of the tissue in either direction, into the lumen or blood, was with a concentration gradient. The amino acids diffuse across the membrane toward an area of lower concentration.

From the previous discussion, it appears that amino acids are transported from the intestinal lumen by an active transport system. The system transports amino acids from an area of low concentration, the lumen, to one of high concentration, the tissue. Movement from

the tissue to the blood can be accomplished by simple diffusion. The first step, transport of amino acids into the tissue, requires energy which can be provided by glycoylsis or oxidative phosphorylation depending on the oxygen supply. The second step, diffusion of amino acids into the blood, is driven by a concentration gradient, thus does not require energy.

Characteristics of the Active Transport Carrier. The active transport system is composed of two components, an energy system and a carrier. The first component, the energy system, converts chemical energy to osmotic energy and is nonspecific, while the second component must be able to recognize the substance to be transported and is very specific. These two components are intimately related, but just how is not known (Csoky, 1961).

Some characteristics of the carrier involved in amino acid transport across the mucosal epithelium have been described, but the carrier itself has not. Alvarado (1966) theorized that in order to be absorbed, amino acids must form a ternary complex with the sodium ion and the carrier. Thus, sodium was necessary for amino acid absorption (Cohen and Huang, 1964; Shultz et al., 1967). Removing the sodium from the incubation medium resulted in decreased uptake of amino acids present in the medium (Csoky, 1961). Reiser and Christiansen (1967) reported that a minimum sodium concentration of 30 millimolar was necessary for optimum amino acid absorption. Below this concentration amino acid absorption would decrease, while above this it would not increase. They also found that one-half of the sodium chloride in the medium could be

replaced with choline chloride or tris-chloride, but this replacement resulted in a sodium concentration reduction of only 59 millimolar, 118 to 59 millimolar. The test solution still contained almost twice the sodium concentration necessary for optimum absorption of amino acids. Conversely, when lithium or potassium chloride replaced one-half of the sodium chloride in the medium, absorption dropped. These ions must have some inhibitory effect on amino acid absorption. Nathans et al. (1960) reported that substituting lithium, potassium, calcium or magnesium for sodium resulted in amino acid absorption dropping to zero. These observations were due to the inability of the cations to replace sodium as opposed to their inhibitory action. Sodium concentration effects upon absorption are evident in the medium on the mucosal side of the tissue, only. Reduction of sodium concentration on the serosal side of the intestine (Cohen and Huang, 1964) or inside the mucosal cell (Shultz et al., 1967) had no effect on amino acid absorption. Intracellular sodium concentration does change during the absorption of amino acids. Reiser and Christiansen (1967) measured a pre-incubation intracellular sodium concentration of 28 millimolar and a post-incubation concentration of over 84 millimolar. Even with a three-fold increase, extracellular concentration remained higher than intracellular concentrations. These concentration differences favor the dissociation of any complex containing sodium, once it reaches the intracellular membrane.

Potassium has been shown to be related to amino acid absorption (Christensen and Oxender, 1960; Riggs et al., 1958). Nathans et al.

(1960) removed the potassium from the incubation medium without affecting the absorption of monoiodotyrosine, but increasing the potassium concentration to 0.02 molar or above did decrease uptake. From Lineweaver-Burk plots, potassium appeared to act as a competitive inhibitor of monoiodotyrosine absorption. Christensen and Oxender (1960) studied the association between potassium and glycine absorption. By forming a layer of tumor cells between two incubation fluids, effects of potassium imbalances could be studied. Equal concentrations of glycine were established on each side of the cells, but one side had a high concentration of potassium while the other had a low concentration. Glycine molecules were transported into the cells and exchanged for potassium from the high concentration side. When potassium alone was placed on one side and only glycine on the other side, the exchange of potassium for glycine occurred. After 30 minutes of incubation, potassium concentration of each side was approximately equal. Glycine ratios between the two sides revealed that a small concentration gradient had developed on the high potassium side. Correlation between potassium concentration in the cell and glycine absorption by that cell was 0.81 (Riggs et al., 1958). Lowering intracellular concentration of potassium lowers the amount of glycine absorbed. Potassium could be interacting with the sodium ion. As sodium enters the intracellular fluids, potassium moves to the extracellular fluid to maintain an electrostatic or ionic balance. Potassium could be an intracellular driving force for amino acid absorption. As amino acids are absorbed, the intracellular sodium concentration increases, while potassium decreases. Thus, the cell may not be

able to absorb more amino acids than it has potassium to exchange (Christensen and Oxender, 1960).

Process of Amino Acid Absorption

Two approaches to the study of amino acid transport were described by Alvarado (1970). The first was the analytic approach which was described as studying as many separate mutually exclusive transport systems as possible. The other approach was the synthetic method. This approach seeks common links between systems claimed to be different. Alvarado (1970) prefers the latter because previous data shows that absorption of amino acids are interrelated with each other as well as with other nutrients.

Although the intestine is a complex structure, Munck and Shultz (1969b) simplified the structure to a one compartment model to explain the process of amino acid absorption. The total system was considered to be composed of three compartments, serosal, tissue and mucosal. The intestinal compartment was described as a divisional barrier between the two fluids, mucosal and serosal. With this concept, they theorized that the intestinal compartment was subject to four fluxes, two of which moved amino acids into the compartment and two which moved amino acids out. Thus, the two fluids separated by the tissue could have amino acids removed by active transport or diffusion as well as receive amino acids from the tissue by similar processes. The tissue could obtain amino acids from two sources as well as lose them to the same two sources. Amino acid movement into the tissue could be greater than determined experimentally if the one parameter measured was simply

removal of amino acid after a set period of time. This value would be net absorption which is the net result of the four fluxes. By altering any one of the four fluxes, net absorption would be affected. Munck and Shultz (1969b) described the uptake of amino acids as a two step procedure. First, the active transport mechanism would move amino acids from the mucosal fluid into the cell, until a large enough concentration gradient was developed to initiate the second step. Amino acids could then diffuse from the high concentration within the cell to the lower concentration of the serosal fluid or circulatory system. The rate limiting step of this sequence of events would be the first step, active transport.

Newey et al. (1970) retained the idea of four fluxes, but they felt that the intestine was more complex than one compartment. They described the intestinal tissue as two compartments. The first was referred to as the mucosa and the second as the submucosa. Amino acids would be actively transported into the mucosa and then diffuse through the submucosa to the serosal side. The submucosa represented the muscular portion or monabsorptive portion of the intestine. Newey et al. (1970) felt that this portion of the intestine could be a barrier against movement out of the intestine. This concept adds the permeability of the submucosa as a factor to be considered. Absorption of amino acids as described by Munck and Shultz (1969b) would be true if the permeability of the submucosa was high. The amino acids would diffuse from the mucosa to the serosal fluid as fast as they were removed from the mucosal fluid. But, if the permeability was low, flux patterns would shift. Amino acids

would be concentrated in the mucosa and begin to diffuse through the submucosa but diffusion would proceed much slower than the active transport phase. As a result, amino acids would become more concentrated in the mucosa and would eventually reach a concentration at which they would begin to diffuse back into the mucosal fluid. Under these conditions the limiting step would be the diffusion through the submucosa. Thus, the permeability of the submucosa would determine which step was limiting and how much amino acid was absorbed.

Shultz et al. (1967) described two barriers which they felt amino acids must cross when being absorbed. The first was the brush border or epithelium cell membrane and the second was a combined serosal and lateral membrane, similar to the submucosa. These membranes can differ in structure and function. The actual structure of the intestinal mucosa is quite similar to the theoretical structures proposed. The intestine is lined with villi, which are fingerlike projections on the valvulae conniventees or folds. The epithelial cells which line the villi are characterized by a brush border which consists of approximately 600 microvilli that are 1.0 micron long with a diameter of 0.1 micron. The epithelial cells are long column-like with the cell walls of other cells on two sides and the brush border on the third side. The fourth side forms the basement membrane, which separates the interior of the epithelial cells from the vascular system (Guyton, 1971). The model described by Newey et al. (1970) takes into account the basement membrane.

Movement of amino acids from the serosal side to the intestinal lumen has been reported. This type of movement would exemplify the flux

from the serosal to tissue and tissue to lumen. Jacobs (1965) injected labeled aminoisobutyric acid into the vena cava. Shortly after injection the presence of the model amino acid was detected in the lumen. The level remained at a steady concentration for 25 minutes before dropping. The imbalance between blood and tissue moved the amino acid into the tissue which created another imbalance between tissue and lumen. Thus, 25 minutes were required to establish the distribution pattern. Schedl et al. (1969) also used aminoisobutyric acid to study movement of amino acids into the lumen. Aminoisobutyric acid secretions did reach a steady concentration after two hours. At this time, the flux into the lumen was equal to the absorption, thus maintaining a constant concentration.

Christensen (1963) used 1-amino-cyclopentane as a model amino acid injected into the blood system. Its release into the lumen was stimulated by the addition of methionine to the lumen. Jacobs (1965) conducted extensive experiments to ascertain the effects of lumen amino acid content and concentration on effluxes from the tissue. He concluded that intestinal transport was a balance between the lumen contents, mucosal cells and the extra-mucosal amino acid pools. Employing the perfusion technique with isolated intestine, he could alter the lumenal concentration of amino acids. Perfusion of saline for 60 minutes resulted in the release of alanine, valine, phenylalanine, methionine, glycine, serine, threonine, leucine, isoleucine, aspartic acid, glutamic acid and tyrosine into the lumen in different amounts, but none were greater than one micromolar. Addition of alanine to the

perfusion solution elicitated release of the same amino acids, but in greater amounts. Subsequent addition to the perfusion solution of amino acids previously found to be released when saline or alanine was perfused resulted in a response similar to saline plus alanine. In order to prove that these amino acids are not the result of sloughing of the intestinal villi or breakdown of residual succus entericus, gastric juices, continuous perfusion of 60 minutes were divided into six 10-minute periods. Amino acid concentration did not decrease between periods. Similar results have been reported by Agar et al. (1954). Intestinal rings were allowed to incubate in a 25 millimolar solution of histidine, removed, and incubated in buffered medium without amino acid. Concentration of histidine began to increase in the incubation medium for 50 minutes, at which time equilibration had been reached. Hume et al. (1972) measured amino acid concentrations of blood entering and leaving the portal system of the sheep. Net absorption of amino acids was determined by differences between arterial and portal blood times flow rate. Net absorption was positive at 2, 6 and 10 hours after feeding when sheep were fed once daily. Negative absorption of amino acids, which occurred just before and several hours after feeding, indicates removal of amino acids from the blood by the intestine. Increasing feeding frequency to every two hours resulted in a more uniform rate of absorption and maintained a positive net absorption value. Removal of amino acids from the blood or the tissue was just part of the dynamic flux of amino acids between the luminal contents, the intestinal mucosa, the circulatory system and the amino acid pools.

Amino Acid Transport Systems

Amino acids have been classified as members of one of three groups, basic, acid, and neutral, based on differences in structure. Amino acids contain a common structural unit, but vary in side chain components and structure. These structural differences have been shown to be a factor in their absorption from the small intestine. Orten (1963) divided amino acids into three absorption groups, fast, medium, and slow, based on their absorption rates when perfused through the human ileum as an amino acid mixture.

If it is assumed that absorption was a two-step process, then each step can be represented by an equation similar to those used in enzyme kinetics. The first step would represent the formation of the amino acid carrier complex between the free amino acid and the unbound carrier at the mucosa brush border. This step would contain two dissociation constants. One for the formation of the complex, k_1, and another for the dissociation of this complex to yield the free amino acid and the unbound carrier, k_{-1}. All of which would occur at the intestinal lumen mucosa membrane. The second step, dissociation of the complex to release the amino acid into the epithelial cell, would be represented by the third dissociation constant, k_2. If we assume that k_2 is equal for all amino acids, then the differences in the amounts of each amino acid absorbed would be due to the k_1 and k_{-1} constants or the affinity of the amino acid for the transport carrier.

Acidic Amino Acids. Wiseman (1953) used everted sacs to study the absorption characteristics of glutamic and aspartic acids. He did

not observe active transport of these amino acids, using concentration gradient as a criteria. Although the concentration of the mucosal and serosal fluid decreased, no buildup of these amino acids in the tissue or serosal fluid was noted. Wiseman (1954) also studied the inhibitory characteristics of glutamic acid which indicates its affinity for transport sites and the different transport system. When the inhibitory effects of glutamic acid were compared to proline, histidine, and methionine, he concluded that its affinity for the transport system was very low. When glutamic acid and aspartic acid were incubated in an in vitro system for 1 hour, alanine was found in both the serosal and mucosal fluids (Matthews and Wiseman, 1953). Once again mucosal and serosal concentrations of these amino acids declined, but neither was found to increase in the tissue. The appearance of alanine, which was more concentrated in the serosal fluid, indicates that a transformation of aspartic and glutamic acid to alanine may be taking place. Other amino acids incubated under similar conditions did not result in alanine formation. This phenomenon appears to be limited to aspartic and glutamic acids. Neame and Wiseman (1957) used various concentrations of glutamic or aspartic acid in perfusion solutions to determine their effects on blood concentrations of glutamic acid, aspartic acid and alanine. Increasing the glutamic acid perfusion concentration from 0.15 to 10% resulted in an increase in glutamic acid appearance in the blood. Concentrations less than 0.5% resulted in little absorption, while a 2% concentration gave the quickest increase in venous glutamic acid concentration. When a concentration of 10% was used, a higher peak venous

glutamic acid concentration was achieved without a dramatic increase in alanine concentration as compared to the 2% level. Alanine concentration increased as the perfusion and venous concentrations of glutamic acid became greater. Aspartic acid concentration had to be increased to 0.5% before any absorption was noted. Alanine concentration increased also but not as rapidly as when glutamic acid was used. With aspartic acid, a two-fold increase in venous blood aspartic acid concentration resulted in a 0.5-fold increase in venous alanine concentration, while a similar increase in venous glutamic acid concentration resulted in a two-fold increase in alanine concentration. It appears that glutamic acid is more susceptible to transamination or decarboxylation than aspartic acid.

Neutral Amino Acids. One of the largest groups of amino acids is the neutral group, which use more than one transport system. Baker and George (1971) used seven different neutral amino acids to ascertain the different transport systems. They found that two systems existed, N_1 and N_2, due to different transport rates and inhibitory abilities. The neutral amino acids can be subdivided according to these two systems. The first system, N_1, transports neutral amino acids, such as glycine and methionine, which do not have any substituted groups on the amine group. The affinity for this system or the transport rate was influenced by the chain length of these amino acids, with increasing chain length being more favorable. Addition of substituted groups to the alpha amine, such as betaine, sarcosine and aminoisobutyric acid, decreased their absorption potential by the N_1 system, but permitted absorption by the

N_2 system. Hagihira et al. (1962) reported that methionine had no effect on the transport of betaine, N_1N- dimethylglycine or sarcosine. Since methionine was a strong inhibitor of amino acids for the N_1 system, its failure to inhibit the substituted form of neutral amino acids was evidence of separate systems. Similar observations were reported by Larsen et al. (1964). They found that the absorption rates of substituted amino acids were much lower than nonsubstituted forms, but no competition existed between the two systems, but dibasic amino acids could inhibit both neutral amino acid systems. The lipophilic side chain was once again determined to be important with regards to absorption rate of neutral amino acids.

Proline was found to be transported by the same system as sarcosine, betaine and N_1N-dimethylglycine, as well as the other neutral amino acid transport system (Lin et al., 1962). Glycine was also found to utilize both systems (Munck, 1966a). Munck (1966a) reported that the imino amino acids, hydroxyproline, sarcosine and betaine, could not use the N_1 amino acid system, but glycine and proline could use both. This seemed logical since two of the imino acids, sarcosine and betaine, are derivatives of glycine. Studies of glycine and proline absorption by inhibition become more difficult since they can use two different systems. Newey and Smyth (1964) theorized that the association between methionine, glycine and proline with regards to the N_1 and N_2 systems may be explained two ways. First, there are two carriers, one for methionine or nonsubstituted amino acids and one for glycine and proline or substituted amino acids. The first carrier is receptive to all three

amino acids while the second is exclusively for N-substituted amino acids. The second theory simply combines the two carriers to form one carrier with two active sites. One site reserved for glycine, proline and other N-substituted amino acids while the second one is available for the other neutral amino acids plus glycine and proline.

Basic Amino Acids. Basic amino acids are actively transported by the small intestine against concentration and electrostatic gradients (Hagihira et al., 1961; Larsen et al., 1964). This system is not as rapid as the neutral amino acid transport system. Hagihira et al. (1961) reported absorption values for lysine, ornithine and arginine of 5 to 10% of neutral amino acid values. They also compared the intestine system to that of the kidney tubules and found them to be similar. Human kidney tubules can possess a genetic defect, cystinuria, which is the absence of the basic amino acid transport system. The patient fails to reabsorb basic amino acids which results in poor protein utilization. Under these conditions perfusion of dibasic amino acids in a peptide form results in increased absorption of the amino acid (Hellier et al., 1970). Once the dipeptide has been absorbed the epithelial cells have the potential to hydrolyze it to amino acids.

The failure of the basic amino acid system, without effects on the absorption of other amino acids, indicates that it was a separate system. This system has been reported to overlap with the neutral amino acid system. Larsen et al. (1964) reported that dibasic amino acids had an inhibitory effect on both neutral amino acid systems. Dibasic amino acids do develop a concentration gradient in the tissue, but it

is slow. McLeod and Tyor (1967) studied arginine and ornithine absorption rates by different sections of the small intestine. This transport mechanism was present throughout the entire length of the intestine, but more so in the lower portion. Munck (1966b) reported that leucine can inhibit lysine absorption due to the operlapping of the neutral and dibasic systems. Reiser and Christiansen (1971) found that histidine was transported by the neutral amino acid system, N_1, and that some neutral amino acids can stimulate the absorption of basic amino acids. Leucine was both inhibitory and stimulatory of lysine absorption, depending upon the concentration of each. The apparent mechanism involved with this phenomenon was a rapid accumulation of leucine in the tissue, since its absorption rate is much greater than the dibasic amino acid, and its subsequently providing the driving force for lysine absorption. Due to its high tissue concentration leucine would drive the lysine absorption system by its movement from the tissue to the mucosal fluid.

Factors Which Affect Amino Acid Absorption

The absorption of amino acids are affected by many different factors, some of which are the structural differences of amino acid side chains. The side chain, by which amino acids differ the most, the alpha carbon, the alpha hydrogen, and the primary amine are used by the carrier to recognize the specific amino acid to be absorbed or to determine the amino acid affinity for the transport system. Consequently, these individual factors have been studied to determine their effect on absorption. Lin *et al.* (1962) proposed that the alpha amine, alpha hydrogen and a carboxyl group are needed by the amino acid so that the carrier can recognize it.

Structure of the Amino Acid. Amino acid side chains can vary in length, structure and charge. Agar et al. (1956) studied the effects of polar side chains on histidine absorption. The amino acids with non-polar side chains were the strongest inhibitors, indicative of a higher affinity for the system, while amino acids with polar side chains were weakly inhibitory. Basic amino acid absorption was determined to be slower than neutral amino acids, due to the charge of the side chain (Hagihira et al., 1961). Lineweaver-Burk plots were utilized by Matthews and Laster (1965) to determine that inhibition among the monoaminomonocarboxyl amino acids was competitive inhibition. Branched chained amino acids are absorbed more rapidly than straight chain amino acids from amino acid mixtures (Adibi et al., 1967). It was also noted that essential amino acids were absorbed much faster than non-essential. The greatest effect of side chain structure on absorption of amino acids from one group would be the neutral amino acids.

Daniels et al. (1969) used methionine and sarcosine as representatives of the two neutral amino acids transport systems, N_1 and N_2, respectively. The N_1 system was more susceptible to the large more lipid soluble amino acids while the N_2 was inhibited by small, more water soluble amino acids. Increasing chain length resulted in greater inhibition of the N_1 system with little effect on the N_2 system. Fleshler and Caligado (1967) reported that chain length was not a common rate limiting factor shown by all neutral amino acids, because isoleucine failed to inhibit glycine absorption in human small intestine. The methionine system, N_1, was inhibited more by the straight

chain amino acids than branches, although they were more effective on the N_2 system. Chain length was shown to be additive to the inhibitory effects of altering the position of the amine group on the N_1 system. Increasing the distance between the carboxyl group and the secondary amine would decrease the inhibition of the methionine system, but increase its inhibitory effect of the sarcosine system. Proline possess more affinity for the N_2 system than betaine, which contains three methyl groups attached to the amine group (Hagihira et al., 1962). Replacing the amine group of glycine with hydroxyl groups or forming two derivatives with tertiary and quaternary amino groups resulted in no absorption (Lin et al., 1962). Removal of the amine group inactivated the amino acid for absorption. Lin et al. (1962) also reported that replacement of the alpha hydrogen with a methyl group reduced the absorption rate, but this could have been due to the increase in molecule size. The presence of the alpha hydrogen may not be absolutely essential, but replacing it with a methyl group does reduce the absorption rate (Akedo and Christensen, 1962; Spencer, 1969). Spencer et al. (1962) tested twenty-six modified structural forms of glycine to ascertain what points were critical for absorption. Molecules were altered at the alpha hydrogen, carboxyl group and amine group, singly and together. Only modification of the alpha hydrogen proved effective in reducing absorption rates. A free carboxyl group must be present for active transport (Spencer, 1969). Modification of the carboxyl group, such as replacing it with a methanol or forming a methyl ester, would prevent active transport (Lin et al., 1962).

Replacing the alpha hydrogen with a large group may physically prevent the amino acid from being absorbed or alter the total charge in such a way as to prevent absorption.

Amino acids must cross the lipid rich epithelial cell membrane to enter the cell and be absorbed. Reiser and Christiansen (1968) explored the possible existence of an amino acid lipid complex which would facilitate crossing the epithelial membrane by increasing the solubility. They reported finding such a complex using valine as the amino acid. This complex was energy independent and forms very quickly. Although the complex did not require a specific lipid, phosphoglycerides carried 100 times more valine than other lipids tested. Amino acids, previously shown to be inhibitory to valine absorption, were also inhibitory to the valine lipid complex, while non-inhibitory amino acids had no effect. The function of this complex is not known, but its rapid dissociation upon contact with water is not characteristic of the active transport system, although it may function as an intermediate step.

Concentration. Andrews et al. (1936) varied the amount of cystine or methionine used to perfuse the Thiry-Vella loops of the dog. Cystine was absorbed at a constant amount independent of concentration, but the amount of methionine absorbed increased 1.5 fold each time the concentration doubled. Metionine concentrations of 5, 10 or 15 mM were used by Agar et al. (1954) to study its effects on the amount of methionine absorbed by intestinal rings. Each increase in methionine concentration resulted in a 7.1 micromole increase in absorption. The amount of any substance transported by diffusion is dependent upon the initial

concentration. The higher the concentration the greater the amount diffused. Active transport is also dependent upon concentration, but in a different sense. The formation of the amino acid carrier complex is dependent upon the concentration of both the amino acid and the carrier. The concentration of the carrier can be assumed to be constant, thus the concentration of the amino acid will determine how much of the complex will be formed. Each increase in amino acid concentration will result in a subsequent increase in the amount absorbed but there is a limit. When the carrier or the system has been saturated, due to an excess of substrate or amino acid in this case, then an additional increase in amino acid concentration does not produce a corresponding increase in the amount absorbed.

Jervis and Smyth (1959) compared urea, which is absorbed by diffusion, and methionine, which is absorbed by active transport, at different concentrations. As the concentration of urea or methionine was increased, the amount absorbed continued to rise, but once the methionine concentration reached 50 millimolar the system became saturated and the amount absorbed did not increase proportionally to concentration as before. Urea absorption continued to increase with each concentration increase. Cheng and Matthews (1970) reported a methionine saturation point of 10 millimolar. Plotting absorption against concentration resulted in an initially linear portion, then the saturation point and a plateau. Increasing methionine concentration from 10 to 20 millimolar resulted in a slope decline from 1.36 to 0.35. A lower saturation point was reported for sheep small intestine by Johns and

Bergen (1973). They reported that methionine and lysine were saturated at 5 millimolar, while glycine remained linear even at 10 millimolar. Flesher et al. (1966) used the intubation procedure to study the concentration effects on glycine and alanine absorption. These amino acids required perfusion concentration above 300 millimolar before absorption ceased to be linear. Tryptophan absorption was saturated at a relatively low concentration, 3 millimolar, as compared to other amino acids (Cohen and Huang, 1964). It was evident that each amino acid could respond differently to a wide range of concentrations, but Orten (1963) did not observe any changes in amino acid absorption pattern when the concentration of the amino acid mixture was decreased from 9.0 to 5.4 millimolar. Initial concentration can influence certain types of observations. In vitro data are usually expressed in concentration ratios of mucosal to serosal fluid. Lower initial concentration can result in higher mucosal to serosal ratios (Matthews and Laster, 1965).

Effects of Ions Present. High concentrations of calcium carbonate in diets have been shown to decrease gains in cattle (Jay and Ray, 1972). These decreases may be due to a decrease in amino acid absorption. The cation may alter mucosal membrane permeability or the active transport system. Jay and Ray (1972) used intestinal loops of the small intestine of heifers to ascertain the effects of calcium, magnesium, potassium and zinc on amino acid absorption and alkaline and acid phosphatase activity. Calcium and zinc were inhibitory at 50, 100 or 200 millimolar while magnesium was inhibitory only at the 200 millimolar level.

