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ABSTRACT

The ruminant with its extensive synthesis of amino acids by symbiotic microorganisms presents unique problems to studies of amino acid nutrition. Protein quality is dependent upon the available amino acids leaving the rumen, rather than those in the ingested diet. Protected proteins or amino acids may be fed to the animals or alternatively nitrogenous compounds may be administered postruminally to study ruminant amino acid nutrition. There is both a theoretical and practical interest in defining ruminant amino acid requirements under various production conditions. The relatively high content of the sulphur amino acids in ruminant products compared to that present in the rumen microorganisms suggests that these amino acids may be a limiting factor in ruminant production.

In this study the essential sulphur containing amino acid, D,L-methionine, was infused into the abomasum of two growing lambs. Graded levels of methionine were infused for three days at each level. A jugular blood sample was collected on the third day of each infusion level. Plasma free amino acids were determined for each infusion level. One lamb initially had an increase in plasma methionine concentration with each increase in the level of abomasal methionine. This indicates that methionine was not limiting for this lamb. For the other lamb an inflection point on the methionine response curve was observed just below 2.0 gm of infused abomasal methionine. This suggested that methionine may have been limiting for this lamb. One lamb developed diarrhoea at the higher infusion
levels so a comparison of the two lambs was not permitted.

Taurine increased with increasing methionine infusions and cystine rose to a constant level. At this infusion level the non-essential amino acids – serine, glycine and alanine – fell to constant levels. This suggests that methionine conversion to cystine was impaired due to a decreased concentration of serine.

Generally, high methionine concentrations depressed the plasma concentrations of most amino acids.

A tracer dose of $^{35}$S-L-methionine was injected into a jugular vein of one lamb at levels below and above the limiting methionine infusion. Serial jugular blood samples were collected at various intervals to 24 hour post-injection. Total urine was collected once daily for four days post-injection. The radioactivity associated with the plasma proteins and other sulphur containing compounds of plasma were expressed as percentages. A plasma methionine specific activity curve indicated some kinetic parameters of plasma methionine.

When methionine was suspected of being limiting, all of the activity of plasma was in the plasma protein fraction after 24 hours. There was no detectable conversion of methionine sulphur to other sulphur containing compounds in deproteinized plasma. Urinary excretion of the administered label was 13% after four days.

During the infusion level when methionine was not limiting, only one half of the activity of plasma was in the protein fraction after 24 hours. At this level there was radioactivity in methionine, taurine, cystine, cystathionine and methionine
sulfoxides of free plasma. The urinary excretion of radioactivity after four days was one-half of the injected dose.

The plasma methionine kinetics indicated that the elevated methionine pool size was not due to an increase in total entry rate but due to a smaller increase in irreversible loss. This agrees favourably with impaired methionine metabolism.

It was concluded that a tracer dose of methionine gave a dynamic picture of methionine metabolism. Below its limiting level, methionine is mainly involved in anabolic processes. Above the level of limiting methionine, catabolic metabolism of methionine becomes increasingly important. Therefore, it is suggested that $^{35}$S-methionine tracer techniques may provide a relatively quick and reliable tool for evaluation of the sulphur amino acid nutrition of ruminants.
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INTRODUCTION

The microbial cells in the rumen have a modifying affect on the diet of the ruminant. The sources of nutrients available to the host animal include unaltered feed, microbial cells and microbial metabolites. Therefore, the classical methods used in nutritional experiments with the simple stomached animal may not be applicable for studies with the ruminant.

Methionine has been shown to be limiting for both sheep and cattle tissue synthesis. Changes in PAA patterns due to post-ruminal infusions are the techniques used for the estimation of the methionine requirement. Changes in PAA levels are modified by both the liver and muscle tissues. A relatively small change in PAA levels represents a much greater change in tissue levels. A dynamic picture of amino acid nutrition can be studied by using radioactive amino acids.

The object of this thesis is to study the effects of abomasally infused methionine in the growing lamb. The lambs will be fed a standard diet of hay and barley. Jugular blood samples will be collected at increasing levels of abomasal methionine infusions. The changes in the PAA profiles will be measured at the various methionine infusion levels. The plasma methionine response curve should indicate if methionine is limiting for these lambs. Another objective of this research is to obtain a dynamic picture of methionine metabolism by employing a radioactive tracer. Rather than using a carbon labelled tracer this study will use a tracer containing radioactive sulphur. Collection of the oxidative products in this
case will be from urine which will eliminate the problems of expired CO₂ collection. Some plasma parameters will be estimated from the plasma methionine specific activity curves. The incorporation of radioactivity into plasma proteins as well as other sulphur containing compounds of plasma should indicate the general metabolic fate of methionine. It is hoped that changes in the PAA profiles and the kinetics of the radioactive sulphur containing tracer will indicate the metabolic fate of methionine in the lamb.
I. SULPHUR IN RUMINANT NUTRITION

The amount and possible forms of plant sulphur that may ultimately be ingested by ruminant animals have been documented (Garrigus, 1970; Moir, 1970; and Playne, 1975). Information on sulphur content of feeds, on sulphur requirements of livestock and on sulphur metabolism in ruminants has been limited until recent years, largely because of problems and uncertainties in the determination of sulphur (Beaton et al., 1968). Of the total sulphur in plant material most is organic in composition (Moir, 1970). Sulphur concentrations in whole plant tops cannot be used to make reliable estimates of sulphur intake by animals grazing pastures because they graze selectively. Playne (1975) has shown that species, season and fertilization affect the sulphur content of plant material.

Fermentation in the rumen, on protein-rich diets, may lead to nitrogen and sulphur waste via the production and absorption of ammonia and sulphide. However, the synthesis of microbial protein from non-protein nitrogen and sulphur may enhance the nutritive value of poor quality diets.

In addition to the dietary sources of sulphur there is some recycling of sulphur within the body of the ruminant. Bray (1969) was able to demonstrate this phenomenon in sheep by intravenous injections of S-labelled sulphate followed by the recovery of the labelled sulphate in rumen. He showed that the labelled sulphate crossed the rumen wall from blood to the rumen. It was also shown that some sulphate is
recycled via the saliva. Moir (1970) reported a high correlation between levels of blood sulphate and salivary sulphate. Efficient recycling of sulphur through pancreatic and bile secretions also conserves sulphur in sheep (Bird, 1972).

The sulphate recycled to the rumen is subjected to reduction and is then available for protein synthesis, in much the same way as ammonia is released from recycled urea, thus providing a valuable conservation mechanism when feed is low in sulphur. Sulphide and sulphate form a recycling system that is in many ways similar to the ammonia-urea systems (Bray and Till, 1975). Even though sulphur is recycled to the reticulo-rumen the N:S ratio of recycled nitrogen to recycled sulphur may be very wide so that microbial growth is limited due to insufficient sulphur (Moir, 1970).

Kennedy et al. (1975) quantitated the recycling of sulphate to the rumen by employing radiotracer techniques. They concluded that sulphate recycling to the rumen of sheep is considerably less than that of cattle due mainly to relatively low concentration of inorganic sulphate in blood. They suggest that the difference in the inorganic sulphate in blood reflects the high sulphur content of wool in sheep.

The gut can be divided into two main systems in terms of sulphur metabolism in the ruminant. The first is the reticulo-rumen where microbes reduce dietary and recycled sulphur and convert this into microbial protein. The second major system is post-ruminal and concerns the overall process of digestion and absorption. Each system has an effect on the other and has its own nutrient requirement.
Rumen Sulphur Metabolism

Sulphur is required for microbial protein synthesis in the rumen (Bird, 1972). With respect to sulphur metabolism three groups of bacteria can be distinguished in the rumen. Those bacteria that cannot meet their own needs for reduced sulphur form the first group. At least two strains of Strep-tococcus bovis are unable to use sulphate or sulphite for growth (Bray and Till, 1975). The second group of microorganisms which reduce sulphate to the sulphide level and incorporate the sulphur into cellular materials without the production of any free detectable sulphides and are termed assimilatory sulphate-reducing microorganisms (Bray and Till, 1975). The initial activation of sulphate requires adenosine triphosphate (ATP) from which adenosine-5'-phospho-sulphate (APS) is formed. APS is then phosphorylated in the 3' position by ATP to yield 3'-phosphoadenosine-5'-phosphosulphate (PAPS), which is the activated form reduced to the sulphide level.

A third group of microorganisms utilizes sulphate as the terminal electron acceptor and produces massive amounts of hydrogen sulphide (H_2S), and these are termed dissimilatory sulphate reducing organisms. APS, rather than PAPS, is the activated form of sulphate reduced by these microorganisms (Siegel, 1975).

Dissimilatory sulphate reducers have been found in concentrations of 10^2-10^8/ml of rumen liquor (Huisingh, 1973; cited by Bray and Till, 1975). Huisingh has also shown that Desulfovibrio ruminus can reduce sulphate at the rate of 9.4 micromoles/hr/10^{10} cells.
Recently Merricks and Salsburg (1976) have shown that the rumen protozoa also have an effect on methionine-sulphur metabolism.

A compartmental model of sulphur metabolism in the rumen has been proposed by Bray and Till (1975). Sulphide is the central intermediate between the incoming sulphur from dietary and recycled sources and the outgoing sulphur made up of sulphur incorporated into microbial cells and other losses from the system. Recently Gawthorne and Nader (1976) have shown indirectly that methionine sulphur can be incorporated into microbial cells (43–48%) without entering the sulphide pool. Thus, the extent of direct incorporation of sulphur amino acids by rumen microorganisms appears to be greater than generally believed (Whanger, 1972).

