AMINO ACID TRANSPORT IN THE SMALL INTESTINE. CHARACTERISTICS OF THE SYSTEMS IN SHEEP SMALL INTESTINE AND A COMPARISON OF THE SYSTEMS FOUND IN EFA DEFICIENT AND NORMAL RATS

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ABSTRACT

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By

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Experiment 1

The active transport systems of the small intestine are responsible for absorption of amino acids from dietary protein. Much is known about the properties and characteristics of these systems in monogastric animals; however, very little work has been done with the systems in the ruminant animal. This study was designed to determine some properties of intestinal amino acid transport in sheep.

Glycine, methionine and lysine transport in sheep jejunum was studied by an in vitro method. Slices of jejunum were prepared from mature ewes after an overnight fast. The slices were incubated under an atmosphere of 95% O_2/5%CO_2 in Krebs-Ringer bicarbonate buffer containing the appropriate ^{14}C labeled amino acid at 37°C. ^{14}C inulin was used to determine extracellular space in jejunum from sheep intestine. Extracellular space ranged from 5% wet tissue weight after a 10 minute incubation to 11.3% wet tissue weight after a 60 minute incubation. Uptake of 5 mM lysine in sheep intestine increased as distance from the pylorus

increased such that maximum uptake was obtained in the ileum. Respiration derived energy dependence of transport was shown by comparing distribution ratios (DR) (CPM per ml intracellular fluid/CPM per ml medium) for methionine and lysine determined in an atmosphere of oxygen. Methionine DR after 10 and 60 minutes of incubation in an atmosphere of nitrogen ranged from .30 to 1.4 while respective values for an oxygen atmosphere ranged from 2.63 to 7.79. Lysine DR after 10 and 60 minutes of incubation in a nitrogen atmosphere ranged from .54 to 1.1 while respective values for an oxygen atmosphere were 2.04 and 5.0. The kinetic constants of transport (Km and Vmax) were determined for methionine, glycine and lysine by use of a Lineweaver-Burk plot. Km (mM) values were 2.43, 33.3 and 1.52 while Vmax (μmole amino acid/100 mg wet tissue/.5 hour) values were .0058, .67 and .0098 for methionine, glycine and lysine, respectively. Leucine competition of lysine uptake was studied in sheep jejunum. One mM lysine was incubated with either 1, 3 or 4 mM leucine while 5 mM lysine was incubated with 5, 15 or 20 mM leucine. Uptake of 1 mM lysine was significantly decreased ($P<.05$) approximately 24% by all concentrations of leucine. Uptake of 5 mM lysine was decreased ($P<.005$) 24% by 5 mM leucine while 15 and 20 mM leucine caused additional decreases ($P<.005$) in uptake of 5 mM lysine of 49 and 48%, respectively.

Experiment 2

Any factor affecting the intestinal active transport systems in a manner that would decrease the amount of amino

acids absorbed may eventually affect every metabolic function of the body. Fatty acids appear to be essential in maintaining normal membrane integrity and, therefore, the normal functioning of the active transport systems. Thus, this study was designed to determine the effect of an essential fatty acid (EFA) deficiency on mucosal phospholipid fatty acid composition and in vitro amino acid uptake in rat jejunum. EFA deficiency produced lower levels of palmitoleic (16:1), oleic (18:1) and arachidonic (20:4) with higher levels of linoleic (18:2) and eicosatrienoic (20:3) acids in phospholipid fatty acids isolated from intestinal mucosa of rats. The in vitro techniques used to study amino acid uptake by rat intestine were the same as those used in the previous experiment. Extracellular space as a % of wet tissue weight of EFA deficient rats after a 10 and 60 minute incubation ranged from 8.60 to 16.38 while the respective values for normal rats were 9.48 and 12.42. After a 60 minute incubation, extracellular space of EFA deficient rats was significantly greater ($P<.05$) than extracellular space of normal rats. Methionine DR after a 10 and 60 minute incubation for EFA deficient rats ranged from .92 to 1.37 while the respective values for normal rats were .85 and 5.82. The DR obtained after 60 minutes was significantly greater ($P<.01$) for rats on the normal diet. Lysine DR for EFA deficient animals after 10 and 60 minutes of incubation were .30 and 1.02. Respective values for normal animals were .43 and 1.06 and .51 and 2.07 for animals on a

commercial diet. DR obtained after 60 minutes for animals on the commercial diet were significantly greater ($P<.05$) than DR obtained for animals on the other two diets. Km (mM) values for methionine transport in EFA deficient and normal rats were 8.69 and 6.89 while the respective values for lysine transport were 11.10 and 7.14. Values of Vmax (μmoles amino acid/100 mg wet tissue/.5 hour) for methionine transport in EFA deficient and normal rats were 21.2 and 16.6 while respective values for lysine were 2.0 and 1.4. Methionine uptake at concentrations of .5, 1.0, 2.0 and 10.0 mM in the presence of 10 and 50 mM glucose was studied in intestine from EFA deficient and normal fed rats. The EFA deficiency had no effect on glucose-methionine transport interactions. Glucose was found to be a competitive inhibitor of methionine transport in the intestine for both dietary groups.

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INTRODUCTION

Quantitatively, proteins are the major component of animal tissue dry matter. They are intimately related with all bodily functions, serving as structural elements, hormones, oxygen carriers, hereditary factors, antibodies and enzymes to name just a few. Proteins are necessary for most all important physiological functions, thus the importance of protein synthesis in the body becomes apparent. Protein synthesis is dependent on a supply of amino acids. Amino acids enter the body, thus becoming available for metabolic reactions, via the active transport systems of the small intestine. Many studies have been devoted to determining the properties and characteristics of these transport systems in the monogastric but little work has been done in the ruminant animal. Thus a portion of the present study was devoted to a determination of some of the properties of intestinal amino acid transport in sheep.

The mechanisms providing for active transport are located on or in the membrane of the intestinal epithelial cell in a highly specific manner. Factors affecting the active transport mechanisms so as to reduce the amount of

amino acids absorbed have the capacity to ultimately affect every metabolic function in the body. Fatty acids have been implicated in the maintenance of normal membrane integrity and, therefore, the normal functioning of the active transport systems. Thus the remainder of this study was devoted to determining the effect of an essential fatty acid deficiency on mucosal phospholipid-fatty acid composition and amino acid transport in the small intestine of the rat.

LITERATURE REVIEW

Classification of the Movement of Molecules Across Membranes

Evolutionary processes have led to the development of abilities for the movement of molecules across cell membranes. Maintenance of life would be impossible without this important body function.

Lehninger (1970) has classed this movement into the two categories of simple diffusion and mediated transport. Simple diffusion may be considered as movement in response to an electrochemical gradient. This type of movement will always be with the gradient and never against it. Mediated transport may be thought of as the translocation of molecules across a membrane by means of a carrier system. Two types of mediated transport, passive and active, occur. Only movement down an electrochemical gradient can be obtained with passive mediated transport. Rosenberg (1948) has defined active transport as a process that brings about net transfer of a substance against an electrochemical potential difference of that substance.

Importance and Characteristics of Active Transport Systems

Active transport enables a cell to absorb substrates present in low concentration and concentrate the compound inside the cell such that it may be utilized rapidly and efficiently. These systems provide the major basis for the intestinal absorption of amino acids, sugars and most minerals.

Lehninger (1970) has outlined characteristics exhibited by most active transport systems. They must show saturation kinetics, indicating binding of a compound with a specific site on a carrier molecule. This carrier molecule must be able to cause movement of a compound against an electrochemical gradient while showing a dependence on metabolic energy. This movement must be unidirectional and the carrier system should show some substrate specificity. The compound being transported must also meet certain structural requirements. Lin et al. (1962) have presented evidence that amino acids must possess a free carboxyl group, an α amino group and an uncharged side chain in order to be actively transported in hamster small intestine. The α hydrogen atom was necessary to maintain normal rates of transport, but not active transport. Randall and Evered (1964) and De La Noüe et al. (1971) could not substantiate the need for an α amino group in rat small intestine as they obtained active transport with the amino group in the β position.

Both D and L amino acids may be actively transported, but the carrier molecules prefer the L form (Gibson and Wiseman, 1951; Jervis and Smyth, 1959a; Randall and Evered, 1964; De La Noüe et al., 1969, 1971). Inhibition studies with the D and L form of the same amino acid indicate that both forms are carried by the same mechanism with the L form having a much higher affinity (Jervis and Smyth, 1959a, 1959b, 1960).

Sodium ion is also known to be a requirement for intestinal amino acid transport. Deletion of sodium from the incubation media or substitution of ammonium ion or lithium ion for sodium have been inhibitory to active transport (Faust et al., 1970; Nelson and Lerner, 1970; Cohen and Huang, 1964). Schultz et al. (1967) gave evidence that amino acid influx in rabbit ileum is dependent on the extracellular sodium concentration and relatively unaffected by the intracellular sodium concentration; however, more recent evidence utilizing rat small intestine, a shorter experimental period and more sensitive experimental technique has shown amino acid uptake to be dependent on the intracellular sodium concentration (Newey et al., 1970b). Curran et al. (1967) have predicted a model for influx involving the combination of sodium and amino acid with a single carrier molecule in the cell membrane leading to the penetration of both solutes. The decrease in carrier affinity for valine and leucine in sodium free incubation media tends to

substantiate the above model (Curran, 1968). Even though it is apparent that sodium is intimately related with amino acid transport, more evidence is needed before a definite model of interaction can be concluded.

Amino acid active transport is known to be dependent on cellular oxygen and metabolic energy. Wilson and Wiseman (1954), Nathans et al., (1960) and Newey and Smyth (1962) have shown anaerobic conditions to be inhibitory to amino acid transport in both rat and hamster small intestine. Many workers have shown inhibitors of energy metabolism such as sodium cyanide, 2, 4-dinitrophenol and others to be inhibitory to active transport of amino acids (Agar et al., 1954; Nathans et al., 1960; Jervis and Smyth, 1960; Finch and Hird, 1960a; Reiser and Christiansen, 1971a).

Location of the Amino Acid Carrier

Competition studies with amino acids and with ATP have shown the amino acid transport systems to have an external membrane location (Finch and Hird, 1960b; Reiser and Christiansen, 1971b). Crane and Mandelstam (1960) compared mucosal and serosal sheets of hamster intestine and concluded that only the mucosal sheet contained active transport systems. Orten (1963), working with human ileum, determined that the principal sites of amino acid absorption were contained in the columnar epithelial cells.

Methods for Studying Intestinal Absorption

Many methods, both *in vivo* and *in vitro*, have been developed for the study of intestinal absorption. This review shall be limited to the more common *in vitro* methods utilizing surviving tissue.

Techniques involving a continuous circulation of an oxygenated amino acid-containing buffer through an intestinal segment have been developed (Wiseman, 1953). The segment is attached to two tubes, completing the route of circulation. The serosal side of the intestine is bathed with buffer containing the same concentration of amino acid as the fluid circulating through the lumen. Absorption is expressed as the increase in serosal amino acid concentration after circulation as compared to the beginning serosal amino acid concentration.

Another method that has proven most useful in the study of absorption has been the preparation of everted sacs of intestine (Wilson and Wiseman, 1954; Wiseman, 1954, 1961). The animal is killed and the desired section of intestine quickly removed. Eversion is started by using a stainless steel or glass rod to push the ileal end into the lumen until is appears at the duodenal opening. Eversion is completed by rolling the proximal half of the intestine onto the rod. The intestine can now be removed from the rod and a thread ligature tied around one end. The sac thus formed is filled via syringe with buffer containing the appropriate

amino acid and tying off is completed. The intestinal sac is incubated in a flask containing oxygenated buffer and the same concentration of amino acid as the solution on the serosal side. Absorption may be determined by the amount of amino acid on the serosal side at the end of incubation in excess of that initially present per unit of intestine per unit of time. The major objection with this method has been the inability to sample the serosal fluid throughout the incubation. Efforts to overcome this problem have resulted in several methods for cannulation of the everted sac. Crane and Wilson (1958) tied one end of everted hamster intestine to a cannula inserted through a rubber stopper and closed the other end with a thread ligature. The complete apparatus was placed in a test tube containing oxygenated buffer and the test compound. Oxygenation was continued by way of a syringe inserted through the stopper to the bottom of the tube. The sac was filled and samples taken through the cannula by use of a syringe with a section of polyethylene tubing on the end. Jorgensen et al. (1961) modified this method slightly by tying a polyethylene tube into the end of the intestinal segment that was previously left closed. Intermediate sampling is now done via the polyethylene tube. These methods allow the sac to be used for more than one incubation; therefore, each sac may serve as its own control in determining transport responses.

Absorption by active transport, as measured by the everted sac method, must be influenced by the diffusion of the compound through several cell layers as it passes into the serosal compartment of the sac. Agar et al., 1954 and Crane and Mandelstam (1960) developed a method to by-pass this problem. The desired segment of intestine is removed and cut into small rings 2 to 4 mm in length and incubated in oxygenated buffer containing the test compound. Accumulation of the compound in the intestinal wall is used as a measure of the rate of absorption.

Other methods, such as preparations of whole sheets of mucosa or preparations of intestinal villi, have been used but have not been totally satisfactory as these tissue preparations have been too fragile for routine use.