Potassium had no significant effect at any concentration. Acid phosphatase activity was not affected by any cation at any of the concentrations, but alkaline phosphatase activity was reduced by the addition of calcium or zinc at a concentration of 50 millimolar. Jay and Ray (1972) felt that the cation inhibition of amino acid absorption may be partially the result of decreasing alkaline phosphatase activity, thereby affecting the active transport system. Changes in physiological state of the animal, such as pregnancy, does increase extracellular fluid volume in the tissue and the absorption of amino acids, but the fluid accumulation was independent of the amino acid absorption (Hutcheson et al., 1975). Fasting of pregnant guinea pigs for 24 hours did not increase the transport of amino acids into the intestine. Kershaw et al. (1960) reported that rats on restricted intake must lose 20% of their body weight before absorption rates of glucose and histidine are increased. These rats will maintain a higher absorption rate for seven days after ad libitum feeding has been resumed. This increased uptake may be due to a thinner intestinal wall, shorter intestinal length, or greater uptake capacity as the result of a restricted diet. It has also been shown that age of the animal does not have a deterrent effect on intestinal wall uptake of amino acids, but overall transfer from the intestinal lumen to the portal vein is altered (Ludorf and Gallo-Torris, 1975).

Stereochemical Selectivity. One of the most documented characteristics of the amino acid transport system is its ability to distinguish between amino acid stereoisomers (Aroska and Berg, 1962; Clarke et al.,

1951; Jervis and Smuth, 1960; Kuroda and Gimbel, 1954; Lerner et al, 1969; Paine et al., 1959). This characteristic indicates that the amino acid transport is more than diffusion (Kuroda and Gimbel, 1954). Jervis and Smyth (1960) noted a decrease in D-methionine absorption from 48 to 19 micromoles per gram of tissue when L-methionine was added to the solution. The active transport system could absorb D-methionine but is more favorable toward the natural L-form. The small intestine of the chicken absorbs L-methionine at a rate two times faster than the D-form except at low concentrations, such as 0.67 millimolar (Lerner et al., 1969). Addition of metabolic poisons does inhibit the absorption of L-methionine, but has no effect on D-methionine. Thus, the absorption of D-methionine does not require energy, but it has been shown to be inhibitory to other L-amino acids which are actively transported. Although it may not be transported by that system, it can block absorption by occupying the active sites (Paine et al., 1959). The L-form of other amino acids have also been reported to be absorbed in greater amounts and to be inhibitory of their stereoisomers (Aroskar and Berg, 1962).

Kinetics of Transport Systems. Kinetic studies have been used by researchers to determine the type of inhibition observed during amino acid absorption studies (Finch and Hird, 1960) to estimate amino acid absorption rates according to their affinities for the system (Schedl and Clifton, 1963) and to identify amino acid transport systems (Spencer and Samiy, 1961). Amounts of amino acid absorbed are determined at various concentrations and plotted versus the concentration. From the

plot, the effects of concentration on amino acid absorption can be seen. These data can also be plotted in the Lineweaver-Burk form with axis composed of the reciprocal of the amino acid concentration as the abscissa and of the amount absorbed as the ordinate. This will result in a straight line plot which crosses the positive portion of the ordinate and the negative portion of the abscissa. The point at which the plotted line crosses the ordinate will yield the reciprocal of the V_{max} and the abscissa yields the negative reciprocal of the K_m value. The maximum velocity of the system or the amount absorbed when the transport or active sites are saturated was represented by the V_{max} (Lehninger, 1970). A large V_{max} value indicates that the system has a high capacity to absorb that particular amino acid. The substrate concentration required to reach one half of the maximum velocity was presented at the K_m value. A low K_m value indicates that the amino acid has a high affinity for the transport system (Fleshler et al., 1966). Thus, at low concentrations it would occupy a majority of the active transport sites as opposed to an amino acid with a high K_m. Amino acids with low K_m values do not respond with increasing amounts being absorbed to increase in concentration over a wide range. At high concentration a larger K_m will result in more amino acid being absorbed, unless an amino acid with a low K_m was present to occupy the transport sites and inhibit its absorption (Christensen, 1963). Finch and Hird (1960) used the Lineweaver Burk plot to determine the type of inhibition observed between amino acids. By the alteration in K_m values with inhibition,

they determined that the inhibition was competitive, which can be overcome by increasing substrate concentration. Although a low K_m for an amino acid was indicative of a greater inhibitory effect toward other amino acids, a higher K_m was more inhibitory toward glucose absorption (Hindmarsh et al., 1966). Nathans et al. (1960) and Schedl et al. (1968) determined K_m and V_{max} values and observed that they varied between individuals as well as intestinal site. Munck and Shultz (1969b) felt that the Lineweaver-Burk plot was not applicable to amino acid absorption because there are four different fluxes moving amino acids in and out of the tissue.

Site of Absorption. Kinetics have also been calculated for different portions of the intestine to acertain if absorption varies with intestinal site. Schedl and Clifton (1963) and Schedl et al. (1968) reported K_m and V_{max} values for methionine absorption in the proximal and distal small intestine of man. Maximum velocity and K_m values for the proximal intestine were four times greater than those reported for the distal intestine. The values indicate that the proximal intestine of man has a system which has a high capacity and low affinity for methionine while the distal intestine has a low capacity and high affinity. To maximize methionine absorption, its concentration should be high in the proximal intestine and low in the distal. This seems logical with regards to amino acid release during digestion. Schedl et al. (1969) measured distribution patterns of alpha aminoisobutyric acid at steady state in the proximal and middle intestine of man. The middle intestine section developed higher tissue and serum levels in proportion to the lumenal concentrations. The

middle portion of the intestine appears to possess absorption capabilities to remove more amino acids from the lumen and release it to the serum. Cohen and Huang (1964) followed tryptophan absorption at different intestinal sites in the small intestine of the rat. Plotting absorption against distance from the pylorus, values increased with distance from the pylorus, until the lower jejunum was reached, at which time it began to decline. The duodenal section of the small intestine of the rat was the slowest absorption site observed by Matthews and Laster (1965) for neutral amino acids, but absorption increased distantly to a peak absorption site located at a point 80% of the total small intestinal length from the pylorus. Baker and George (1971) and Larsen et al. (1964) reported the middle intestine as the most active absorption site in the small intestine of the rat, but absorption values vary between animals. Different observations concerning absorption were reported by Nathans et al. (1960). They found the terminal ileum to be the site of maximum absorption of tyrosine. Tyrosine absorptive rates increased as the distance from the pylorus increased in both hamster and rat intestine, but it was more prominent in the hamster. Spencer and Samiy (1961) used small intestine of the hamster to study phenylalanine absorption. They observed more absorption occurring in the middle portion of the intestine than the terminal ileum. The lack of agreement between observed values by different researchers may be due to differences in species used (Alvarado, 1968).

Only a few experiments using ruminant small intestine have been reported. Williams (1969) ranked amino acids in the order by which

they were absorbed from intestinal loops in the sheep at different positions. Amino acid order varied with position, indicating different affinities for the transport systems at each site. Lysine absorption by sheep intestinal rings increased with distance from the pylorus with maximum absorption occurring in the ileum, but Johns and Bergen (1973) felt that this was an artifact of the in vitro system. Additional in vitro information using the small intestine of the sheep has been reported by Phillips et al. (1976). Instead of intestinal rings, everted sacs from the duodenum, jejunum and ileum were used to study the uptake of threonine, valine and methionine, individually. The ileum proved to be the most efficient site of threonine and valine absorption while methionine was absorbed at similar rates by both the jejunum and ileum. Amino acid concentration in digesta increases once it reaches the duodenum due to endogenous amino acids and amino acids released by digestive processes. Thus, it appears logical that the jejunal tissue would possess a system with high absorption capabilities. Once most of the amino acids have been removed and competition reduced, then a system with lower capacity but higher affinity can be used to absorb those amino acids missed by the middle intestine (Christensen, 1963). But this sequence may vary in position along the tract with species. The lower intestine may, in some cases, be able to absorb large amounts of amino acids, but it may not be the site of maximum absorption. Sites may be more numerous in an effort to be more efficient in absorbing those amino acids previously missed.

Competition and Inhibition of Amino Acid Absorption

Competition with Sugars. Amino acids are not the only digestive products in the lumen of the small intestine that are ready for absorption. Simple sugars are present and can influence amino acid absorption. Glucose absorption has been shown to be dependent upon concentration and the volume or amount present in order to increase surface area contact (Raudin et al., 1933). Blood glucose levels had no effect on methionine absorption (Cummins, 1952), but the presence of methionine or histidine in the lumen can inhibit glucose absorption (Hindmarsh et al., 1966). Higher concentrations of methionine or histidine were increasingly inhibitory up to 10 millimolar. It appears that glucose inhibition had been maximized at this point. This type of inhibition was different from inhibition of other amino acids because the amino acid with a higher K_m value was more inhibitory (Hindmarsh et al., 1966). If amino acids and sugars competed for the same transport sites, lower affinities would be more inhibitory. Hindmarsh et al. (1966) also noted that sugars which were passively absorbed were not inhibited by amino acids. Of the seven amino acids they studied, only two, methionine and histidine, were inhibitory in the D-form.

Hopfer and Murer (1975) used isolated mucosa to study the interaction between glucose and alanine. When both were present in the incubation medium, glucose absorption dropped, but they could not determine if the effect was mediated on the membrane or inside the tissue. To determine where the inhibition occurred, mucosal cells were incubated in glucose medium without amino acid. This allowed the tissue

to absorb glucose and developed a glucose concentration intracellularly. When alanine was added and began to be absorbed into the tissue, glucose began to efflux into the lumen. The absorption of alanine increased the efflux of glucose which decreased net glucose absorption. This interaction may be electrically related or both substances may require sodium as part of the carrier.

Sugars can also be inhibitory toward amino acid absorption. Glucose and galactose inhibited phenylalanine uptake in both the rat and the hamster (Luisier and Robinson, 1975). Alvarado (1966) studied the effects of three sugars, galactose, methyl alpha glucoside and glucose on neutral amino acid absorption. Galactose was the most inhibitory, while glucose was the least and methyl alpha glucoside was intermediate. The mode of inhibition could be direct competition at the active transport sites or intracellular competition for sodium or energy (Alvarado, 1966; 1968). Neither Alvarado (1970) or Luisier and Robinson (1975) supported the energy competition concept but felt that not enough information was available to decide between the two. The movement of sugars across the intestinal membrane could result in a potassium and sodium imbalance, which would have an effect on amino acid absorption (Alvarado, 1970).

Amino acid absorption rates were shown to decrease in man when glucose was added, but this reduction was uniform with regards to amino acid (Orten, 1963). Although the amount was reduced, the absorption pattern of the 18 amino acids used remained the same.

Interaction with Peptides. During the digestive phases, proteins

are hydrolyzed to amino acids and peptides. These peptides are hydrolyzed to amino acids, but may also be absorbed. Glycine can be absorbed as the free amino acid and as the dipeptide (Agar et al., 1953). When the dipeptide was the only form used, free glycine and glycyl-glycine were found in the serosal fluid. The cell or brush border must contain dipeptidase to hydrolyze the dipeptide to free amino acids. Agar et al. (1953) also reported transfer of a glycyl-leucine dipeptide. Kim et al. (1974) stated that the hydrolysis procedure proceeds faster than the amino acids can be absorbed. This results in a buildup of amino acids at the mucosa which will maximize the transport system rate, but peptides vary in susceptibility to hydrolysis.

Similar amounts of methionine were absorbed from incubation medium independent of the form, free amino acid or dipeptide (Cheng and Matthews, 1970). The addition of the methionine dipeptide to medium containing free methionine stimulated the absorption of the free form. Dipeptides have proven valuable in preventing malnutrition in cases of cystinuria (Hellier et al., 1970). Genetic failure to establish a transport system for basic amino acids results in cystinuria. Hellier et al. (1970) used a dipeptide, glycyl-lysine, to observe its absorption under these conditions. Lysine was poorly absorbed and glycine was readily absorbed in patients with cystinuria. Addition of glycyl-lysine resulted in an increase in the amount of lysine absorbed, but not all of the lysine absorbed remained in the tissue. After the dipeptide had been perfused, free

glycine and lysine were found in the lumen. Glycine levels were equal to those normally found, but lysine levels were higher than normal. After absorption and hydrolysis some lysine leaked back. Thus, dibasic amino acids can be absorbed in the dipeptide form.

Competition Among Amino Acids. One of the most complex features of amino acid absorption is the ability of one amino acid to compete with another amino acid for absorption. The carriers or active sites involved in amino acid transport can segregate amino acids into groups or families based on their structure, but amino acids within these groups must compete with each other for absorption. Some overlapping does exist; amino acids may be inhibitors to more than one transport system. Amino acids with negative charges, aspartic and glutamic acid, are poorly absorbed from mixtures where they are the only amino acids present (Adibi et al., 1967; Matthews and Wiseman, 1953). Although they do compete with each other for absorption (Orten, 1963), they are poor inhibitors of the absorption of other amino acids (Annegers, 1969) due to the polar side chain which decreases the affinity for other amino acid transport systems (Agar et al., 1956; Nathans et al., 1960). This factor also increases their susceptibility to the inhibitory effects of other amino acids. Tasaki and Takahashi (1966) reported a 32% decrease in glutamic acid absorption when methionine was added to the medium.

Basic amino acids comprise another group of amino acids which are transported by their own system (Hagihira et al., 1961; Larsen et al., 1964). These amino acids do compete for transport within their own

group (Orten, 1963). Adibi et al., (1967) reported that the presence of arginine decreased lysine absorption by 28%. Arginine was the most powerful inhibitor of lysine absorption observed by Reiser and Christiansen (1969). Larsen et al. (1964) stated that the best inhibitor of any group was a member of that group. They also noted dibasic amino acids can inhibit neutral amino acids and N-substituted amino acids with equal effectiveness. Neither tryptophan nor histidine was affected by the presence of arginine (Pinsky and Geiger, 1952). Amino acids with polar side chains are weak inhibitors of neutral amino acids (Agar et al., 1956). Neutral amino acids can inhibit basic amino acids in a manner proportional to their affinity for the neutral amino acid transport system (Reiser and Christiansen, 1969). Leucine has been shown to be inhibitory toward lysine, but at a concentration of 2 millimolar, it did not decrease arginine or ornithine absorption (Munck, 1966b). Lysine can be inhibitory and also be subject to inhibition by neutral amino acids, as well as arginine. Lysine reduced leucine absorption by 20% when it was present at a concentration of 10 millimolar, five times the leucine concentration. When leucine concentrations are greater than lysine, leucine becomes inhibitory. Lysine was held constant at 1 millimolar and leucine concentration was increased from 1 to 4 millimolar; each increase resulted in a 25% reduction in lysine absorption (Johns and Bergen, 1973). Johns and Bergen (1973) also reported reductions in lysine absorption of 48% when leucine concentration was increased to 3 or 4 times the lysine concentration, 15 and 20 millimolar, respectively.

Two systems are available for neutral amino acid absorption, but these systems overlap within the group and with other systems. Methionine does not use the N-substituted amino acid transport system, N_2, and has been shown to be ineffective in inhibiting absorption of betaine, dimethylglycine and sarcosine (Hagihira et al., 1962). At low concentrations, 2 mM, proline stimulated sarcosine absorption, but increasing the concentration to 8 mM while sarcosine remained at 2 mM resulted in inhibition. Betaine, which was transported by this system, was also inhibitory toward sarcosine (Munck, 1966a). Although phenylalanine, leucine and methionine had little effect on sarcosine and betaine absorption, dibasic amino acids did exhibit an inhibitory effect (Larsen et al., 1964). Amino acids which use the N_2 system must compete with each other as well as dibasic amino acids. Glycine can utilize both transport systems, N_1 and N_2, but it was considered to be one of the slowly absorbed amino acids (Adibi et al., 1967; Munck, 1966a). The glycine absorption rate was indicative of its inhibitory action. Glycine was observed to have little effect on neutral or dibasic amino acids, because of its monlipophilic side chain (Adibi et al., 1967; Reiser and Christiansen, 1965, 1969). Some neutral amino acids are susceptible to glycine inhibition. Although glycine absorption was inhibited by histidine and methionine, glycine was inhibitory to only methionine (Annegers, 1969). Fleshler et al. (1966) reported K_m and V_{max} values for glycine and alanine. Both amino acids had similar V_{max} values, 14 and 15, but the K_m value for glycine was two times greater than alanine. Alanine and glycine can reach

the same maximum velocity, but alanine has a stronger affinity for the transport system, which was demonstrated by its ability to inhibit glycine absorption.

The N_2 system was capable of absorbing proline, which proved inhibitory toward sarcosine and glycine. Betaine can inhibit proline absorption when the concentration ratio of the two amino acids was in favor of betaine (Hagihira et al., 1962), but reversing the ratios will reverse the effects (Munck, 1966a). Proline can also use the N_1 system and can be inhibitory to amino acids using this system. At high concentrations, proline will inhibit valine absorption (Hagihira et al., 1962) but have only a slight effect on monoidotyrosine (Nathans et al., 1960). Other members of the N_1 group do inhibit proline absorption, such as leucine and methionine (Baker and George, 1971; Munck, 1966a). Proline and glycine share both systems, N_1 and N_2. To inhibit glycine absorption, proline concentration must be increased to four times that of the glycine present (Munck, 1966a). Reiser and Christiansen (1965) used valine as a representative of the N_1 system and listed leucine, isoleucine, methionine, tryptophan and phenylalanine as the most inhibitory amino acids of the N_1 system. They noted that the inhibitory amino acids possessed the following characteristics: were the L-form, were neutral amino acids, contained a lipophilic side chain and have one carboxyl and one amine group. Histidine and alanine were only slightly inhibitory because they contained a partial charge and weakly lipophilic side chain. Valine can inhibit dibasic amino acids such as lysine (Reiser and

Christiansen, 1969). Reciprocal inhibition between valine and leucine was reported by Habihira et al. (1960). Using absorption values determined when each amino acid was present alone, Hagihira et al. (1960) observed a 60% decrease in valine absorption and a 50% decrease in leucine absorption. Leucine and isoleucine are not as effective inhibitors of valine absorption as methionine. Reduction in valine absorption with the addition of leucine or isoleucine was only 25% of control values while methionine reduced absorption by 50%. Leucine was also inhibitory of other neutral amino acids. A 2 mM concentration of leucine was adequate to reduce alanine absorption by 46%, even when alanine was present at a concentration of 20 mM (Matthews and Laster, 1965). Leucine appears to be effective in inhibiting most neutral amino acids, with the exception of methionine, as well as basic amino acids (Tasaki and Takahashi, 1966; Munck, 1966b). Tryptophan and phenylalanine are not as effective inhibitors as other neutral amino acids. Tryptophan does inhibit histidine absorption when both are present at the same concentrations, but neither is affected by the addition of phenylalanine (Pinsky and Geiger, 1952). Cohen and Huang (1964) used tyrosine and phenylalanine to reduce tryptophan absorption, but tyrosine was more inhibitory than phenylalanine. Methionine has been shown to be the most inhibitory amino acid for the neutral amino acid system, but it was also susceptible to inhibition. Methionine inhibitory effects have been reported toward glycine, histidine, monoidotyrosine, valine, leucine, alanine, glutamic acid and proline (Annegers, 1969; Baker and George, 1971; Larsen et al., 1964; Matthews

and Laster, 1965; Nathans et al., 1960; Paine et al., 1959; Reiser and Christiansen, 1965; Tasaki and Takahashi, 1966). Histdine absorption was decreased by 30% when an equal concentration of methionine was added. Reducing the methionine concentration reduced the inhibition to 12% (Hagihira et al., 1960). Methionine was also effective in inhibiting glucose absorption. But glycine inhibited methionine absorption when glycine concentrations were higher (Annegers, 1969). Multiple linear regression equations were developed by Annegers (1969) to predict the amount of amino acid that would be absorbed when an inhibitor was present. The model contained the intercept, the effects of the test amino acid concentration, the effects of the inhibitor concentration and the effects of fluid movement across the intestine. Fluid movement was significant for all equations. Alanine, methionine, histidine, glycine and lysine were significant inhibitor components of regression equations for glycine, methionine and histidine prediction equations. R square values ranged from 0.82 to 0.96. Most inhibition research was conducted with only the amino acids of interest present. Aroskar and Berg (1962) noted that individual amino acids were more inhibitory than the addition of an amino acid mixture. Amino acid competition reported from in vitro procedures may be larger than those observed in vivo. As digesta moves through the tract, amino acids are removed, which should reduce the amount of competition (Christensen, 1963).

Stimulation of Amino Acid Absorption

Not all interactions between amino acids result in inhibition, as some amino acids can stimulate absorption of other amino acids. The addition of proline to an incubation medium containing threonine, sarcosine, glycine and proline resulted in a significant increase in absorption of each amino acid (Munck 1966a). Threonine, sarcosine and glycine absorption was increased by 17.2%, 31.4% and 18.2%, respectively. Ornithine, arginine, and lysine absorption was increased with the addition of leucine (Munck, 1966b). Leucine concentrations of 0.5 to 5.0 millimolar increased the amount of lysine removed from a 10 millimolar lysine solution. Leucine increased the influx of lysine from the lumen into the tissue without affecting the efflux from the tissue to the lumen (Munck and Shultz, 1969a). This results in an increase in net lysine absorption. Christensen (1963) theorized that one amino acid could provide the driving force necessary for active transport of another amino acid by a second system. The first amino acid would be absorbed quickly by the first carrier and develop a high tissue to lumen concentration gradient. This gradient plus the affinity for the second carrier system would drive the absorption of the second amino acid.

Absorption from Amino Acid Mixtures

To ascertain the absorption characteristics of individual amino acids, researchers have usually studied each amino acid alone or in the presence of two or three other amino acids. Under physiological

conditions amino acids would be absorbed from mixtures containing many amino acids. Orten (1963) looked at absorption rates and patterns of 18 amino acids in an equal molar mixture by the human ileum. Essential amino acids (EAA) were absorbed in the same pattern when nonessential amino acids (NEAA) were present as when NEAA were not present. Absorption patterns of NEAA were different when EAA were present. Alanine and serine absorption decreased with the addition of EAA. Thus, NEAA must compete with EAA for absorption. Doubling EAA concentrations in the presence of NEAA increased EAA absorption by 12%, but no change was noted in absorption patterns. Orten (1963) divided 18 amino acids into three groups using the half life of the amino acid in the lumen as the criteria. The fast group was composed of arginine, isoleucine, methionine and leucine, while threonine, histidine, glycine and glutamate were in the slow group. Valine, lysine, phenylalanine, cystine, tyrosine, tryptophan, proline, aspartate, alanine and serine were intermediate. The average half life of an amino acid in the fast, intermediate and slow group were 24, 59 and 93 minutes, respectively. When the amino acids in the mixture were no longer maintained at equal concentrations, the absorption pattern remained the same unless there was an overwhelming preponderance or near absence of one amino acid.

Basic and acidic amino acids compete with each other for absorption, but reducing the concentration of one member of the group removes the inhibition and allows increased absorption of the other members of the group. Aroskar and Berg (1962) reported that methionine

was the strongest inhibitor of the absorption of amino acids from a seven amino acid mixture. Tasaki and Takahashi (1966) noted competition effects, similar to those described by Orten (1963), being present in the ileum of the fowl. Amino acids with large nonpolar side chains, methionine, isoleucine, valine, leucine and tryptophan, were absorbed in the greatest amounts. Aspartate and glutamate were poorly absorbed due to the polarity of their side chains. Absorption data which utilized ruminant tissue is limited, but absorption patterns reported have been similar to previously reported monogastric research (Williams, 1969). Infusing leucine into the abomasum of sheep resulted in a decrease of valine, isoleucine, glycine and alanine in both the carotid and portal blood, but only lysine net absorption was altered by the addition of leucine (Hume et al., 1972). Variation between animals within a species can be as great as variations between species. The differences noted between individuals could be a reflection of efficiency with which an animal utilizes amino acids (Williams, 1969). The absorption rate of an amino acid from a mixture was dependent upon its relative concentration and interrelationships with other amino acids that are present. Other factors, noted by Orten (1963), which altered absorption were dietary intake, anger or psychological tension, dehydration, alcohol, and antibiotics. During the experimental period, the intestinal loop which Orten used was accidentally exposed to 0.2 N hydrochloric acid which stripped the intestinal epithelium. Absorption rate dropped and patterns were altered for 96 hours, during which time the mucosal

epithelium was being replaced with new cells. He theorized that absorption was performed by mature epithelial cells on the villus as opposed to new or immature cells.

Transport systems found in the intestine may be the same systems used to transport amino acids across the brain barrier and into the liver. Lacy (1970) observed that amounts of amino acid absorbed by the liver of the rat changed with length of perfusion, but the pattern remained the same. This pattern differed from those reported by Orten (1963). Alanine and glycine were absorbed rapidly while isoleucine, leucine, and valine resulted in negative absorption. Amino acid movement across the brain barrier yielded another pattern (Oldendorf, 1973). Valine, lysine, threonine and arginine were poorly absorbed, but over 50% of the phenylalanine, tyrosine and leucine present was absorbed.

Ruminants are capable of utilizing low quality energy and protein sources because of the symbiotic relation which exists between the animal and the microorganisms of the rumen. But the type of nutrients the ruminant consumes does influence amino acid absorption. Sniffen and Jacobson (1975) studied the effects of good and low quality alfalfa hay on amino acid adsorption by Holstein steers as measured by portal-carotid amino acid concentration differences. The greatest amount of amino acids absorbed was measured at 0 to 6 hours and 6 to 12 hours for good and low quality hay, respectively. Steers fed good quality hay consumed 1.6 times more amino acids, absorbed 4.9 times more EAA, 5.9 times more NEAA and 5.3 times more total amino acids than

those fed low quality hay. The feeding of low quality hay resulted in 85% of the EAA consumed being absorbed as compared to 248% when good quality hay was fed. Essential amino acid absorption patterns in sheep have been reported to be similar to composition of bacteria, but NEAA patterns were different (Hume et al., 1972).