In vitro and in vivo availability studies of different organic and inorganic forms of sulphur (Kahlon et al., 1975a and 1975b) have shown that they are all available in various proportions to the microbes for incorporation into microbial cells. L-methionine was best for fixation into microbial protein and the hydroxy analogs of methionine were poorly utilized. Lambs were fed semi-purified diets and the addition of any of the sulphur forms resulted in a faster rate of gain, increased consumption of dry matter and greater retention of both nitrogen and sulphur (Kahlon, et al., 1975b). Sulphur supplementation increased in vitro digestion of forage cellulose by rumen microorganisms (Spears et al., 1976). They found elemental sulphur and methionine were equally effective for increased cellulose digestion of Ky 31 and Kenby tall fescue.
Due to the metabolism of the microbes in the forestomach of the ruminant the digesta passing to the abomasum is presented to the host in an altered form from that originally consumed. In addition to synthesizing sulphur amino acids (i.e. microbial cell protein), the microorganisms also synthesize sulphur containing vitamins (thiamine and biotin) which have nutritional value for the host (Garrigus, 1970). Since vitamins are produced in trace amounts relative to the synthesis of protein, they do not quantitatively play a large role in the sulphur balance of the producing ruminant. Increases in both thiamine and biotin levels have been observed in rumen samples when diets have been supplemented with elemental sulphur (Albert, 1954).

Deficiencies of thiamine in ruminants have not been thought to exist under field conditions but the Nutrition Foundation, Inc. (1969) have reported that clinical symptoms of thiamine deficiency in young cattle and sheep were cured after treatment with thiamine.

When considering the sulphur requirements of the ruminant animal and the different sources and chemical forms of sulphur in the diet it is difficult to estimate the exact level of dietary sulphur-containing compounds required for both the microbes and the host. Since most of the natural sulphur containing compounds enter the "sulphide pool" (due to the rumen microbial activity) it suggests that only the total sulphur level in the diet be considered for nutritional studies.

The overall amino acid composition of rumen bacteria or mixed bacteria and protozoa is not greatly affected by the
feed consumed (Purser and Buechler, 1966; Leibholz, 1972). However, these workers have shown that the sulphur containing amino acids (methionine and cystine) of the rumen bacteria vary to a greater degree than the other amino acids. The addition of methionine to the basal ration for sheep brought about a higher content of arginine, aspartic acid and serine in bacterial protein, and a slight decrease in isoleucine and leucine contents (Kurilov et al., 1976).

The hind gut in sheep may also play a role in sulphur metabolism (Bray and Till, 1975). Judson et al. (1975) studied the digestion and utilization of $^{35}$S-labelled bacteria that were placed in the caecum of sheep. Most of the activity was excreted in the feces (70%) or the urine (21%). Approximately 10% of the activity was retained of which 3% was detected in the fleece.

Interrelationships of Sulphur with other Minerals

The interrelationships of sulphur with other minerals have been reviewed (Muth and Oldfield, 1970; Whanger, 1972). Therefore, only more recent evidence will be discussed here.

The interactions of copper, molybdenum, selenium and sulphur have been studied by many workers. Suttle (1975) observed that molybdenum and sulphur inhibit the repletion of plasma copper levels. Sulphur alone inhibited repletion slightly while molybdenum alone had no effect. Huisingh et al. (1973) proposed that copper becomes unavailable via two routes: (1) the formation of cupric molybdate which is absorbed and excreted rendering both copper and molybdate less available;
and (2) the formation of an insoluble cupric sulphide in the 
rumen, intestines or tissues. Regarding the interaction of 
sulphate and molybdate they proposed that several sites are 
involved with different effects. Dick et al. (1975) have 
suggested there may be a blocking of copper transport across 
membranes which is controlled by the molybdenum intake. 
According to them the elevated blood-copper values which are 
observed with high molybdenum diets are a result of mobilized 
tissue copper and not due to absorption from the rumen.

Gawthorne and Nader (1976) reported that molybdenum 
decreased reduction of sulphate to sulphide in the rumen by 
competing for the first enzyme, ATP-sulphurylase. Under these 
conditions there was an increase in concentration of sulphide 
in the rumen which was due to reduction in absorption of 
sulphide from the rumen. Bryden and Bray (1972) observed that 
molybdenum severely depress rumen sulphide levels. Huisingh 
et al. (1975) reported that 50 ppm of molybdenum in the diet 
significantly inhibited sulphide production from sulphate but 
hindered significantly the production of sulphide from 
methionine. The reasons for this observation are not known.

Selenium may compete with sulphur in the synthesis of 
sulphur containing amino acids and possible other sulphur 
containing compounds in the rumen. In poultry Cantor et al. 
(1975) found selenomethionine to have low availability when 
incorporated into a poultry ration. Selenomethionine may be 
produced by the same pathway as methionine and if incorporated 
into proteins may affect systems where -SH is required.

Godwin et al. (1971) found inorganic $^{75}$SeO$_3^{2-}$ given as Na$_2$
$^{75}\text{SeO}_3$ had about 3% of $^{75}\text{Se}$ incorporated into milk of sheep as selenomethionine.

Selenium competes with sulphur in the following reactions (De Meiro, 1975):

\[
\begin{align*}
\text{ATP} + \text{SO}_4^{-2} & \quad \text{APS} \\
\text{or} & \quad \text{ATP-sulphurylase} \quad \text{or} \\
\text{SeO}_4^{-4} & \quad \text{APSe} \\
\text{ATP} + \text{APS} & \quad \text{PAPS} \\
\text{or} & \quad \text{APSe} \quad \text{or} \\
\text{APSe} & \quad \text{PAPSe}
\end{align*}
\]

Selenium in PAPSe can take the place of sulphur in all reactions involving sulphurylation.

Recently Fuss and Godwin (1975) studied the fate of selenium, given as $\text{Na}_2^{75}\text{SeO}_3$, or $^{75}\text{Se}$ selenomethionine, administered intravenously to ewes and lambs. Small, though significant amounts of selenium, derived from $\text{Na}_2^{75}\text{SeO}_3$, were incorporated as selenoamino acids into the proteins of liver, kidney and pancreas, as well as into the proteins of milk and plasma. The activity could be detected in both selenomethionine and selenocystine chromatographic fractions.

Preston et al. (1974) reported an interaction between sulphur and potassium in cattle diets. Rate of gain in steers was depressed by increasing the inorganic sulphur content of the diet from 0.10 to 0.14%, but the addition of potassium appeared to overcome this depression.
Post-ruminal Sulphur Metabolism

Sulphur is required by the animal because it is a vital constituent of certain amino acids (i.e. methionine and cystine) and therefore of proteins. In addition, sulphur is a constituent of certain vitamins (i.e. thiamine and biotin). Thiamine is involved in the "one carbon pool" and specifically carbon dioxide metabolism (Ledger, 1975). Biotin is also involved in the "one carbon pool" and is involved in decarboxylation reactions (Eisenberg, 1975). Sulphur is also a component of certain co-enzymes (Co-enzyme A, Abiko, 1975; and lipoic acid, Koike and Koike, 1975). Glutathione (Meister, 1975) is also a sulphur containing compound and is important in mammalian metabolism.

The sulphur amino acids found in protein are L-methionine, L-cystine, and L-cysteine. Methionine is considered the most important in the diet of a monogastric animal, since the others can be synthesized from methionine (Maynard and Loosli, 1969).

Methionine, like most amino acids can be incorporated into protein but also has other functions. Its conversion to cystathionine, cystine and cysteine can supply the animal's needs for these sulphur containing amino acids. Methionine may form S-adenosylmethionine (SAM), which is a universal biological transmethylating agent. Abundant evidence has accumulated that protein synthesis is initiated by the formulation of methionine to N-formylmethionine (Lehninger, 1972). The multifunctional nature of the methionine requirement makes it difficult to determine the exact level required in the
diet. Other dietary compounds may spare the methionine from some of the above roles (Molitosis and Baker, 1976).

Inorganic sulphur in its oxidized form, sulphate, plays a role in detoxifying and excretion of many compounds (De Meio, 1975). Sulphate is the end product of sulphur amino acid oxidation and is excreted in the urine.

When reviewing the published results of the sulphur requirements of the ruminant one is impressed with the wide range and lack of agreement. Some of the difference could be attributed to the type of animals being studied. It seems very possible that sheep, beef and dairy cattle may have different requirements. Also the physiological status or type of production criteria being measured (i.e. wool growth or rate of gain in sheep) may affect the required dietary sulphur level. Other considerations which may have an effect on the estimation of the sulphur requirement are type of diet (natural or semi-purified), source of sulphur (organic or inorganic), source of nitrogen (natural or nonprotein nitrogen) and the type of experimental design.

Much of the early research attempting to establish the sulphur requirement of the ruminant was conducted with sheep. One of the first studies reported (Whiting et al., 1954) used mature range ewes fed various sources of supplemental sulphur at different levels added to a diet low in sulphur. They concluded that the sulphur requirement was less than 0.1% of the diet, which is lower than levels which have been published subsequently. Albert et al. (1956) used growing lambs and concluded that the sulphur requirement was met when 0.138%
sulphur was added as methionine. Perhaps the highest sulphur requirement was reported by Evans and Davis (1961) who demonstrated that on the basis of cellulose digestion in the rumen, the optimum level of sulphur in the diet was 0.29%. This higher level based upon in vivo results is similar to some of the optimum levels reported from in vitro results (Barton et al., 1971; Bull and Vandersall, 1973).