Neutral Amino Acid Transport Systems: Two Carriers

Ample evidence has been accumulated to show at least two separate transport pathways for neutral amino acids in the small intestine of rat, hamster, rabbit and fowl (Akedo and Christensen, 1962; Baker and George, 1971; Hagihira et al., 1962; Burns and Faust, 1969; Schultz and MarkscheidKaspi, 1971; Tasaki and Takahashi, 1966). The two pathways now referred to are most commonly known as the methionine and sarcosine carriers. The remainder of the neutral amino acids are carried by both systems but with differing affinities. Work by Hagihira et al. (1962) indicated that proline and hydroxyproline showed greater binding affinity for the

sarcosine carrier than the methionine carrier while L-valine exhibited the greatest affinity for the methionine carrier in hamster small intestine. Tasaki and Takahashi (1966) reported a common pathway for methionine, isoleucine and valine in the small intestine of domestic fowl. Glycine appeared to be absorbed mainly by a different pathway. Akedo and Christensen (1962) defined two distinct transport systems based on rate of transport in rat small intestine. Glycine and α amino isobuytric acid exhibited a slow rate of transport but were not able to saturate their carrier system. Valine, leucine, methionine and the model amino acid 1-aminocyclopentane-1-carboxylic acid exhibited a very rapid rate of transport and quickly saturated their carrier, thus reaching a steady state distribution in a short time. Baker and George (1971) used betaine instead of sarcosine but could still show two distinctly different transport systems for the neutral amino acids in rat small intestine. One carrier possessed high affinity for betaine. Leucine, alanine, proline, glycine and α-amino isobutyric acid exhibited varying affinity for both systems with leucine and alanine transport mainly by the methionine carrier and proline, glycine and α-aminoisobutyric acid transport mainly via the betaine carrier. This substantiates previous evidence based on inhibition studies that glycine and proline share a common pathway in rat small intestine (Evered and Randall, 1963). Pinsky and Geiger (1952), using inhibition studies, reported that

histidine and tryptophan are actively transported by the neutral amino acid pathways of rat small intestine. Agar et al. (1956) confirmed the report for tryptophan and also presented evidence that phenylalanine follows these same pathways. Work by Wiseman (1954) indicates that histidine may prefer the methionine carrier. Phenylalanine also appears to have the greatest affinity for the methionine carrier (Spencer and Samiy, 1961).

The two systems of transport for neutral amino acids may account for the varying characteristics that have previously been noted. Daniels et al. (1969a) found no requirement by the sarcosine transporter for an α-amino group on the substrate as α, β and γ amino acids were equally well transported by the sarcosine carrier while the methionine carrier exhibited specificity only for α amino acids. Daniels et al. (1969b) reported that the sarcosine carrier may be responsible for the observed transport of D-amino acids as the methionine carrier had no affinity for D-amino acids. Thompson et al. (1970) reported a significant increase in transport of most neutral amino acids when pH of the incubation buffer was lowered from 7.3 to 6.3. The increase in transfer was due to an increased functioning of the sarcosine and not the methionine system.

The two pathways just discussed correspond rather well to the A and L system of the ehrlich cell as defined by Christensen (1969). The methionine carrier is similar in

most respects to the L system. Both systems have minimal sensitivity to pH changes and both are reactive in some degree to most neutral amino acids. The systems differ in that no sodium dependency is known for the L system while transport via the methionine system seems to be sodium dependent. Newey et al. (1970b) observed no methionine uptake in everted sacs of rat small intestine when sodium was deleted from the incubation medium. Tryptophan (Cohen and Huang, 1964), histidine (Faust et al., 1970), valine and leucine (Curran, 1968), and alanine (Schultz et al., 1967) are all transported by the methionine system and are known to be sodium dependent. The A system of the ehrlich cell corresponds most closely with the sarcosine carrier. Both systems are sensitive to pH changes and are most reactive to glycine, proline, α aminoisobutyric acid and sarcosine although transport of most neutral amino acids by this system is known. Transport by both systems is sodium dependent.

Rates, Gradients and Kinetic Constants of Neutral Amino Acid Transport

Data from Wiseman (1956) indicated that hamster small intestine has the ability to move threonine, alanine, serine, valine, hydroxyproline, phenylalanine, isoleucine, leucine and tryptophan against a concentration gradient, leading to an intracellular accumulation of the amino acids. Proline and threonine exhibited the greatest rate of transport (μl/mg dry weight/hour) while tryptophan exhibited the least.

Agar et al. (1956), using rat small intestine, observed histidine and phenylalanine movement against a concentration gradient. Phenylalanine transport was great enough to allow a tissue to media ratio greater than 2:1 to be reached.

Nathans et al. (1960) reported that rate of transport for L-monoiodotyrosine in rat small intestine varied depending upon the intestinal segment used. The segment having the greatest rate of transport was consistently found to be the terminal ileum while the proximal jejunum was found to have the lowest rate of transport. The workers attributed this difference to a greater mucosal efflux of the amino acid in the proximal intestine.

Finch and Hird (1960b) reported that the rate of amino acid uptake in rat small intestine depends on the original concentration of the amino acid being studied. At 10 mM the more lipophilic amino acids exhibited lower rates of uptake than did amino acids with smaller side chains as evidenced by leucine having the least rate of uptake and serine the greatest. At 1 mM the order of uptake was distinctly reversed with leucine exhibiting the greatest rate of uptake and glycine the least. Leucine showed the greatest apparent affinity for uptake as measured by a Km of 0.65 mM. Methionine had the next greatest affinity with an apparent Km of 0.91 mM. Phenylalanine exhibited an apparent Km of 3.3 mM while respective values for proline and histidine were 10.0 and 10.4 mM. Glycine possessed the least affinity as shown

by an apparent Km of 34.0 mM. The other neutral amino acids exhibited Km values intermediate to this range.

The experimental method used for tissue accumulation may influence the apparent kinetic parameters obtained. Spencer and Samiy (1961), using everted sacs of hamster small intestine, reported an apparent Km of 1.8 mM for phenylalanine, while Samiy and Spencer (1961), using whole rings of hamster small intestine, reported an apparent Km of 7.1 mM and a Vmax of 4.4 μmoles/100 mg wet tissue/20 minutes for phenylalanine. In both cases, phenylalanine was moved against a concentration gradient and mid-jejunum was found to be the area of highest transport activity.

Larsen et al. (1964), studying amino acid transport with everted sacs of rat small intestine, reported that the lower jejunum-upper ileum was the area of maximum transport for every amino acid studied. These workers found the amount of amino acid transported to be proportional to affinity for the carrier at low amino acid concentrations but inversely proportional at high concentrations. The apparent Km values reported in mM were: monoiodotyrosine, 4; phenylalanine, 14; leucine, 22; valine, 33; methionine, 53; alanine, 63 and glycine, 100.

Laster and Matthews (1965), using everted sacs of hamster ileum, derived apparent Km values (mM) of 1.5 for leucine, 1.9 for valine, 7.5 for alanine and 43.2 for glycine. The differences may be species related as the experimental technique was the same as reported above.

Schedl et al. (1968) found that Km and Vmax for methionine varied between the proximal and distal small intestine of the human. Km ranged from 12-28 mM for the proximal intestine and 2-5.7 mM for the distal intestine. Vmax values for the proximal intestine ranged from 16-33 mMoles/hour/segment while values in the distal intestine were found to be 5-6 mMoles/hour/segment.

Williams (1969) measured the rate of absorption for amino acids in sheep small intestine and reported that isoleucine, methionine and valine had the most rapid rate of absorption while glycine had the least rapid.

Reiser and Christiansen (1971a) reported that inhibitors of protein synthesis had no effect on the rate of uptake of L-leucine by isolated intestinal epithelial cells. The apparent Km of L-leucine uptake was calculated to be 3.2 mM.

Ling and Morin (1971) injected rats with the protein synthesis inhibitor, tetracycline, 12 hours before sacrifice. Alanine and cycloleucine uptake by intestinal rings from treated animals had a significant decrease in Vmax and the distribution ratio resulting from a decrease in amino acid influx to the tissue. Km for alanine uptake remained unchanged at 5 mM.

Newey and Smyth (1962) reported the rate of uptake for glycylglycine and glycine by everted sacs of rat small intestine to be equal both in vivo and in vitro. Lis et al. (1971) introduced 100 mM L-methionyl-L-methionine and 200 mM

L-methionine into tied off loops of rat small intestine. Methionine from the dipeptide was absorbed at twice the rate of the free amino acid.

The Basic Amino Acid Transport System

Most workers originally thought that only the neutral amino acids could be actively transported against a concentration gradient as Wiseman (1954) was not able to show active accumulation of L-lysine or L-ornithine in hamster small intestine. Di Bella (1960) gave evidence for active accumulation of basic amino acids with the report that rat small intestine has the capacity to actively accumulate L-lysine. Cystinuria has clearly established the importance of the basic amino acid transport system in humans. Cystinuria has been shown to be a disorder of basic amino acid transport in both the kidney and small intestine (Thien and Segal, 1972). The disease is expressed clinically by formation of calculi in the urinary tract mainly as a result of kidney inability to reabsorb cystine. The calculi may ultimately lead to renal insufficiency.

Although the literature contains many conflicting reports basic amino acids are generally thought to be transported by only one major pathway but some overlap of specificity may be observed between the neutral and basic amino acids. Hagihira *et al.* (1961), using competition studies in hamster and rat small intestine, observed that lysine and arginine could compete with glycine for transport

while methionine competed with lysine for transport. Neame (1966) reported L-lysine to be a competitive inhibitor of L-histidine in rat small intestine. Agar et al. (1956) observed that equimolar quantities of lysine and arginine would inhibit histidine transport in rat small intestine by 17 and 21%, respectively, as compared to controls. Munck (1966) observed that addition of 1 mM leucine to the incubation fluid increased the rate of transport and the final serosal concentration of lysine in rat small intestine but increasing leucine to 15 mM inhibited lysine transport. There was no effect of lysine on leucine transport. The results may be explained on the basis of three assumptions: (1) leucine exhibits an intermediate Km for the lysine carrier (2) lysine exhibits a very high Km for the leucine carrier (3) leucine exhibits a counterflow effect on lysine (inhibition of lysine efflux by intracellular leucine). Fifteen mM leucine may have been in excess of the intracellular concentration, thus overcoming the counterflow effect and explaining the inhibition of lysine transport. Contrary to Munck's work, Reiser and Christiansen (1971a, 1972) found that lysine was inhibitory to leucine uptake as well as alanine, methionine, phenylalanine, threonine and histidine uptake by isolated intestinal epithelial cells. One mM leucine, methionine, alanine and phenylalanine were stimulatory to uptake of 1 mM lysine and arginine while 1 mM isoleucine and tryptophan were inhibitory (Reiser and

Christiansen, 1971c). Leucine, valine and proline at 12.5 mM were found to be inhibitory to the uptake of 1 mM lysine (Reiser and Christiansen, 1969), thus somewhat confirming the observations of Munck (1966).

Rates and Kinetic Constants of the Basic Amino Acid Transport System

Hagihira et al. (1961) observed that everted sacs of hamster small intestine could absorb lysine faster than ornithine and ornithine faster than arginine when each amino acid was studied separately. Each amino acid had a final serosal to mucosal concentration ratio greater than one. Finch and Hird (1960a) found no difference in rate of uptake by rat small intestine for any of the three amino acids. Apparent Km values (mM) of .55 for lysine, 6.0 for ornithine, and 1.5 for arginine indicate a difference in affinity of the amino acids for the carrier. Rates of absorption change such that arginine is greater than lysine when the amino acids are presented to the tissue in a mixture rather than as single amino acids, (Robinson and Felber, 1964).

Larsen et al. (1964) found apparent Km (mM) values of 7.0 for DL-ornithine, 7.0 for lysine and 12.0 for arginine. Maximum uptakes of arginine and lysine were equal and approximately twice the value for DL-ornithine.

Acidic Amino Acid Transport

There is no direct evidence for active transport of acidic amino acids in the intestine (Neame, 1965). Acidic

amino acids are assumed to be transformed into their respective keto-acids during the transport process, thus creating a situation in which direct evidence of transport is very difficult to obtain (Rasaswamy and Radhakrishnan, 1966).

Experiment 2

Defects in normal function of the amino acid transport systems may occur as noted earlier with cystinuria. However, all functional disorders are not the result of genetic abnormalities. Essential fatty acid deficiencies have caused changes in mitochondrial membrane permeability and function (Levin et al., 1957; Ito and Johnson, 1964, 1968). Incomplete structural differentiation of cells lining the microvilli of rat jejunum has also been observed. Although lipids play many important roles in the body, they are especially important in maintaining normal membrane structure and function, thus helping to mediate the movement of sugars and amino acids across cell membranes.

Lipid-Amino Acid Complexes

Reiser and Christiansen (1968) reported that valine and glucose could form a complex with lipid isolated from the mucosa of rat small intestine. The valine-lipid complex formation was very rapid and leucine acted as a competitive inhibitor. Glucose did not compete with valine for complex formation, indicating specific combining sites for both sugars and amino acids in the lipid component of the membrane. Phosphoglycerides were determined to be the major lipid

fraction showing binding ability for the amino acid. LeFevre et al. (1964) observed that phospholipids extracted from membranes of human red cell ghosts could complex with glucose. Compounds that decrease sugar transport with in vitro tissue preparations would decrease the sugar-phospholipid complexing to a similar degree. Frizzell and Schultz (1970) reported that preincubation of rabbit ileum in bile salts resulted in a decreased in vitro influx of alanine. An increase in the trichloroacetic acid precipitable material was found in the incubation medium and the workers concluded that bile salts were acting as a detergent--removing lipoprotein structures from the membrane, thus damaging the transport process.

Effects of an Essential Fatty Acid Deficiency on Fatty Acid Composition, Cellular Structure and Function

Enser and Bartley (1962) studied the effects of an essential fatty acid deficiency on the fatty acid composition in different sections of rat intestine and colon. In mucosa from both proximal and mid ileum, an increase in palmitoleic (16:1) and eicosatrienoic acids (20:3), a decrease in linoleic (18:2) and arachidonic (20:4) acids and no change in oleic (18:1) acid were observed when comparing tissue from deficient animals with tissue from control animals. Mucosa isolated from the colon had the same pattern of change with the exception of an increase in oleic acid caused by the deficiency. Imami et al. (1969) also observed a decrease in linoleic and arachidonic with an increase in eicosatrienoic

but unlike the previous work, an increase in oleic was also observed in the small intestinal mucosa of essential fatty acid deficient rats. Yurkowski and Walker (1970) also found a decrease in linoleic and arachidonic with an increase in palmitoleic, oleic and eicosatrienoic acids in the small intestinal mucosa of essential fatty acid deficient rats. Hayashida and Portman (1960) found essential fatty acid composition in rat liver mitochondria similar to that reported for intestinal mucosa.