Once amino acids leave the intestinal lumen, the proportions and amounts which appear in the portal blood may be altered. Hume et al. (1972) noted a positive net absorption rate at 2, 6 and 10 hours after feeding in sheep, fed one time per day. Negative absorption was observed during the other periods. By decreasing the feeding interval to two hours, negative net absorption was eliminated. This feeding sequence provided a more constant and uniform absorption rate. Wolff et al. (1972) estimated that only 50% to 67% of the total amino acids removed from the intestinal lumen of the sheep appears in the portal system and as much as 1.45 millimoles of glutamate was utilized by the intestinal tissue each hour. The intestine also produced alanine and glycine at the rate of 2.3 and 1.6 millimoles per hour, respectively. Production of alanine, glycine and serine by the viscera and liver of the sheep was demonstrated by Wolff and Bergman (1972a). Five percent of the labeled alanine was collected as glutamate and aspartate. This indicated that conversion of amino acids to other amino acids was possible. Seventy-five to 110% of the glycine, serine, proline, tyrosine, threonine, leucine and phenylalanine removed from the intestinal lumen appeared in the portal blood as compared to 56-62% of the valine, isoleucine, lysine and histidine removed (Wolff

et al., 1972). The amino acid pattern of the plasma can be altered by the liver. Gluconeogenesis of alanine, aspartate, glutamate, glycine and serine by the liver can account for 11% of the blood glucose found in sheep fed a near maintenance diet (Wolff and Bergman 1972b). Glycine, alanine and glutamate alone can account for 9.8% of the blood glucose and 50% of the total alpha amino nitrogen loss to the liver (Wolff and Bergman, 1972a,b). They also calculated that the total hepatic uptake of amino acids could account for no more than 30% of the glucose produced.

OBJECTIVES

This experiment was conducted to determine the absorption characteristics of three structurally different amino acids by the jejunum and the ileum of the sheep.

The specific objectives were:

(1) To establish functional double re-entrant cannulae in the small intestine of the sheep.

(2) To determine the absorption characteristics of the jejunum and the ileum of the sheep for the amino acids studied.

(3) To determine the absorption rates of threonine, valine and methionine and how these amino acids interact at different concentrations for the transport systems of the small intestine.

EXPERIMENTAL PROCEDURE

Two-year-old wethers equipped with double re-entrant cannulae located in either the upper jejunum or lower ileum were used in this study.

Preparation of Animals

Cannulae were constructed from polypropylene "T" shaped tubing connectors with an internal diameter of 1.02 cm. The horizontal portion of the cannula was 5.40 cm in length and was cut in half horizontally to form a concave section perpendicular to the vertical portion, which was 4.20 cm long (figure 1). These cannulae are similar to those described by Phillipson (1952). All surfaces, which were cut, were polished to insure a smooth surface. Other components of the cannula are shown in figure 1 also. A dacron mesh patch,[1] approximately 3.8 cm x 3.8 cm, was used to add reinforcement to the point at which the cannula entered the intestine. Rings, diameter 5 cm, were cut from polypropylene sheets and used to hold the cannula in place until adhesions between the intestine and abdominal wall had been established, after which time they prevented the cannula from being pushed in or pulled out. Silastic brand medical grade tubing,[2]

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[1] E. I. DuPont de Nemours and Company, Incorporated, Wilmington, Delaware.

[2] Dow Corning Corporation, Medical Products Div., Midland, Michigan.

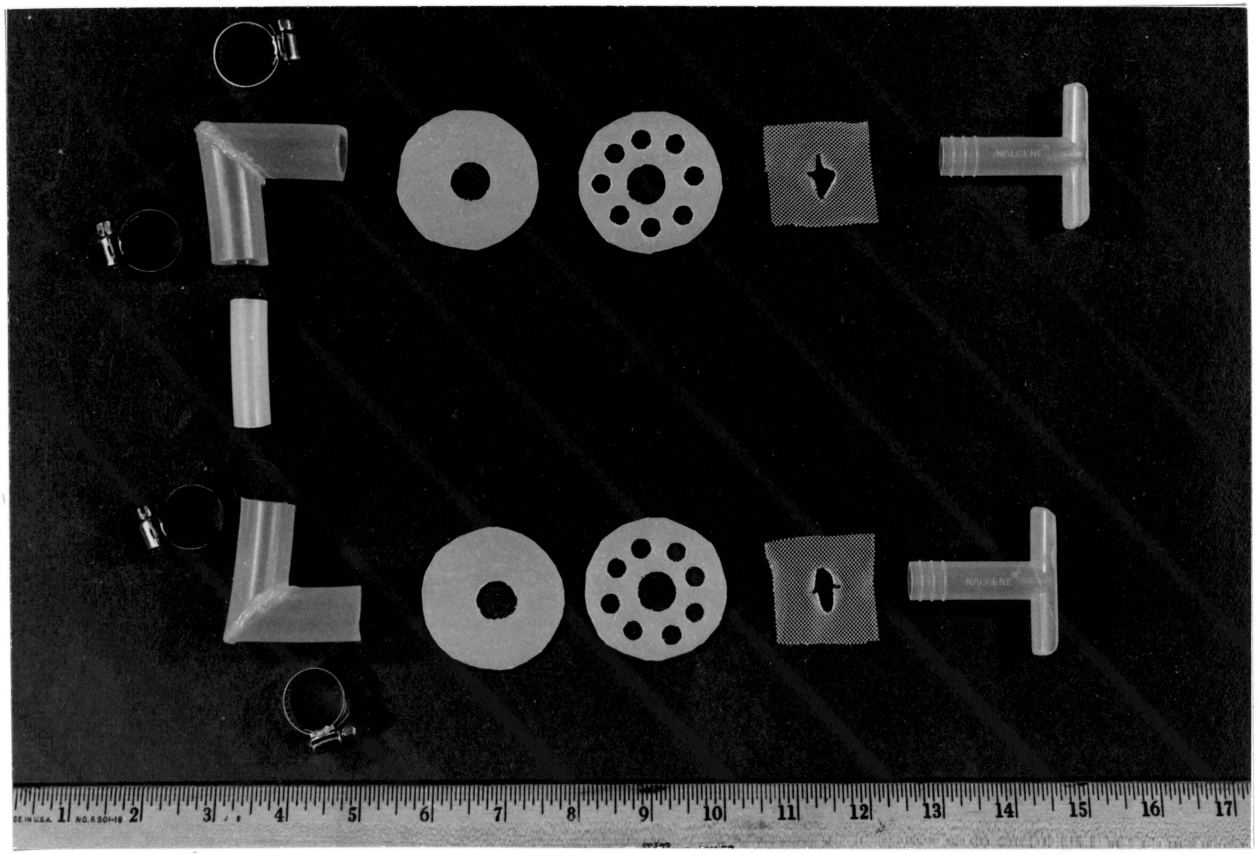

FIGURE 1. COMPONENTS OF CANNULAE DISMANTLED.

inside diameter of 1.27 cm, was formed into right angle elbow joints to serve as connectors between the cannulae. One connector was placed on each cannula and held in place by a metal worm gear clamp. Each pair of silastic connectors was mated by polypropylene internal sleeve and two metal clamps.

All components described in figure 1 are connected together and shown in figure 2. This is one of the two cannulae pairs installed in each animal, with one pair being located at each of the two points at which the intestine is divided.

Prior to cannulation, wethers were treated for internal parasites, housed as a group on wire mesh flooring and fed a maintenance level of a grass-legume hay and corn ration. Wethers were fasted for 24 hr with access to water, prior to surgery. The jugular vein was catheterized to allow the administration of the anesthetic (sodium pentobarbital) and an electrolyte solution containing 5% glucose during surgery. Approximately 1 liter of the electrolyte-glucose solution was administered during the operation in an effort to prevent post-operative shock. A working incision was made in the lower portion of the right flank to obtain access to the small intestine (Brown et al., 1968). The intestinal section, jejunum or ileum, was located and a 100 cm section measured. The jejunal section began 2.5 to 3.0 m from the pylorus and extended 100 cm distally. The ileal section ended 2.0 m from the ileocecal valve and extended proximally 100 centimeters. Before the intestine was transected at both the beginning and end of the 100 cm segment, blood vessels in the mesentery were ligated by

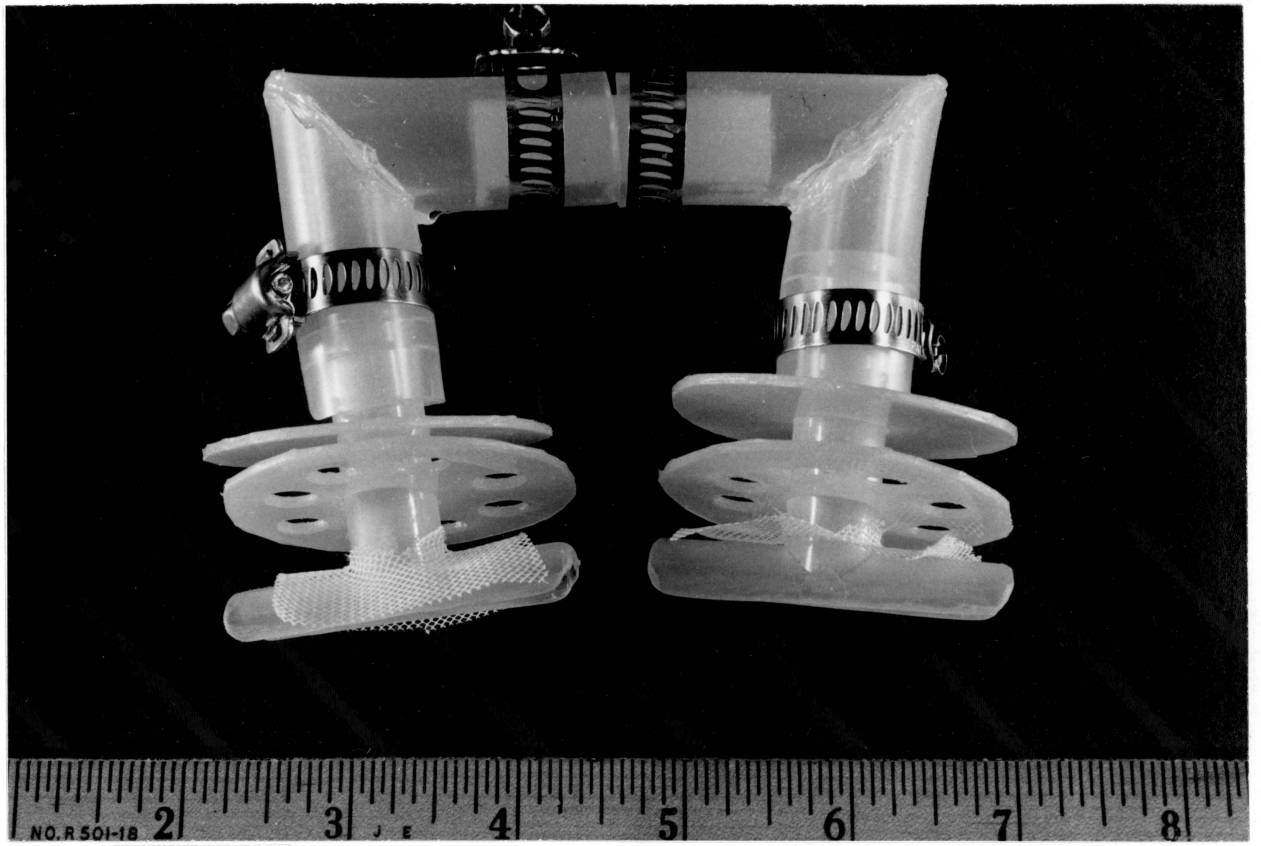

FIGURE 2. COMPONENTS OF CANNULAE CONNECTED.

single silk sutures[3] to reduce hemorrhaging. It was found necessary to cut the mesentery to provide adequate spacing between each pair of cannulae. Once divided, each end of the intestine was stumped by oversewing a clamp placed on the end of the intestine according to the Parker-Kerr method as described by Markowitz et al. (1964) (figure 3). The clamp was removed slowly while tension was applied to the first row of sutures.[4] The end of the intestine was then inverted and the suture continued in the opposite direction as shown in the right hand portion of figure 3. The end of the second row of sutures and the beginning of the first row were at the same point so a tie was made at this point.

A longitudinal incision, 3 to 4 cm long, was made on the anti-mesenteric side of the intestine (figure 4). The cannula was inserted and the opening was closed with two purse string sutures[2] to reduce the amount of exposed mucosa. To add reinforcement and to prevent leakage, a dacron mesh patch,[1] 3.8 cm x 3.8 cm, was slipped over the cannula and secured to the intestine by a continuous silk suture[2] (figure 5).

In order to reduce adhesion of the intestine to the abdominal wall, the omentum was draped over the cannula and each cannula was brought through the omentum by blunt dissection (Ash, 1962). A stainless steel trocar was used to exteriorize the cannula above the working incision.

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[3]Size 0 surgical suture, Ethicon, Inc., Sommerville, New Jersey.

[4]Size 1 chronic surgical gut, Ethicon, Inc., Sommerville, New Jersey.

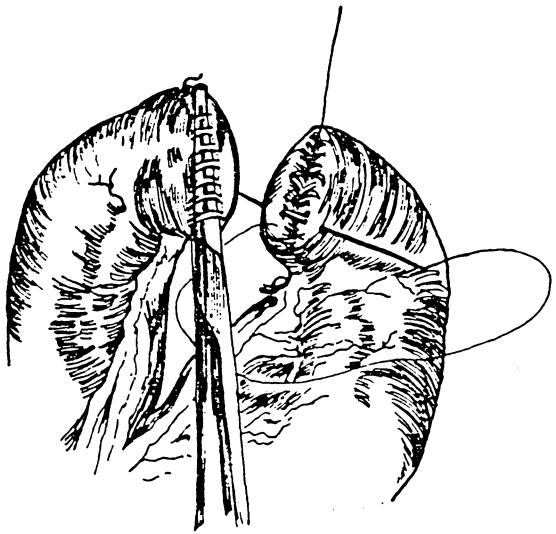

FIGURE 3. STUMPING OF INTESTINAL ENDS AFTER TRANSECTION.

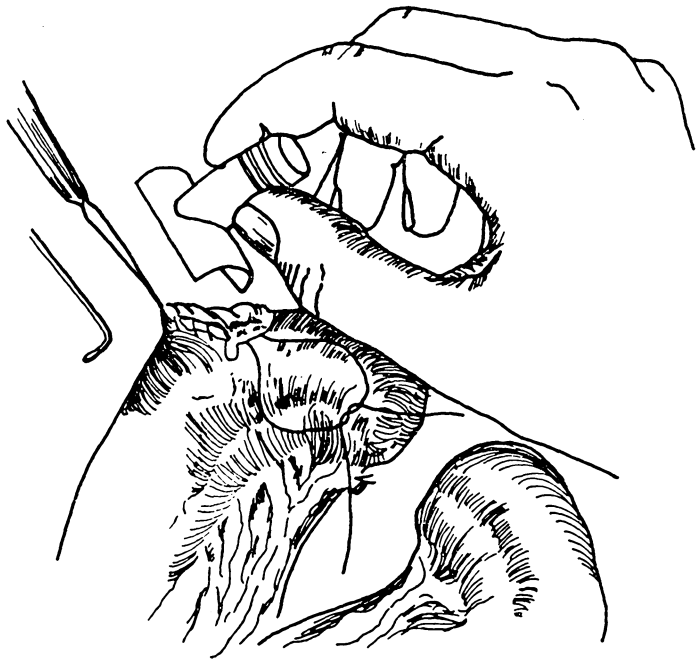

FIGURE 4. LONGITUDINAL INCISION WITH PURSE STRING FOR INSERTION OF CANNULA.

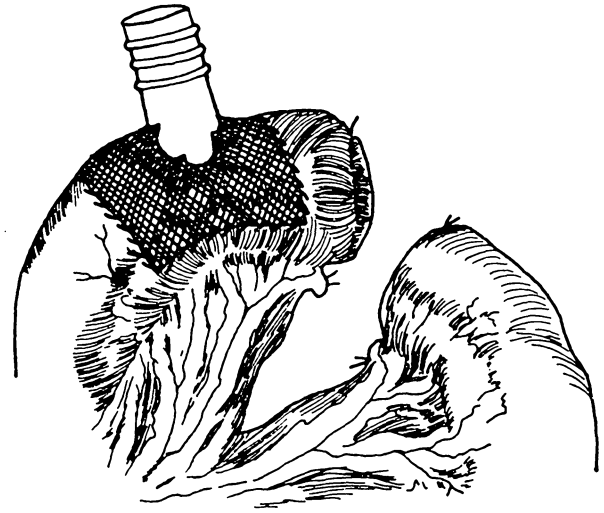

FIGURE 5. CANNULA SECURED IN PLACE WITH DACRON MESH PATCH FOR REINFORCEMENT.

The four cannulae and the isolated segment are shown diagrammatically in figure 6. The omentum would be located between the dacron mesh patch and the first polypropylene ring on each cannula. This ring was perforated to allow adhesions to the abdominal wall to add support. The second ring is solid and located between the fistula and the silastic connector. In order to facilitate the description of the digesta flow, cannulae are numbered one through four from right to left in figure 6. Digesta from the upper tract, right of figure 6, flowed out of cannula No. 1 through the connector and into cannula No. 2, which is the beginning of the 100 cm isolated segment. After passing through the isolated segment, the digesta flowed from cannula No. 3 into No. 4 and down the tract. To isolate the 100 cm segment for perfusion purposes, cannula No. 1 and No. 4 are connected to by-pass digesta during the perfusion period. Solutions were perfused into cannula No. 2, passed through the isolated segment and collected from cannula No. 3. In this experiment, isolated segments of this type were established in either the upper jejunum or the lower ileum.

One of the wethers used in this experiment is shown in figure 7. This animal was cannulated for over 6 months before this photograph was taken. Placement of cannulae pair resulted in a downward slope from the forward to the rear cannula. This facilitated the flow of digesta from one cannula to the next. The placement of cannulae in the animal differed from the linear model of figure 6 (figure 7). The first cannulae pair, No. 1 and 2, is on top and the second cannulae

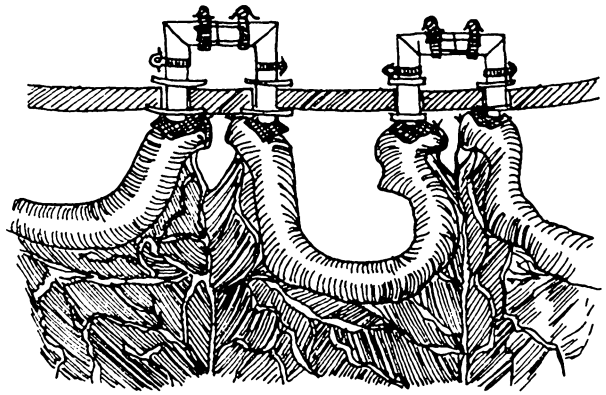

FIGURE 6. FOUR CANNULAE, CONNECTORS AND ISOLATED SEGMENT.

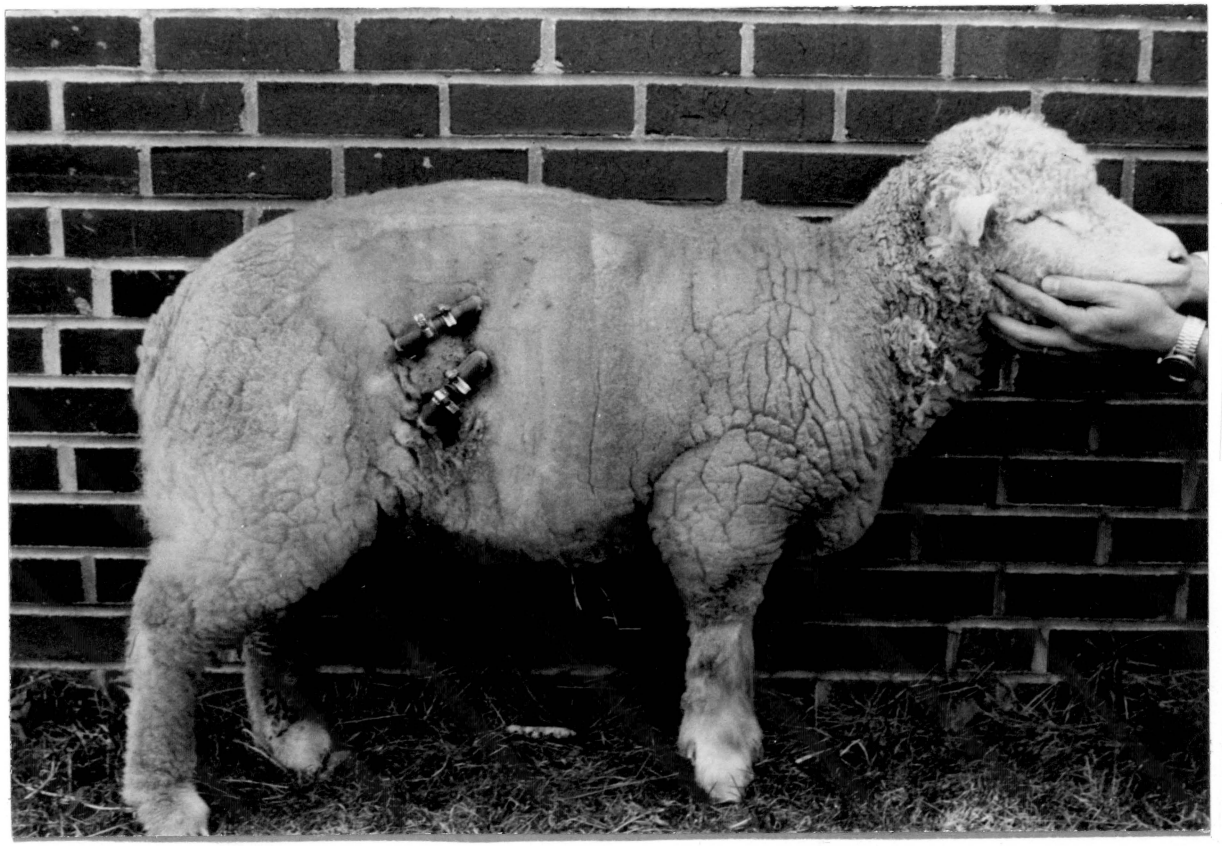

FIGURE 7. WETHER WITH DOUBLE RE-ENTRANT CANNULAE.

pair, No. 3 and 4, is on the bottom.

Feeding of Animals

Wethers were offered feed and water upon recovery from anesthesia which was usually 3 to 4 hr postoperative. A combination of stomach, appetite and intestinal stimulants[5,6] was occasionally needed to get some animals to begin eating again. Stimulants were administered 48 hrs after cannulation if the wether had not consumed any feed. Cannulae were checked daily and the area around the cannulae was washed with warm soapy water weekly. Wool around the cannulae was shorn regularly to reduce matting. Antibiotics[7] were administered daily by intra-muscular injection for 7 days after cannulation, then the amount was slowly decreased over a second period of 7 days until zero was reached. Cannulae were allowed at least 2 weeks to seal before using the animal. Wethers were housed in individual pens, 1.21 m x 1.21 m, with expanded metal flooring. The experimental ration was fed in equal parts at 12-hr intervals. Each feeding consisted of 350 g of alfalfa hay and 100 g of cracked shelled corn. The composition of the hay and corn fed is shown in table 1. This ration was calculated to provide a maintenance level of energy with excess crude protein. Trace mineralized salt and water were available ad libitum. An attempt was made to feed complete ground rations, but resulted in

[5]Bos-o-Tone, Fort Dodge Laboratories, Fort Dodge, Iowa.

[6]Carmilax, Norden Laboratories, Lincoln, Nebraska.

[7]Combiotic, Pfizer Agriculture Division, New York, New York

TABLE 1. COMPOSITION OF RATION INGREDIENTS

Item	Alfalfa hay	Corn
IRN	1-00-059	4-02-931
Dry matter, %	90.5	89.6
Composition of dry matter, %		
Crude protein	20.8	9.2
Ether extract	1.9	4.7
Crude fiber	27.9	3.5
Ash	8.3	1.4
NFE	41.1	8.12

cannulae blockage (Ash, 1962). Wethers refused to eat if cannulae were blocked. These blockages were treated immediately by opening each connector and flushing with warm electrolyte solution[8] (Horney et al., 1972). If the blockage had created undesirable pressure due to digesta backed up in the tract, this digesta was removed, because excessive pressure can result in leakage around the cannulae. Once the blockage had been corrected animals quickly returned to normal and consumed the experimental ration readily. Long alfalfa hay and cracked shelled corn resulted in only a few blocked cannulae. Each wether was weighed at the beginning and the end of the experimental period to insure that each wether at least maintained its body weight.

Preliminary Experiments

Perfusions of one jejunal and one ileal cannulated wether were conducted initially to determine if the concentration of amino acids in the effluent varied within the 1 hr perfusion period and to establish how many hours after feeding the perfusion period should begin. Wethers were cleaned and equilibrated as described later in the amino acid perfusions. Samples of effluent were taken at the beginning of the 1-hour perfusion period (0 min.) at the middle (30 min.) and at the end (60 min.). The 1 hr perfusion period began at either 2, 4, 6, or 8 hr after feeding. Time periods were randomly allotted to days and both wethers received the same treatment on the same day. Each treatment was replicated twice. The initial concentration of

[8]Eltrodd IV - 4000, Haver-Lockhart, Shawnee, Kansas.

threonine, valine and methionine in the perfusion solution was 2.46, 2.36 and 2.27 μmoles/ml., respectively.