Bray (1965) concluded that a sulphur level of 0.14% was adequate for maximum nitrogen retention in sheep. Bird (1972) has shown that the ratio of N:S in sheep tissue is about 13.5:1, and considered that a narrower ratio than this was necessary for optimal usage of dietary nitrogen by sheep. Bird (1973) observed the ratio of nitrogen to sulphur in rumen bacteria to be about 20:1; he concluded that the microbes were deficient in sulphur amino acids with respect to the host's tissue synthesis. Moir et al. (1967) concluded that the optimum nitrogen to sulphur ratio is about 10:1. Kennedy et al. (1975) also showed that a N:S of 10:1 is optimum for sheep, and animals on low quality roughages may have N:S ratios as wide as 50:1 and at best 12:1 which includes the recycled sulphur. This relationship between dietary nitrogen and sulphur is preferred by many workers and considers the sulphur requirement as a function of the nitrogen content of the diet. Kennedy and Siebert (1975), suggest feeding molasses with urea supplemented ration to decrease the N:S ratio towards the optimum. Molasses contains approximately 10 gm S/Kg DM and may have contributed to the positive results in molasses-urea rations reported throughout the literature.
Some studies using dairy cattle have failed to show any improvement when natural diets were supplemented with sulphur. Jacobson et al. (1969) reported no improvement in dairy cows when natural diets containing about 0.10% sulphur were supplemented with sodium sulphate to raise sulphur to 0.18%. Grieve et al. (1973a) observed no improvement in feed intake or milk yield when sodium sulphate was added to corn silage diets containing between 0.11 and 0.13% sulphur. Nitrogen utilization was not improved by the addition of sodium sulphate (Grieve et al., 1973b). In these studies it appeared that the sulphur requirement was met by 0.11-0.13% sulphur present in the basal diet.

Bouchard and Conrad (1973) found that the addition of sulphur to a low sulphur diet improved the production of dairy cows as measured by various criteria. Based upon regression analysis they concluded that sulphur balance could be accomplished by 0.12% dietary sulphur and that 0.18% sulphur would allow for a positive sulphur balance of 4.0 gm daily in producing cows.

Chalupa et al. (1973) reported an improved growth rate in Angus steers when sodium sulphate or elemental sulphur was added to bring dietary sulphur from 0.05 to 0.13%, but there was no improvement measured at higher sulphur levels. Thus, the 0.13% dietary sulphur appeared to meet the steers' requirement.

The incorporation of inorganic sulphur containing compounds into ruminant rations as well as absorbed and
recycled inorganic sulphate may have "sulphur-amino acid sparing action". This effect has been observed in poultry (Ross and Harms, 1970; and Martin, 1972) in which supplemental sulphate gave positive responses in growth rate. Hinton and Harms (1972) identified sulphate as the unidentified growth factor in fish solubles in poultry diets. In man dietary inorganic sulphate has been shown to increase nitrogen retention (Zezulka and Calloway, 1976). Sulphate effectively replaced up to 50% of the dietary methionine requirement as measured by collagen metabolism and growth in the young pig (Robel, 1976). Therefore, the beneficial reports of sulphate supplementation in the diet may not be completely due to ruminal biosynthesis of sulphur amino acids but may be attributed to the "sulphur-amino-acid sparing action" of sulphate for the synthesis of various sulphated compounds.

The NRC estimates of sulphur requirements of sheep (1975), beef cattle (1976) and dairy cattle (1971) are 0.14 - 0.18%, 0.10% and 0.20% respectively. Elam (1975) has reviewed the sulphur requirements of the ruminant and supports the NRC recommendations.

Effects of Sulphur Deficiency and Excess

The effects of sulphur deficiency in sheep have been reviewed by Whanger and Matrone (1970). Symptoms of a sulphur deficiency are not specific and may be difficult to identify. Some of the symptoms include loss of appetite, loss of weight, excessive lacrimation, weakness, dullness, emaciation and death.
Gram positive organisms are predominant in the rumen of sulphur-deficient sheep (Whanger and Matrone, 1970). Hume and Bird (1970) found decreased microbial protein synthesis in the rumen when sulphur was deficient. Slyter et al. (1971) reported lower total rumen bacteria counts for calves fed low (0.04%) or high (1.72%) levels of sulphur compared to calves fed 0.34% sulphur.

Further evidence of the critical nature of sulphur in the rumen is the result of various workers that the digestibility of certain ration constituents are reduced when sulphur is limiting in the diet. Starch digestion by rumen microbes was increased by the addition of various forms of sulphur (Kennedy et al., 1968). Martin et al. (1964) reported that in steers cellulose digestion was lower for rations that were deficient in sulphur. Similar effects were reported in sheep by Bray and Hemsley (1969). Barton et al. (1971) used in vitro techniques to show the marked influence of sulphur concentrations on cellulose digestion by rumen microbes. Additional in vitro studies by Bull and Vandersall (1973) indicate the addition of sodium sulphate, calcium sulphate, and D,L-methionine, were equal at equal sulphur content in promoting cellulose digestion. The optimum sulphur level was 0.16 to 0.24%.

Additional ruminal effects to sulphur deficient diets have been found in the form of end products of microbial action. Rumen microorganisms from sheep fed a sulphur-deficient diet formed more acetate and propionate on a purified diet than those from sheep fed the diet with sulphur added (Whanger and
Matrone, 1965). Further, there was an accumulation of lactate in the rumen of the sulphur deficient sheep. The cause of these effects on lactate and VFA production has not been fully elucidated.

One of the common indications of a sulphur-deficiency is reduced feed consumption. It is suspected that the reduced feed intake is the result of a reduced microbial population and the subsequent reduction in rate of digestion of feed components in the rumen. A lower rate of digestion would be expected to reduce rate of passage of feed through the digestive tract and thereby cause a reduction in feed intake by the animal. Reports of lower feed intake by animals fed diets low in sulphur have been made for sheep (Kahlon et al., 1973) beef cattle (Chalupa et al., 1973) and dairy cattle (Chalupa et al., 1971; Leibholz and Kang, 1973).

There is abundant evidence that the sulphur containing amino acid methionine is one of the most toxic amino acids. Largely on the basis of experiments with rats, Harper et al. (1970) concluded that consumption of methionine at four times its requirement results in growth depression and tissue damage when it is incorporated into a diet low in protein. Numerous attempts have been made to overcome the growth-depressing effects of a methionine-induced toxicity. Katz and Baker (1975) studied the effects of adding supplemental glycine or threonine to a methionine excess ration in young chicks. They found that glycine was partially effective in alleviating the growth depression caused by excess methionine. The addition of threonine together with glycine improved performance still further.
In dairy cattle excess methionine and methionine hydroxy analog have both been shown to reduce feed intake (Satter et al., 1975). In sheep, excess methionine has reduced both feed intake (Kelly and Thomas, 1975) and wool growth (Reis et al., 1973a). In contrast to this effect of methionine, excess cystine did not appear to affect wool growth adversely. Reis et al. (1973a) concluded that the effects of high levels of methionine on wool growth are due specifically to the inability of the animal to metabolize this amino acid rapidly enough or to store it in tissue by a mechanism such as that possible with cystine, namely thiol-disulphide reactions with proteins. This conclusion is supported by plasma amino acid data (Reis et al., 1973b) which showed that plasma methionine levels increase steeply with increasing amounts of abomasally infused methionine whereas equimolar amounts of cystine caused only a slight rise in plasma cystine.

Benevenga (1974) reported methionine toxicity is not due to methionine per se but involves the metabolism of the methionine methyl group. His work does not support the suggestion by Reis et al. (1973b) that the toxic effects of excessive dietary methionine can be ascribed to its effect on transport of other amino acids.

**Sulphur Supplementation**

Since it appears that ruminant diets may be less than optimum in their sulphur content, the addition of supplemental sulphur may provide beneficial increases in production. It has been shown that low sulphur diets may depress both the
growth rate of the rumen microbes and the host. On occasions the rumen microbes' sulphur requirement has been met yet the host requirement is still suboptimal (Fenderson and Bergen, 1975). Therefore in any ration formulation the choice of supplementation of a sulphur containing compound should be determined by either the microbes, the host or both of their requirement for sulphur.

If sulphur is deficient for microbial growth the choice should be a sulphur containing compound that is readily available to the micro-organisms. This then should increase the biological value of the digesta entering the abomasum. If only the host requirement for sulphur containing compounds is not met then the preferred choice of supplementation will be compounds which are poorly utilized by the microbes but have high biological value to the host.

Compounds that are available to the microbes include L-methionine, DL-methionine, sodium sulphate, ammonia sulphate, calcium sulphate and elemental sulphur (Kahlon et al., 1975a). It has been suggested that ammonia sulphate may have broader application for it can supply both sulphur and nitrogen (Elam, 1975).

The number of compounds that bypass rumen fermentation and provide the sulphur requirement of the animal is increasing. Methionine hydroxy analog is the most popular (Baker, 1975) and has a positive effect on milk yield and composition which was equal to abomasal methionine (Olson and Grubough, 1974). Other compounds include N-hydroxymethyl-DL-methionine (Cheeke and Whanger, 1976), DL-homocysteine thiolactone (Amos et al.,
1974), N-acetyl-DL-methionine and S-methyl thiobutane-1,2-diol (Steadman et al., 1975).