Essential fatty acids have been implicated in the maintenance of membrane integrity and structure. The changes in fatty acid composition that occur in essential fatty acid deficiency must affect cellular functions that depend on a normal membrane structure. Levin et al. (1957) found that mitochondria from essential fatty acid deficient rats could oxidize α-ketoglutarate, succinate, malate and NADH at a much higher rate than mitochondria from control animals, indicating a change in mitochondrial membrane permeability. These workers were also able to observe a physical difference in the two mitochondria. Mitochondria isolated from deficient animals were much larger and less opaque than those from normal tissue.

Ito and Johnson (1964) reported that after aging (an in vitro incubation at 30°C) mitochondria from essential fatty acid deficient rats lost respiratory control and ATP-orthophosphate exchange activity more readily than did

mitochondria from control animals. Mitochondria from deficient animals also lost respiratory control more readily in the presence of Crotalus adamanteus venom or digitonin than did mitochondria from control animals. Apparently the deficiency altered mitochondrial structure as to increase vulnerability to the action of these compounds and to the aging process.

Intact mitochondria do not oxidize NADH; however, suspension in a hypotonic medium will allow oxidation to occur by altering the mitochondrial membrane permeability. Ito and Johnson (1968) reported that mitochondria from essential fatty acid deficient rats did not exhibit the increased rate of NADH oxidation when suspended in hypotonic medium whereas mitochondria from control animals did exhibit the phenomena. The data were explained by the assumption that for mitochondria to be permeable to NADH under hypotonic conditions, certain steric factors that are dependent on the presence of essential fatty acids in membrane phospholipids are required. This requirement could not be met in the deficiency state.

Morphological changes have been observed in intestinal mucosa from rats and mice with essential fatty acid deficiency. Mitochondria from jejunal epithelial cells exhibit a change in shape and a reduction in matrix density. On occasion, the inner membrane may appear to be pulled away from the outer membrane. Abnormal microvilli were observed with

the epithelial cells being in a state of incomplete structural differentiation. Transport phenomena were also affected to some degree as total fat absorption decreased from 97% for control animals to 83% for deficient animals (Snipes, 1968).

Imami et al. (1969) observed that an essential fatty acid deficiency significantly decreased valine and α-methyl-D-glucoside transport by rat intestine. Tissue uptake was not affected but rate of transfer from the uptake site to the serosal medium was decreased. The role of essential fatty acids may have been indirect, due to an impaired mitochondrial electron transport and ATP synthesis or directly due to an effect on the epithelial cell membrane allowing an increased efflux of valine and glucoside to the mucosal medium.

Ahmed and Walker (1972) reported that essential fatty acid deficiency significantly reduced the uptake of phenylalanine and leucine per cm of intestine in rats. No effect on lysine transport was observed. The effects of the deficiency were attributed to either a change in the mucosal cell membrane structure or a decrease in the energy yielding capacity of the mitochondria.

Sugar-Amino Acid Interactions in Intestinal Absorption

Many reports providing evidence for sugar-amino acid interactions of both a stimulatory and inhibitory nature have been published. No single mechanism has yet been

conclusively proven but many hypotheses have been formulated to explain these interactions. Work by Saunders and Isselbacher (1965) indicated that sugars may inhibit amino acid transport by formation of a toxic metabolite. Galactose was a noncompetitive inhibitor of *in vitro* alanine transport. There was no galactose effect on ATP levels but the tissue accumulation curve of galactose-1-phosphate followed the inhibition curve of alanine, indicating that galactose-1-phosphate was inhibitory to amino acid transport.

Considering that active transport of both sugars and amino acids requires metabolic energy, a hypothesis attributing the interaction of sugars and amino acids to a competition for energy has been formed. Munck (1968a) reported that galactose inhibited the small intestinal transport of proline and valine in the rat. Addition of glucose to the incubation medium eliminated the inhibition and he concluded that glucose supplied an energy supplement enabling the sugar and amino acid carriers to function optimally at the same time. Newey and Smyth (1964) reported that glucose stimulated and galactose inhibited glycine uptake by rat small intestine. Nonactively transported sugars had no effect on glycine transport, thus a limitation of energy availability for transport was assumed to be responsible for the inhibition. Hindmarsh *et al.* (1966) reported that methionine and histidine were inhibitory to the active transport of D-glucose, D-galactose and 3-O-methyl-D-glucose and attributed this to a competition for available energy.

Hardcastle et al. (1968) observed both a stimulation and inhibition of glycine and leucine transport by glucose. In the presence of iodoacetate, which prevented glucose metabolism, the sugar competed for a limited energy supply, thus becoming inhibitory. Without iodoacetate present, glucose was metabolized for energy, thus stimulating amino acid transport. De La Noüe (1970) observed that glucose increased the transport of alanine and methionine in rat small intestine by 100% in an anaerobic situation. The increased alanine transport was shown to be due to an increased energy supply to the methionine carrier. Genel et al. (1971) found a mutual noncompetitive inhibition between α-amino isobutyric acid and α-methyl-D-glucoside in rat kidney cortex slices that could be interpreted as competition for a common energy supply.

Data from Orten (1961) and Cook (1971) tend to refute competition for energy as a viable hypothesis. Glucose was observed to inhibit intestinal amino acid transport in vivo when energy supplies should be adequate.

The hypothesis of mutual stimulation of substrate efflux from the cell has been postulated to explain the mutual inhibition of sugars and amino acids. Chez et al. (1966) found that total alanine transport and cellular accumulation, but not influx, across the mucosal border of rabbit ileum were inhibited by glucose and galactose. The observations are most easily explained by a glucose-galactose enhancement of alanine efflux. The hypothesis lacks popularity as Nathans

et al. (1960) reported that glucose enhanced monoiodotyrosine transport by decreasing mucosal surface efflux of the amino acid. Munck (1968b) studied efflux by preloading intestinal tissue with either sugars or amino acids and then observing the effect of adding the unpreloaded compound to the incubation media. D-glucose and α-methyl glucoside had no effect on leucine efflux while arginine, histidine and lysine had no effect on galactose efflux.

Sugar-amino acid interactions at the brush border membrane have led to the hypothesis of allosteric effects due to proximity of related binding sites in a polyfunctional matrix. Alvarado (1966) postulated the transporter to be a macromolecular unit containing binding sites for sugars, neutral and basic amino acids and sodium. Galactose and arginine demonstrated allosteric effects, behaving as partially competitive inhibitors of cycloleucine transport. A decrease in apparent affinity of the transporter for cycloleucine without a change in Vmax was interpreted as inhibitor binding at a site different from but close to the active site of transport. Duthie and Hindmarsh (1966), observing that D and L-histidine and L-methionine were inhibitory to the transport of D-xylose in hamster small intestine, postulated an allosteric inhibition due to different binding sites on the same carrier. Robinson and Alvarado (1971), in a comparative study of hamster, mouse, guinea-pig, rat and rabbit, concluded that in all species an allosteric interaction between sugars and amino acids occurred at the surface

of the brush border membrane due to the proximity of the respective binding sites. Alvarado (1971) demonstrated countertransport of L-tyrosine as elicited by methyl-α-glucoside in hamster intestine and concluded that the two compounds share a common mobile carrier.

Other workers do not agree with the hypothesis of allosteric inhibition. Reiser and Christiansen (1969) observed no effect of galactose and α-methyl-D-glucoside on the steady state distribution of lysine between mucosal, serosal and tissue water and concluded that sugars and amino acids do not interact at the carrier level. Faust et al. (1968) found no effect of amino acids on the binding of D-glucose to brush border membrane from hamster jejunum while Burns and Faust (1969) found no effect of D-glucose on amino acid binding to identical brush borders.

Due to the common dependence of sugar and amino acid transport systems on sodium, it has been hypothesized that sugar-amino acid interactions are a result of the common effect of two separate carrier systems on sodium influx to the cell. Semenza (1971) has postulated that sodium influx due to both sugars and amino acids is additive and increases the intracellular sodium concentration, thus affecting other sodium dependent carriers by: (1) stimulation of the sodium dependent substrate efflux or (2) inhibition of the sodium dependent substrate influx mechanism. Frizzell and Schultz (1971) were able to demonstrate a difference in the effects of galactose and phenylalanine on alanine uptake by rabbit

ileum. Galactose appeared to be acting intracellularly on the sodium concentration rather than at the carrier level as was phenylalanine, thus indicating distinct carrier systems for sugars and amino acids with both being sodium dependent.

Interactions of sugars and amino acids during intestinal transport occur in most species. The interaction has ranged from a severe inhibition to mutual stimulation and may reflect species differences. Sodium is apparently involved in the interaction but more research is needed to determine the transport step at which interactions occur and the exact involvement of sodium.

MATERIALS AND METHODS

Experiment 1

Twenty commercial ewes were maintained on a pasture of mixed grasses during the experimental period. Each animal was fasted overnight before slaughter the following morning. No device for stunning was used and the animals were killed by exsanguination. The small intestine was immediately removed and a distance of approximately 20 feet was measured distally from the pyloric end. This area was assumed to be upper jejunum. A one foot section was excised, immediately placed in cold oxygenated saline and transported to the laboratory. Total elapsed time from slaughter to arrival in the laboratory was approximately 5 minutes. Upon arrival in the laboratory, the intestinal segment was flushed free of digesta and cut into rings which were opened lengthwise and placed in a dish of cold oxygenated saline. Rings were picked at random and cut into strips weighing from 10 to 50 mg. each for the *in vitro* incubations. Strips were picked randomly and placed in triplicate into 25 ml. Erlenmyer flasks containing the oxygenated media. Media for amino acid uptake studies consisted of Krebs-Ringer bicarbonate buffer

(composition shown in Table 1) and unlabeled and labeled amino acids at a concentration of .5, 1, 2, 5 or 10 mM in a total volume of 5 ml. Enough glucose was added to make a .3% solution and pH was adjusted to 7.4. The labeled and unlabeded amino acid solutions were added after a preincubation period of 10 minutes. All flasks were shaken on a Gyrotory Water Bath Shaker (New Brunswick Scientific, New Brunswick, New Jersey), gassed continuously with O_2/CO_2 (95-5%) and maintained at 37°C for the desired period of incubation. Radioactivity was added at the rate of 1.25 or 2.50 microcuries per flask. Labeled compounds used were L-methionine-methyl-^{14}C (SA=34.5mc/mM), ^{14}C glycine U.L. (SA=84.0 mc/mM), ^{14}C L-lysine U.L. (SA=231.0 and 228.0 mc/mM) and ^{14}C inulin U.L. (SA=1-3 mc/gm). All compounds were stated by the manufacturer to have >99% purity. Inulin was checked for impurities by paper chromatography. No impurities were found as all radioactivity was confined to the inulin spot. Incubations were stopped by placing the flasks in ice and immediately removing the tissue strips. Each strip was washed in cold saline, blotted gently on filter paper, weighed on a microgram balance and placed in a tube containing 2 ml of .1N nitric acid. The strips were extracted for 2 hours at 70°C. One tenth aliquots of the media and .2 ml aliquots of the tissue extracts were placed in scintillation vials, mixed with 10 ml of the scintillation fluid shown in Table 2 and counted in a Nuclear-Chicago liquid scintillation counter model 6848 at 77% efficiency.

TABLE 1

Composition of Krebs-Ringer Bicarbonate Buffer

Ingredient	% of Buffer
.9% NaCl	76.93
1.15% KCl	3.08
.60% $CaCl_2$	2.30
2.11% KH_2PO_4	.77
3.82% $MgSO_4 \cdot 7H_2O$	.77
1.30% $NaHCO_3$[1]	16.15

[1]Previously gassed with CO_2 for 1 hour.

TABLE 2

Composition of Scintillation Fluid

Ingredient	Amount
P-Dioxane	385 ml
Xylene	385 ml
Absolute Ethanol	230 ml
2, 5-Diphenyloxazole	5 gm
Naphthene	80 gm
Cabosil[1]	25 gm
1, 4-bis-[2-(4-Methyl-5-Phenyloxazolyl)]-Benzene	100 mg

[1]Added to fluid when counting inulin.

Experiments were conducted to demonstrate respiration derived energy dependence of methionine and lysine uptake by strips of sheep intestine. Tissue incubations were conducted as described above with the exception that nitrogen was used in place of oxygen. The extraction and counting procedures were identical with those previously described. Methionine and lysine distribution ratios (described below) obtained with nitrogen were compared to ratios obtained with oxygen in order to demonstrate energy dependence.

For dry matter determinations, incubations were carried out as described above except that no radioactivity was added. The difference in volume was made up with additional KRB buffer. Tissue strips were washed, blotted and weighed as above and then dried in a forced air oven at 100°C for 24 hours.

Extracellular space measurements were determined by carrying out incubations as described above with inulin used in place of amino acids. Extraction procedures were also as previously described. The equations used for calculations were those of Rosenberg et al. (1961).

Competition for uptake between neutral and basic amino acids in sheep intestine was studied with leucine and lysine. Tissue incubations using intestinal rings were conducted as previously described. Lysine was used as the labeled amino acid. Unlabeled lysine was added to attain a final concentration of 1 and 5 mM. Enough unlabeled leucine was added to each lysine concentration to form final leucine to

lysine ratios of 1, 3, and 4 to 1. In all cases, lysine uptake was compared to incubations containing no leucine.