Amino Acid Perfusions

The design of this experiment was a three-dimensional central composite with three replications as described by Gardiner et al. (1967). The design consists of three amino acids each at five concentration levels. The 15 treatments dictated by this design are presented in table 2. By choosing five concentrations of each of the three amino acids and creating 15 treatments combinations, the effects of all possible combinations of the three amino acids can be predicted within the concentration limits of this study.

Phillipson (1952) reported the volume of flow of digesta through the duodenum of sheep to be variable and range from 10.2 liters to 14.4 liters per day. Webb et al. (1973) determined the grams of nine essential amino acids which reached the abomasum in 24 hr in sheep fed a conventional diet. Using these two sources of data, an estimate of amino acid concentration which could reach the intestinal tract of the sheep could be made. The average concentration calculated was 2.46 micromoles per milliliter. Thus, a concentration of 2.50 micromoles per milliliter was selected as the midpoint concentration and two levels below, 0.625 and 1.25, and two levels above, 5.0 and 10.0, were established. This resulted in a doubling of concentration between each concentration level. The actual concentrations of the amino acids perfused as determined by analysis are presented in table 3 for each treatment. The concentrations ranged from 0.6 to 8.4, 7.6 and 7.4 mM for

TABLE 2. TREATMENT COMBINATIONS OF AMINO ACIDS

Treatment	Amino acid		
	Threonine	Valine	Methionine
1	-1[a]	-1	-1
2	-1	-1	+1
3	-1	+1	-1
4	-1	+1	+1
5	+1	-1	-1
6	+1	-1	+1
7	+1	+1	-1
8	+1	+1	+1
9	0	0	0
10	0	0	-2
11	0	0	+2
12	0	-2	0
13	0	+2	0
14	-2	0	0
15	+2	0	0

[a]Values are coded as follows: -2 = 0.6; -1 = 1.2; 0 = 2.1; +1 = 4.3; and +2 = 7.8 micromoles per milliliter. These values represent the mean concentrations of the three amino acids as determined by analysis.

TABLE 3. CONCENTRATION OF AMINO ACIDS IN PERFUSION SOLUTIONS

Treatment	Amino Acid		
	Threonine	Valine	Methionine
	µmoles/ml[a]		
1	1.16	1.14	1.05
2	1.25	1.21	4.36
3	1.14	4.37	1.18
4	1.34	4.53	4.22
5	4.19	1.11	1.11
6	3.95	1.08	3.80
7	4.54	4.31	1.14
8	4.73	4.66	4.47
9	2.08	2.02	1.93
10	2.10	2.04	0.56
11	2.28	2.25	7.40
12	2.23	0.59	2.02
13	2.15	7.61	1.99
14	0.65	2.30	2.17
15	8.44	2.24	2.19

[a]Concentration as determined by analysis.

threonine, valine and methionine, respectively.

Initially, amino acid solutions were prepared in Krebs-Ringer bicarbonate buffer, pH 7.4, in sufficient quantities to perform all perfusions. Each solution was prepared, divided into aliquots and frozen until needed. One hr perfusions were conducted and these began 4 hr after feeding. Two wethers, one with an isolated jejunal and one with an isolated ileal, were placed in stalls 45 min prior to the perfusion period. Cannual No. 1 and No. 4 were connected, allowing digesta to bypass the isolated 100 cm section. The isolated sections were then cleaned of digesta by flushing with Krebs-Ringer bicarbonate buffer. First, 200 ml were administered by syringe, then additional buffer was perfused by a peristaltic pump for 10 min at a rate of 20 ml/minute. The section was then charged with 150 ml of the amino acid mixture and allowed to equilibrate by perfusing the same solution for 15 min at 10 ml/minute. This rate was maintained throughout the perfusinn period. At the end of the equilibration period, the distal end of the isolated section, cannula No. 3, was connected to a reservoir for total collection of all effluent during the 1 hr perfusion period. All solutions were maintained at 39 C in a water bath. All wethers were perfused with one treatment on the same day and treatments were randomly allotted to days for each replication. Four wethers, two jejunal and two ileal, were used in replication 1, but only three of the wethers were used in replication 2. One of the ileal cannulated wethers

developed a leak around one cannula and was dropped from the trial. Only one wether of each cannulation type, jejunum and ileum, was used in replication 3. This was due to a lack of sufficient solution to perfuse more than two animals.

Two samples were taken from the perfusion solution, one at the beginning and one at the end of the perfusion period. The total volume of effluent collected during the perfusion period was determined by allowing the solution to come to room temperature and measuring it in a graduated cylinder. A sample of effluent from each wether was taken for amino acid analysis. Protein-free filtrates of perfusion solution and effluent were prepared for analysis by adding 1 ml of a 20% sulfosalicylic acid solution to 4 ml of the sample. These samples were cooled for at least 6 hours, then centrifuged and filtered through fine glass wool. The filtrates were sealed under nitrogen and stored frozen at -10 C for later analysis. Amino acid analyses of all samples were conducted in duplicate using an automatic amino acid analyzer.[9]

Statistical Analysis

Values are expressed as micromoles of each amino acid absorbed during 1 hour. Total micromoles of each amino acid perfused during the 1 hr period were calculated as the product of amino acid concentration, as determined by analysis, and the 600

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[9]Model TSM, Technicon Instruments, Tarrytown, New York

ml/hr perfusion rate. The micromoles of amino acids not absorbed were calculated as the product of effluent volume and concentrations of the amino acids in the effluent. The micromoles of absorbed amino acids were calculated as the difference in the micromoles perfused and the micromoles in the effluent.

Data were statistically analyzed by the least squares analysis of variance procedure of Barr and Goodnight (1972) and the response surface statistical method (Gardiner et al., 1967).

Three different statistical models were written to analyze these data. Sources of variation for the first model were replication, site, treatment, site x treatment and replication x treatment. Effluent volume was used as a covariant. Means for a particular variable were adjusted for all remaining sources of variation within the model. Two additional models were constructed and used to determine linear, quadratic and interaction components of the prediction equation for each amino acid after the data had been transformed to the logarithm base 10. The covariant, volume of effluent collected, was the only difference between these two models, but its presence proved important. Significant differences between means were determined using the student T test (Dixon and Massey, 1969).

RESULTS AND DISCUSSION

Preliminary Experiments

Time of Sampling. Mean concentrations of threonine, valine and methionine in the effluent for each sampling time and each wether are presented in table 4. Concentrations remained relatively constant through the perfusion period for each amino acid within each site. No significant differences were noted between sampling times for each amino acid within animal. No attempt was made to compare concentration changes between sites, because effluent volume was different for each site and was confounded with individual differences. The lack of a significant concentration change between the 0 and 30 or 60 min. sample indicates that charging the loop with 150 ml of solution and pumping the same solution for 15 min at a flow rate of 10 ml per min was adequate to equilibrate the isolated loop. There was no indication that amino acid removal fluctuated substantially ($P > .05$) during the perfusion period. Thus, the intestine was removing amino acids at a constant rate.

Amounts of each amino acid absorbed during the 1 hr perfusion period at different times after feeding are presented in table 5 for the jejunal cannula and table 6 for the ileal cannula. Although there were no significant differences between amounts

TABLE 4. CONCENTRATIONS OF AMINO ACIDS AT VARIOUS SAMPLING TIMES DURING PERFUSION PERIOD

Amino Acid	Jejunum			Ileum		
	Time of sampling (min)[a]					
	0	30	60	0	30	60
	umoles/ml[b]					
Threonine	1.66	1.72	1.67	1.45	1.23	1.23
Valine	1.30	1.34	1.43	0.85	0.70	0.73
Methionine	1.07	1.19	1.18	0.68	0.53	0.52

[a]Time sample was taken during perfusion period

[b]Initial concentration of perfusion solution was 2.46, 2,36 and 2.27 for threonine, valine and methionine, respectively.

TABLE 5. ABSORPTION OF AMINO ACIDS AT DIFFERENT PERFUSION TIMES BY THE JEJUNUM (REPLICATION I AND II)

Hours after[a] feeding	Amino acids					
	Threonine		Valine		Methionine	
	I	II	I	II	I	II
	umoles/hr/100 cm					
2	307	257	432	461	453	538
4	233	51	507	356	566	501
6	126	-274	312	-184	398	0
8	387	292	543	560	594	711

[a]Hours after feeeding at which perfusion period began

removed by the jejunal loop at different times after feeding, it was noted that at 8 hr after feeding the highest amount was removed. The lowest absorption rate was observed at 6 hr after feeding. The other two times were intermediate, with 4 hr being larger than 2 hr except for threonine. The ileal loop removed larger amounts of each amino acid over the various perfusion periods than the jejunal loop (tables 5 and 6). Time after feeding was not different ($P > .05$) within amino acid for the ileal loop, but trends were evident. Six hours after feeding once again resulted in the lowest amounts of each amino acid being absored, with the exception of methionine during the second replication. The highest amounts were absorbed when perfusions were conducted at 4 hrs after feeding, but values collected at 8 hrs were similar. Although absorption values were not different ($P > .05$) for either intestinal site, 4 or 8 hrs after feeding tended to be the highest values and both were quite similar for each amino acid within a site.

Rumen volatile fatty acid production has been shown to peak at four hours after feeding when sheep are fed alfalfa hay once daily (Church, 1969). Portal blood flow has also been shown to increase from feeding to 7 hr after feeding when sheep are given one feeding of alfalfa hay each day, but this pattern can become more consistent if feeding frequency was at 2 hr intervals (Hume et al., 1972). Hume et al. (1972) also reported that portal-carotid

TABLE 6. ABSORPTION OF AMINO ACIDS AT DIFFERENT PERFUSION TIMES BY THE ILEUM (REPLICATION I AND II)

Hours after[a] feeding	Amino Acid					
	Threonine		Valine		Methionine	
	I	II	I	II	I	II
	µmoles/hr/100 cm					
2	1024	321	1335	391	1254	475
4	1180	975	1353	1160	1383	1170
6	783	201	1087	308	1100	515
8	996	1038	1262	1280	1243	1332

[a]Hours afer feeding at which perfusion period began

concentration differences of total amino acids were positive at 2, 6, 8, and 10 hrs after feeding and negative the other sampling times, 4 and 14 through 22 hr after feeding, when sheep were fed one time each day. A 2 hr feeding interval also removed this fluctation and resulted in a relatively constant positive portal-carotid total amino acid concentration difference. From these data and the results of the preliminary trial, 4 hr after feeding was selected as the best time after feeding to begin the perfusion period for all subsequent perfusions. This period of time offered maximum portal blood flow and one of the highest uptake periods for each intestinal site. Movement of amino acids from the tissue of the upper tract to the portal blood would also be minimized during this time. This would reduce the possibility of interference from high portal amino acid concentrations as a result of absorption from sections proximal to the isolated loop upon the abosrption potential of the loop.

Effluent Collection. Internal markers or indicators were not added to the perfusion solution in this experiement because their effects upon absorption are not known. Calibration of the perfusion pump was completed each day prior to the perfusion period. Thus, an accurate measurement of total milliliters perfused could be obtained and standardized by rigid adherence to a 1 hr perfusion period. Total collection of effluent was possible since all fluid from cannula No. 3, the end of the isolated loop, could be channeled to a plastic container.

A total of four wethers, two with jejunal and two with ileal double reentrant cannulae, were used to conduct a second preliminary experiement to determine if the amount perfused into cannula No. 2 could be collected from cannulae No. 3. After cleaning and equilibrating the loop, buffered medium without amino acids was perfused for 2 hr beginning 4 hr after feeding. Effluent volumes collected from each wether for four consecutive 30-min periods are shown in table 7. Standard deviations of the volumes collected range from 16 to 47 ml and were lower for the two jejunal wethers than the two ileal wethers. Values do not vary more than 1.1 standard deviations from the mean for observation within each animal. Average effluent volume collected each 30 min over the 2-hr period from animals number 2, 3 and 4 were 276, 298 and 271 ml, respectively. These are below the 300 ml perfused into cannulae No. 2 for the same period of time. Animal No. 1, used in the first preliminary trial, consistently produced more effluent than was perfused. During the cleaning process 400 ml of buffer was administered by syringe and perfusion pump to remove digesta from the loop. An additional 300 ml of buffer was used to charge and equilibrate the isolated loop. If all of the fluid administered was retained until the collection of effluent began this would add 700 ml to the 1200 ml perfused during the 2-hr period. This would account for only 1900 of the 2100 ml collected if all the fluid administered was collected, but this was not the case. Fluid was emitted from cannula No. 2 during the cleaning and equilibration

TABLE 7. VOLUME OF EFFLUENT COLLECTED AT 30 MINUTE INTERVALS

	Jejunum		Ileum	
	Animal Number			
Time (min.)	1	2	3	4
	---------- µmoles/hr/100 cm ----------			
0 - 30	500[b]	270	318	216
30 - 60	550	260	-	300
60 - 90	500	290	322	296
90 - 120	540	282	255	-
$\bar{x}$	522	276	298	271
S.D.	26.3	16.2	37.6	47.4

[a]Perfusion rate was 100 milliliters per minute.

[b]Values are expressed as milliliters

process. This response was much quicker than that observed when the ileal cannulated wethers were being cleaned and equilibrated. The effluent collected from animal No. 1 did contain digesta and was dark in color while the effluent collected from the other three wethers was almost clear. This digesta was present throughout the perfusion period of 2 hours. No other explanation, other than the presence of digesta or addition of water by the intestine, can be given for the higher effluent volumes collected, but the flow rate remained constant and the amino acid concentration did not fluctuate (table 4 and 7).

Mean values for the amount of effluent collected from each wether during the perfusion of the fifteen treatments used in this experiement are presented in table 8. During the experimental period the ileal wethers maintained a smaller standard deviation than the jejunal wethers. The mean volume collected were less than the 600 ml perfused into the isolated loop with the exception of animal No. 1. This wether consistently had more effluent collected than perfused which resulted in an average of 1023 ml per hour. Effluent volume was entered into the statistical analysis as a covariant and will be discussed with regards to that analysis later.

TABLE 8. VOLUME OF EFFLUENT COLLECTED DURING ONE HOUR PERFUSION

	Intestinal section			
	Jejunum		Ileum	
	Animal number			
Treatment	1	2	3	4
	ml[a]			
1	1095	516	542	580
2	1038	627	550	635
3	932	650	508	580
4	1005	520	482	610
5	1195	412	537	570
6	1010	495	525	590
7	895	470	568	555
8	1070	375	485	505
9	1075	528	553	600
10	1062	612	507	585
11	918	535	498	595
12	1050	565	473	590
13	1040	592	597	565
14	988	590	583	530
15	972	710	537	595
$\bar{x}$	1023	546	530	579
S.E.	77	89	37	32

[a]Mean of three replications for animals number 2 and 3, two replications for animal number 1 and one replication for animal number 4.

Amounts of Amino Acids Absorbed by Replications and Different Intestinal Sites

The first statistical model, model 1, was written to determine variation due to the main effects (site, replication and treatment) and the interactions (site x treatment and replication x treatment) within each amino acid. Treatments were different ($P < .01$) as well as effluent volume ($P < .01$), which was entered as a covariant. Two of the amino acids studied, threonine and methionine, contained significant interaction components. Site x treatment ($P < .05$) and replication x treatment ($P < .01$) were sources of variation in the analysis of threonine.

Absorption of Amino Acids by Replication. Adjusted means are presented in table 9 to show the differences in the amounts of each amino acid absorbed during each replication. Values are adjusted for the two remaining main effects, site and treatment, and the interactions contained in model 1. The amounts abosrbed during the third replication were higher ($P < .05$) than the first replication for all three amino acids while the second replication was intermediate. Absorption of threonine was similar for the first and second replication but both were lower ($P < .05$) than the third replication. Valine absorption during replications two and three was not different but more ($P < .05$) was absorbed during the third replication. During all three replications, methionine absorption was different ($P < .01$).

TABLE 9. AMOUNTS OF AMINO ACID ABSORBED DURING EACH REPLICATION

Item	Replication I	Replication II	Replication III
Number of observations	60	45	27
Amino acid[a]	---------- μmoles/hr/100 cm ----------		
Threonine	529.66[b]	598.58[b]	760.87[c]
	(42.38)[d]	(48.94)	(63.18)
Valine	703.56[b]	867.42[c]	992.62[c]
	(53.61)	(61.91)	(79.92)
Methionine	690.85[e]	859.29[f]	1059.88[g]
	(44.04)	(50.86)	(65.66)

[a]Values are means adjusted for site, treatment, site x treatment, replication x treatment and effluent volume.

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

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

[e,f,g]Means in the same row with different superscripts are significantly different ($P < .01$).

A total of 59 days were required to complete all three replications. During this time the intestine not only maintained its ability to absorb amino acids but actually increased. Andrews et al. (1936) reported a decrease in the absorption of methionine by the small intestine of the dog over a 2-year period, once a thiry loop had been established. After two years the absorption was one third of the initial values. Thiry loops permanently isolate the loop from the intestinal tract and allow no digesta to flow through the loop when not in use. The loops or isolated segments used in this study functioned as normal intestine during the majority of the time and were isolated only when needed. Keren and Elliott (1973) theorized that some factor was present in the chyme which prevented alteration of the intestinal mucosa and its absorption capacity. Huan and Hung (1972) reported no changes in the absorption rate of Thiry-Vella loops established in the small intentine of the rat for the first 2 months, but atrophy was noted 6 months after cannulation. All wethers used in this study had been cannulated for less than 4 months at the beginning of the experiment. Significant increases in the absorption of amino acids with time indicates that the isolated loops used in this experiment remained normal.

Absorption of Amino Acids by Different Intestinal Sites. Amounts of each amino acid absorbed by either the jejunal or the ileal isolated loops are presented in table 10. These values are adjusted means derived from the model 1 analysis and are adjusted

TABLE 10. AMOUNTS OF AMINO ACID ABSORBED DURING EACH REPLICATION

Item	Intestinal Segment	
	Jejunum	Ileum
Number of observations	72	60
Amino acids[a]	--- μmoles/hr/100 cm ----	
Threonine	564.49[b] (38.69)[d]	694.93[c] (42.38)
Valine	760.07[e] (48.94)	919.00[f] (53.61)
Methionine	804.19[e] (40.21)	935.83[f] (44.04)

[a]Values are means adjusted for replication, treatment, site x treatment, replication x treatment and effluent volume.

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

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

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

for replication, treatment and the interactions. The ileal loop absorbed larger ($P < .05$) amounts of threonine, valine and methionine from the perfusion solution than the loop located in the jejunum. Johns and Bergen (1973) observed a similar response in an *in vitro* system employing intestinal rings from the small intestine of mature sheep. They noted that lysine uptake by the rings increased as the distance from the pylorus increased. Maximum absorption occurred at a point 21 m from the pylorus, which was the mid-ileum. Phillips *et al.* (1976) used everted sacs to study the absorption of valine, threonine and methionine by jejunal and ileal segments of the small intestine from young growing wethers. They reported the the ileum absorbed more valine and threonine than sacs prepared from the jejunal section. Methionine absorption was found to be similar for the two distal sections. These observations differ somewhat from the previously reported data using monogastric tissue. Baker and George (1971) studied the uptake of neutral amino acids by the small intestine of the rat. Maximum absorption of these amino acids occurred in the mid intestine, corresponding to the middle jejunum to upper ileum. But methionine absorption was as high in the ileum as in the jejunum. The other amino acids studied had peak absorption in the lower jejunum with a decline in the absorption rate in the ileum. Larsen *et al.* (1964) reported the the lower jejunum and upper ileum were the sites of the maxiumum absorpiton of neutral amino acids by everted sacs prepared from the small intestine of the rat. Samiy and Spencer

(1961) reported that phenylalanine uptake by hamster small intestine increased from the duodenum to the jejunum, but peaked in the jejunum and declined through the ileum. Absorption of methionine by human small intestine was similar to the hamster (Schedl and Clifton, 1963). They characterized the proximal intestine as having a methionine absorption system of high capacity but low affinity. The distal intestine contained a system which transported methionine at a lower capacity but the affinity for the transport system was greater. These observations of the methionine transport system in the small intestine of man were the result of K_m and V_{max} determinations.

Within each intestinal site, the amount of amino acid absorbed increased from threonine to valine and to methionine. Thus, it appears that methionine absorption proceeds at a faster rate than threonine absorption. Larsen et al. (1964) determined K_m and V_{max} values for each of 18 amino acids absorbed by everted sacs of small intestine from the rat. The threonine K_m value was 13 mM which was 2.5 and 4 times larger than the K_m values determined for valine and methionine. This shows that valine and methionine have a stronger affinity for the transport system than threonine V_{max} values were similar for both threonine and methionine, 6.7 and 6.5 umoles, but 2 times larger than that reported for valine. Thus, in the rat intestine threonine was absorbed by a system at a maximum rate similar to methionine,but the affinity for the system was lower. Valine has less affinity for the system than

methionine, but was stronger than threonine. Christensen (1963) theorized that amino acids are removed from the lumen of the small intestine rapidly by the proximal intestine because of the high capacity of the absorption systems found there. This reduces the possibility of a high concentration of any one particular amino acid and minimizes the inhibitory effects of this amino acid on other amino acids. The lower or distal intestine would function as the last opportunity to absorb the amino acids released by further digestive processes or those which were not absorbed in the proximal intestine. The absorption system in this area would not require as much capacity as the proximal intestine, but a high affinity was needed to insure absorption and maximum efficiency of amino acid absorption.

Neutral amino acids are transported by two systems, N_1 and N_2, in the small intestine of the rat (Baker and George, 1971). Methionine, valine and threonine are transported by the N_1 system. Baker and George (1971) noted that increasing the length of the side chain of the neutral amino acid would increase the absorption rate. This pattern was evident in table 10. As chain length increased, moving down the table from threonine to methionine, the amount removed by each intestinal site increased.

Prediction Equations for Amino Acid Absorption

Two additional statistical models, model 2 and 3, were constructed and used to derive regression coefficients for

absorption prediction equations for each of the three amino acids studied. Each model contained linear, quadratic and interaction components of the three amino acids, as well as a covariant, effluent volume, in order to predict the amount of each amino acid that would be absorbed when all three were present. Model 2 contained all the components listed above except the covariant, effluent volume. As a result, the R^2 values were much lower for model 2 than those presented in table 11 for model 3. Without the presence of the covariant in the model, site, became a significant component ($P < .01$) of each prediction equation. Upon its addition, the covariant became a highly significant ($P < .01$) component of the prediction equations for each amino acid, while the importance of the site component was lost except in the case of methionine ($P < .10$).

Effluent volume was different for the two intestinal sites. The overall average of effluent collected during the 3 replications was 653 ml per hr. Jejunal wethers average 784 ml per hr which was 230 ml greater than the 554 ml per hr collected from the ileal wethers. It appears that site differences may be due to flow rate. R-square values were increased upon the addition of the covariant to the model by 12, 7 and 8 percentage units for threonine, valine and methionine prediction equations, respectively.

From model 1 analysis of threonine, it was determined that the site x treatment interaction was significant ($P < .05$). As a result of this interaction, additional components were added

TABLE 11. REGRESSION COEFFICIENTS[a] AND R^2 FOR AMINO ACID ABSORPTION PREDICTION EQUATIONS

Item	Amino Acid		
	Threonine	Valine	Methionine
Site	- 53.22	60.26	56.35[d]
b_o	1217.53	1137.22	1144.36
b_1	571.50	- 517.22	- 410.37
b_2	- 519.30	557.02	- 462.31
b_3	- 382.06	- 304.42	671.55[c]
b_{11}	4271.35[d]	424.74	310.55
b_{22}	29.67	1526.86[b]	297.77
b_{33}	755.69	122.04	1106.01[b]
b_{12}	- 24.60	377.78	292.58
b_{13}	-2913.34	- 190.30	- 270.91
b_{23}	378.73	- 253.44	267.20
sb_1	- 74.32	--	--
sb_2	- 292.27	--	--
sb_3	- 30.01	--	--
sb_{11}	-1493.77	--	--
sb_{22}	295.16	--	--
sb_{33}	- 369.69	--	--
sb_{12}	611.22	--	--
sb_{13}	1153.24	--	--
sb_{23}	269.12	--	--
XV	- 1.06[b]	- 0.94[b]	- 0.86[b]
R^2	.652	.714	.708

[a] b_o = intercept; b_1, b_2, b_3 = linear effects of initial concentrations of threonine, valine and methionine, respectively; b_{11}, b_{22}, b_{33} = quadratic effects of each amino acid; b_{12}, b_{13}, b_{23} = interactions between initial concentration of threonine and valine, threonine and methionine, valine and methionine, respectively; s = site, $sb_1 \ldots sb_{23}$ = interaction between site and linear, quadratic and interaction components; XV = effluent volume collected.

[b] $P < .01$ [c] $P < .05$ [d] $P < .10$

to the threonine prediction equation to define these interactions. These components are also presented in table 11 and are coded with the letter S for site. The addition of these 9 interaction components increased the R^2 value for the threonine prediction equation from 61.2% to 65.2%, indicating their importance. Only two of the 21 components which make up the threonine prediction equation were significant. The quadratic effect of threonine was not significant ($P < .10$) but the covariant ($P < .01$), effluent volume, was highly significant.