Rees et al. (1974) have compared the merits of sulphur fertilization of pasture with sulphur supplementation of the animal and have found that intakes of digestible energy by sheep were higher with sulphur-fertilized grass than with direct supplementation. With sulphur fertilization, more DM digestion took place in the rumen. However, in most pasture conditions only low levels of fertilizer will be used, and that additional nutrient requirements should be met by direct supplementation to the grazing animal.
II. NITROGEN IN RUMINANT NUTRITION

The protein nutrition of ruminant animals must be considered in terms of amino acids absorbed from the gastrointestinal tract in relation to amino acids required for productive purposes. Amino acids available for absorption are of a different spectrum than those present in the feed proteins. The proteolytic activity of the rumen microorganisms and the synthesis of microbial protein greatly alter the dietary protein, so that the protein present in the digesta post-ruminally is made up of undegraded feed protein, microbial protein and endogenous protein secretions. Another characteristic of ruminant protein nutrition is the beneficial effects of non-protein nitrogen (NPN) in the diet. This capability of changing NPN into protein nitrogen is also a function attributed to the rumen microorganisms. These characteristics allow the ruminant animal to be an integral link in the human food chain because poor quality protein sources can be fed to ruminants and converted into animal protein of high biological value.

The effects of the rumen microbes on dietary protein have been reviewed (Blackburn, 1965; Hutton, 1972; Church, 1974). The extent of degradation of dietary protein is largely dependent on the solubility of the material in rumen liquor (Wohlt et al., 1973) and any process which reduces solubility will result in a decreased ruminal breakdown of food protein. Such processes include heating (Tagari et al., 1965), treatment with vegetable tannins (Wohlt, et al., 1973) and treat-
ment with formaldehyde (Reis and Tunks, 1969; Faichney, 1975). Some dietary protein usually escapes rumen breakdown and there is evidence that both feed intake (Orskov et al., 1971) and processing of the feed (Coelho da Silva et al., 1972) influence this proportion. Satter and Roffler (1974) feel that 40% of the dietary protein escapes rumen degradation under most dairy and feedlot management systems. This value of rumen by-passing of protein is much higher than most workers have obtained (Smith, 1975) and a more realistic range is from 15 to 35%.

Since a large proportion of the nitrogen entering the rumen is incorporated into microbial cells, the production or growth rate of rumen microorganisms is of great nutritional interest. Ultimately the microbial protein will be the major source of protein to the host. To quantitate the amount of microbial protein being produced, workers are employing many methods such as the bacterial markers diamino pimelic acid (DAP) and amino ethyl phosphoric acid (AEP) (Ibrahim and Ingalls, 1972) and also microbial nucleic acids (Smith and McAllan, 1970). The more recent in vivo methods are employing isotopes. $^{15}$N (Mathison and Milligan, 1971), $^{35}$S (Walker et al., 1975) and $^{14}$C (Singh et al., 1974) have been reported to be successful.

Thomas (1973) has reviewed microbial protein synthesis and discussed the significance and limitations of optimal microbial growth. He concludes that ruminants have an important role in the production of protein of high biological value.

Another characteristic of ruminant nitrogen metabolism is the recycling of nitrogen. It is well accepted that blood urea nitrogen enters the rumen. It was felt that both saliva
and direct transport of urea across the rumen wall contributed to this nitrogen recycling. Isolated preparations have shown that urea from blood can enter the rumen through the epithelium (Thorlacius et al., 1971). Nolan et al. (1973) suggest that saliva is the main route in the intact animal and transport directly from blood only occurs at non-physiological blood urea levels.

The post-ruminal digestion of nitrogenous compounds has not been studied in great detail. Church (1974) showed that intestinal protein digestion is similar in all mammalian species. Armstrong and Hutton (1975) discuss protein digestion by comparing the ruminant to the monogastric animal. The ruminant appears to possess the same complement of proteases which are present in the monogastric. There is a difference in the acid conditions of the upper part of the small intestine. In the ruminant extension of the acidic conditions exist due in part to the copious secretion of acid by the abomasum and in part to the weakly alkaline nature of the bile and the pancreatic secretions (Kay, 1969).

The use of re-entrant cannulas in sheep by Ben-Ghedalia et al. (1974) has indicated that the sites of digestion and absorption of proteins are the small intestine. In calves the flow of digesta protein and the pancreatic secretions has been studied with various artificial milks (Ternouth et al., 1975). Like the monogastric, amino acids and small peptides are the end products of proteolytic digestion. There is also increasing evidence to suggest that quantitatively absorption of dipeptides is more important than individual amino acids.
(Mathews, 1974; Kim et al., 1974). Under normal conditions nutritionally important amounts of amino acids are not absorbed from the rumen (Leibholz, 1971).

An area which has only recently been studied is the function of the large intestine in the ruminant. The role of the caecum in ruminant nitrogen metabolism is now being appreciated for it appears that it may be the major site of urea degradation in sheep (Nolan et al., 1973). Ruminal bacteria that were labelled with $^{35}$S and injected into the caecum of sheep and 3% of the injected activity was retained in the fleece (Judson et al., 1975).

Recent tracer studies of nitrogen metabolism in ruminants using the stable isotope $^{15}$N have demonstrated the potential of isotope tracer techniques as a means of studying quantitative nitrogen transactions in the body of the normal feeding animal. Quantitative models of nitrogen metabolism in sheep are considering dietary protein, microbial activity, recycling of nitrogen, post-ruminal nitrogen metabolism as well as the host protein requirement (Nolan and Leng, 1974). As more information is being collected these models are becoming more complete (Nolan, 1975; Mazanov and Nolan, 1976). These models describe both the biochemistry and the significance of rumen microbial nitrogen metabolism in terms of the host nitrogen metabolism.

The amount of material available on ruminant nitrogen nutrition is very large and the result is that certain fields of ruminant nitrogen nutrition are emerging. Thomas (1976) and van Es (1975) have both reviewed the effect of diet on
milk protein production. They indicate that milk protein is very important in human nutrition, especially of growing children. The protection of dietary proteins and amino acids against rumen microbial fermentation has also recently been reviewed (Phillipson, 1972; Ferguson, 1975). They concluded that the protection of dietary components can increase production under certain conditions. The utilization of non-protein nitrogen in ruminant rations is one of the most beneficial applications of applied nutrition. Again this area has been extensively reviewed (Chalupa, 1973; Bull, 1973; Huber, 1975; Kertz and Everett, 1975; Satter and Roffler, 1974). The addition of NPN is of greatest benefit when protein is the limiting nutrient.

Due to the special characteristics of ruminant metabolism it appears that the nutritional methods used for monogastrics may not be appropriate. Blaxter (1975) suggests that an alternative method for estimating protein requirements be established utilizing the energy-protein relationships. Miller (1973) has devised a system for the evaluation of feeds as sources of nitrogen and amino acids. His scheme takes into account the metabolism and growth rate of the rumen microorganisms. Another approach is the biological value of protein in ruminants (Black and Colebrook, 1976) which compares favourably with the more conventional methods. The most practical method for estimating dairy cattle nitrogen requirements is the metabolizable protein (MP) feeding standard (Burroughs et al., 1975). The MP feeding standard for lactating cows represents a balance between animal requirements
for metabolizable amino acids (MAA) and their fulfillment by diets composed of a wide variety of feedstuffs after rumen microbial activity.
III. PLASMA AMINO ACIDS AND RUMINANT NUTRITION

The plasma free amino acids represent a balance between input from intestinal absorption and body protein catabolism and output by protein synthesis and amino acid catabolism. Munro (1970) has suggested that free amino acids are the currency of protein metabolism. He also suggests that changes in plasma amino acid (PAA) concentrations may be a sensitive indicator of amino acid metabolism.

The use of free amino acids as an indicator of protein nutrition status has been reviewed (McLaughlan, 1975; Shelford, 1974) and it appears that, as yet, there is not convincing evidence that PAA levels are useful for evaluating protein quality per se. However, many investigators have reported that feeding diets deficient in one essential amino acid results in a relatively low level of that amino acid in the plasma. This facet of PAA levels is useful as a nutritional indicator.

Several workers have shown that in monogastrics a suboptimal dietary content of an essential amino acid results in a low plasma concentration, and that a dietary excess results in a high plasma level (Zimmerman and Scott, 1965; Boomgaardt and Baker, 1973; Itoh et al., 1974). When increasing amounts of the first limiting amino acid were fed, the plasma concentrations remained low until the amount required for maximum growth was exceeded, then a sharp and linear increase in concentration was observed. The point of intersection of the two lines obtained when amino acid intake was plotted against PAA con-
centration has been accepted as a measure of amino acid requirement under the overall conditions of the experiment. Favorable comparisons of animal performance in the pig (Keith et al., 1972) and the chick (Zimmerman and Scott, 1965) provide support for the validity of the procedure.

The use of PAA profiles to determine the quality of dietary protein has been employed in numerous species. It has been used to assess the amino acid requirement of poultry (Zimmerman and Scott, 1965), swine (Davey et al., 1973), man (Irwin and Hegsted, 1971), rat (Stockland et al., 1970), crocodilia (Herbert and Coulson, 1975), sheep (Reis et al., 1973b), and cattle (Derrig et al., 1974).

The ruminant with its extensive synthesis of amino acids by symbiotic microorganisms presents unique problems to studies of amino acid nutrition. Protein quality is dependent upon the available amino acids leaving the rumen, rather than those in the ingested diet. Protected proteins or amino acids may be fed to the animals or alternatively nitrogenous compounds may be administered postruminally to study ruminant amino acid nutrition. Protected proteins (Broderick et al., 1974) and post-ruminal infusions of amino acids (Burris et al., 1976) have both been used in conjunction with PAA levels. Other routes of administration for the determination of ruminant amino acid nutrition are intraperitoneal (Barry, 1976) and intravenous infusions (Kelly and Thomas, 1975). However, these administration routes bypass the intestinal mucosa and liver so that their nutritional significance is limited.