Amino acid uptake data were expressed as both a distribution ratio (CPM per ml intracellular fluid/CPM per ml medium) and as μmoles of amino acid per 100 mg wet tissue per .5 hour. The kinetic constants, Km and Vmax, were determined for each amino acid by plotting the data in the manner of Lineweaver and Burk (1934). Data for extracellular space measurements were expressed as ml of inulin space, % of wet tissue weight. Differences in means were analyzed for significance by analysis of variance and Duncan's multiple range test or a t test for the difference of 2 means.

Experiment 2

Eighty eight weanling male rats, Sprague-Dawley strain (Spartan Research Animals, Haslett, Michigan), were divided equally among the two diets shown in Table 3. Symptoms of an essential fatty acid deficiency appeared after 12-14 weeks of feeding at which time the experiments were conducted. The animals were stunned with a blow to the head and then decapitated. The body cavity was opened with a mid-line incision and a 10 cm section of mid jejunum was removed and handled in the same manner as described in experiment 1 except that the gut segments were left as rings rather than being cut into strips. Tissue incubations and extraction procedures for amino acid uptake, dry matter and

TABLE 3

Diet Composition

Ingredients	EFA Deficient (%)	Normal (%)
Casein[1]	21	21
Non Nutritive Fiber[2]	16	16
Rogers-Harper Salt Mix[3]	4	4
Vitamin Mix[4] (Cat. No. 40060)	1	1
Glucose Monohydrate	58	53
Corn Oil[5]	--	5

[1]General Biochemicals, Chagrin Falls, Ohio.

[2]General Biochemicals, Chagrin Falls, Ohio.

[3]General Biochemicals, Chagrin Falls, Ohio.

[4]General Biochemicals, Chagrin Falls, Ohio.

[5]Columbus Foods Co. Inc., Chicago, Illinois.

extracellular space measurements were done in the same manner as described in experiment 1.

Interactions of sugars and amino acids in the transport processes have been well documented (Nathans et al., 1960; Segal et al., 1962; Thier et al., 1964; De La Noüe, 1970; Newey et al., 1970a). In an effort to provide more information on the nature of these interactions, observations on methionine uptake at .5, 1.0, 2.0 and 10.0 mM in the presence of 10 and 50 mM glucose were made in both normal and EFA deficient rats. Methods of incubation, tissue extraction and counting were the same as done previously.

Expression of all data and statistical tests for significance were done in the same manner as described in experiment 1.

Intestines to be used for fatty acid analysis were frozen in liquid nitrogen immediately after removal from the animal and were stored under nitrogen at -60°C until analysis. At the time of analysis, the segments were allowed to thaw, were opened lengthwise and the mucosa removed by scraping with a glass slide and then immediately refrozen. Total lipids were removed by extracting overnight in a covered beaker under an atmosphere of nitrogen with 20 ml of chloroform-methanol (2:1 v/v) per gram of lyophilized mucosa. The extracts were filtered through a fritted glass Buchner funnel and the nonlipid impurities removed by the salt wash procedure of Folch et al. (1957). Total lipid was determined

by evaporating the extracting solvent to a constant weight under a stream of nitrogen.

Isolation of Mucosal Phospholipids

The dry lipid was taken up in a small amount of chloroform and added to a column of coarse mesh silicic acid at the rate of 30 mg of lipid per gram of absorbant. A flow rate of about 3 ml per minute was maintained by applying pressure from a stream of nitrogen to the column. Neutral lipids were eluted with 10 column volumes of chloroform while the phospholipids were eluted with 10 column volumes of absolute methanol. The completeness of neutral and phospholipid separation by this method was checked by thin layer chromatography. Thin layer plates, .5 mm thick, were prepared with Silica Gel G, spotted and then developed in petroleum ether, diethyl ether, 90:10. Lipids were detected with a 50% sulfuric acid spray while a molybdenum blue reagent spray was used to detect phospholipids. Thin layer chromatograms indicated complete separation of neutral and phospholipids. Total phospholipid was determined in the same manner as total lipid.

Preparation of Methyl Esters of Fatty Acids

Fatty acids were esterified by the method of McGinnis and Dugan (1965) with slight modification. A 125 ml Erlenmyer flask was used to dissolve 20 mg of phospholipid in 20 ml of peroxide free diethyl ether. The solution was cooled to -60°C with a dry ice-acetone bath and magnetic

stirrer. After cooling, 2 ml of concentrated sulfuric acid were added at the rate of 1 ml per minute and the solution allowed to reach -10°C over a ten minute interval. After cooling to -60°C again, 15 ml of absolute methanol were added and the solution allowed to stand in the cooling bath at least 20 minutes. After standing, 13 ml of 35% methanolic KOH were added and the solution was removed from the cooling bath and stirred until it reached room temperature. The solution was quantitatively transferred to a 500 ml separatory funnel containing 150 ml of water. Any precipitated KOH was redissolved with extra additions of water and by gentle swirling if necessary. Methyl esters were recovered by extracting 3 times with petroleum ether (30 ml first and 15 ml the two remaining times). The petroleum ether extract was dried over anhydrous sodium sulfate and concentrated to the proper volume by evaporation under nitrogen before storage in graduated test tubes. Analysis by gas chromatography was conducted within 12 hours after storage.

Gas Chromatography Analysis

Methyl esters were analyzed by using a F & M 810 gas chromatograph equipped with a hydrogen flame ionization detector. The samples were injected into a 72 x 1/4 inch stainless steel column packed with Chromosorb W, 80-100 mesh, as solid support with 15% DEGS and 3% phosphoric acid as the liquid support. Helium, flow rate of 35 ml per minute, was used as the carrier gas. The column was used isothermally

at 190°C with detector temperature of 260°C and injector temperature of 250°C. Fatty acids were identified by comparison with standards purchased from the ANSPEC CO., Inc., Ann Arbor, Michigan. The fatty acids were calculated as a percent of the total fatty acids by calculating the peak area and expressing it as a percent of the total area from all peaks of the methyl esters on the chromatogram.

RESULTS

Experiment 1

Uptake of 5 mM lysine by different areas of sheep intestine is presented in Table 4. Mean uptake varied from 68.8 to 273.0 (μmoles lysine/100 mg wet tissue/.5 hr.) X 10^{-6}. The proximal intestine had the lowest uptake while the distal intestine had the highest uptake. Uptake of mid to lower intestine was intermediate to these areas. There was no significant difference in uptake between segments from 3 and 20 feet distal to the pylorus. Uptake at 50 feet was significantly greater ($P<.01$) than that at 3 and 20 feet but significantly less ($P<.01$) than uptake at 70 feet. Total length of the intestine was approximately 75 feet.

The dry matter and total tissue water of sheep intestinal strips is presented in Table 5. Total dry matter ranged from 16% at zero time to 18% after 60 minutes of incubation. Total tissue water varied from 84% at zero time to 82% after 60 minutes of incubation. None of the differences were significant.

Measurements of extracellular space in sheep intestine are presented in Table 6. Inulin space (% of wet weight) ranged from .84 at initiation of the incubation to 11.25

TABLE 4

Uptake of 5 mM Lysine in Different Sites of Intestine in Sheep

Distance from Pylorus (Feet)	Lysine Uptake[1]
3	68.8±4.0[2]
20	85.4±2.6[2]
50	127.7±7.2[3]
70	273.0±12.8[4]

[1] (μmole of lysine/100 mg wet tissue/.5 hr.) X 10^{-6}, mean ± standard error of 12 determinations on 2 sheep.

[2, 3, 4] Values not sharing common superscripts differ significantly ($P<.01$).

TABLE 5

Total Dry Matter and Tissue Water of Intestinal Strips from Sheep Jejunum[1]

Time[2]	% Total Dry Matter	% Total Tissue Water
0	16.11±.42	83.89±.42
10	16.07±.57	83.93±.57
20	15.45±.46	84.55±.46
30	17.42±.31	82.58±.31
40	18.66±.20	81.34±.20
60	18.49±.48	81.51±.48

[1]Mean ± standard error of 6 determinations on 2 sheep.

[2]Minutes of incubation.

TABLE 6

Extracellular Space of Intestinal Strips
from Sheep Jejunum

Time[1]	Ml of Inulin Space (% of Wet Tissue Weight)
0	.84
10	5.51
20	8.25
30	9.19
40	9.35
60	11.25

[1]Minutes of incubation.

after 60 minutes of incubation. The pattern of inulin equilibration with extracellular water is shown in Figure 1. The initial rate of inulin accumulation was very rapid but decreased with time, almost reaching a steady state at 60 minutes.

Table 7 presents the distribution ratios for methionine and lysine at 1 mM after an in vitro incubation in a nitrogen atmosphere. Methionine ratios varied from .39 after 10 minutes of incubation to 1.40 after 60 minutes of incubation. Ratios for lysine ranged from .54 after 10 minutes to 1.06 after 60 minutes of incubation. The distribution patterns of methionine and lysine are shown in Figure 2. Initial rate of accumulation for both amino acids was linear with time for the first minute after which accumulation rate for lysine decreased, reaching an apparent steady state after 40 minutes of incubation. Rate of accumulation of methionine slowed but did not reach a steady state level during the incubation.

Distribution ratios (CPM per ml of intracellular fluid/ CPM per ml medium) for methionine, lysine and glycine at 1 mM after an in vitro incubation of sheep proximal jejunum in an atmosphere of oxygen are shown in Table 8. D.R. for methionine ranged from 2.63 after 10 minutes to 7.79 after 60 minutes of incubation. Lysine D.R. varied from 2.04 after 10 minutes to 5.0 after 60 minutes while D.R. for glycine were lower, ranging from .81 after 10 minutes to 2.56 after 60 minutes of incubation. The distribution patterns for the amino acids are shown in Figure 3. Both glycine and

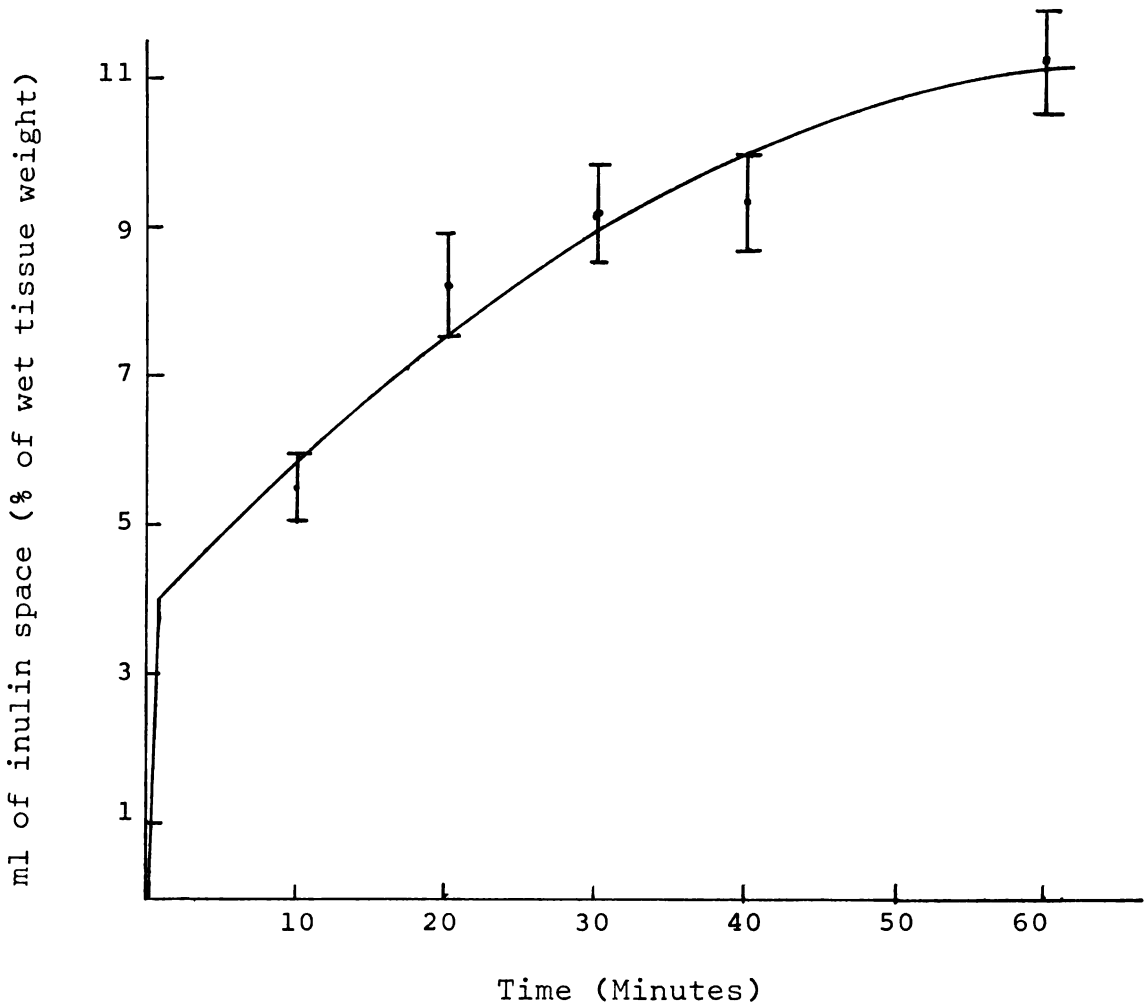

Figure 1. Inulin space in proximal jejunum of sheep.

TABLE 7

Methionine and Lysine Distribution Ratios from Proximal Jejunum of Sheep Incubated Under an Atmosphere of Nitrogen[1]

Time[2]	Methionine Distribution Ratio	Lysine Distribution Ratio
10	.39±.08	.54±.05
20	.75±.08	.88±.13
30	.85±.08	.87±.13
40	.92±.08	1.14±.10
60	1.40±.28	1.06±.17

[1]Mean ± standard error of 6 determinations at each time period.