Analysis of valine absorption by model 1 revealed no significant interactions, thus only 12 components were used to construct the prediction equation for valine absorption shown in table 11. Effluent volume was a highly significant ($P < .01$) component, while the quadratic effect of valine concentration was the only other significant ($P < .01$) component.

The interaction, replication x treatment, for methionine model 1 analysis was not significant ($P < .15$), therefore, no change in the prediction equation was made as in the case of threonine. Four of the twelve components presented in table 11 and used to describe the absorption of methionine were significant. Site remained an important component ($P < .10$) after the addition of the covariant, but this was not true for the other two amino acids. Linear and quadratic components of methionine concentration were significant ($P < .05$) as well as the effluent volume ($P < .01$).

Effluent volume was a significant component of the three prediction equations constructed to predict the absorption of threonine, valine and methionine when all three amino acids are present at known concentrations. The quadratic components of each amino acid were important components ($P < .10$) for the prediction of the absorption response for that amino acid. The methionine prediction equation was the only equation which contained a significant ($P < .05$) linear component, which was the initial concentration of methionine. Linear components of intial concentration of each amino acid exerted a negative influence upon the absorption of the other amino acids. This was indicative of the fact that amino acids compete for transportation sites and the presences of other amino acids can inhibit absorption.

Stepwise regression could have been performed on this data to limit each prediction equation to the smallest number of components needed to predict absorption. But the model was established at the beginning of the trial; thus, all components must remain in the model. All regression coefficients listed in table 11 were retained and used to calculate the amounts of each amino acid absorbed when all three amino acids are present at various concentrations.

Effects of Concentration on Absorption of Amino Acids

Regression equations presented in table 11 were used to calculate the umoles of each amino acid which would be absorbed at

various concentrations of that amino acid by jejunal or ileal sections of the small intestine of sheep. Each prediction equation was reduced to only those components which represented concentrations effects of the amino acid in question by setting the other two amino acids at concentrations corresponding to the midpoint of the 5 concentrations used in this study (table 3). Midpoints of threonine, valine and methionine were 2.2, 2.2 and 2.1 umoles per ml, respectively. Thus, each equation derived to predict the effects of concentration upon absorption contained the linear and the quadratic components of the amino acid as well as the intercept. Two equations were reduced for each amino acid to predict the effects of concentration upon absorption at two intestinal sites. By entering a +1 or a -1 in the site component, listed as site in table 11, and entering a +1 or +2 for s in any interaction components in the case of threonine, the equation would predict the absorption of each amino acid by the ileum or jejunum, respectively, Effluent volume was different between sites and was a significant ($P < .01$) component of each regression equation. Equations used to predict the amounts absorbed by the ileum contained 554 ml times the regression coefficient for effluent volume, XV, while those used to predict absorption in the jejunmum used 784 ml times the regression coefficient for effluent volume.

To determine how much threonine would be absorbed at various concentrations of threonine by the ileum the following substitutions would be made. Valine and methionine would be set at their

midpoint concentration and entered into the threonine prediction equation. The site component would be set at +1 and all s's which appear in the equation would be set at +1. All like terms would be combined and a three component equation would result. This equation would contain an intercept and the linear and quadratic effects of the initial concentration of threonine upon threonine absorption.

Amounts of threonine absorbed in 1 hr by a 100 cm jejunal or ileal section at threonine concentrations ranging from 0.6 to 8.4 umoles per ml are shown in figure 8. Absorption by either section increased as the concentration of threonine increased, but the ileal section absorbed more threonine than the jejunal section at all concentrations. The increase in the absorption of threonine by the ileal section became more prominent as the threonine concentration increased. Concentration increases above 2 umoles per ml resulted in progressively smaller increases of threonine absorption by the jejunal section. The quadratic effect was not as prominent in the ileal section.

Valine absorption curves at two intestinal locations at various concentrations of valine are shown in figure 9. Prediction equations used to construct these lines are identical except for the intercept; thus, the two lines are parallel. Absorption of valine by the ileum was 337 umoles greater than that absorbed by the jejunum. Absorption of valine by either section does not appear to begin to plateau or reach saturation.

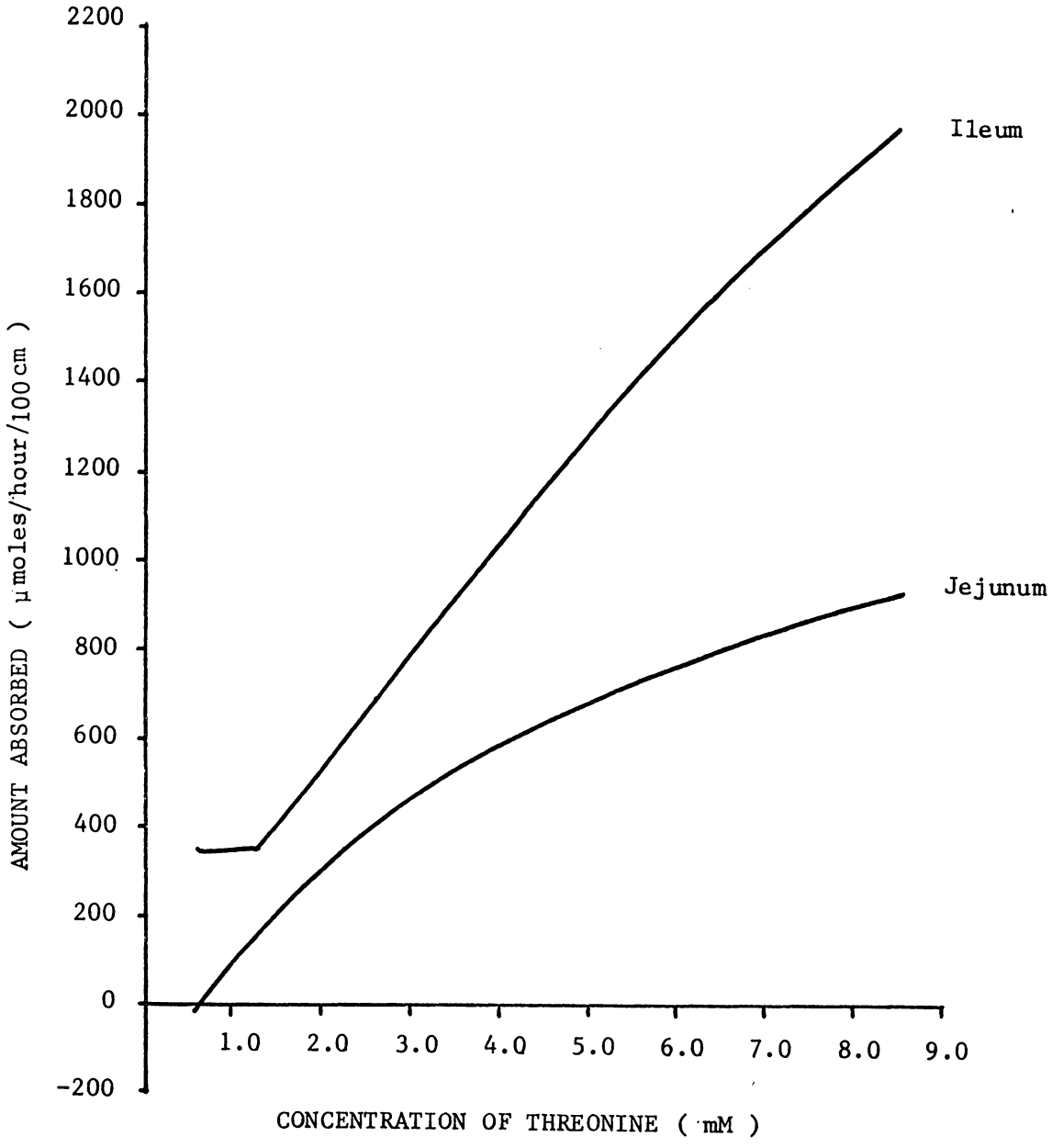

FIGURE 8. ABSORPTION OF THREONINE AT VARIOUS CONCENTRATIONS OF THREONINE BY TWO INTESTINAL SITES.

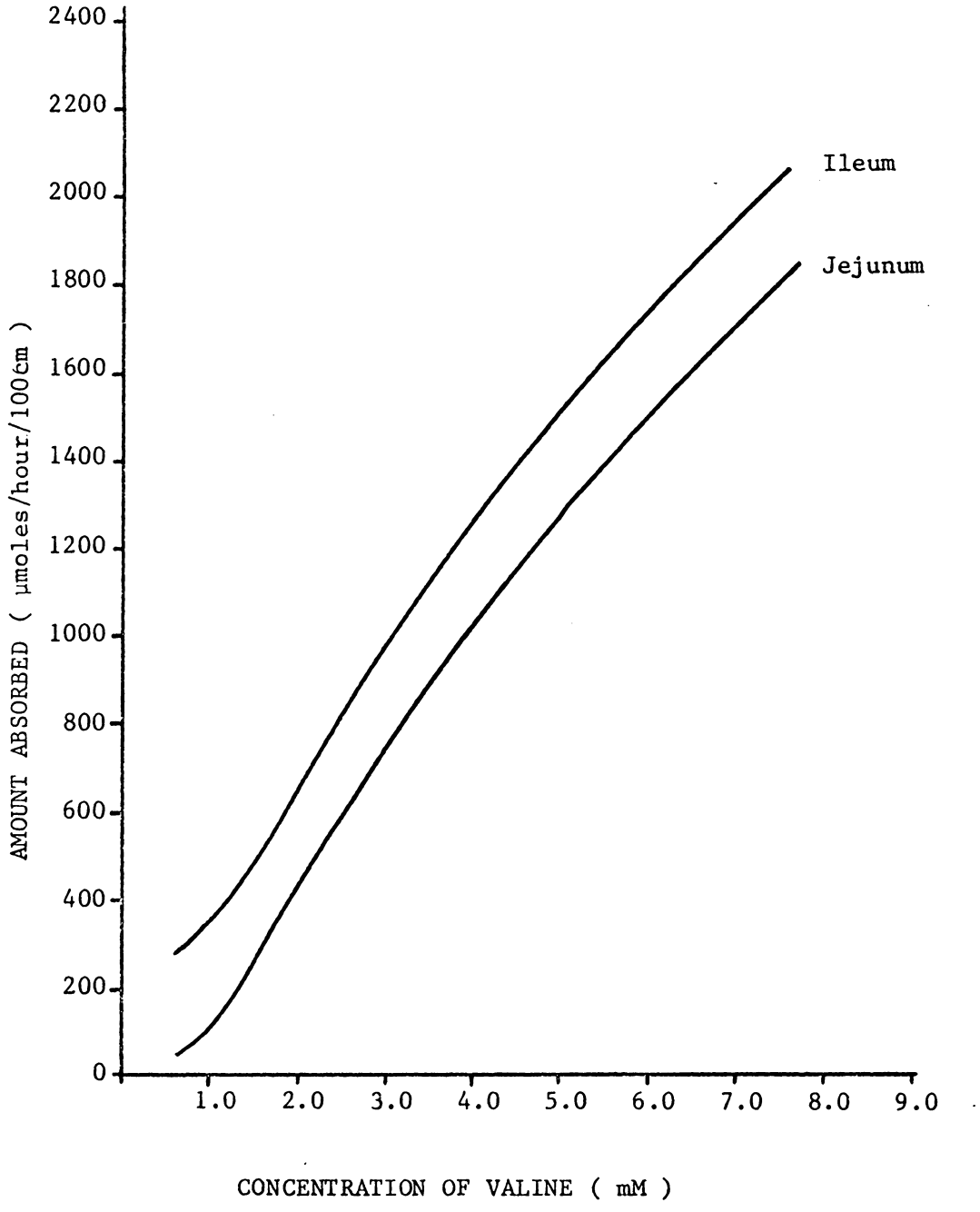

FIGURE 9. ABSORPTION OF VALINE AT VARIOUS CONCENTRATIONS OF VALINE BY TWO INTESTINAL SITES.

Methionine absorption followed a similar pattern (figure 10). Increasing the methionine concentration increased the amounts of methionine absorbed. Amounts absorbed by the two intestinal sections were different ($P < .10$). Methionine absorption curves are similar to those presented in figure 9 for valine and do not show a saturation point.

Analysis of the data by model 2, which contained no covariant, resulted in a significant ($P < .10$) site component for each prediction equation. Thus, under model 2 analysis sites were different ($P < .01$). Upon adding the covariant to the model to form model 3, the importance of the site component was lost except in the methionine equation. The covariant, effluent volume, could account for the variability originally contributed to sites in the prediction equations for the absorption of valine and threonine, but not for methionine. The differences in the amounts of methionine absorbed by the two intestinal sites were still different ($P < .10$) after addition of the covariant. Effluent volume was much higher from wethers with cannulae in the jejunum than the ileum. But this higher average was due to animal No. 1 which collected large volumes of effluent during each perfusion. Amounts absorbed by different intestinal sections shown in figures 8 and 9 are the result of effluent volume differences instead of site differences. But the increased amounts of methionine absorbed by the ileal section were the result of site, as well as effluent volume.

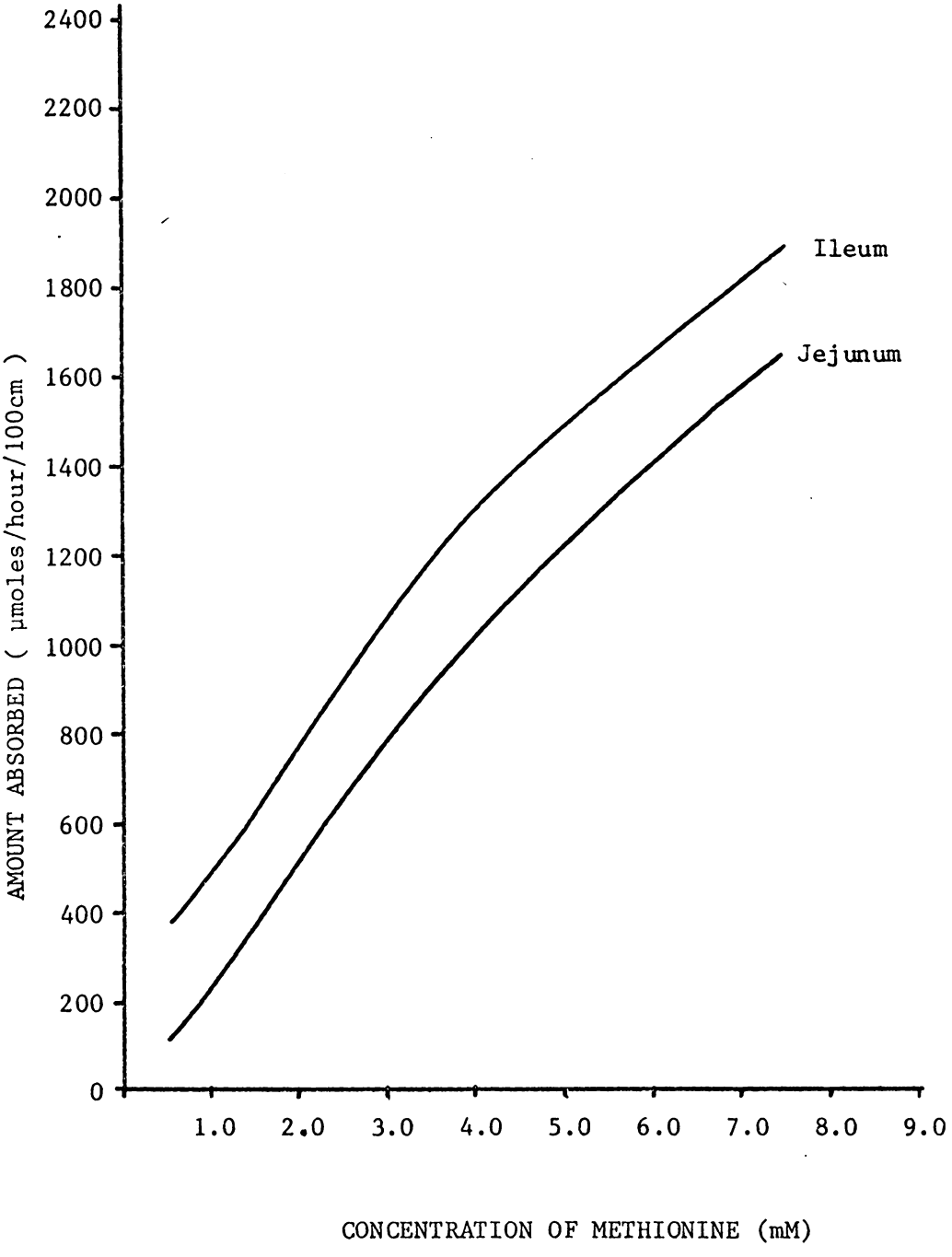

FIGURE 10. ABSORPTION OF METHIONINE AT VARIOUS CONCENTRATIONS OF METHIONINE BY TWO INTESTINAL SITES.

The regression coefficients for effluent volume were negative for each amino acid. As effluent volume increased the amount of each amino acid absorbed would decrease. Church (1969) has estimated digesta flow in the jejunum and the ileum of the sheep. He estimated 300 to 1000 ml of digesta passed out of the duodenum and into the jejunum each hour, while only 90 to 180 ml passed through the ileum each hour. Phillipson (1952) collected digesta as it passed into the duodenum from the abomasum of sheep. He thought that the flow rate was between 350-800 ml per hr and that this rate was dependent upon the ration. The absorption rate of a particular amino acid may be influenced more by intestinal flow rate than intestinal site. As flow rate increases, the number of contacts between amino acids and active sites decreases, thus reducing the formation of the carrier-amino acid complexes.

Independent of the differences in sites, the patterns of amino acid absorption remained the same over the range of concentrations used in this experiment. Saturation of the transport system can be noted by a plateau in the absorption curve plotted against various concentrations. Absorption curves presented in figures 8, 9 and 10 did not show the saturation response, with the exception of the absorption of threonine by the jejunum. Lerner et al. (1969) found that the methionine transport system in the small intestine of fowl became saturated at a concentration between 5 and 10 mM. Cheng and Matthews (1970) reported higher methionine saturation points in the small intestine of the rat. They found that a

concentration of 10 to 20 mM were needed. Work with ruminants has revealed values similar to those reported for the fowl. Johns and Bergen (1973) reported that concentrations of 5 mM would saturate both the methionine and lysine systems in the small intestine of the sheep. Matthews and Laster (1965) studied the uptake of five neutral amino acids by the small intestine of the rat as a function of concentration. Valine absorption peaked at a concentration of 15 to 20 mM. The shorter the chain length of the amino acid the higher the concentration required to reach the saturation point, because the affinity for the system decreases with chain length (Fleshler and Coligado, 1967; Nathans et al., 1960). From the data observed in this experiment it appears that the concentration of threonine, valine and methionine must be increased above 8.4, 7.6 and 7.4 mM, respectively, to saturate the neutral amino acid transport system in the small intestine of the sheep.

Inhibition of Amino Acid Absorption by Other Amino Acids

Inhibition of Threonine Absorption. Inhibitor effects of valine and methionine upon the absorption of threonine by jejunal and ileal tissue are shown in figures 11, 12, 13 and 14. By setting one amino acid at a constant concentration and varying the concentrations of the second, its effects upon threonine absorption can be determined. Although valine and methionine were not significant components of the threonine prediction equation, some trends can be noted.

The effects of valine upon threonine absorption by the jejunum are shown in figure 11. Methionine concentration was held constant at the midpoint and the concentrations of threonine and valine were varied. Each line represents the amounts of threonine which would be absorbed at various concentrations of threonine in the presence of valine, X_2, at the indicated concentrations. Jejunal tissue responded to increases in threonine concentration by increasing the amount of threonine absorbed independent of valine concentration. At low concentrations of threonine, valine became inhibitory of threonine absorption. Negative uptake values were predicted when valine concentrations were increased above 2.2 mM. At higher concentrations of threonine, increasing the valine concentration resulted in increased amounts of threonine absorbed. Valine appears to be inhibitory at low threonine concentrations, but stimulatory when threonine concentrations are above 5 millimolar.

Valine had little if any effect upon threonine absorption by the ileum (figure 12). The inhibitory effects of valine on threonine absorption are evident at low concentrations of threonine, but no trends develop once the threonine concentration has been increased above 3 mM. Valine appears to have more effect upon threonine absorption in the jejunum than the ileum and the response may be inhibitory or stimulatory depending upon the threonine concentration. The lack of competition between valine and threonine in the ileum may be due to the presence of more active transport sites. This would limit the effectiveness of an amino acid inhibitor.

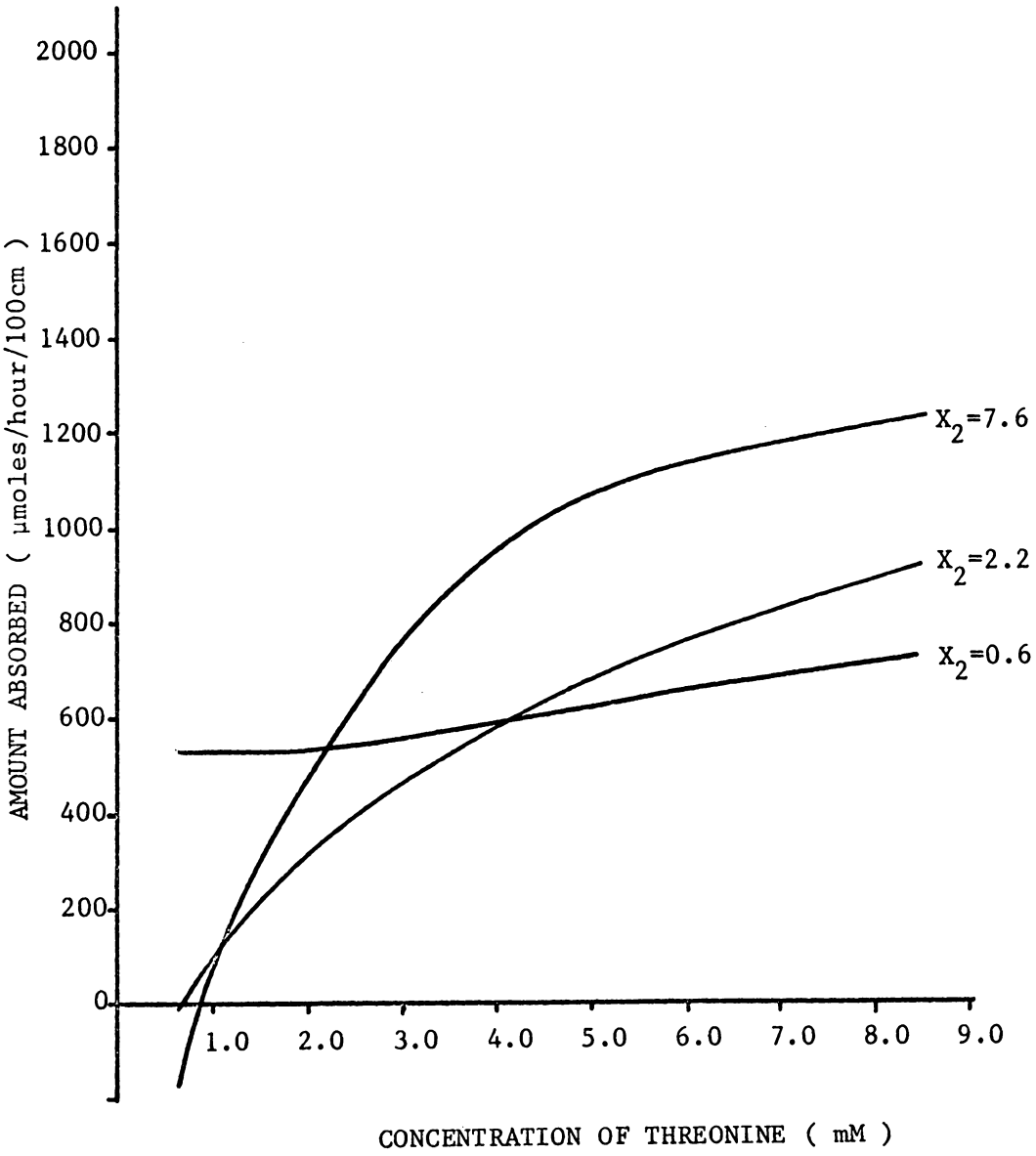

FIGURE 11. ABSORPTION OF THREONINE AT VARIOUS CONCENTRATIONS OF VALINE BY THE JEJUNUM. X_2 = CONCENTRATION OF VALINE (mM).