The special features of nitrogen metabolism in ruminants,
which stem from the extensive breakdown of dietary protein and the synthesis of microbial protein imply the absence of specific dietary requirements for essential amino acids. However, there is considerable theoretical and practical interest in defining the amino acid requirements of ruminant tissues in animals of known nutritional and physiological status. Wakeeling et al. (1970) were the first investigators who utilized PAA levels in ruminant amino acid nutrition. They conducted a series of experiments in which graded levels of L-methionine were infused into the duodenum of sheep; the plasma methionine levels responded in a sigmoid manner. Similar experiments with lysine showed that plasma lysine concentration increased linearly with increasing passage of lysine into the duodenum over the whole range of infusion. These results suggest that methionine, but not lysine, was limiting under the imposed experimental conditions.

The high content of sulphur containing amino acids in wool suggests that methionine may be limiting for maximum production in sheep. The effect of abomasal methionine infusions on the PAA response curve (Reis et al., 1973a) and wool growth (Reis et al., 1973b) confirm that sulphur containing amino acids may be limiting. Kelly and Thomas (1975) concluded that methionine may be limiting for protein synthesis in the tissues but is not involved in the control of feed intake. Barry (1976) has confirmed the increased wool growth response to supplementary methionine, but indicated that methionine may have a role in the control of voluntary intake. The influence of abomasal supplements of zein and lysine on wool growth rate
and PAA profiles suggests that in some conditions lysine may be the limiting amino acid for sheep production (Reis and Tunks, 1976).

In the growing calf methionine has been shown to be the first limiting amino acid (Williams and Smith, 1974). In the preruminant calf graded levels of methionine in the diet caused a sigmoid PAA curve indicating that methionine may be limiting (Williams and Smith, 1975).

Only methionine infusion resulted in a two-phase PAA response curve and the quantity of threonine, lysine and tryptophan available from digesta met the requirement for growing steers (Fenderson and Bergen, 1975). The improved amino acid balance of opaque-2 corn did not affect the PAA concentrations in steers (Redd et al., 1975). This latter information suggests that PAA profiles may be of value for the evaluation of new feeds for ruminants.

The use of PAA profiles for the determination of nutritional status is considered to be most sensitive for the growing animal. For the high milk producing ruminant PAA levels also provide a sensitive measurement of amino acid nutrition. Linzell and Mepham (1974) suggest that milk protein synthesis may be limited by the availability of either methionine or tryptophan in the lactating goat. Vik-Mo et al. (1974) found methionine to be the first limiting amino acid followed by phenylalanine and lysine. Methionine has been shown to be limiting for dairy cows by Broderick et al. (1974) and Derrig et al. (1974). Broderick et al. (1974) suggests that valine and lysine are the next limiting amino acids. Methionine
and lysine have recently been shown to limit milk protein synthesis in lactating cows in that order (Spires et al., 1975).

Kellaway et al. (1974) have reported results indicating that threonine is the first limiting amino acid followed by phenylalanine and methionine in the lactating cow. Shelford (1974) has shown that essential amino acids may be limiting milk protein synthesis. Of the amino acids, abomasal lysine infusion was observed to cause greatest increase in milk protein production.

When comparing PAA levels reported by various investigators many factors should be considered. Time of sampling has been shown to have a marked effect on PAA levels in swine (Davey et al., 1973), man (Marrs, 1975), sheep (Cross et al., 1975) and the lactating goat (Mepham and Linzell, 1974). The location of the sampling site is important. Hormonal affects on PAA levels has been reviewed by Munro (1970). Davis (1972) and Tao et al. (1974) have indicated that prolactin, growth hormone and insulin are involved in sheep amino acid metabolism and are reflected by changes in PAA levels. In the lactating dairy cow PAA concentrations have been shown to be affected by the estrous cycle (Mason et al., 1973) and the stage of lactation (Fisher et al., 1975). Other considerations are the participation of muscle tissue in maintaining PAA homeostasis in ruminants (Cross et al., 1974) and acute changes in environmental temperature (Bell et al., 1975).

The dietary levels of protein and energy affect PAA
levels in the calf (Leibholz, 1975). Eskeland et al. (1974) have compared different sources of energy for protein formation in the lamb. By measuring changes in PAA levels they concluded that glucose and propionate are the best energy sources. Continuously fed steers had a different PAA profile than those fed twice a day (Boila and Devlin, 1975). High fiber-urea diets also affect PAA profiles in dairy cattle (Bouchard and Conrad, 1975).

Wolfrom et al. (1976) suggest that free amino acids in whole blood as well as plasma should be determined in future studies of amino acid metabolism in sheep. They found small but significant difference in methionine level in whole blood and plasma. They also indicated that the route of intravenous infusion is very important.

An alternative approach to the measurement of amino acid requirements utilizes radioactive amino acids. The basis for this method is similar to that previously discussed using PAA levels to estimate amino acid requirements.

Since amino acids absorbed from the digestive tract cannot be stored in major quantities; they are distributed as free amino acids in blood and tissues. The raised amino acid concentration in blood and tissues automatically causes an increased rate of amino acid degradation. This is because the $K_m$ values of the enzymes initiating amino acid degradation are in general high, much higher than the concentration of amino acids in the tissues (Krebs, 1972). If the extent of oxidation of essential amino acids is significantly increased when their supply exceeds tissue requirements, the pattern of re-
lease of $^{14}\text{CO}_2$ following the administration of a labelled amino acid to an animal should provide an alternative procedure for the estimation of essential amino acid requirements.

The use of radioactive amino acids for nutritional studies has mainly been conducted on monogastrics. Benevenga et al. (1968) concluded that the oxidation of $^{14}\text{C}$-essential amino acids is a reliable technique and compares favourably with growth rate and carcass analysis. Neale and Waterlow (1974a) found no difference in oxidation rate between intragastric and intravenous administration. However, they did suggest that interpretation of the results presents many pitfalls and that the skin of the rat has a significant role in the protein metabolism of the whole body. Neale and Waterlow (1974b) critically evaluated the amino acid oxidation method for determining the amino acid requirement. The choice and position of the label are very important for the estimation of endogenous losses of amino acids (Neale, 1976).

Newport et al. (1976) reported that the $^{14}\text{CO}_2$ output from amino acid oxidation is reliable for the estimation of amino acid requirements in small pigs. They found favourable results for both lysine and methionine. Perry (1975) has shown that the breed of pig should be considered when calculating the proportion of the label incorporated into the muscle protein.

Armstrong and Annison (1973) compared the amino acid requirements of sheep measured by PAA profiles and $^{14}\text{CO}_2$ output procedures. Comparable results with both methods were obtained for threonine and methionine. Cross et al. (1975) studied the uptake of radioactivity from labelled leucine
injected intravenously into wethers. They calculated the plasma half-time and the proportion of the activity incorporated into plasma protein. Fasting did not have a significant effect on plasma half-time values. In ruminants the collection of CO₂ is complicated because it originates from the rumen and the respiratory system. Cross et al. (1975) suggest that plasma parameters be studied in ruminants when radioactive amino acids are administered, so that the difficulties involved in collection of respired CO₂ from large animals be eliminated. The experiments of Birt and Clark (1976) indicate that radioactive amino acids with rats may assist in interpreting the role of the liver and muscle in modifying the PAA response to dietary amino acids.
IV. PLASMA UREA AND RUMINANT NUTRITION

Mercer and Miller (1973) suggested that the response of plasma urea (PU) to abomasal infusion of methionine may be of value in estimating the methionine requirements of sheep. In contrast, Williams and Smith (1974) found that PU concentration and abomasal infusions of amino acids are not reliable methods for estimating the amino acid requirements in the calf.

Young et al. (1973) has shown that PU increased with increasing protein intake for a given protein, but is dependent on the nature of the protein. They indicated that this dependence is related to the extent to which ammonia is formed from a protein in the rumen rather than the quality of the protein. In the monogastric animals elevated PU concentrations are due to increased amino acid catabolism (Lewis and Speer, 1973). This suggestion that elevated PU concentrations in sheep are due to higher ruminal ammonia levels is further supported by Pfander et al. (1975) and Wohlt et al. (1976) who measured PU with varying protein allowances. The elevated plasma urea concentrations in steers fed a high protein diet were due to high ruminal ammonia levels (Fenderson and Bergen, 1976).

Williams and Smith (1975) studied PAA and PU concentrations in the pre-ruminant calf. The PAA and PU concentrations decreased soon after feeding, which surprisingly is in contrast to the simple-stomached animals. They did show that PU concentration may be of value in determining the methionine requirement, for these calves had their lowest PU concentration at the optimum dietary methionine level. Mehra (1976) noted that the blood urea concentration of buffalo and dairy calves
responds to varying protein levels in a similar manner. Torell et al. (1974) have discussed the factors affecting PU and its use as a nutritional index for sheep. They concluded that neither ages nor sampling time had any significant effect on PU level but variation between animals with age class was significant.