[2]Minutes of incubation.

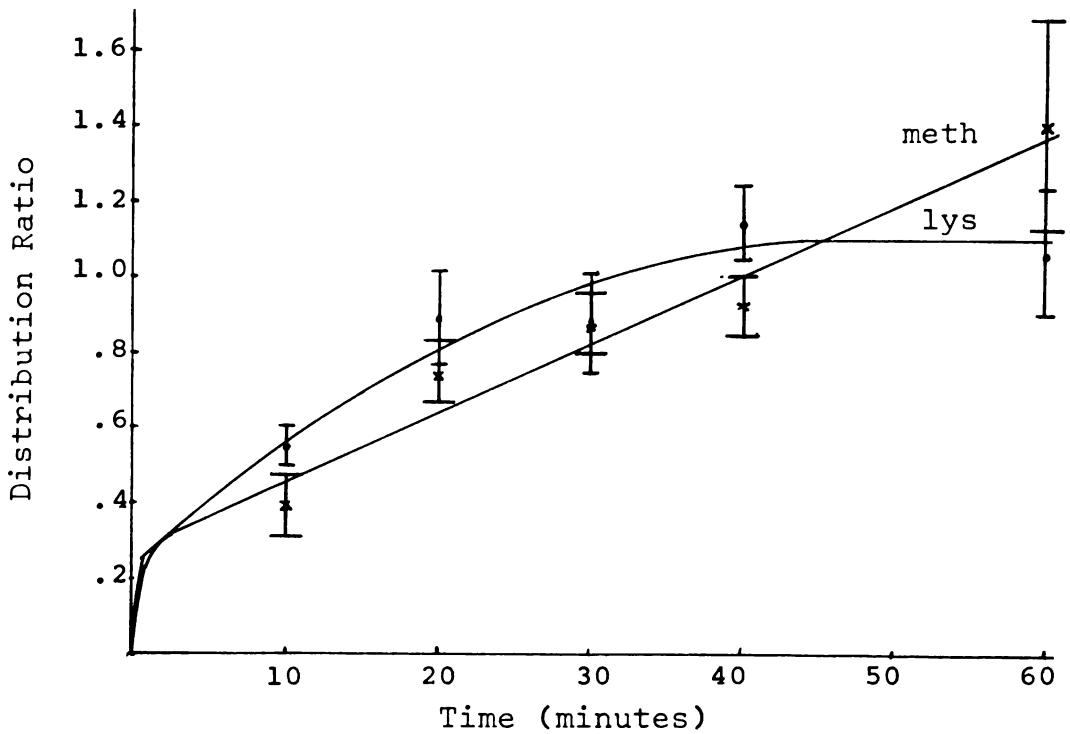

Figure 2. Changes in distribution ratio of lysine (I) and methionine (X) in ovine proximal jejunum during a 60 minute incubation under nitrogen.

TABLE 8

Methionine, Lysine and Glycine Distribution Ratios from Proximal Jejunum of Sheep Incubated in an Atmosphere of Oxygen[1]

Time[2]	Methionine Distribution Ratio	Lysine Distribution Ratio	Glycine Distribution Ratio
10	2.63±.06	2.04±.11	.81±.08
20	6.28±.06	2.35±.23	1.50±.09
30	7.71±.33	4.57±.38	2.03±.04
40	7.15±.10	3.58±.29	2.50±.03
60	7.79±1.0	5.0 ±.42	2.56±.30

[1]Mean ± standard error of 12 determinations at each time period.

[2]Minutes of incubation.

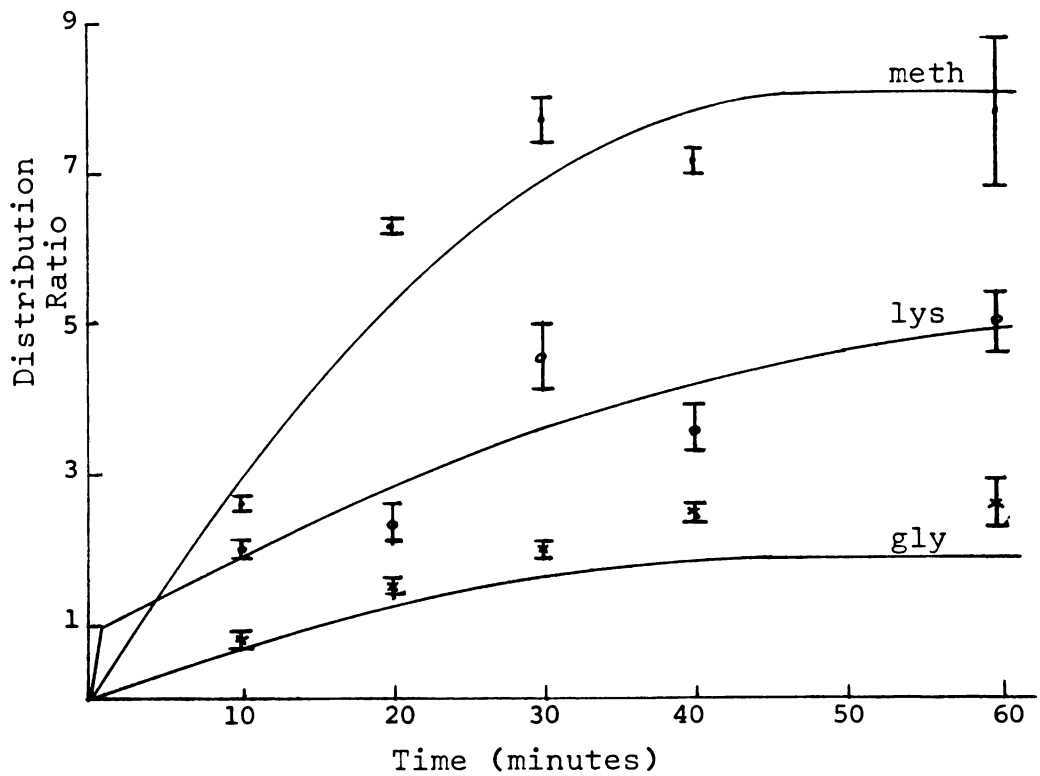

Figure 3. Changes in distribution ratio of methionine (⊡), lysine (⊕) and glycine (⊠) in ovine proximal jejunum during a 60 minute incubation under oxygen.

methionine reached an apparent steady state after 40 minutes of incubation and remained constant for the remainder of the incubation. Lysine accumulation decreased from the initial rate but did not reach a steady state during the incubation period.

The kinetic constants, Km and Vmax, presented in Table 9 were determined for each amino acid by a Lineweaver-Burk plot (Figure 4). Of the two neutral amino acids, glycine had much less affinity for the transporter than did methionine as indicated by the larger Km value for glycine; however, glycine had a much greater capacity to be transported than did methionine as indicated by the larger Vmax value for glycine. The basic amino acid lysine exhibited a great affinity for its carrier but showed a very small capacity for transport.

The results of leucine competition on the uptake of lysine are presented in Table 10. The addition of 1, 3 or 4 mM leucine significantly decreased ($P<.05$) the uptake of 1 mM lysine by approximately 25% in all cases. When lysine concentrations were increased to 5 mM, the addition of 5 mM leucine caused a significant ($P<.005$) decrease in uptake of 24%. Leucine concentrations of 15 and 20 mM increased the inhibition of 5 mM lysine uptake to 49 and 48%, respectively. The extent of inhibition was significantly greater ($P<.005$) than that observed when using 5 mM leucine.

TABLE 9

Kinetic Constants of Transport Systems in the Proximal Jejunum of Sheep[1]

Amino Acid	Km	Vmax (10^{-3})
Glycine	33.30	670.00
Methionine	2.43	5.80
Lysine	1.52	.87

[1]Units for Km are millimolar and units for Vmax are μmoles of A.A./100 mg of wet tissue/.5 hr.

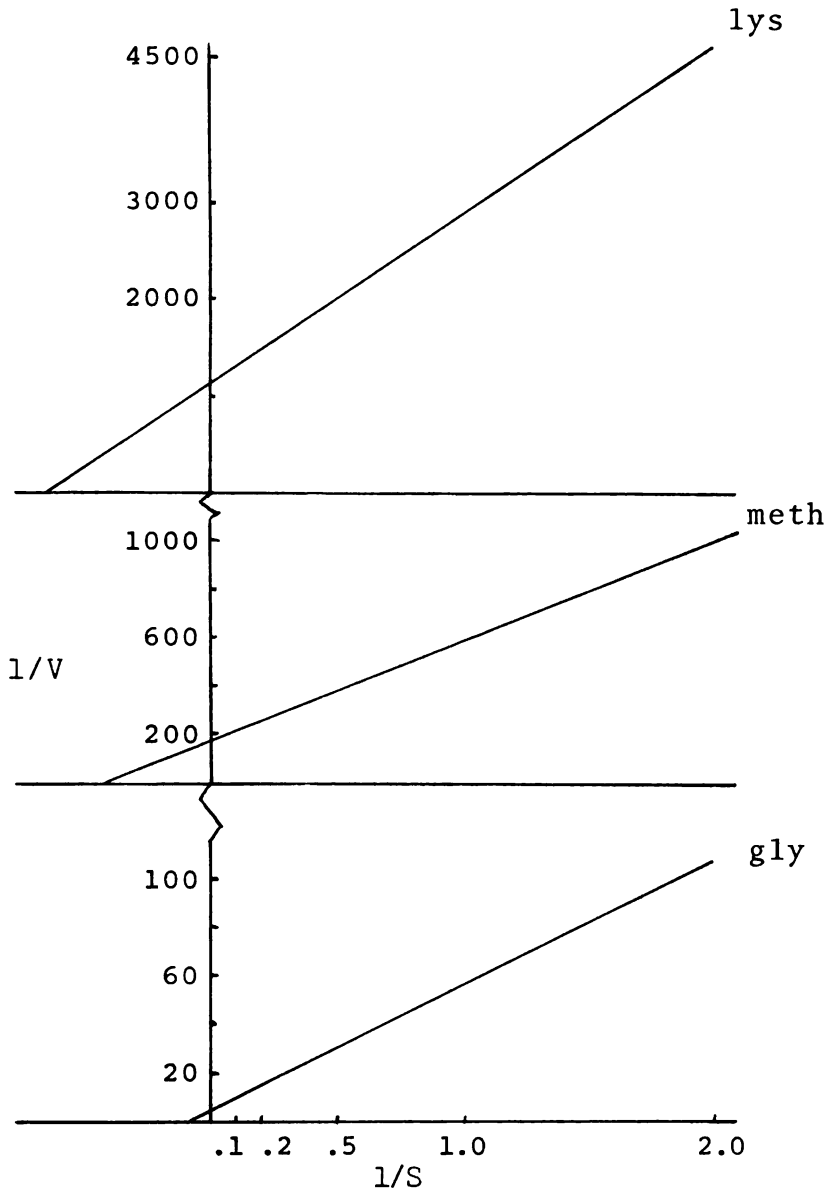

Figure 4. Lineweaver-Burk plots for glycine, methionine and lysine transport in proximal jejunum of sheep.

TABLE 10

Effect of Leucine on the Uptake of Lysine in Proximal Jejunum of Sheep

Amino Acid	Addition	Lysine Uptake[1]	% Decrease
Lys 1 mM	None	401±38[2]	--
Lys 1 mM	Leu 1 mM	303±23[3]	24
Lys 1 mM	Leu 3 mM	299±23[3]	25
Lys 1 mM	Leu 4 mM	301±27[3]	25
Lys 5 mM	None	838±57[4]	--
Lys 5 mM	Leu 5 mM	637±27[5]	24
Lys 5 mM	Leu 15 mM	430±11[6]	49
Lys 5 mM	Leu 20 mM	439±11[6]	48

[1](μmole lysine/100 mg wet tissue/.5 hr) x 10^{-6}.

[2,3]Means not sharing common superscripts are significantly different ($P<.05$).

[4,5,6]Means not sharing common superscripts are significantly different ($P<.005$).

Experiment 2

Differences in body weights of rats fed EFA deficient and normal diets are shown in Table 11. The body weights of rats on the normal diet were significantly greater after 6 ($P<.05$) and 12 ($P<.001$) weeks of feeding time than body weights of rats on the EFA deficient diet. By 12-14 weeks of feeding time, symptoms of EFA deficiency such as dullness of hair coat, loss of hair, scaliness of the skin and skin lesions of the front and hind paws were visible. In a few of the more severly effected animals, the tip of the tail was broken off. With the appearance of these symptoms, the animals were assumed to be EFA deficient and the animals slaughtered for the experimental gut tissue samples.

The fatty acid composition of phospholipids isolated from the small intestinal mucosa of EFA deficient and normal rats is presented in Table 12. Phospholipids isolated from rats on the EFA deficient diet had lower levels of palmitoleic (16:1), oleic (18:1) and arachidonic (20:4) but higher levels of linoleic (18:2) and eicosatrienoic (20:3) acids than did phospholipids from normal rats. Palmitic (16:0) and stearic (18:0) acids were not different in EFA deficient or normal rats.

Total tissue water and dry matter of rings of rat jejunum from animals on both the EFA deficient and normal diets are presented in Table 13. Total dry matter for EFA deficient and normal rats at the initiation and after 60

TABLE 11

Dietary Effects on Rat Body Weight[1]

Feeding Period	EFA Deficient	Normal
6 weeks	164.3±1.89[2]	171.7±2.3[3]
12 weeks	260.5±2.82[4]	300.0±2.14[5]

[1]Body weight in grams. Mean ± standard error for 44 animals.

[2,3]Values on the same line not sharing common superscripts differ significantly ($P<.05$).

[4,5]Values on the same line not sharing common superscripts differ significantly ($P<.001$).