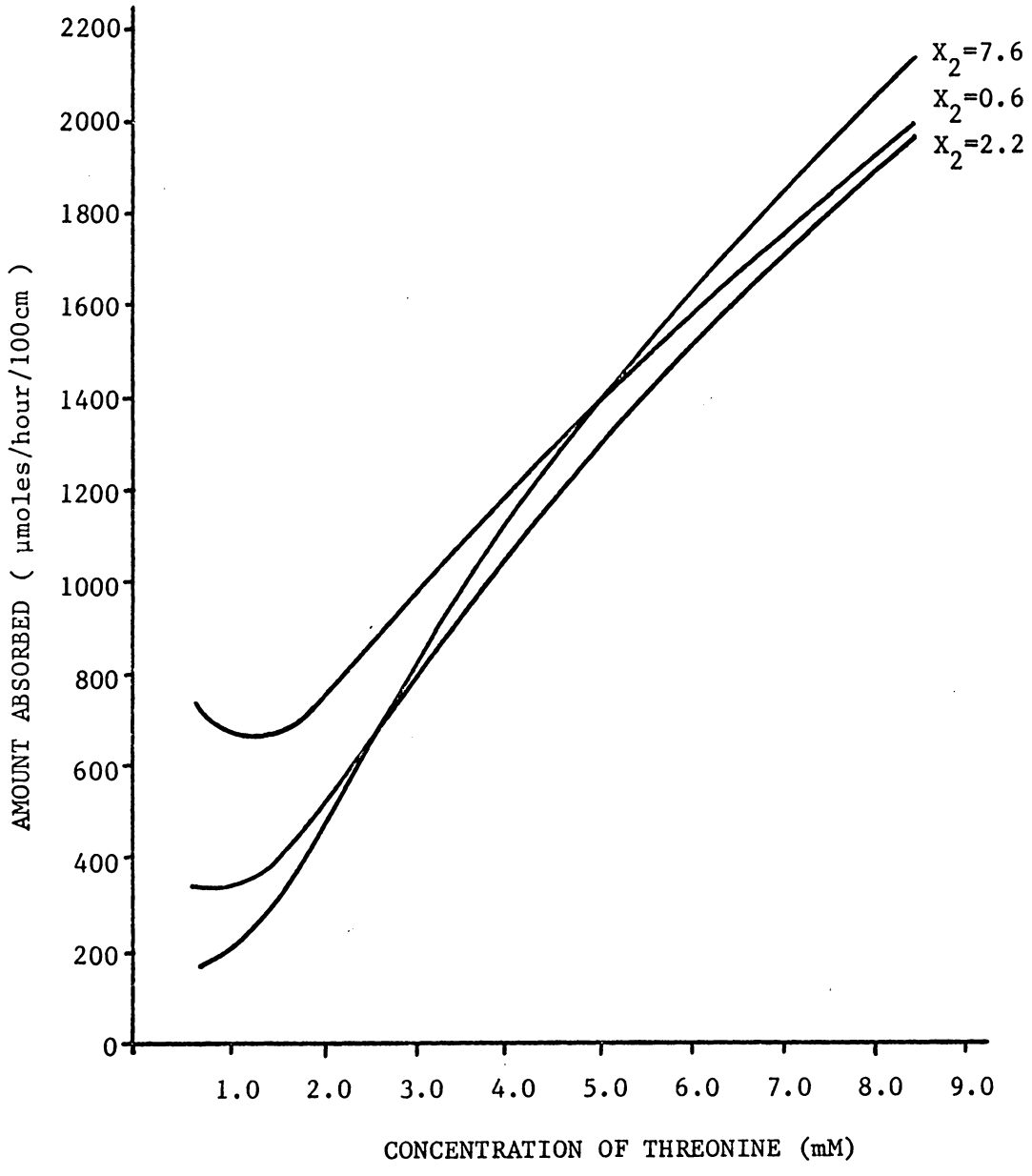

FIGURE 12. ABSORPTION OF THREONINE AT VARIOUS CONCENTRATIONS OF VALINE BY THE ILEUM. X_2= CONCENTRATION OF VALINE (mM).

To determine the interaction between threonine and methionine for transport sites, valine concentration was held constant at the midpoint, 2.2 mM, in the threonine prediction equation. The predicted absorption values for threonine at various concentrations of methionine, X_3, are presented in figure 13 for the jejunal section and figure 14 for the ileal section. At low concentrations of threonine similar amounts of threonine were absorbed at each methionine concentration. As the threonine concentration was increased from 0.6 to 8.4 mM, methionine became progressively more inhibitory and this trend was present at both intestinal sites. Increasing methionine concentration from 0.6 to 7.4 mM in the presence of 8.4 mM of threonine resulted in a decrease of threonine absorption by the jejunal section of 767 umoles and 1773 umoles by the ileal section. Methionine was not a significant component of the regression equation to predict threonine absorption, but at concentrations of threonine above 2 mM there was a trend for methionine to be inhibitory at both intestinal sites.

Kuroda and Gimbel (1954) reported that 57% of the threonine presented to human ileum could be absorbed in 30 min. But threonine has been listed among those amino acids which are absorbed at a slow rate by human ileum (Orten, 1963). These rates are determined by using amino acid mixtures with each amino acid at the same concentration. Increasing the concentration of the mixture from 5.4 mM to 9.0 mM resulted in a 10% increase in threonine absorption, from 40% to 50%. Although this was a small absolute increase,

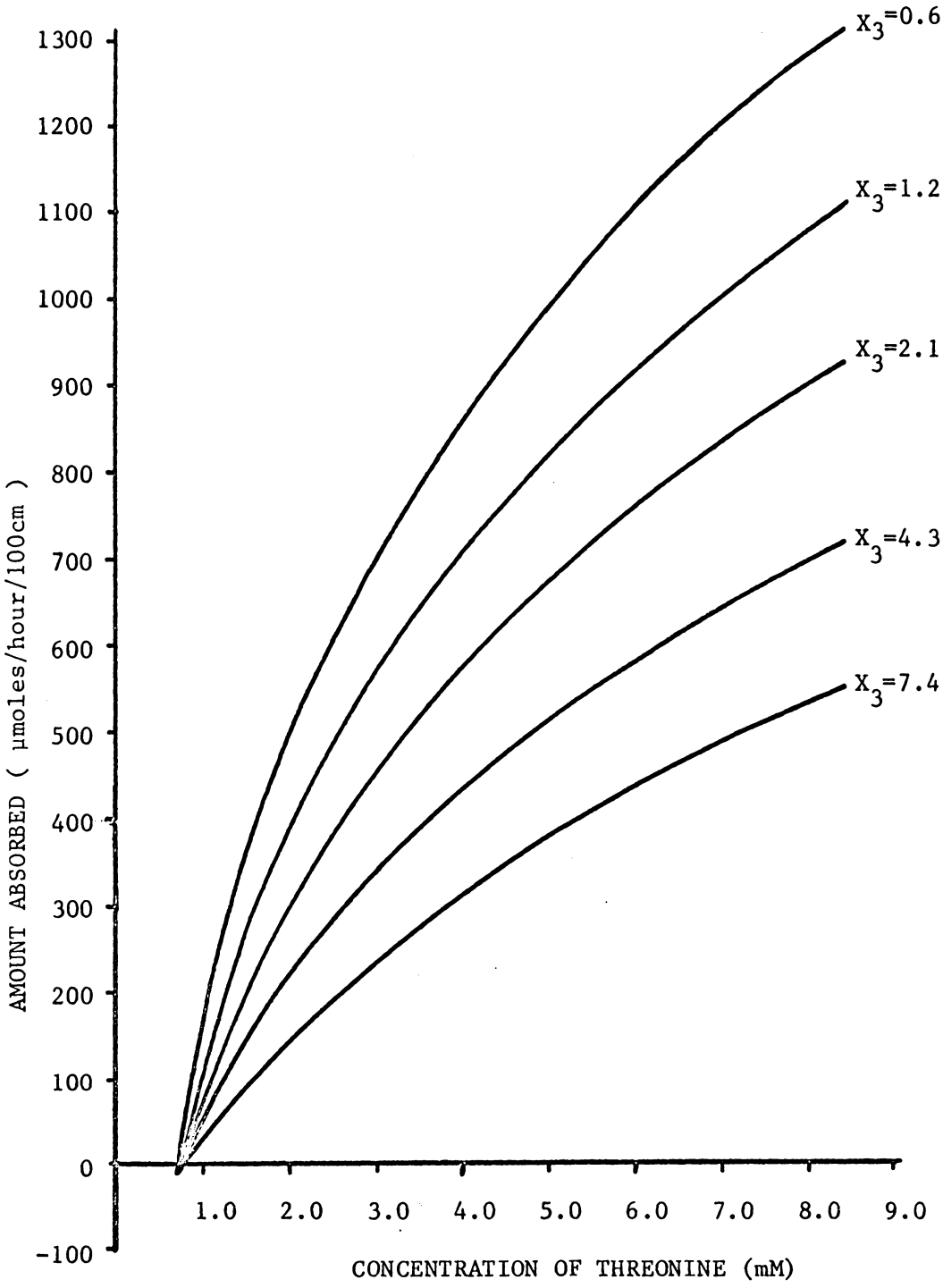

FIGURE 13. ABSORPTION OF THREONINE AT VARIOUS CONCENTRATIONS OF METHIONINE BY THE JEJUNUM. X_3= CONCENTRATION OF METHIONINE (mM).

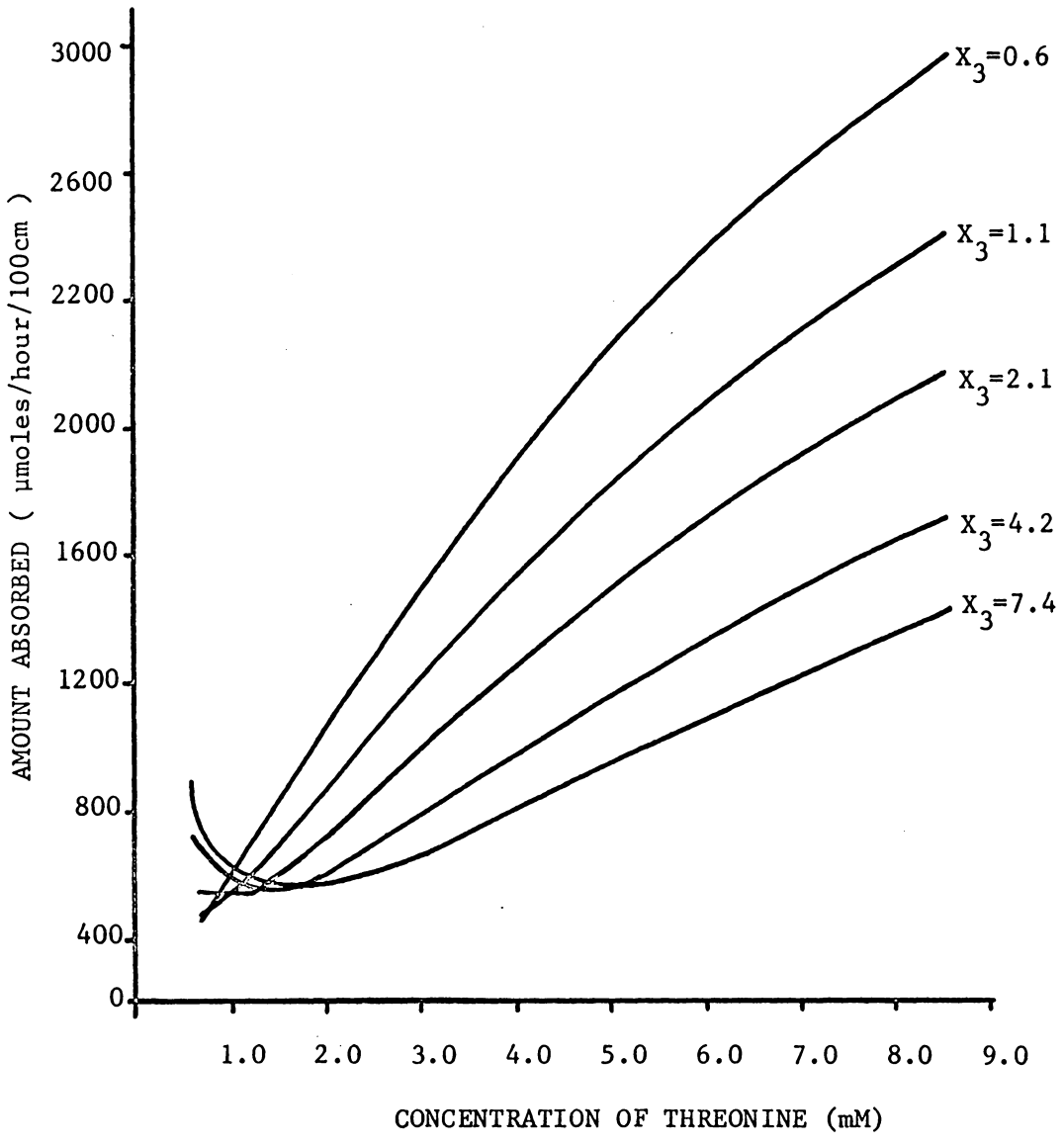

FIGURE 14. ABSORPTION OF THREONINE AT VARIOUS CONCENTRATIONS OF METHIONINE BY THE ILEUM. X_3= CONCENTRATION OF METHIONINE (mM).

it would be larger if expressed as a relative amount. Tasaki and Takahashi (1966) used lower initial concentrations of threonine, 1.25 mM, and reported an absorption rate of 78% by the ileum of the chicken. Threonine was susceptible to inhibition by long chain neutral amino acids, because the longer the chain the more affinity that amino acid has for the neutral amino acid transport system. Leucine has been shown to be inhibitory toward the absorption of threonine as well as other shorter chained amino acids such as glycine and proline. Glycine, proline, and alanine at concentrations of 20 mM were reported to have been inhibitory to threonine absorption, when added to a solution containing 2 mM threonine (Munck, 1966a). Threonine was low in amounts absorbed as well as sensitive to inhibition by longer chained amino acids, such as valine and methionine.

Orten (1963) and Adibi et al. (1967) reported that valine and methionine are absorbed from equal molar amino acid mixtures in greater quantities and at faster rates than threonine by human ileum. Methionine has been observed as a strong inhibitor of the neutral amino acid transport system in the small intestine of monogastrics (Agar et al., 1956; Baker and George, 1971; Reiser and Christiansen, 1965; Tasaki and Takahashi, 1966). Valine can also be inhibitory to neutral amino acid absorption, but it was not as strong as methionine (Hagihira et al., 1960; Kinter and Wilson, 1965; Reiser and Christiansen, 1965). These trends were also observed during this experiment. Valine was inhibitory to threonine

absorption at low concentrations but stimulatory at higher concentrations. Stimulation may be due to rapid uptake of valine and a subsequent exchange of valine from inside the mucosa cell for threonine in the intestinal lumen. Methionine was much more effective as an inhibitor of threonine absorption. The effects of methionine were more prominent than valine and were more consistent across sites.

If the ileum possesses more transport sites than the jejunum, this would explain some of the differences noted between the two sites with regards to inhibition of threonine absorption by valine or methionine. Valine has less affinity for the transport system than methionine. The additional transport sites found in the ileum would reduce the inhibitory effect of valine, but have a smaller effect on the ability of methionine to inhibit threonine absorption.

Inhibition of Valine Absorption. The absorption of valine at various concentrations and how the amounts of valine absorbed were affected by the presence of threonine or methionine are presented in figures 15 and 16. The only significant components of the valine absorption prediction equation by model 3 analysis were effluent volume and the quadratic effect of the valine concentration. Previous analysis revealed no significant interactions as pointed out in the discussion of threonine.

The amounts of valine absorbed by different intestinal sites are not shown, instead zero was entered for the site component to remove the effect of site and an average effluent volume,

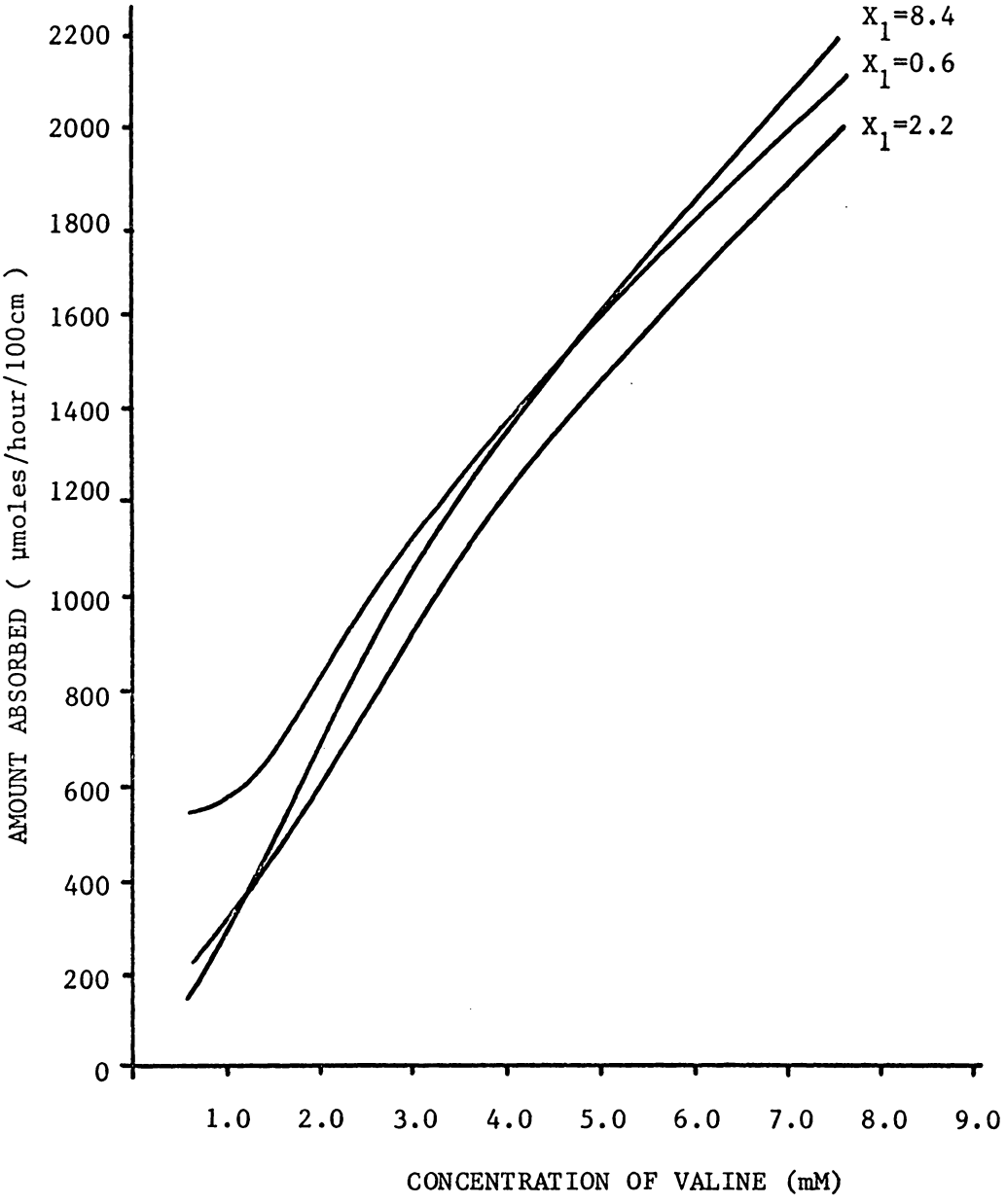

FIGURE 15. ABSORPTION OF VALINE AT VARIOUS CONCENTRATIONS OF THREONINE. X_1= CONCENTRATION OF THREONINE (mM).

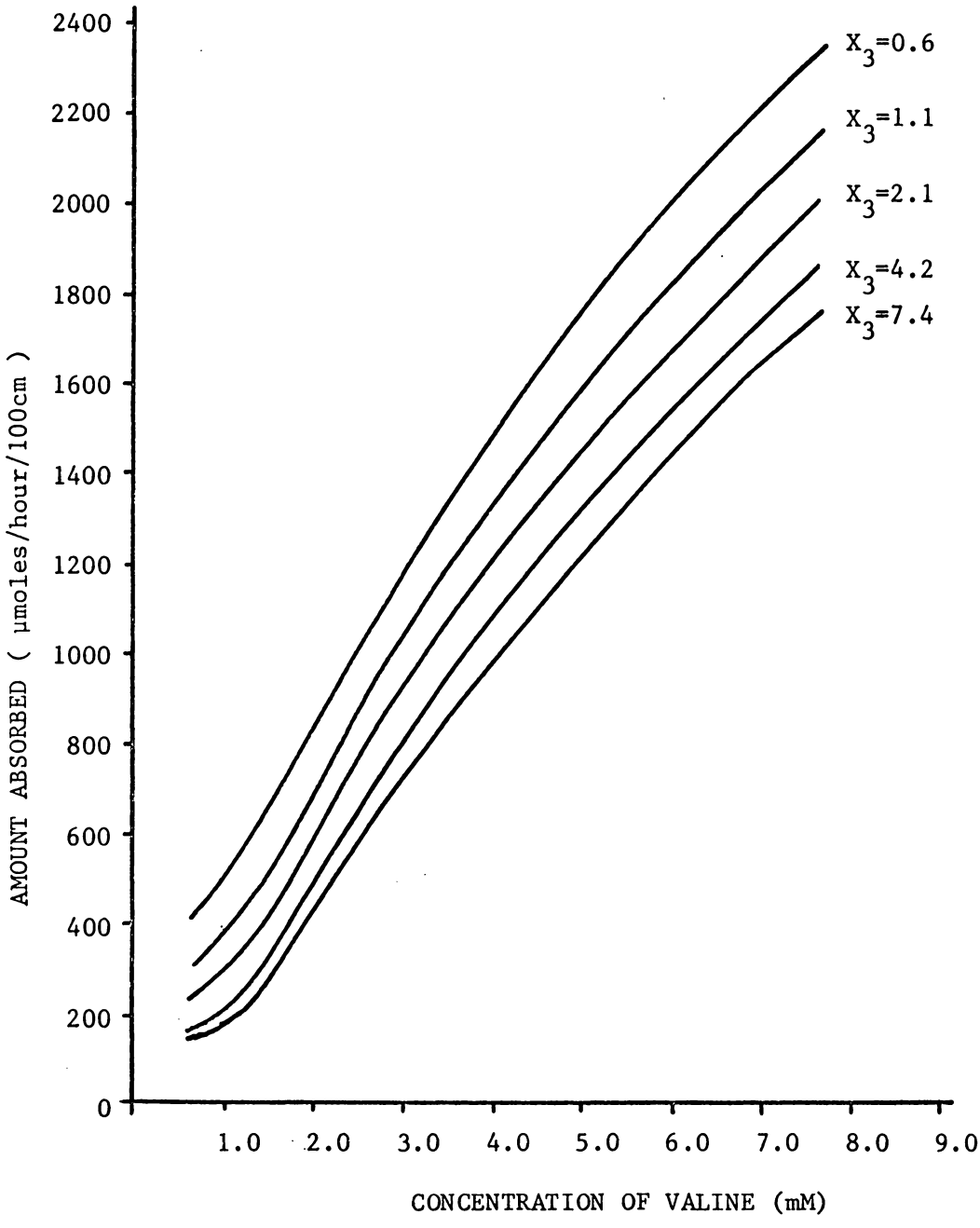

FIGURE 16. ABSORPTION OF VALINE AT VARIOUS CONCENTRATIONS OF METHIONINE. X_3= CONCENTRATION OF METHIONINE (mM).

653 ml, was used for the covariant. Sites were not different ($P < .12$) by model 3 analysis. Absorption patterns of valine are identical whether a numerical factor or zero was entered for the site component. Only the intercept would be different in the prediction equations derived for the absorption of valine by the jejunum, the ileum or no site. Jejunal absorption of valine would lie 183 umoles below the curves presented in the two figures and ileal absorption would lie 153 umoles above each curve. This would result in a margin of 336 umoles between the lines for the two sites.

Threonine had little effect on the absorption of valine at various concentrations of each amino acid (figure 15). Methionine concentration was held constant at 2.1 mM while the concentration of threonine was increased from 0.6 to 8.4 millimolar. At the lowest concentration of valine, a slight pattern did appear. As the concentration of threonine was increased, the absorption of valine decreased. At higher concentrations of valine, threonine concentrations above 2.2 mM showed a stimulatory trend.

Methionine exerted more influence upon the amount of valine absorbed at different concentrations of each amino acid (figure 16). Although somewhat less at lower concentrations of valine, methionine was inhibitory toward valine absorption because each increase in methionine concentration resulted in a decrease in the amount of valine absorbed. Methionine appears to have more inhibitory effects on the absorption of valine than threonine, although neither were statistically significant ($P > .05$).

Aroskar and Berg (1962) measured the uptake of valine and methionine by the rat using the Cori technique. Valine was absorbed at a rate equal to or slightly higher than methionine. This was similar to results found in this study. Valine absorption, depending upon concentration, ranged from 165 umoles per hr to 1947 umoles per hr, while the absorption of methionine ranged from 250 umoles per hr to 2072 umoles per hour. Yamada et al. (1967) used jejunal loops of the small intestine of the rat to measure valine absorption. They reported 68 to 79% of the valine presented to the intestine was absorbed. A similar rate of valine absorption from an equal molar amino acid mixture was reported by Orten (1963) by a human, ileal, blind loop. Although the concentration of each amino acid in the mixture was increased from 5.4 to 9 mM, 80% of the valine was absorbed over a 90 min period at each concentration. Valine absorption in the present study was not as high. Absorption as a percentage of the amount perfused was 65% at 0.6 mM and 44% at 7.6 mM. Orten (1963) also observed that valine was absorbed at a faster rate than threonine, but slower than methionine. Methionine has been classified as very inhibitory toward valine absorption while threonine was considered noninhibitory (Reiser and Christiansen, 1965). Hagihira et al. (1960) found that methionine could decrease the uptake of valine by as much as 50%. They also reported that the presence of leucine and isoleucine could reduce valine absorption to 72% of normal values. Kinter and Wilson (1965)

studied the site of valine inhibition by methionine in the small intestine of the hamster. Methionine inhibited both transepithelial movement and columnar cell accumulation of valine. The site of action was determined to be the brush border of the columnar cells.

Inhibition of Methionine Absorption. Absorption of methionine by different intestinal sites was different ($P < .10$), but the interactions between site and treatment was not significant ($P < .12$). Thus, no site effect was entered into the prediction equation to construct the graphs shown in figures 17 and 18. Absorption patterns of the two intestinal sites would be identical to those presented in figures 17 and 18, as discussed with regards to valine absorption. The differents in amounts of methionine absorbed by the two intestinal sites would be 310 µmoles. The jejunal section would absorb 169 µmoles less than the amounts shown in the two figures and the ileal section would absorb 141 µmoles more.