Kirk and Walker (1976a) suggest that PU can be a reliable indicator of protein quality in the preruminant lamb. Protein quality and urinary nitrogen constituents have recently been discussed in terms of PU levels in sheep (Kirk and Walker, 1976b). They concluded that PU is a valuable criteria to estimate protein quality but has limited use for estimating ruminant amino acid requirements.
EXPERIMENTS

Experiment I - Effect of Abomasal Methionine Infusion on Plasma Amino Acid Profiles and Plasma Urea Concentrations

In this experiment graded levels of methionine will be infused into the abomasum of two growing lambs. Jugular blood samples will be obtained at each infusion level and plasma free amino acids and plasma urea will be determined by an amino acid analyser. The plasma methionine response curve should indicate if methionine is limiting production for these lambs. The changes in the PAA profile will hopefully indicate the effect of increasing methionine infusions.

(A) Materials and Methods

Two Dorset-Suffolk ewe lambs, #13 (lamb #1) and #31 (lamb #2), weighed 28 and 34 kg respectively during the experimental period. Each lamb was surgically fitted with an abomasal cannula during the first week post-weaning. The cannula (Medical-Grade Silastic Tubing, Dow-Corning) had an internal diameter of 2.6 mm. At one end a silastic collar was attached to the cannula and sutured into the abomasum. The abomasum was sutured to the abdominal wall where the cannula was passed under the skin to the midback. The sutures were removed one week after the operation. The experimental work commenced two weeks post-operation.

The animals were fed 400 gm of hay (Timothy-orchard grass mixture) and 165 gm barley twice daily. Both animals were accustomed to being handled and had been on the above diet for
over two weeks before the commencement of the experiment. During the experimental period the animals were placed in metabolic cages. Water and a colbalt-iodized salt lick were available throughout the experimental period. The lambs remained in the metabolic cages until the final blood sample was collected at the highest infusion level.

The percentage nitrogen (Technicon, 1974a) and phosphorus (Technicon, 1974b) of the diet were determined by an auto-analyser colourimetric method. The percentage sulphur was determined after wet ashing with magnesium acetate (Piper, 1950) and by a colourimetric method (Tabataba, 1974).

Each animal was abomasally infused with graded levels (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 gm/day) of D,L-methionine (Nutritional Biochemical Corporation) in physiological saline at an infusion rate of 250 ml/day. On the third day of each infusion level jugular blood was collected into heparinized vacutainer tubes (Becton, Dickinson & Co. Canada Ltd.) at 12:00 noon.

Jugular blood plasma was obtained from the centrifugation of whole blood at 5,000 rpm for 10 minutes. The plasma was deproteinized with sulfosalicylic acid (50 mg/ml plasma) at 15,000 rpm for 20 minutes at 4°C. The supernatant was frozen until analyzed. One ml of this solution was applied directly to the column of a Hitachi KLA3B amino acid analyzer for the determination of the neutral and acidic amino acids (Spackman et al., 1958). Plasma urea was also determined in this analysis. The basic amino acids were not determined as workers in previous studies have indicated that the effect of
methionine infusion on these amino acids was not of metabolic significance.

(B) Results

The feed was consumed at all the infusion levels of both lambs. The percentage nitrogen, phosphorus and sulphur of the grass (1.22%, 0.26% and 0.13%) and the barley (2.16%, 0.77% and 0.44%) were expressed on dry matter basis respectively.

The plasma free amino acids were expressed as micromoles per 100 ml of blood plasma. Lamb #1 developed diarrhoea after the 3.0 gm D,L-methionine infusion per day. It was observed that this lamb was consuming all of its feed and the infusion was continued. The amino acid profile for lamb #1 (Table 1) indicates that the diarrhoea drastically affected all plasma amino acids. Therefore, this prevented a comparison to be made between the two experimental lambs at the 4.0 and 5.0 gm per day of methionine infusion.

The plasma methionine response curve for lamb #1 (Fig. 1) increased at increasing concentrations of the methionine infusion. After this lamb developed diarrhoea the plasma methionine dropped to a lower level. The linear response at the first infusion levels suggests that methionine was not limiting in the digesta available to this lamb. Lamb #2 had a different plasma methionine response curve (Fig. 2). An inflection point was present just below the 2.0 gm methionine level. This response is typical of a limiting amino acid and suggests that methionine was below its optimal level in the digesta available to this lamb.
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</tr>
<tr>
<td>Leucine</td>
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<td>4.95</td>
<td>5.19</td>
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<tr>
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<td>7.77</td>
</tr>
<tr>
<td>Cystathionine</td>
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<td>0.67</td>
<td>0.81</td>
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<td>0.16</td>
</tr>
<tr>
<td>Amino Acid</td>
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<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>---------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
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</tr>
<tr>
<td>Taurine</td>
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<td>3.11</td>
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<td>9.49</td>
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<tr>
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<td>4.51</td>
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<td>2.85</td>
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<td>3.04</td>
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<td>2.66</td>
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<td>3.01</td>
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<td>19.45</td>
<td>24.36</td>
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<tr>
<td>Alanine</td>
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<td>10.45</td>
<td>10.04</td>
<td>10.45</td>
<td>13.84</td>
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<td>Valine</td>
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<td>Leucine</td>
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<td>7.92</td>
<td>7.16</td>
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<td>6.89</td>
<td>6.71</td>
<td>2.81</td>
<td>7.84</td>
</tr>
</tbody>
</table>
The other sulphur containing compounds-cystine, cystathionine and taurine-were affected by the infusion. Plasma taurine increased with each increase in the methionine infusion level for lamb #2 and until 4.0 gm/day methionine infusion for lamb #1. Plasma cystine increased with the first infusions but leveled off with further increases in methionine infusions. This was observed in lamb #2 (Fig. 2) and lamb #1 (Fig. 1; 3.0 gm/day methionine). Cystathionine was only measured in the plasma for lamb #1. It increased through all increasing infusion levels until the diarrhoea developed, whereafter it fell to trace amounts.

Methionine sulfoxides were not included in the standard so it was not possible to quantify this amino acid. In both lambs a peak was observed where the methionine sulfoxides were expected to occur. This peak increased with increasing methionine infusions.

The non-essential amino acids - alanine, glycine and serine - tended to decrease with increasing methionine infusion levels in both lambs. Glutamic acid was observed to decrease in lamb #2, but not in lamb #1, with increasing methionine infusions. Plasma glutamic acid levels were constant in lamb #1.

The branch-chained amino acids - isoleucine and leucine - decreased with increasing methionine infusions for both lambs. Valine was detected in trace amounts in the plasma of lamb #1. In lamb #2 plasma valine decreased with increasing methionine infusions.

The plasma concentration of phenylalanine and tyrosine
Fig. 1. Effects of abomasal methionine infusions on the concentration of sulphur amino acids in plasma in lamb #1.
Fig. 2. Effects of abomasal methionine infusions on the concentration of sulphur amino acids in plasma in lamb #2.
Fig. 3. Effects of abomasal methionine infusions on the concentrations of some non-essential amino acids in plasma in lamb #2.
Fig. 4. Effects of abomasal methionine infusions on the concentration of the branch-chained amino acids in plasma in lamb #2.
### TABLE 3. EFFECT OF ABOMASAL INFUSION OF METHIONINE ON THE CONCENTRATION OF UREA IN PLASMA

<table>
<thead>
<tr>
<th>INFUSION LEVEL (GM/DAY)</th>
<th>PLASMA UREA CONCENTRATION (µ MOLE/100 ML)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LAMB #1</td>
</tr>
<tr>
<td>0.0</td>
<td>171.0</td>
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<td>1.0</td>
<td>368.0</td>
</tr>
<tr>
<td>2.0</td>
<td>255.0</td>
</tr>
<tr>
<td>3.0</td>
<td>215.0</td>
</tr>
<tr>
<td>4.0</td>
<td>292.0</td>
</tr>
<tr>
<td>5.0</td>
<td>243.0</td>
</tr>
</tbody>
</table>
appeared to have increased with increasing infusion levels. Threonine decreased in plasma concentrations for both lambs at the increasing infusion levels. Proline was only measured in the plasma of lamb #2 and was constant.

The plasma urea concentration for lamb #2 (Table 3) peaked at the 3.0 gm/day methionine infusion level after which the plasma urea concentration decreased. This peak in plasma urea was also observed for lamb #1 at the 1.0 gm/day methionine infusion level.

(C) Discussion

It has been proposed that the requirement for a limiting amino acid can be determined from the inflexion point of a curve relating the plasma concentration of an amino acid to the intestinal supply. The plasma methionine curve for lamb #1 (Fig. 1) increased linearly for the first infusion levels and suggests that methionine was not the limiting amino acid. The plasma methionine curve for lamb #2 (Fig. 2) had an inflexion point just below the 2.0 gm methionine infusion level. This indicated that methionine was limiting for this lamb.

The reason for the different plasma methionine curves is not clear. The lambs were of the same sex, same breed, similar weight and age, and received the same quantity and type of feeds. Weight gains since birth indicate that lamb #2 had a higher growth rate than lamb #1. This higher growth rate of lamb #2 may explain the difference in the plasma response curve.
Plasmas cystine increased with increasing methionine infusion levels but plateaued at the higher infusion levels. This effect of methionine on plasma cystine has been reported by Tao et al. (1974). Reis et al. (1973b) found that plasma cystine increased at each increasing level of methionine infusion. Tao et al. (1974) suggest that the route of infusion plays a key role in amino acid metabolism. They used the intravenous infusion method and Reis et al. (1973b) used post-ruminal infusions. Tao et al. (1974) suggest that the gut tissue and liver, which were by-passed when the nutrient solution was administered parenterally via jugular veins, play an important role in the biosynthesis of cystine. In growing pigs Keith et al. (1972) found that plasma cystine leveled off at the higher dietary methionine levels. Therefore, it appears that the plasma cystine responses may in fact be explained by infusion route.