TABLE 12

Fatty Acid Composition of Phospholipids Isolated from Jejunal Mucosa of EFA Deficient and Normal Rats

	% of Total F.A.	
F.A.	EFA Def.	Normal
16:0	32.62	30.16
16:1	5.03	13.37
18:0	6.52	7.87
18:1	20.85	35.55
18:2	29.79	5.73
20:3	10.35	-----
20:4	-----	7.31

TABLE 13

Total Dry Matter and Tissue Water of Jejunum[1] From Rats Fed an EFA Deficient or Normal Diet

Time[2]	% Total Dry Matter		% Total Tissue Water	
	EFA Deficient	Normal	EFA Deficient	Normal
0	19.58±.76	19.21±.24	80.42±.76	80.79±.24
10	18.31±.42	18.74±.35	81.69±.42	81.26±.35
20	17.00±.93	17.68±.58	83.00±.93	82.32±.58
30	15.13±.45	18.48±.43	84.87±.45	81.52±.43
40	16.33±.28	16.21±.73	83.67±.28	83.79±.73
60	17.85±.97	16.59±.47	82.15±.97	83.41±.47

[1]Mean ± standard error of 6 determinations on 2 rats per diet.

[2]Minutes of incubation.

minutes of incubation ranged from 19 to 17% and 19 to 16% respectively. Total tissue water for EFA deficient and normal rats at the initiation and after 60 minutes of incubation ranged from 80 to 82% and 80 to 83% respectively. No significant differences in tissue water or dry matter due to diet were found.

Dietary effects on the extracellular space of rat intestine are shown in Table 14. Inulin space (% of wet weight) for EFA deficient rats at initiation and after 60 minutes of incubation increased from 2.78 to 16.38 and respective values for normal rats were 3.0 and 12.42. After 60 minutes of incubation, inulin space for EFA deficient rats was significantly ($P<.05$) greater than inulin space for rats on the normal diet. The pattern of inulin equilibration with the tissue as affected by diet can be seen in Figure 5. Inulin reached an equilibration with the intestinal tissue of rats on the normal diet after 30 to 40 minutes and remained constant for the rest of the incubation. With tissue from EFA deficient rats, inulin did not reach an equilibration, increasing instead throughout the incubation period.

The DR for methionine and lysine at 5 mM from EFA deficient and normal rats are presented in Table 15. Methionine distribution ratios for EFA deficient animals after 10 and 60 minutes of incubation were .92 and 1.37 while respective values for normal animals were .85 and 5.82. The ratio reached after 60 minutes of incubation using intestine from

TABLE 14

Extracellular Space of Rat Jejunum from Animals Fed an EFA Deficient or Normal Diet

Time[1]	Ml of Inulin Space[2]	
	EFA Deficient	Normal
0	2.78	3.00
10	8.60	9.48
20	10.86	12.42
30	11.61	11.95
40	14.18	12.72
60	16.38[3]	12.42[4]

[1]Minutes of incubation.

[2]Percent of wet tissue weight.

[3,4]Values on the same line not sharing common superscripts differ significantly ($P<.05$). Only 60 minute values were tested for significance.

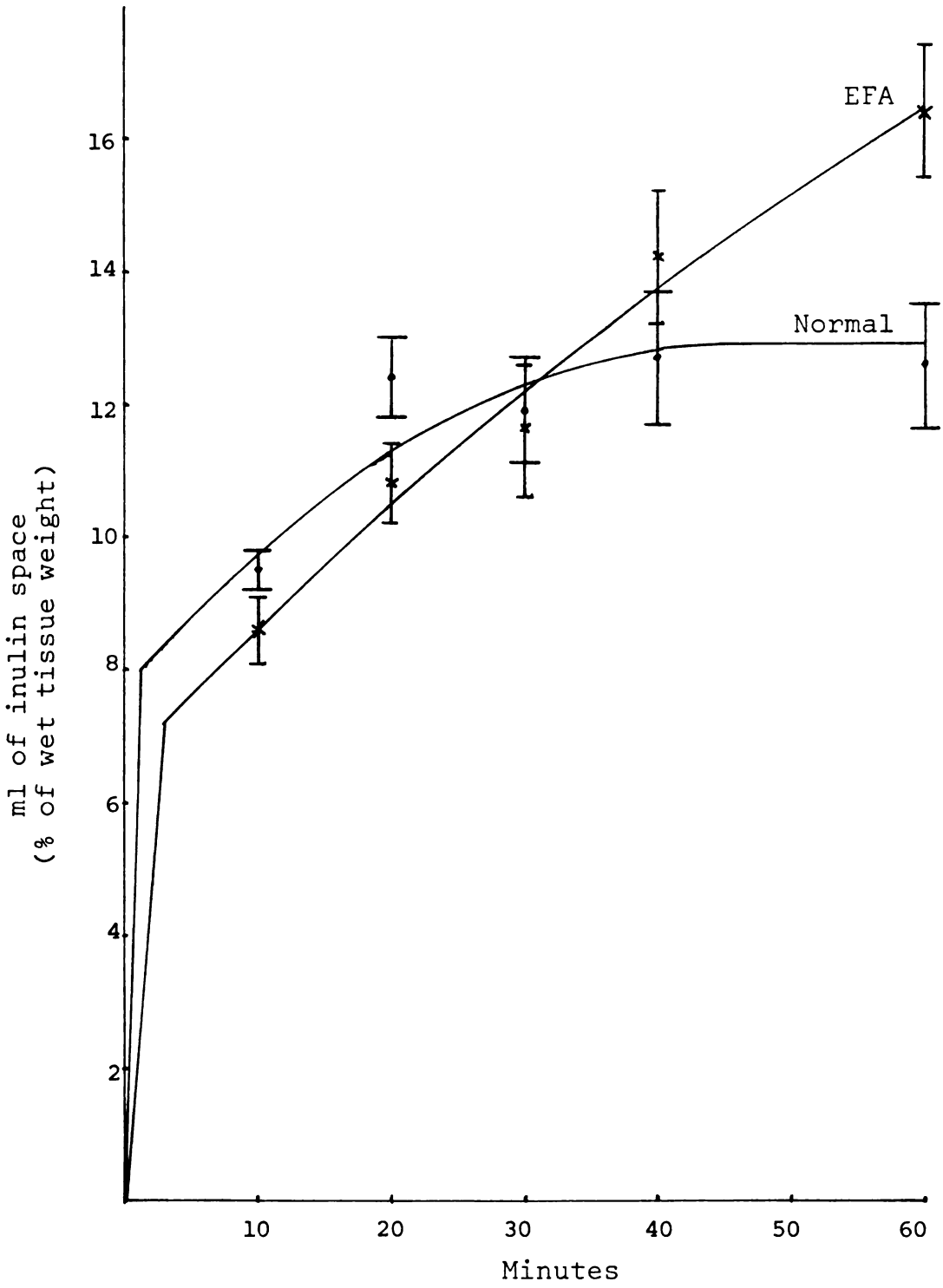

Figure 5. Inulin space in jejunum of rats fed an EFA deficient (⧗) or normal (I) diet during a 60 minute incubation.

TABLE 15

Methionine and Lysine Distribution Ratios in Jejunum of Rats Fed an EFA Deficient, Normal or Commercial Diet[1]

Time[2]	Methionine Distribution Ratios		Lysine Distribution Ratios		
	EFA Deficient	Normal	EFA Deficient	Normal	Commercial[3]
10	.92±.08	.85±.05	.30±.03	.43±.01	.51±.03
20	1.08±.06	1.12±.04	.56±.01	.66±.02	.96±.14
30	1.34±.12	2.85±.86	.68±.01	.82±.03	-------
40	1.30±.03	5.60±.60	.86±.07	.87±.01	1.32±.09
60	1.37±.06[4]	5.82±.02[5]	1.02±.01[6]	1.06±.02[6]	2.07±.11[7]

[1]Mean ± standard error of 12 determinations at each time period. Only DR determined after a 60 minute incubation were tested for significance.

[2]Minutes of incubation.

[3]Composition listed in Appendix Table 1.

[4,5]Values on the same line not sharing common superscripts differ significantly ($P<.01$).

[6,7]Values on the same line not sharing common superscripts differ significantly ($P<.05$).

rats on the normal diet was significantly greater ($P<.01$) than the 60 minute ratio for EFA deficient animals. Lysine DR at 5 mM for EFA deficient animals after 10 and 60 minutes of incubation were .30 and 1.02. Respective values for normal-fed animals were .43 and 1.06 and .51 and 2.07 for animals on a commercial diet. No significant difference was found between the 60 minute ratios for animals on the EFA deficient or normal diets; however, 60 minute ratios for animals on the commercial diet were significantly greater ($P<.05$) than the other two. The pattern of methionine and lysine distribution for the intestinal rings can be seen in Figures 6 and 7 respectively. After 2 or 3 minutes, intracellular methionine accumulation plateaued in intestine from EFA deficient animals and remained relatively constant for the rest of the incubation period. Methionine accumulation in intestine from normal animals increased for the entire incubation period. Lysine accumulation in intestine from commercial, EFA deficient or normal fed rats occurred continuously during the 60 minutes of incubation; however, the rate of lysine accumulation was much greater for intestine from the commercial than from EFA deficient or normal-fed rats.

The kinetic constants, Km and Vmax (Table 16), were determined for methionine and lysine within diets by use of a Lineweaver-Burk plot (Figures 8 and 9). A trend toward decreased carrier affinity for the amino acid as indicated

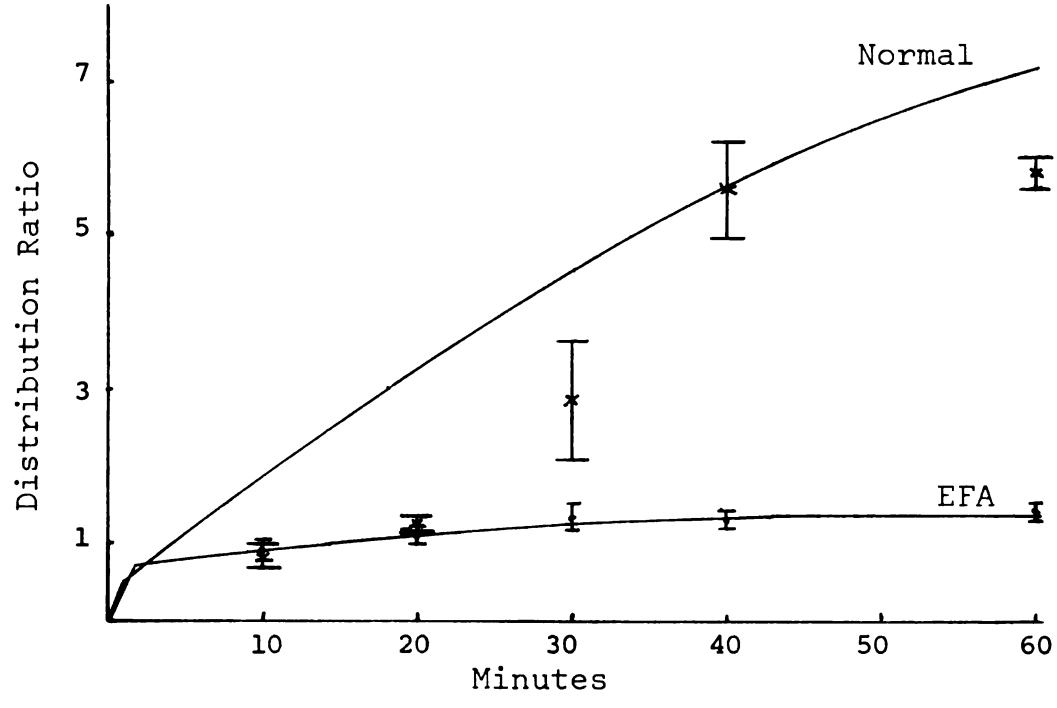

Figure 6. Changes in methionine distribution ratio in jejunum of rats fed an EFA deficient (I) or normal diet (⊛) during a 60 minute incubation.

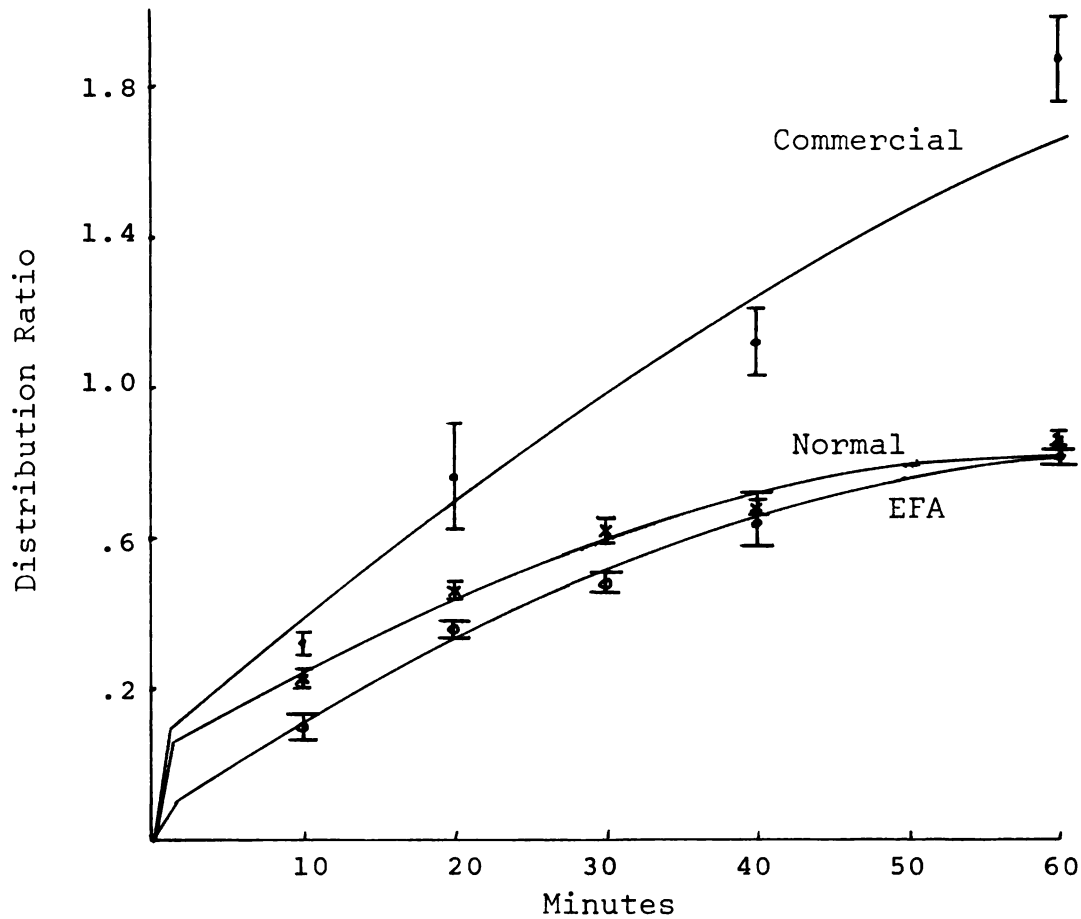

Figure 7. Changes in lysine distribution ratio in jejunum of rats fed an EFA deficient (⊕), normal (✱) or commercial (I) diet during a 60 minute incubation.