Threonine (figure 17) had more effect upon methionine absorption than valine (figure 18). At the higher concentrations of methionine, threonine exhibited an inhibitory effect upon absorption, while valine had very little effect.

Methionine was considered to have a strong affility for the neutral amino acid transport system in the small intestine of monogastrics (Aroskar and Berg, 1962; Orten, 1963; Tasaki and Takahashi, 1966). Glycine has been reported to be inhibitory of methionine absorption, when the concentration of glycine was greater

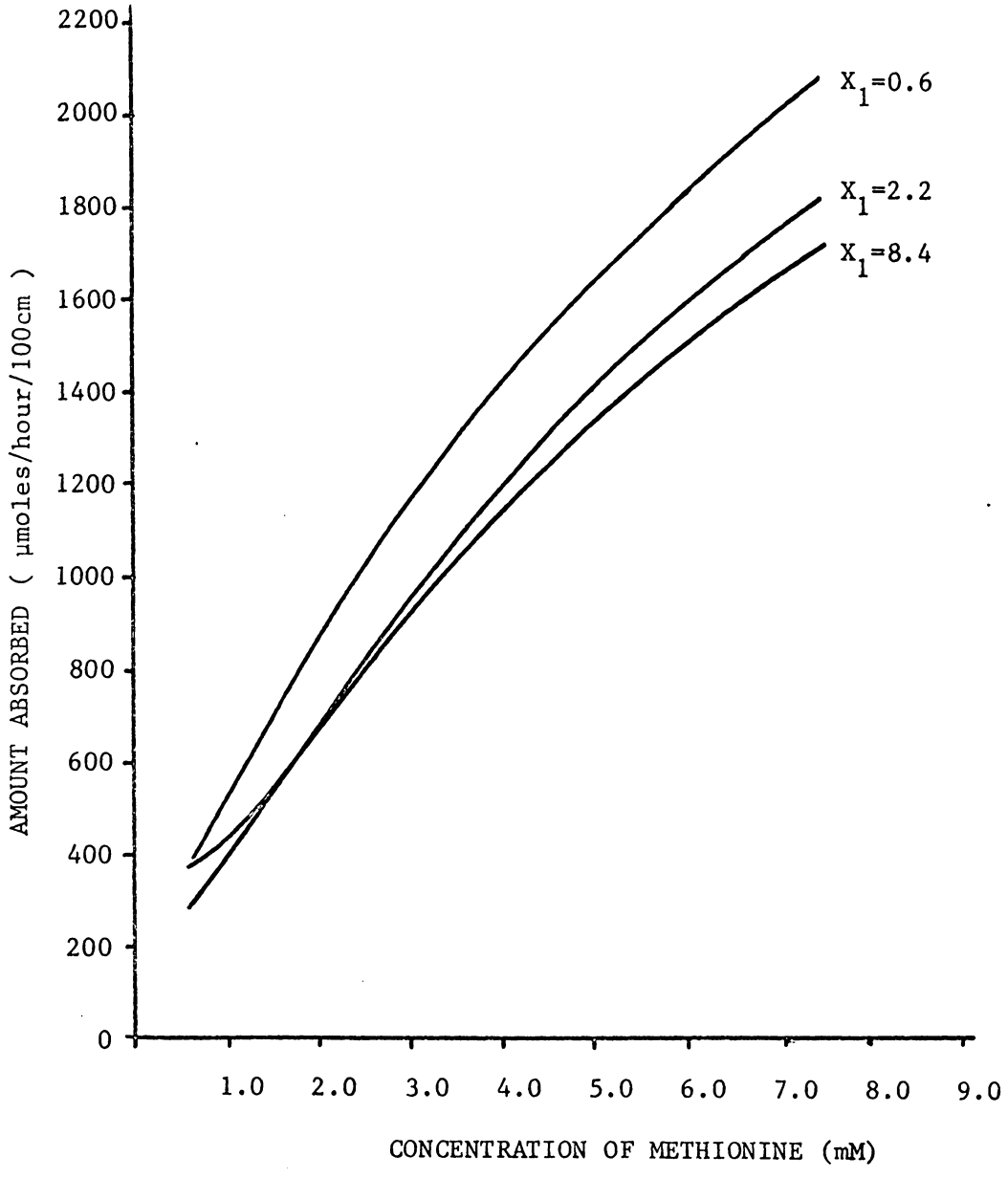

FIGURE 17. ABSORPTION OF METHIONINE AT VARIOUS CONCENTRATIONS OF THREONINE. X_1= CONCENTRATION OF THREONINE (mM).

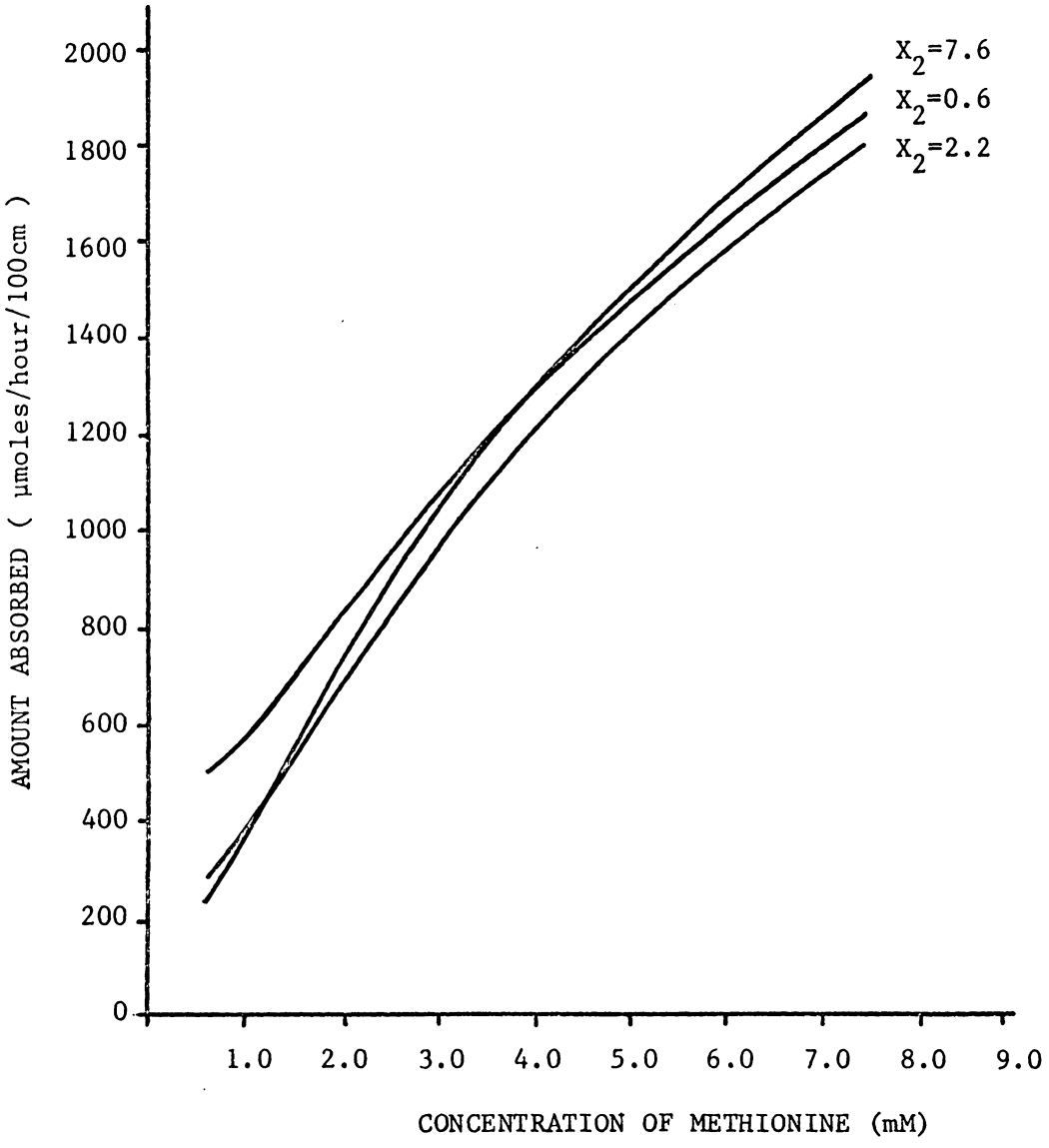

FIGURE 18. ABSORPTION OF METHIONINE AT VARIOUS CONCENTRATIONS OF VALINE. X_2= CONCENTRATION OF VALINE (mM).

than the methionine concentration. Leucine can inhibit threonine absorption in mixtures containing equal concentrations of each (Munck, 1966a) and methionine can inhibit the absorption of leucine (Tasaki and Takahashi, 1966). Thus, methionine possesses more affinity for the transport system than threonine.

Paine, et al. (1959) used permanent Thiry-Vella loops located in the middle of the intestine to study the uptake of methionine and histidine individually and together. Absorption of methionine alone was 41±13.2 μmoles per 15 minutes. Histidine absorption when perfused alone was 32.2±2.8 μmoles per 15 minutes. When both amino acids were perfused at the same time, the absorption of histidine declined to 23.4±5.3 μmoles per minute while methionine absorption measured 25.6±2.9 moles per minute. The absorption of methionine by the intestine was not affected by the presence of histidine due to the large variability associated with methionine absorption. The effects upon histidine were more pronounced than the effects of histidine on methionine absorption. Methionine cannot possess an affinity high enough to be unaffected by the presence of other amino acids, but their presence was not as detrimental upon methionine absorption as the inhibition exhibited by methionine.

Annegers (1969) used Thiry-Vella loops to determine the effect of glycine, alanine, lysine and glutamic acid on the absorption of methionine. Glycine was a highly significant component of the regression equation derived to predict the amounts of methionine absorbed. Glycine exerted a negative effect on methionine

absorption because the regression coefficient was -.012. They also determined that methionine was a highly significant component of the glycine prediction equation, but the inhibitory effect of methionine on glycine absorption was much larger than the inhibition of methionine absorption by glycine. Observations from the present experiement followed this pattern. Threonine absorption was reduced by an average of 190 umoles in the jejunum and 443 umoles in the ileum, each time the methionine concentration was doubled. But similiar increases in threonine concentration resulted in an average decrease of only 88 umoles in the absorption of methionine. Thus, the absorption of methionine was affected by the addition of threonine, but methionine was much more inhibitory on threonine absorption.

Absorption of Amino Acids from Amino Acid Mixtures

Regression equations derived to predict the amounts of each amino acid absorbed were used to determine how much amino acid would be absorbed when the concentration of each of the three amino acids studied were varied from 0.6 to 7.4 millimolar. In order to show how the absorption of one amino acid was affected by the presence of two other amino acids, a three dimensional figure, a cube,was employed. All edges of the cube are of equal length, thus the integrations of concentration range must be equal among amino acids. The concentration ranges used in this experimant began at 0.6 mM but ended at 8.4, 7.6 and 7.4 mM of threonine,

valine and methionine, respectively. A concentration range of 0.6 to 7.4 mM was established as the length of each edge of the cube, since this range was common to each of the three amino acids.

Each cube was composed of three sets of parallel lines which were perpendicular to form the six surfaces. Each set of lines would represent the concentration range of one amino acid. Thus, each surface would be formed by two amino acids at varying concentrations, 0.6 to 7.4 mM, while the third amino acid was held constant at 0.6 or 7.4 mM. Since the concentration of one amino acid was known, this would reduce the original prediction equation to two unknown X values. These unknown values would be the initial concentrations of the amino acids which form that surface. By setting one of the remaining amino acids at various concentrations within the 0.6 to 7.4 mM range, the prediction equation would simplify to a quadratic equation. Contour lines representing umoles of amino acid absorbed per hr per 100 cm of intestine could be determined for each surface by setting the predict Y at the desired response value and solving the quadratic equation. These contour lines would yield the concentrations of the two amino acids used to form the surface, which would result in absorption equal to the value of the contour line when the third amino acid was held constant at 0.6 or 7.4 mM. A common contour line on perpendicular surfaces would yield a plane through the cube. All points which laid within the plane would represent the concentration combinations of the three amino acids which would result in an absorption rate

equal to the contour line value.

Absorption of Threonine. Three dimensional figures representing the absorption of threonine and how the amounts absorbed are affected by various concentrations of threonine, valine and methionine are shown in figures 19 and 20. Absorption by the jejunal and ileal sections are shown separately due to significant ($P < .05$) interaction between site and treatment.

Three absorption planes equal to 250, 500 or 1000 umoles of threonine absorbed per hr per 100 cm of the jejunal section are shown in figure 19. Increasing the concentration of threonine resulted in more threonine being absorbed. At low concentrations of valine and methionine, valine was inhibitory to the absorption of threonine. As the concentration of valine was increased, the inhibition it formerly exerted diminished and changed into a stimulatory effect. When the concentration of methionine was 7.4 mM, valine did not inhibit the absorption of threonine, but was stimulatory at all concentrations.

The bottom and top surfaces of the cube represent the effects of methionine concentration on the absorption of threonine when valine was at a low concentration of 0.6 mM or a high concentration of 7.4 mM. Methionine proved to be inhibitory at low valine concentrations and stimulatory at high concentrations of valine. The stimulation responses, noted on the top surfaces, were more evident in the 250 and 500 umoles contour lines, but not present when absorption reached 1000 umoles. When the concentration of

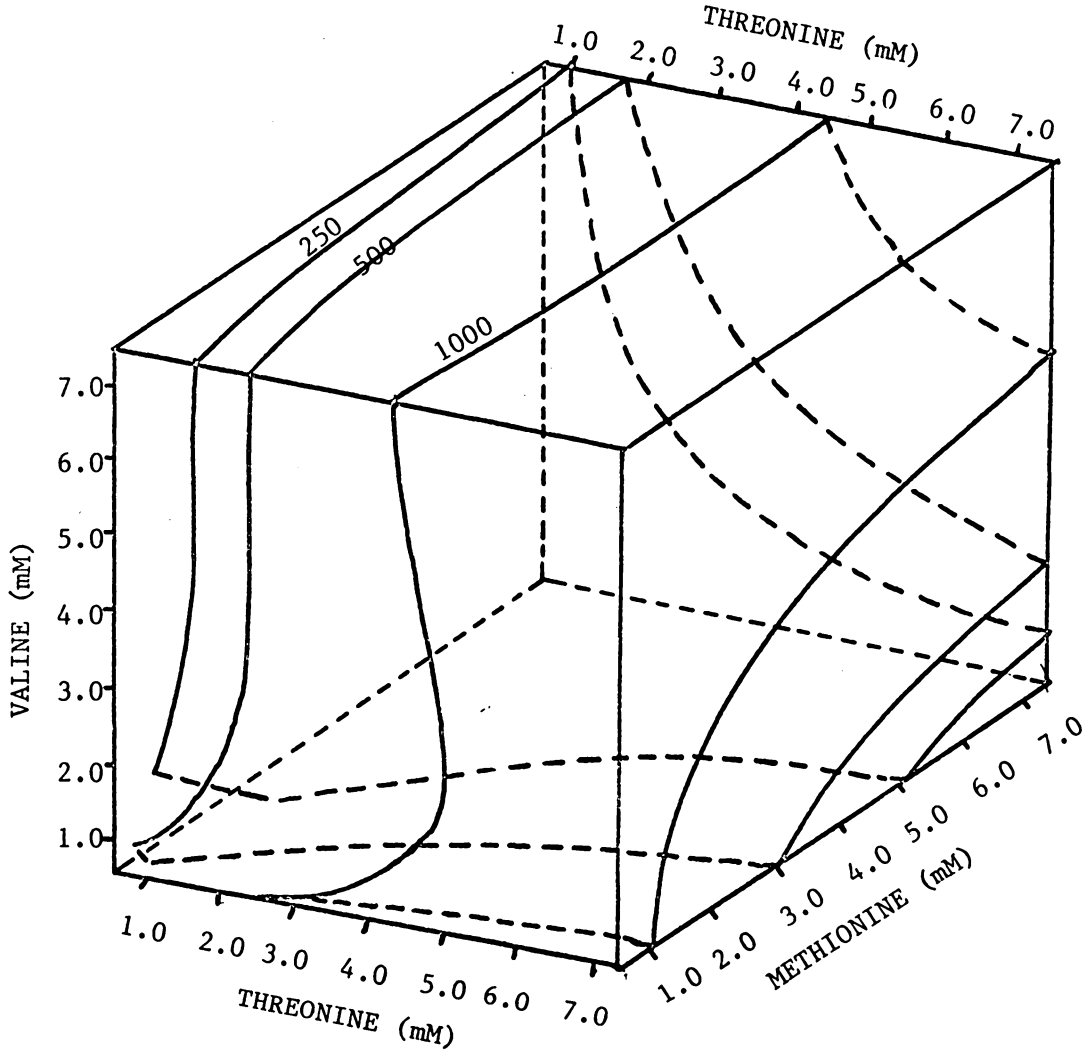

FIGURE 19. ABSORPTION OF THREONINE FROM AMINO ACID MIXTURES BY THE JEJUNUM.

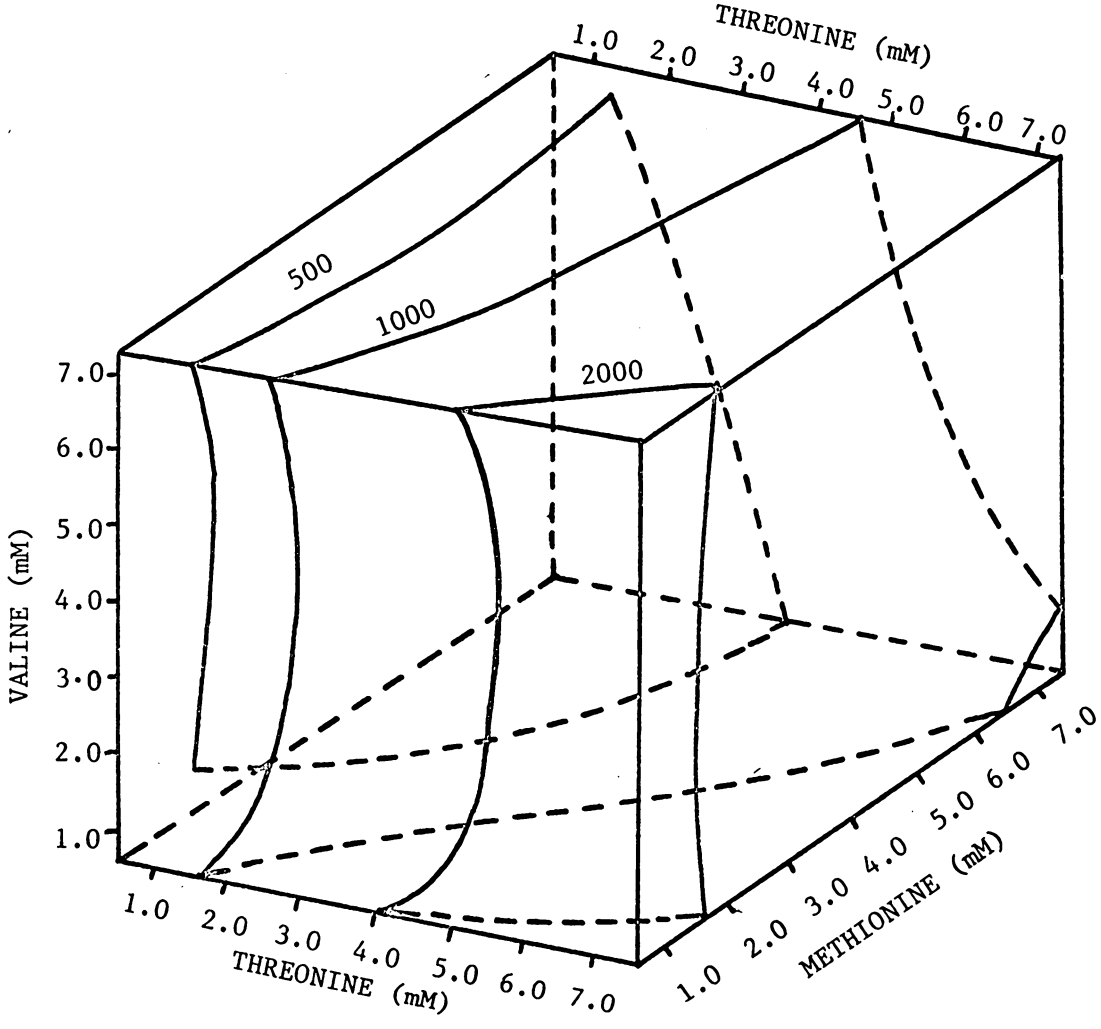

FIGURE 20. ABSORPTION OF THREONINE FROM AMINO ACID MIXTURES BY THE ILEUM.

valine was 7.4 mM and the methionine concentration was 0.6 mM, 250 and 500 umoles of threonine would be absorbed when the threonine concentrations were 1.7 and 2.35 mM, respectively. As the methionine concentration was increased across the top surface smaller concentrations of threonine were needed to maintain an absorption rate of 250 and 500 umoles per hr per 100 cm. When the methionine concentration reached 7.4 mM, a threonine concentration of only 1.0 mM was needed to achieve an absorption rate of 250 umoles per hr per 100 cm and 1.7 mM would result in 500 umoles of threonine being absorbed. The 1000 umole contour line changed very little when the concentration of methionine was increased from 0.6 to 7.4 mM. The amount of threonine absorbed by the jejunum was decreased when methionine was present in increasing amounts, but this inhibition can be overcome by increasing the amount of valine present in the solution.

Threonine absorption by the ileum was similar to that by the jejunum but the amounts absorbed were larger (figure 20). The presence of valine at low concentrations, below 2 mM, was inhibitory to threonine absorption, but once valine concentrations had been raised above 6 mM, stimulation of threonine absorption was noted. The stimulatory effects of valine on threonine absorption were more prominent when methionine concentration was increased to 7.4 mM, although the amounts absorbed were less. Unlike the jejunum, the stimulatory effects of the presence of valine did not override the inhibitory effects of methionine. Methionine

inhibited threonine absorption at all concentrations of valine. The stimulatory effects of valine were present in the ileal section as well as the jejunal, but the stimulation of threonine absorption by valine did not cancel out the inhibitory effects of methionine in the ileal section.

Methionine was inhibitory to threonine absorption by both intestinal sections, but the inhibition was more prominent in the jejunal section than the ileal when valine was held at a low concentration. Methionine possesses the ability to decrease threonine absorption because the greater affinity it possess for the transport system than threonine (Reiser and Christiansen, 1965). As the concentration of methionine was increased, more of the active transport sites were being occupied by methionine. This would decrease the number of active transport sites available for threonine absorption.

Neutral amino acids have been shown to possess the ability to stimulate or increase the amounts of basic amino acids (Reiser and Christiansen, 1971). The rapid accumulation of the neutral amino acids by the epithelial cells resulted in a very high concentration gradient between the intracellular and extracellular neutral amino acids. The gradient, which develops in 1 to 2 min, would provide the driving force to propel the carrier of the basic amino acids to move these amino acids into the cell. The neutral amino acid would complex with the carrier intracellularly, move to the outside of the cellular membrane and dissociate. The

basic amino acid could complex with its carrier and be transported into the cell. This would increase the net absorption of the basic amino acid and decrease the net absorption of the neutral amino acid, because the intracellular neutral amino acid was being exchanged for the extracellular basic amino acid. Not all neutral amino acids could function in this manner. Leucine, methionine, phenylalanine and alanine were found to be stimulatory toward the absorption of lysine, while valine, isoleucine and histidine were not. Reiser and Christiansen (1971) felt that these later amino acids could not participate in the exchange system for basic amino acids. Cell accumulation of 1 mM valine or histidine was higher than 1 mM methionine; thus, the power to drive the exchange system was present, but these amino acids must have a poor affinity for the basic amino acid transport system.

Threonine, valine and methionine are neutral amino acids which use the same transport system. Rapid accumulation of either valine or methionine intracellularly could result in the initiation of an exchange system to increase the absorption of threonine at the expense of intracellular valine and methionine. Previous reports have established that methionine and valine are absorbed at a much faster rate than threonine (Adibi et al., 1967; Orten, 1963; Tasaki and Takahashi, 1966), but cell accumulation would also depend upon how fast these amino acids are removed from the cell by the circulatory system. Higher initial concentrations of valine and methionine would result in more of each amino acid

being absorbed, but this does not mean that the intracellular to extracellular concentration gradient would increase for each amino acid. Valine may be the only amino acid capable of participating in the exchange system with threonine or the intracellular methionine concentration may be kept low by rapid removal by the circulatory system. Thus, valine would exert more stimulatory effects on threonine absorption than methionine.

Phillips et al. (1976) studied the absorption, tissue accumulation and release of amino acids by the small intestine of sheep. The ileum absorbed and released significantly larger amounts of amino acids than the jejunum. Tissue accumulation was also significantly larger in the ileum than the jejunum, but recovery of amino acids from the tissue was low. Only 19% of the umoles calculated to be present in the jejunum were recovered, while 40% of the amino acids in the ileum were recovered. Thus, the ileum can absorb large amounts of valine and methionine, but the concentration gradient needed to drive the threonine absorption system did not develop to as large an extent in the ileum as the jejunum, due to the rapid movement of the amino acids through the tissue to the circulatory system. This would explain the more prominent stimulatory effects of valine when present in the jejunal section.