The increasing concentrations of both taurine and cystathionine in plasma when excess amounts of methionine are infused indicate that the lambs are able to convert a proportion of the influx of methionine to cysteine. Reis et al. (1973a) suggest that this conversion of methionine to cysteine is inadequate in sheep and contributes to the elevated plasma concentration of methionine.

The decrease in plasma levels of some of the non-essential amino acids (serine, glycine and alanine) compares favourably with the results of Reis et al. (1973b). Tao et al. (1974) found that plasma serine and glycine decreased but plasma alanine increased in sheep infused with graded levels of
methionine. Serine is involved in cystathionine synthesis and a decreased plasma level may explain the inability of sheep to convert a large influx of methionine to cysteine. Cysteine can readily be removed and is not as toxic as methionine. Toxic amounts of methionine have caused lowered plasma concentrations of glycine and serine in rats (Benevenga and Harper, 1970), swine (Keith et al., 1972) and sheep (Schelling et al., 1973). Katz and Baker (1975) found that glycine was partially effective in alleviating the growth depression caused by excess methionine in chicks. The toxic effects of excess methionine have been discussed in terms of glycine, serine and cysteine metabolism (Benevenga, 1974). He suggests that glycine and serine supply are important for the metabolism of methionine and that low levels may impair its conversion to cysteine. These amino acids (serine, glycine and alanine) are key precursors for glucose synthesis in the ruminant (Bergman, 1973), which places a further demand on their requirement.

The changes in the plasma urea concentration with the graded infusion levels of methionine probably reflect changes in the substrate supply (i.e. amino acids) for urea synthesis. Generally there was a depression in all of the plasma amino acids except for the sulphur containing amino acids which increased in plasma concentration. Plasma urea increased in concentration up to 3.0 gm/day methionine infusion (lamb #2). The plasma cystine concentration plateaued at this infusion level and the plasma concentrations of the non-essential amino acids (alanine, serine and glycine) appeared to have reached a
constant lower concentration (Fig. 3). After this point amino acid supply may have been critical and urea synthesis was depressed. Methionine metabolism was also impaired at the higher infusion levels so that its conversion to urea would also be reduced. Therefore, the decreasing plasma urea concentrations after the plasma urea concentration peak reflects decreasing substrate for urea synthesis.

The reduction in the concentration of the branch chained essential amino acids (valine, leucine and isoleucine) with methionine infusion in sheep has been reported by Reis et al. (1973b), Tao et al. (1974) and Schelling et al. (1973). They suggest that sulphur containing amino acids may be limiting wool growth and a reduction in essential amino acids is consistent with the expected effects of supplying a limiting amino acid. This is only valid up until the requirement is met for the limiting amino acid. Increasing an amino acid beyond its requirement also results in a reduction in plasma levels for most essential and non-essential amino acids. This appears to be true for sheep but in pigs the plasma level of some of the essential amino acids increased when methionine was included in the diet beyond its requirement (Keith et al., 1972). Schelling et al. (1973) have shown by nitrogen balance experiments that protein synthesis can still be increasing even after the plasma level of a limiting amino acid begins to rise. In general, methionine in excess of its requirement depresses most plasma amino acid concentrations.
Experiment II - Abomasal Methionine Infusion and Radioactive Methionine Tracer

The use of radioactive tracers gives a dynamic picture of amino acid metabolism. In this experiment a lamb will receive a trace dose of radioactive sulphur containing methionine under two conditions. One condition will be that methionine is limiting and the other will be that methionine is in excess of the limiting requirement. Urinary excretion of radioactivity, incorporation of radioactivity into plasma proteins and other compounds, and plasma kinetics will be estimated. It is hoped that a comparison of these parameters under the above conditions will indicate the metabolic fate of methionine.

(A) Materials and Methods

Lamb #2 was placed in a metabolic cage and received two continuous infusions. The first was 250 ml of physiological saline per day (zero infusion level). The other contain 5.0 gm of DL-methionine infused in 250 ml of physiological saline per day (high infusion level). The lamb remained in the metabolic cage for one week during each infusion. The diet has already been described in Experiment I.

Two indwelling jugular cannulas were inserted and flushed with heparinized saline (100 units of heparin/ml). At each of the two infusion levels a tracer dose of $^{35}$S-L-methionine (Amersham/Searle SJ.123) was injected via a cannula into the jugular vein. The doses were 93.8 and 83.9 $\mu$Ci at 0.0 and 5.0 gm/methionine infusions respectively. Following the injection the cannula was immediately flushed with 10 ml of
sterile saline.

Serial blood samples (7 ml) were collected via the other jugular cannula. Blood was collected regularly for the first four hours post-injection. A 24 hour blood sample was also collected. Blood was stored in the cold (4°C). Plasma was obtained and deproteinized as in Experiment I. Total urine was collected once daily for four days post-injection.

Plasma, deproteinized plasma and urine were counted for $^{35}$S radioactivity as soon as possible in an Isocap/300 liquid scintillation counter. The channel ratio counting method was utilized and the scintillation cocktail was PCS (Amersham/Searle).

The deproteinized plasma was frozen and stored for amino acid analysis. A Hitachi KLA3B amino acid analyzer was used to determine the neutral and acidic amino acids. A stream splitting device connected at the base of the resin column removed one quarter of the volume leaving the column. This portion was collected by a fraction collector (KBR-Bromma, 7000 Ultrorac Fraction Collector). The fractions were counted for $^{35}$S activity as previously described.

(B) Results

The counting efficiency of $^{35}$S ranged from 68% to 76%. Urine had the lowest and deproteinized plasma had the highest counting efficiencies.

The difference between plasma and deproteinized plasma radioactivity was considered to represent the radioactivity associated with the plasma proteins.
Fig. 5. Percentage of $^{35}\text{S}$ activity of plasma located in the plasma proteins at two abomasal methionine infusion levels.
The proportion of the plasma activity detected in the plasma proteins (Fig. 5) indicated that more of the activity is associated with the plasma proteins at the zero infusion level. After 24 hours all the activity in plasma was detected in the protein fraction at the zero infusion level. At the high infusion level only one half of the plasma activity was associated with plasma proteins after 24 hours.

The cumulative recovery of $^{35}$S in urine expressed as a percentage of administered dose (Fig. 6) indicated a difference of $^{35}$S excretion at the two infusion levels. At the high infusion level over 50% of the injected activity was present in the urine after four days. At the zero infusion level only 13% of the injected dose could be accounted for in the urine after four days.

The S-containing amino acid fractions were counted for $^{35}$S activity. At the zero infusion level all the activity detected in the deproteinized plasma was located in the methionine fraction. At the high infusion level $^{35}$S activity was detected in plasma methionine, cystine, taurine, cystathionine and the fraction collected at the expected location of methionine sulfoxides (Table 4).

The percentage of the $^{35}$S activity located in the methionine fraction decreased with time at the high infusion level. Due to the closeness of the methionine and cystathionine peaks it was difficult to separate these fractions completely. Even so, there was metabolism of methionine sulphur to cysteine, taurine, and the methionine sulfoxides. This was apparent for after ten minutes only 53% of the activity
Fig. 6. Percentage of administered dose detected in urine.
TABLE 4. $^{35}$S ACTIVITY OF THE SULPHUR-CONTAINING AMINO ACIDS, EXPRESSED AS A PERCENTAGE OF THE TOTAL $^{35}$S ACTIVITY IN THE SULPHUR-CONTAINING AMINO ACIDS AT 5.0 GM ABOMASAL METHIONINE.

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Methionine</th>
<th>Cystine</th>
<th>Taurine</th>
<th>Methionine Sulfoxides</th>
<th>Cystathionine</th>
</tr>
</thead>
<tbody>
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<td>0</td>
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</tr>
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</table>
Fig. 7. Plasma methionine specific activity curve at 0.0 gm abomasal methionine infusion.
Fig. 8. Plasma methionine specific activity curve at 5.0 gm abomasal methionine infusion.
## Table 5

**METHIONINE PLASMA KINETICS**

<table>
<thead>
<tr>
<th>Infusion Level (g Methionine/day)</th>
<th>Pool Size (μ Moles)</th>
<th>Irreversible Loss (μ Moles/Min)</th>
<th>Total Entry Rate (μ Moles/Min)</th>
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</thead>
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<td>0.0</td>
<td>81.4</td>
<td>3.85</td>
<td>12.3</td>
</tr>
<tr>
<td>5.0</td>
<td>1290.0</td>
<td>8.4</td>
<td>36.1</td>
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</table>
detected in the protein free plasma was located in the methionine fraction.

The amount of the activity in the cystine fraction was relatively low. The proportion of the activity in taurine and the methionine sulfoxides appeared to level off at about 30% and 20% respectively. The proportion of the activity in the cystathionine fraction increased with each serial blood sample. One third of the activity of protein free plasma was detected in cystathionine after four hours.

The specific activity curves for methionine (zero infusion level, Fig. 7; high infusion level, Fig. 8) can be represented by two exponential components. The pool size, irreversible loss, and total entry rate were determined by the mathematical treatment of isotope dilution data (Leng, 1970). These data (Table 5) were calculated at both infusion levels.

The methionine pool sizes were 81.4 and 1290.0 μmoles respectively at the low and high infusion levels. The total entry rate at the high infusion level was three fold larger than the lower level. The difference for total entry rate at the high and zero infusion levels was 23.8 μmoles/min. Irreversible loss at the higher level was over twice the size of the lower level. The difference for irreversible loss at the high and zero levels was 4.5 μmoles/min.