TABLE 16

Kinetic Constants of Methionine and Lysine Transport Systems in Jejunum of Rats Fed an EFA Deficient or Normal Diet

Amino Acid	Diet	Km[1]	Vmax (10^{-3})[2]
Methionine	EFA Def	8.69	21.2
Methionine	Normal	6.89	16.6
Lysine	EFA Def	11.10	2.0
Lysine	Normal	7.14	1.4

[1]Units for Km are millimolar.

[2]Units for Vmax are µMoles of A.A./100 mg wet tissue/.5 hr.

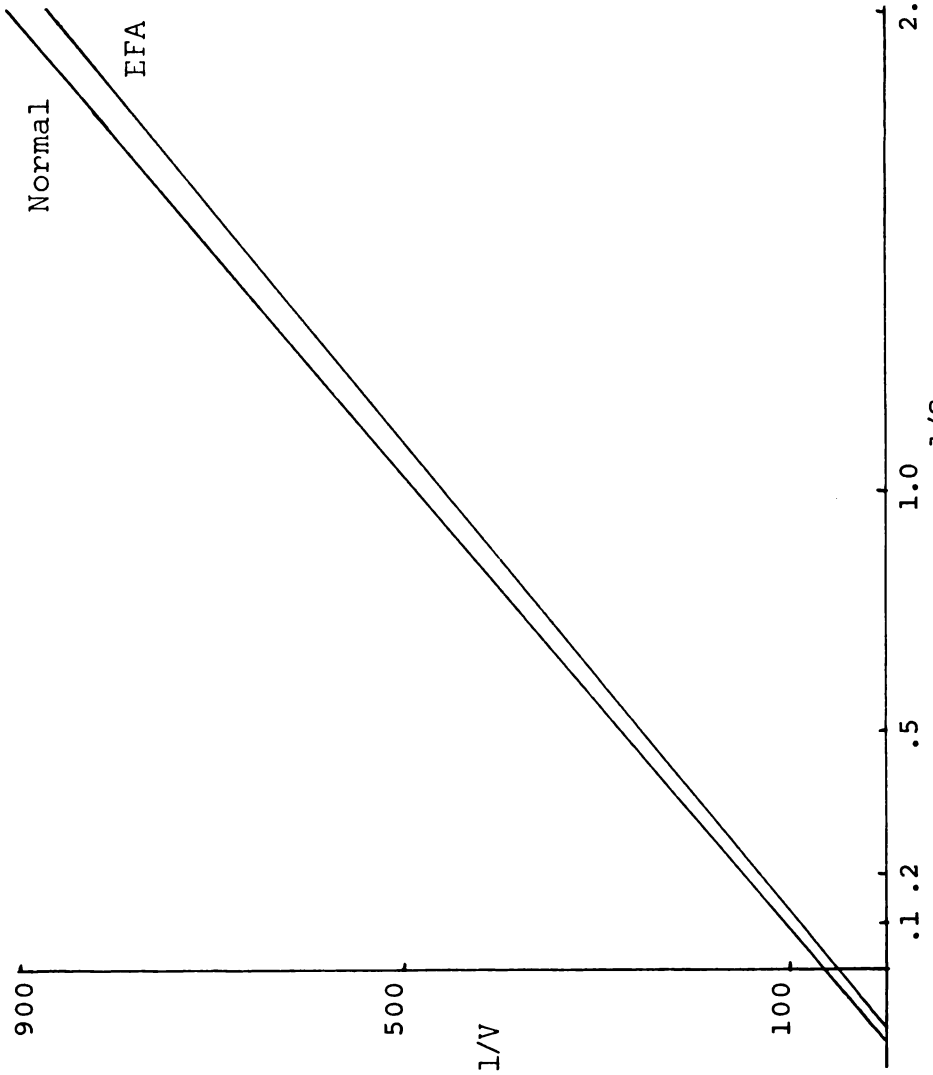

Figure 8. Lineweaver-Burk plot of methionine transport in jejunum of rats fed an EFA deficient or normal diet.

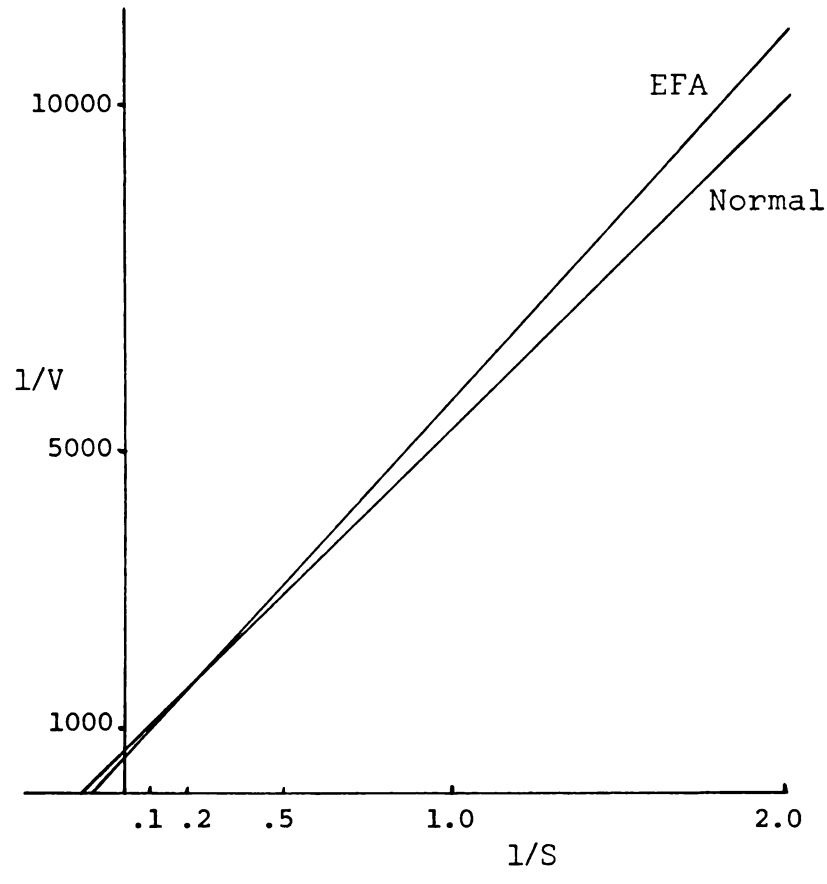

Figure 9. Lineweaver-Burk plot of lysine transport in jejunum of rats fed an EFA deficient or normal diet.

by increased Km was observed in the deficiency state; however, differences were nonsignificant.

Lineweaver-Burk plots of methionine-glucose transport interactions for intestinal rings from EFA deficient and normal animals are depicted in Figures 10 and 11. In both types of intestinal rings significant differences between the intercepts on the Y axis were not found, thus for each graph all lines have a common Y intercept (1/Vmax). The kinetic constants, Km and Vmax, of methionine uptake in the presence of glucose by rat small intestine are presented in Table 17. The EFA deficiency did not alter the form of glucose-methionine transport interactions as glucose was observed to be a competitive inhibitor of methionine transport in the intestine for both dietary groups.

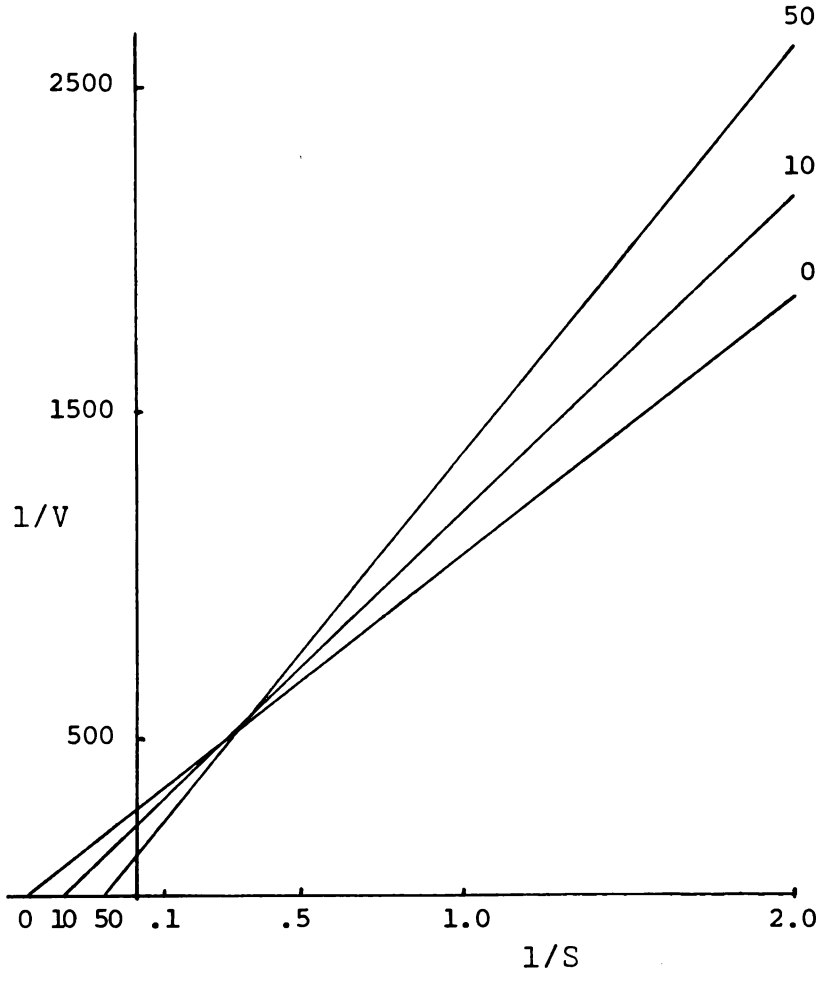

Figure 10. Lineweaver-Burk plot of methionine-glucose transport interactions in jejunum of rats fed an EFA deficient diet.

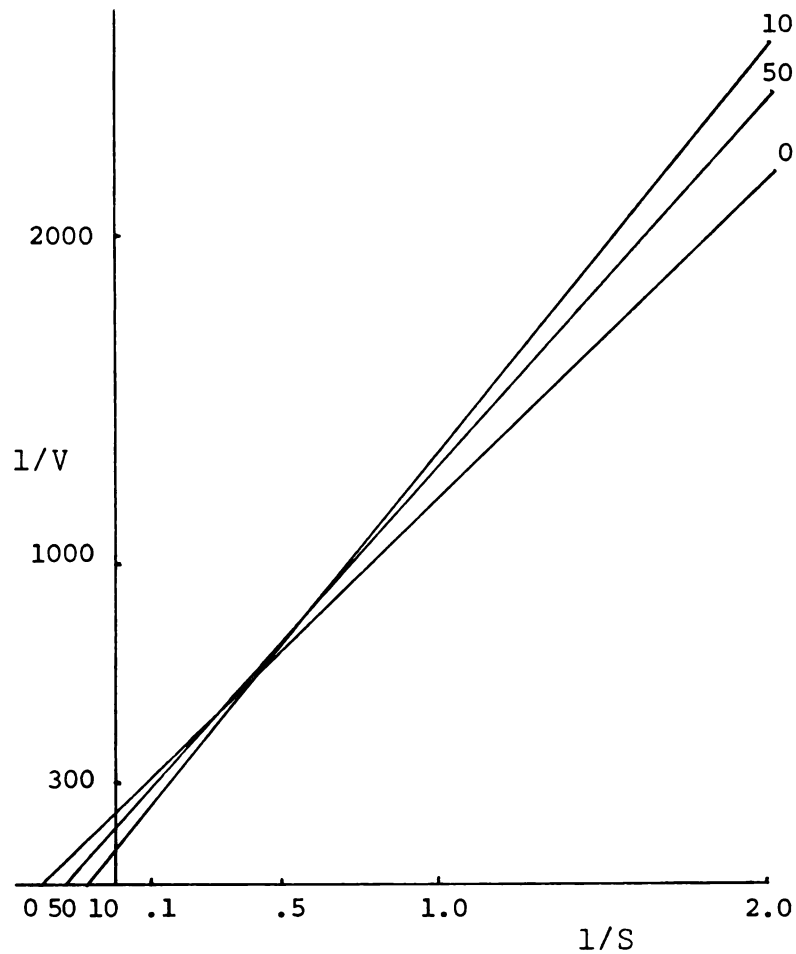

Figure 11. Lineweaver-Burk plot of methionine-glucose transport interactions in jejunum of rats fed a normal diet.