Absorption of Valine. The amounts of valine absorbed at various concentrations of valine, threonine and methionine are shown in figure 21. Three contour lines corresponding to 500,

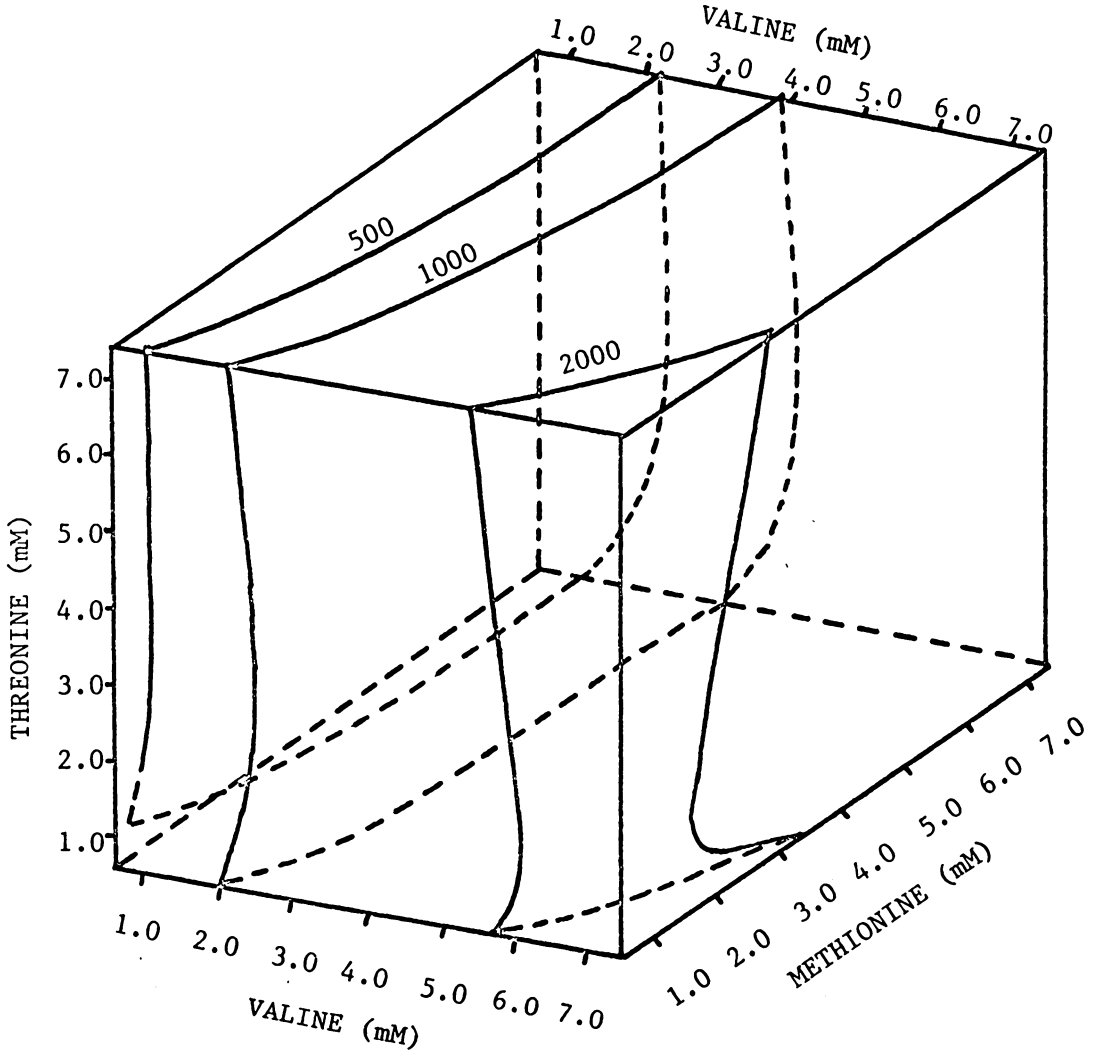

FIGURE 21. ABSORPTION OF VALINE FROM AMINO ACID MIXTURES.

1000 or 2000 umoles of valine absorbed per hr per 100 cm were constructed. Absorption of valine was similar between the two intestinal sites studied, therefore, no site component was entered into the prediction equation.

Threonine had very little if any effect upon valine absorption, but methionine was inhibitory toward valine absorption. Threonine appeared to inhibit valine absorption when present in low concentrations but this effect was diminished as the concentration of threonine was increased above 1 millimolar. The effects of threonine on the amounts of valine absorbed were the same at either low or high concentrations of methionine. The presence of methionine in the perfusion solution resulted in a decrease in the amounts of valine absorbed. As the concentration of methionine was increased, larger concentrations of valine were required to maintain an absorption rate of 500, 1000 or 2000 umoles per hr per 100 cm of intestine. Valine absorption dropped below 2000 umoles when the methionine concentration was above 3 millimolar. Increasing the concentration of threonine did not alter the inhibitory patterns of methionine, except at very low concentrations of threonine. The three planes shown in figure 21 have a similar structure. Each crosses the cube diagonally and folds or bends at the bottom of the cube. The diagonal pattern indicates the inhibitory effect of methionine. The bend at the bottom of the cube shows the inhibitory effects of threonine when present in low

concentrations. As the concentration of threonine was increased from 0.6 to 1.0 mM the concentration of valine required to maintain an absorption response of 500, 1000, or 2000 increased. The right front surface of the cube shown in figure 21 shows the combinations of threonine and methionine which corresponds to 2000 umoles of valine being absorbed when the valine concentration was held constant at 7.4 millimolar. As the concentration of threonine was increased the amount of methionine needed to limit absorption of valine to 2000 umoles decreased. Threonine continued to be able to replace methionine as an inhibitor of valine absorption up to 2 millimolar. At that point threonine lost its inhibitory effect.

Methionine has been shown to be very inhibitory of valine absorption while threonine was considered to be non-inhibitory (Reiser and Christiansen, 1965). Kinter and Wilson (1965) reported that methionine inhibited both transepithelial movement and columnar cell accumulation of valine and the site of this action was near the brush border. The inhibitory effects of methionine were evident in the present study. The inhibition of valine absorption was probably mediated at the active transport site because the inhibition increased with increases in methionine concentration.

It is unlikely that threonine actually inhibited the absorption of valine, but instead increased the efflux of valine from the epithelial cells. This would in turn decrease the net absorption

of valine. The increase in the efflux would be the result of valine being swapped for threonine. As the concentration of threonine was increased above 2 mM, threonine could compete more efficiently at the brush border for absorption and become less dependent on intracellular valine concentration for absorption. As this occurred, the efflux of valine would drop and net absorption would increase.

Absorption of Methionine. The effects of concentration of each amino acid and amino acids present in the solution on the absorption of methionine are shown in figure 22. The effects of site and effluent volume were removed by entering a zero for all site components and an overall effluent volume average.

Threonine did inhibit methionine absorption at low concentrations of threonine and valine, but the threonine inhibition noted at low concentrations of valine diminished when the concentration of valine was increased to 7.4 millimolar. Valine was inhibitory toward methionine absorption when the threonine concentration was low, 0.6 mM, but the inhibition changed to stimulation when threonine was held constant at a concentration of 7.4 millimolar. It was apparant that the function of valine with regards to the absorption of methionine was dependent upon the concentration of threonine. The reduction in the effectiveness of threonine as an inhibitor of methionine absorption decreased when the valine concentration was increased. Thus, the stimulatory effects of valine can override the inhibition of methionine

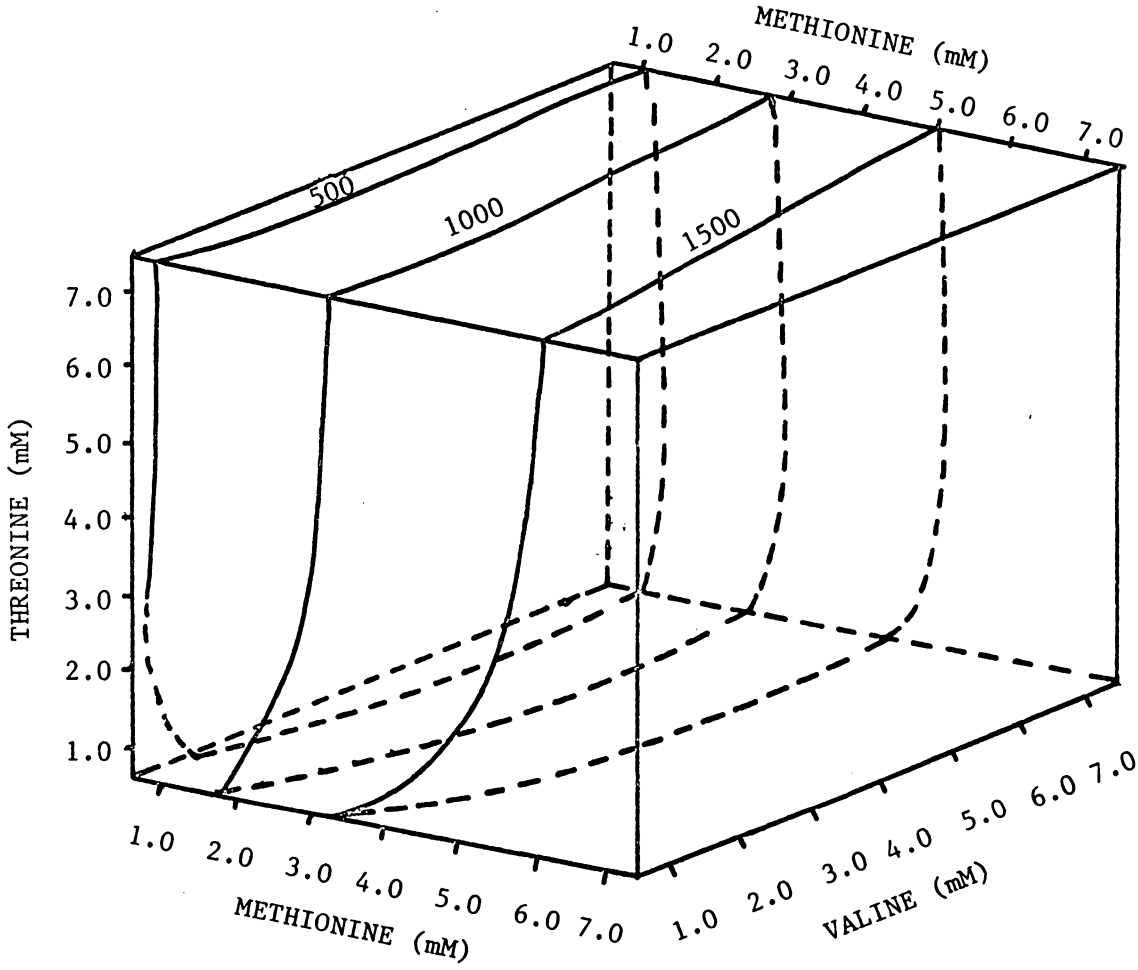

FIGURE 22. ABSORPTION OF METHIONINE FROM AMINO ACID MIXTURES.

absorption by threonine. Although methionine absorption has priority over other amino acids, due to the affinity it has for the transport system, valine absorption was not completely stopped by the presence of methionine, but it was reduced. At low concentrations of threonine, valine could be constantly fluxing in and out of the brush border because it was involved in the exchange system for the absorption of threonine. This would increase the amount of valine available for extracellular competition for active transport sites with methionine. Once the threonine concentration was increased and the exchange system between valine and threonine decreased, intracellular concentrations of threonine and valine would increase and limit their competitive inhibition of methionine at the brush border.

SUMMARY

Wethers fitted with double re-entrant cannulae located in either the jejunal or ileal section of the small intestine were used to study the absorption characteristics of threonine, valine and methionine. Perfusions of the cannulated intestine were conducted 4 hr after feeding for a 1 hr period. Absorption of amino acids was measured as the total amount of amino acid removed from the perfusion solution during the 1 hr period. Concentrations of each amino acid ranged from 0.6 to 7.4 millimolar. The experiment design, a three dimensional central composite design, required only 15 different concentration combinations of the three amino acids used to determine their absorption characteristics.

Removal of amino acid from the perfusion solution was constant over the 1 hr perfusion period, but absorption did vary with time after feeding at which the wethers were perfused. Four or 8 hr after feeding tended to have higher absorption rates than 2 or 6 hours. Absorption of each amino acid increased with each replication indicating that the cannulae remained healthy and functional. Wethers with cannulae located in the ileum absorbed more ($P < .05$) threonine, valine and methionine than those with jejunal cannulae.

Equations were derived for each amino acid to predict the amount which would be absorbed when the initial concentrations of all three amino acids were known. Site of absorption and effluent volume were also part of the absorption prediction equations. Absorption of threonine and valine was affected ($P < .10$) by the quadratic component of their own concentrations. The linear and quadratic components of methionine concentration exerted a positive influence ($P < .05$) on methionine absorption. Site of absorption was not an important component of the prediction equation with the exception of methionine. Effluent volume was a significant ($P < .01$) component of all three absorption prediction equations. The amounts of each amino acid absorbed was dependent upon the initial concentration of that amino acid. As the initial concentration was increased, the amount absorbed increased, but no saturation points were noted.

Absorption, as observed in this experiment, was subject to inhibition and stimulation by the other amino acids present in the perfusion solution. Valine was inhibitory of threonine absorption when present in low concentrations, but increasing the concentration of valine resulted in a stimulatory effect on threonine absorption. Methionine inhibited the absorption of threonine when valine was present at concentrations below 3 mM, but the stimulation of threonine absorption by high concentrations of valine was enough to override the inhibitory effects of methionine.

The amount of valine absorbed was decreased by increasing the methionine concentration. Low concentrations of threonine decreased the amount of valine absorbed while high concentrations had a stimulatory effect at high concentrations of valine and low concentrations of methionine. Valine exerted inhibitory effects on methionine absorption when threonine was present in low concentrations and threonine was also inhibitory if valine was present at low concentrations. When the concentration of threonine was increased to 7.4 mM, each increase in valine concentration resulted in more methionine being absorbed.

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APPENDIX

TABLE 12. COMPOSITION OF THE SURGICAL INTRAVENOUS INFUSION SOLUTION[a]

Item	g/liter
Dextrose	50.00
Sodium lactate	2.60
Potassium chloride	1.30
Magnesium chloride ($6H_2O$)	0.31
Potassium phosphate (dibasic)	0.26
Sodium chloride	0.12

[a]Adjusted to a pH of 7.4.

TABLE 13. COMPOSITION OF PERFUSION BUFFER[a]

Item	g/liter	Molar concentration
NaCl	6.92	0.1180
KCl	0.35	0.0050
$CaCl_2 \cdot 2H_2O$	0.37	0.0025
KH_2PO_4	0.16	0.0012
$MgSO_4 \cdot 7H_2O$	0.29	0.0012
$NaHCO_3$	2.09	0.0249

[a]Adjusted to pH 7.4.

TABLE 14. EXAMPLE OF LEAST SQUARES ANALYSIS--MODEL 1

Source	Degrees of freedom	Sum of squares	Mean squares	F
Total	131	49050581		
Regression	60	41399710	689995	6.40**
replication	2			4.20*
site	1			3.93
treatment	14			13.49**
site x treat	14			1.96*
rep x treat	28			2.31**
XV	1			41.78**
Error	71	7650871	107758	

*P < .05

**P < .01

TABLE 15. EXAMPLE OF LEAST SQUARES ANALYSIS--MODEL 2

Source	Degrees of freedom	Sum of squares	Mean squares	F
Total	131	49050581		
Regression	12	24058616	2004884	9.55**
replication	2			4.45*
site	1			13.67**
LX1	1			1.11
LX2	1			4.81*
LX3	1			1.69
LX1SQ	1			2.19
LX2SQ	1			0.77
LX3SQ	1			0.18
LX1X2	1			2.04
LX1X3	1			2.52
LX2X3	1			2.51
Error	119	24991964	210016	

*P < .05

**P < .01

TABLE 16. EXAMPLE OF LEAST SQUARES ANALYSIS--MODEL 3

Source	Degrees of freedom	Sum of squares	Mean squares	F
Total	131	49050581		
Regression	13	30001293	2307791	14.29**
replication	2			2.85
site	1			1.79
LX1	1			1.08
LX2	1			5.74*
LX3	1			1.54
LX1SQ	1			3.53*
LX2SQ	1			1.04
LX3SQ	1			0.16
LX1X2	1			2.63
LX1X3	1			3.74
LX2X3	1			2.00
XV	1			36.81**
Error	118	19049287	161434	

*P < .05

**P < .01

TABLE 17. VOLUME OF EFFLUENT COLLECTED DURING PERFUSION (REPLICATION I)

	Animal number			
	Jejunum		Ileum	
Treatment	1	2	3	4
1	1030	590	530	580
2	880	605	550	635
3	620	360	460	580
4	1030	510	485	610
5	1130	410	555	570
6	840	545	495	590
7	850	400	605	555
8	975	245	440	505
9	1020	315	610	600
10	1035	335	480	585
11	715	555	565	595
12	995	570	490	590
13	945	605	610	565
14	805	575	640	530
15	895	580	505	595
$\bar{x}$	918	480	535	579

TABLE 18. VOLUME OF EFFLUENT COLLECTED DURING PERFUSION (REPLICATION II AND III)

	Animal number				
	Jejunum			Ileum	
	1	2		3	
Replication	II	II	III	II	III
Treatment					
1	1160	610	350	520	575
2	1195	670	605	550	550
3	1245	710	880	515	550
4	980	655	395	525	435
5	1260	380	445	515	540
6	1180	560	380	585	495
7	940	540	-	525	575
8	1165	360	520	575	440
9	1130	670	600	560	490
10	1090	680	820	500	540
11	1120	515	-	375	555
12	1105	560	-	420	510
13	1135	540	630	615	565
14	1170	625	570	540	570
15	1050	620	930	580	525
$\bar{x}$	1128	580	594	526	529

TABLE 19. AMOUNTS OF AMINO ACID ABSORBED FROM PERFUSED MIXTURES WITH DIFFERENT CONCENTRATIONS

Treatment	Amino acid					
	Threonine		Valine		Methionine	
	Perfusate[a]	Absorbed[b]	Perfusate	Absorbed	Perfusate	Absorbed
1	1.2	393.0	1.1	441.6	1.0	428.7
2	1.2	274.2	1.2	338.4	4.4	1482.3
3	1.1	314.0	4.4	1660.4	1.2	497.5
4	1.3	217.2	4.5	1329.4	4.2	1410.5
5	4.2	1456.2	1.1	432.4	1.1	468.0
6	4.0	685.2	1.1	222.9	3.8	1184.2
7	4.5	1249.2	4.3	1627.5	1.1	454.7
8	4.7	1098.2	4.7	1331.7	4.5	1366.4
9	2.1	428.6	2.0	597.1	1.9	706.3
10	2.1	706.2	2.0	840.6	0.6	250.4
11	2.3	180.9	2.2	431.9	7.4	1745.5
12	2.2	675.7	0.6	207.8	2.0	850.7

Table 19--Continued

Treatment	Amino acid					
	Threonine		Valine		Methionine	
	Perfusate[a]	Absorbed[b]	Perfusate	Absorbed	Perfusate	Absorbed
13	2.2	311.0	7.6	1838.6	2.0	602.8
14	0.6	196.4	2.3	918.6	2.2	921.9
15	8.4	1259.6	2.2	599.1	2.2	680.3

[a]Values for perfusate are expressed as micromoles per milliliter and are means of three observations.

[b]Values for absorbed are expressed as micromoles per hour per 100 centimeter section of intestine and are means of nine observations except for treatments seven, eleven and twelve, which are eight observations. Means are adjusted for replication, site, site x treatment, replication x treatment and effluent volume.

TABLE 20. STANDARD REGRESSION COEFFICIENTS[a] AND R^2 FOR AMINO ACID ABSORPTION PREDICTION EQUATIONS

Item	Amino acid		
	Threonine	Valine	Methionine
site	-0.09	0.09	0.09[d]
b_o	0.00	0.00	0.00
b_1	0.27	-0.22	-0.20
b_2	-0.25	0.24	-0.23
b_3	-0.19	-0.13	0.34[c]
b_{11}	1.25[d]	0.14	0.12
b_{22}	0.01	0.49[b]	0.11
b_{33}	0.25	0.04	0.38[b]
b_{12}	-0.01	0.09	0.08
b_{13}	-0.73	-0.04	-0.07
b_{23}	0.10	-0.06	0.07
sb_1	-0.06	-	-
sb_2	-0.25	-	-
sb_3	-0.02	-	-
sb_{11}	-0.97	-	-
sb_{22}	0.18	-	-
sb_{33}	-0.21	-	-
sb_{12}	0.27	-	-
sb_{13}	0.48	-	-

Table 20--Continued

Item	Amino acid		
	Threonine	Valine	Methionine
sb_{23}	0.12	-	-
XV	-0.40	-0.31[b]	-0.33[b]
R^2	0.652[b]	0.714	0.708

[a] b_o = intercept; b_1, b_2, b_3 = linear effects of initial concentration of threonine, valine and methionine, respectively; b_{11}, b_{22}, b_{33} = quadratic effects of each amino acid; b_{12}, b_{13}, b_{23} = interactions between initial concentration of threonine and valine, threonine and methionine, valine and methionine, respectively; s = site, sb_1 . . . sb_{23} = interaction between site and linear, quadratic and interaction components; XV = effluent volume collected.

[b] $P < .01$

[c] $P < .05$

[d] $P < .10$

TABLE 21. ABSORPTION PREDICTION EQUATIONS

Threonine:

$$Y_1 = 1217.53 - 53.23S + 571.50LX_1 - 519.30LX_2 - 382.06LX_3 + 3271.35LX_1SQ + 29.67LX_2SQ + 755.69LX_3SQ - 24.60LX_1X_2 - 2913.34LX_1X_3 + 378.73LX_2X_3 - 74.32SLX_1 - 292.27SLX_2 - 30.01\ SLX_3 - 1493.77SLX_1SQ + 295.16SLX_2SQ - 369.69SLX_3SQ + 611.22\ SLX_1X_2 + 1153.24SLX_1X_3 + 269.12\ SLX_2X_3 - 1.06XV$$

Valine:

$$Y_2 = 1137.22 + 60.26S - 517.22LX_1 + 557.02LX_2 - 304.42LX_3 + 424.74LX_1SQ + 1526.86LX_2SQ + 122.04LX_3SQ + 377.78LX_1X_2 - 190.30LX_1X_3 - 253.43LX_2X_3 - 0.94XV$$

Methionine:

$$Y_3 = 1144.36 + 56.35S - 410.37LX_1 - 462.31LX_2 + 651.55LX_3 + 310.55LX_1SQ + 297.77LX_2SQ + 1106.01LX_3SQ + 292.58LX_1X_2 - 270.91LX_1X_3 + 267.20LX_2X_3 - 0.86XV.$$

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CHARACTERISTICS OF THREONINE, VALINE AND METHIONINE ABSORPTION IN THE JEJUNUM AND ILEUM OF SHEEP

by

William Allison Phillips

(ABSTRACT)

Wethers fitted with double re-entrant cannulae located in either the jejunal or ileal section of the small intestine were used to study the absorption characteristics of threonine, valine and methionine. Perfusions of the cannulated intestine were conducted 4 hr after feeding for a 1 hr period. Absorption of amino acids was measured as the total amount of amino acid removed from the perfusion solution during the 1 hr period. Concentrations of each amino acid ranged from 0.6 to 7.4 millimolar. The experiment design, a three dimensional central composite design, required only 15 different concentration combinations of the three amino acids used to determine their absorption characteristics.

Removal of amino acid from the perfusion solution was constant over the 1 hr perfusion period, but absorption did vary with time after feeding at which the wethers were perfused. Four or 8 hr after feeding tended to have higher absorption rates than 2 or 6 hours. Absorption of each amino acid increased with each replication indicating that the cannulae remained healthy and functional. Wethers with

cannulae located in the ileum absorbed more ($P < .05$) threonine, valine and methionine than those with jejunal cannulae.

Equations were derived for each amino acid to predict the amount which would be absorbed when the initial concentrations of all three amino acids were known. Site of absorption and effluent volume were also part of the absorption prediction equations. Absorption of threonine and valine was affected by the quadratic component ($P < .10$) of their own concentrations. The linear and quadratic components of methionine concentration exerted a positive influence ($P < .05$) on methionine absorption. Site of absorption was not an important ($P < .10$) component of the prediction equation with the exception of methionine. Effluent volume was a significant ($P < .01$) component of all three absorption prediction equations. The amounts of each amino acid absorbed was dependent upon the initial concentration of that amino acid. As the initial concentration was increased, the amount absorbed increased, but no saturation points were noted.

Absorption, as observed in this experiment, was subject to inhibition and stimulation by the other amino acids present in the perfusion solution. Valine was inhibitory of threonine absorption when present in low concentrations, but increasing the concentration of valine resulted in a stimulatory effect on threonine absorption. Methionine inhibited the absorption of threonine when valine was present at concentrations below 3 mM, but the stimulation of threonine absorption by high concentrations of valine was enough to override the inhibitory effects of methionine.

The amount of valine absorbed was decreased by increasing the methionine concentration. Low concentrations of threonine decreased the amount of valine absorbed while high concentrations had a stimulatory effect at high concentrations of valine and low concentrations of methionine. Valine exerted inhibitory effects on methionine absorption when threonine was present in low concentrations and threonine was also inhibitory if valine was present at low concentrations. When the concentration of threonine was increased to 7.4 mM, each increase in valine concentration resulted in more methionine being absorbed.