(C) Discussion

The choice of the label for amino acid metabolism aids in the interpretation of the results. Reeds (1974), and Neale and Waterlow (1974b) have indicated that the location of the
$^{14}$C-label in essential amino acids is important in the estimation of amino acid oxidation. Carbon labelling of non-essential amino acids is unsuitable for studies of protein turnover and the choice of the position of the label on the molecule is important when labelled essential amino acids are employed (James et al., 1975). Short term changes in amino acid metabolism are evaluated better with amino acids with a small pool size; the equilibration time in the excretory bicarbonate pool is also shorter than in the urea pool so that $^{15}$N is less useful than carbon labelling.

Methionine is an essential amino acid that contains sulphur, carbon, hydrogen, nitrogen and oxygen atoms. The fate of these atoms differs with the metabolism of methionine. When methionine is incorporated into protein a water molecule is lost. The role of methionine in the one carbon pool is to donate a methyl group so that carbon and hydrogen atoms are removed. Only the sulphur atom of methionine is incorporated into cysteine and cystine the other sulphur containing amino acids of protein. When methionine is oxidized the carbon is expired as CO$_2$ and the nitrogen is excreted as urea in the urine. The oxidation of the sulphur containing amino acids results in inorganic sulphate being excreted in the urine. Therefore, the role of methionine in protein metabolism and cysteine synthesis is followed best by $^{35}$S-labelled methionine.

Cross et al. (1975) were able to determine the half time of leucine in plasma of wethers. Fractionation of the plasma was not required for this amino acid. With methionine, it is necessary to fractionate plasma to account for the conversion
of methionine to its metabolites. Birt and Clark (1976) administered (U-$^{14}$C)-L-alanine to rats and could account for 30% to 50% of the activity in free taurine in the tissue. This is not surprising since the primary synthesis of taurine is from cysteine, and the carbon chain of cysteine comes from serine. This potential conversion of methionine carbon to the non-essential amino acids limits the use of $^{14}$C-labelled methionine as a specific tracer for sulphur amino acid metabolism.

The tracer used in this experiment was $^{35}$S-L-methionine and the abomasal supplement was DL-methionine. In the rat (Shannon et al., 1975) and man (Kies et al., 1975; and Zezulka and Calloway, 1976) the metabolism of the D- and L-methionine molecules is different. L-methionine is preferentially incorporated into protein while more D-methionine is excreted in the urine. D-methionine had equal nutritional value of its L-isomer for the chick (Sugahara et al., 1967). Triebwasser et al. (1976) have shown that the dog oxidized L-tryptophan to a greater degree than D-tryptophan. The urinary excretion pattern of the D- and L-tryptophan was different. The dog excretes D-tryptophan intact while L-tryptophan is excreted mainly as tryptophan metabolites. Therefore, the metabolism of amino acids differs not only with species and physiological states but also with the isomer of the amino acid. In this experiment with the lamb the tracer was L-methionine; the effect of DL-methionine supplementation on L-methionine metabolism was studied.
The incorporation of the $^{35}S$ label of methionine into plasma proteins and urinary excretion of $^{35}S$ indicates the metabolic pathways for methionine. At the low infusion level all the activity of plasma was located in plasma proteins after 24 hours and methionine was the only fraction which contained any detectable activity in the protein free plasma. After four days 13% of the injected activity could be accounted for that excreted in the urine. This suggests that methionine was utilized for anabolic processes which includes protein synthesis.

At the high infusion level one half of the activity of plasma was in the protein fraction after 24 hours. The activity in the deproteinized plasma was located in taurine, cystathionine, cystine, methionine sulfoxides and methionine. This indicated a catabolism of methionine for taurine and methionine sulfoxides are both catabolic products of sulphur containing amino acids. This is supported by the higher activity present in the urine. Urinary excretion of the activity accounted for over one half of the injected dose.

The amount of methionine entering the plasma pool per day was calculated as 2.53 gm and 7.60 gm on the zero and high infusion levels respectively. The difference between these values agrees favourably with the supplemental 5.0 gm/day of DL-methionine at the high infusion level. The basic assumption for these calculations is that intestinal amino acids are absorbed at a constant rate.

Since amino acid absorption is never 100% and the L-amino acids are absorbed more efficiently than the D-amino acids
(Mathews, 1974) it is apparent that the total entry rate has a non-intestinal component. This suggests that at the high infusion level protein turnover is greater than the lower level. Therefore, more methionine would be entering the pool by tissue protein breakdown. Reis et al. (1973a) stated that tissue protein synthesis increased at the higher methionine levels but wool synthesis decreased. Waterlow (1975) has shown that protein turnover in the whole body is higher in the animal which is synthesizing more tissue protein. Therefore, at the high infusion level tissue protein synthesis and protein turnover may be greater than those at the low infusion level.

The ratio of the methionine pool size at the high to the low infusion levels is 15.8:1. The methionine concentration in plasma ratio is 12.3:1 for the high to low infusion levels. The difference between these values may be attributed to the tissue concentration of methionine. Amino acids are free in both plasma and tissue and their concentrations vary (Munro, 1970). Plasma methionine concentration was determined on jugular blood which may not reflect the methionine concentration throughout the circulatory system. These characteristics may account for the difference between the ratios of pool size and plasma concentration. The higher ratio of methionine pool size compared to the ratio of plasma methionine concentration supports the suggestion by Munro (1970) that small changes in PAA concentrations reflects larger changes in body amino acid metabolism. Cross et al. (1974) have indicated that muscle tissue is very important in PAA homeostasis in the ruminant.

The larger proportion of the injected activity excreted
in the urine and the larger irreversible loss at the high infusion level indicates catabolism of methionine at this level. Methionine was in excess and methionine sulphur is converted to methionine sulfoxides, cysteine and to taurine. Sulphur is excreted in the urine in three forms: inorganic sulphate, ethereal sulphate and neutral sulphur. The inorganic sulphate of the urine is derived almost entirely from the oxidation of the protein molecule. Therefore, the activity detected in the urine indicates sulphur amino acid oxidation.

The magnitude of the increase in irreversible loss (4.5 u moles/min.) is much smaller than the increase in total entry rate (23.8 u moles/min.). This difference may account for the elevated plasma methionine concentration and methionine pool size at the high infusion level. Urinary loss of methionine sulphur contributes to the irreversible loss difference. At the high infusion level four times as much of the injected dose appeared in the urine compared to the low infusion level which supports the suggestion of increased catabolism of methionine.

In Experiment I the 0.0 gm methionine infusion per day was shown to be limiting for lamb #2. The excretion of activity in the urine at this level may indicate that methionine also has a role to supply sulphate in sheep. Moir (1975) discusses the catabolism of sulphur amino acids for sulphation purposes. The quantitative requirements of sulphate are not known but undoubtedly methionine has "sulphur-amino acid sparing action".
SUMMARY

The PAA profile at the graded abomasal methionine infusions indicates that methionine was not limiting in the digesta available to lamb #1. The plasma methionine curve for lamb #2 had an inflection point just below the 2.0 gm per day of abomasal methionine. The difference between these lambs may be a result of different growth rates. Lamb #2 had a higher growth rate than that of lamb #1. Therefore, one would expect lamb #2 to have a higher methionine requirement compared to lamb #1.

The increased plasma concentrations of taurine and methionine sulfoxides at the higher infusion levels indicates a catabolism of sulphur amino acids. The plateau in the plasma cystine concentration and the lower plasma levels of serine, glycine and alanine suggest that the metabolism of methionine may be impaired. The conversion of methionine to cystathionine is limited by the supply of serine. The elevated plasma concentration of methionine at the higher infusion levels may have been due in part to this limited conversion of methionine to cysteine.

The injection of the labelled methionine gives a dynamic picture of methionine metabolism. Methionine sulphur can be converted to cysteine, taurine, cystathionine and methionine sulfoxides. At the zero methionine infusion no detectable conversion was observed. At the high infusion level activity was detected in the other sulphur containing compounds. This suggests that at the zero infusion level methionine was
involved in anabolic processes; this is supported by the small excretion of label and the large proportion of the activity associated with the proteins of plasma. At the high infusion level methionine is being catabolized. A large proportion of the injected dose was recovered in the urine and less of the activity of plasma was located in the protein fraction.

The plasma parameters estimated from the dilution data also indicate the pathway of methionine metabolism. The ratio of methionine pool size and plasma concentration at the zero and high infusion levels supports the suggestion that changes in plasma concentrations reflects larger changes in tissue levels. The ratio was higher for the methionine pool size than for the plasma concentration.

The elevated methionine plasma concentration at the higher infusion level was not due to an increased entry rate but to a much smaller increase in irreversible loss. This supports the suggestion of impaired methionine metabolism at high plasma concentration of methionine.

The injection of labelled methionine at different levels of sulphur supplementation and the collection of serial blood and urine samples may prove to be a relatively quick and reliable method for the estimation of the sulphur requirement of the ruminant. When methionine is in excess, less of the label will be associated with the protein fraction of plasma and more of the injected dose will appear in the urine. When methionine is limiting in the digesta available to the animal more of the activity of plasma will be located in the protein
fraction and a small proportion of the injected dose will be excreted in the urine.


Davis, S.L. (1972) Plasma levels of prolactin, growth hormone, and insulin in sheep following the infusion of arginine, leucine and phenylalanine. Endo. 91: 549-555.