TABLE 17

Effect of Glucose on the Kinetic Constants of Methionine Uptake in Jejunum of Rats Fed an EFA Deficient or Normal Diet

A.A.	Diet	Addition	Km[1]	Vmax $(10^{-3})^2$
Meth	EFA	None	2.86	3.57
Meth	EFA	10 mM glu	3.85	4.00
Meth	EFA	50 mM glu	7.69	6.25
Meth	Normal	None	3.85	3.85
Meth	Normal	10 mM glu	9.09	7.14
Meth	Normal	50 mM glu	6.67	5.88

[1]Units for Km are millimolar.

[2]Units for Vmax are µmoles/100 mg wet tissue/.5 hr.

DISCUSSION

Experiment 1

Results of this study indicate that distal segments of sheep small intestine possess a greater capacity for uptake of 5 mM lysine than do proximal segments. This observation is in agreement with a report by Nathans et al. (1960) who observed the highest uptake of monoiodotyrosine in terminal ileum of rat small intestine; however, the data are in general disagreement with results of studies from other species. Maximum transport of sugars (Crane and Mandelstam, 1960), L-tryptophan (Spencer and Samiy, 1960), L-phenylalanine (Samiy and Spencer, 1961; Spencer and Samiy, 1961) and L-proline (Spencer and Brody, 1964) have been obtained in segments of mid-gut from hamster small intestine. Larsen et al. (1964) and Baker and George (1971), studying uptake of neutral and basic amino acids in rat small intestine, observed maximum uptake of each amino acid to occur in mid-gut. This report may reflect nothing more than a species difference in the intestinal uptake of 5 mM lysine; however, assuming that other basic amino acids will have the same pattern of uptake, this finding could reflect a more efficient

functioning of the basic amino acid carrier in sheep. It may be suggested from these results that as the amino acid nears the end of the absorptive area, the capacity for its transport is greatly increased.

In vitro uptake of amino acids can be affected by changes in the water composition of the tissue. A change in total tissue water or compartmentation of tissue water can change the effective area with which the accumulating amino acid has to equilibrate. As a consequence a true rate of uptake or distribution ratios under such circumstances cannot be determined. No significant effects of incubation time on total tissue water and distribution were observed in this study. Previous workers have reached similar conclusions (Kipnis and Cori, 1957).

In comparison studies with inulin, sucrose, dulcitol and mannitol in rat kidney cortex (Rosenberg et al., 1962) and inulin and mannitol in rat small intestine (Jackson et al., 1970), only inulin was found to be completely confined to the extracellular space with no leakage into the intracellular fluid. In the present study, the rate of inulin accumulation decreased with time and reached a steady state by 60 minutes of incubation. This finding suggests that inulin did not penetrate the intracellular fluid. Thus the fact that total tissue water did not change and that the intracellular compartment was measured with a high degree of accuracy points to the conclusion that the determined

rates of amino acid uptake and distribution ratios in this study reflect actual physiological situations.

Results from early reports on sugar and amino acid active transport in rat and hamster small intestine led to the conclusion that active transport is dependent on energy derived solely from respiration (Wilson and Wiseman, 1954; Finch and Hird, 1960a; Crane and Mandelstam, 1960; Newey and Smyth, 1962). Although anaerobiosis (nitrogen atmosphere) led to a distinct decrease in the magnitude of methionine and lysine distribution ratios in this study, complete cessation of the active transport of methionine was not accomplished. Recent evidence by De La Noüe (1970) indicated that the methionine component of the neutral amino acid transport system in rat small intestine can draw energy for transport from both aerobic and anaerobic pathways. The methionine transport system in sheep small intestine appears to have the same capability.

Studies by Larsen et al. (1964), to determine the apparent Km values of amino acid active transport systems in rat small intestine have led to the conclusion that the order of amino acid carrier affinity for rat intestine is lysine > methionine > glycine. Examination of the Km values determined for these amino acids in sheep intestine indicates the same order of affinity as above. If we assume saturating concentrations of methionine and lysine but not glycine to be present in the intestine, the kinetic constants (Km and Vmax) indicate that rate of transport would be of

the order methionine > lysine > glycine for sheep intestine. This order is in agreement with the report by Williams (1969), who was studying relative rates of amino acid absorption in sheep small intestine. The present study is also in agreement with the report of Delhumeau et al. (1962), who studied relative rates of amino acid absorption in the small intestine of rats.

A lack of absolute specificity for amino acid binding between neutral and basic amino acid transport systems in the small intestine is known to occur in the hamster (Hagihira et al., 1961) and rat (Larsen et al., 1964; Neame, 1966; Reiser and Christiansen, 1969). Results of Hume et al. (1972), who studied the effects of infusing leucine at 10 and 30 grams per day on the net absorption of other amino acids in sheep small intestine, indicate that only lysine was decreased in net absorption. Results of leucine-lysine transport interactions observed in the present study indicate that at saturating amino acid concentrations, leucine is able to decrease lysine transport by almost one-half. The present results, as well as those of Hume et al. (1972), suggest that leucine and lysine may compete for transport in the small intestine of sheep.

The transport systems of sheep intestine appear to be very similar to those of the rat. Methionine transport in sheep intestine appears able to use energy from glycolysis as well as respiration linked ATP genesis. The relative order of amino acid affinity as well as rates of transport

in sheep intestine appear the same as in the rat. Neutral and basic amino acids may share the same carrier in sheep intestine as well as the small intestine of the rat.

DISCUSSION

Experiment 2

The work of Burr and Burr (1929) first established the need for polyunsaturated fatty acids in the diet of rats. Subsequent studies have indicated that other mammalian species also have a dietary requirement for these acids. These polyunsaturated acids are linoleic (18:2), linolenic (18:3) and arachidonic (20:4) acids and are commonly termed as the essential fatty acids. EFA are contained in most all lipid fractions in the body; however, phospholipids tend to be high in EFA content. EFA appear to have structural functions as both cell and mitochondrial membranes are rich in the EFA containing phospholipids. The mammalian system lacks the capacity to synthesize linoleic and linolenic acids (Lehninger, 1970) and arachidonic acid must be synthesized from dietary linoleic acid (Holman, 1964). Thus a decrease in tissue content of each of the acids would be expected in an essential fatty acid deficiency. Studies of Hayashida and Portman (1960) with total lipid from rat liver mitochondria, Enser and Bartley (1962) and Yurkowski and Walker (1970) with total lipid of rat intestinal mucosa indicate that a decrease in

linoleic and arachidonic acids with an increase in eicosatrienoic acid is observed in an essential fatty acid deficiency state. The present study is in partial agreement with the above work as a decrease in arachidonic and increase in eicosatrienoic acid was observed in phospholipids of intestinal mucosa; however, the increase in linoleic acid observed here was not seen in previous work. The increase in linoleic acid may be explained by the presence of some unknown peak appearing on the chromatograph of EFA deficient samples in the same area as linoleate; however, no evidence to support this explanation can be given. Although all changes in fatty acid composition observed in this study were not as expected, based on differences in body weights and other physical symptoms, there appears little doubt that the animals were in an essential fatty acid deficiency state.

Total water of the intestinal tissue was not affected by the deficiency; however, extracellular space measurements were significantly increased. In view of the mitochondrial membrane alterations produced by EFA deficiency and observed by Snipes (1968) and Ito and Johnson (1968), the increase in extracellular space may be explained most readily by an alteration in the epithelial cell membrane produced by the EFA deficiency. Due to the large molecular weight, inulin is normally excluded from the intracellular space by the cellular membrane; however, examination of Figure 5 reveals a continual diffusion of inulin into the EFA deficient intestinal rings but not into rings from rats on the normal diet.

Rosenberg et al. (1962), in a comparison of inulin, sucrose, dulcitol and mannitol for their ability to mark the extracellular space of rat kidney, have interpreted the continual uptake of marker by tissue during the incubation to represent a leakage of the marker into the intracellular space. In view of this interpretation and knowing that inulin should not pass the cell membrane, the present study suggests that the EFA deficiency produced a membrane alteration allowing abnormal passage of inulin through the cell membrane.

In this study it was assumed that membrane alterations sufficient to affect amino acid uptake would be manifested by changes in the kinetic constants of transport (Ling and Morin, 1971). Although there was a trend for a decreased apparent carrier affinity of both methionine and lysine during EFA deficiency, no significant effect of diet on kinetic constants were found. The 60 minute distribution ratio for methionine in normal tissue was significantly greater ($P<.01$) than the 60 minute ratio in EFA deficient tissue. Although the data do not totally rule out a decrease in amino acid uptake, the abnormal passage of inulin through the cell membrane and the lack of dietary effect on kinetic constants of methionine and lysine transport suggest that differences in methionine distribution ratios between EFA deficient and normal rings may best be explained by an increase in amino acid efflux rather than a decrease in amino acid influx. The lack of dietary effect on kinetic constants of methionine and lysine transport suggests that the efflux

is non-carrier mediated, due only to the inability of the cell membrane to retain the amino acid. No valid conclusions concerning dietary effects on lysine distribution ratios can be reached due to the fact that the 60 minute distribution ratios for EFA deficient and normal rings were not significantly different. Lysine distribution ratios determined with rings from normal rats appeared to be abnormally low as compared to ratios determined with rings from rats on a commercial diet and thus may account for the lack of significance between 60 minute distribution ratios for EFA deficient and normal rings. Assuming that both the neutral and basic transport systems functioned in the same manner, they were not expected to react differently to membrane alterations induced by an EFA deficiency. No explanation for the lack of dietary effect on the basic transport system, other than a masking effect by the abnormally low ratios in normal rings, will be attempted.

Methionine uptake at .5, 1.0, 2.0 and 10.0 mM in the presence of 10 and 50 mM glucose was studied in intestinal rings from both EFA deficient and normal rats in an effort to provide information on sugar-amino acid transport interactions. It was hoped that the EFA deficiency would indicate the level of sugar-amino acid interaction by producing a difference in the glucose-methionine interaction in deficient and normal tissue. However, glucose was observed to be a competitive inhibitor of intestinal methionine uptake in both dietary groups. Since any membrane alteration that may have

been induced by the EFA deficiency apparently effected amino acid transport at the level of efflux and not at the steps of influx or energy input, these results give no further information on the site of sugar-amino acid transport interactions.

GENERAL CONCLUSIONS

Experiment 1

1. Uptake of 5 mM lysine in sheep was affected by section of the intestine; uptake increased as distance from the pylorus increased.

2. Length of incubation had no effect on any tissue water measurements and inulin appeared to give accurate measurements of extracellular space in sheep intestine.

3. Sheep intestinal methionine and lysine transport were largely dependent on respiration derived ATP for energy; however, the methionine system appears to be able to derive some energy for transport from glycolysis.

4. The basic and neutral carrier systems appear to operate in the same manner as those in the monogastric animal.

5. The basic transport system in sheep intestine is not totally specific for basic amino acids as leucine can interfere with the transport of lysine.

Experiment 2

1. Based on weight changes, physical symptoms and the increase in eicosatrienoic and decrease in arachidonic acids, an essential fatty acid deficiency was developed in the rats.

2. Although the deficiency did not affect total tissue water, extracellular space measurements in rat intestine were increased due to an apparent abnormal passage of inulin through the cell membrane.

3. The decrease in rat intestinal concentrating ability for methionine and possibly lysine appears to be due to an increased amino acid efflux.

4. Glucose was observed to be a competitive inhibitor of methionine uptake in rat intestinal tissue. The EFA deficiency did not modify the interaction between glucose-methionine uptake; therefore, no conclusion as to the site of sugar-amino acid interaction could be reached.

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APPENDIX TABLES

APPENDIX

TABLE 1

Composition of Commercial Diet for Rats

Ingredient	Percent of Total Diet
Ground Shelled Corn	48.0
Soybean Meal (49% C.P.)	17.5
Fishmeal	10.0
Dehydrated Alfalfa Meal	5.0
Dried Skim Milk	10.0
Sucrose	5.0
Corn Oil	3.0
Salt	.5
M.S.U. Vitamin Premix	1.0

APPENDIX

TABLE 2

The "t" Test Statistic Used in this Study

$$t = \frac{\hat{Y}_1 - \hat{Y}_2}{S\hat{y}_1 - \hat{y}_2}$$

Where

$\hat{Y}_1$ = $1/Vmax_1$, $\hat{Y}_2$ = $1/Vmax_2$

$$S\hat{y}_1 - \hat{y}_2 = \sqrt{S^2 \left(2/n + \frac{(X_1 - \bar{X}_1)^2}{\Sigma X_1^2} + \frac{(X_1 - \bar{X}_2)^2}{\Sigma X_2^2}\right.}$$

S^2 = average error variance of the two regression lines

n = total number of observations per regression line

X_1 = X_2 = values on the abscissa

APPENDIX

TABLE 3

Inverse Predictions in Simple Linear Regression
Confidence Intervals for Values of Km
Determined in this Study

$$\left.\begin{matrix} L \\ U \end{matrix}\right\} = \bar{X} + \frac{b_1(Y_o-\bar{Y})}{D} \pm \frac{tS_E}{D} \sqrt{B(Y_o-\bar{Y})^2 + D\left(\frac{n+1}{n}\right)}$$

Where

$\bar{X}$ = mean of all values on the abscissa

b_1 = slope of each regression line

Y_o = the observed value of Y for which the estimated value of X is made, in this study Y_o is always = zero

$\bar{Y}$ = mean of observed Y values for each regression line

$D = b_1^2 - t^2 S^2_E B$

t = the appropriate critical value taken from a t table

S^2_E = error variance

$B = 1/\Sigma X^2$

n = total number of observations per regression line