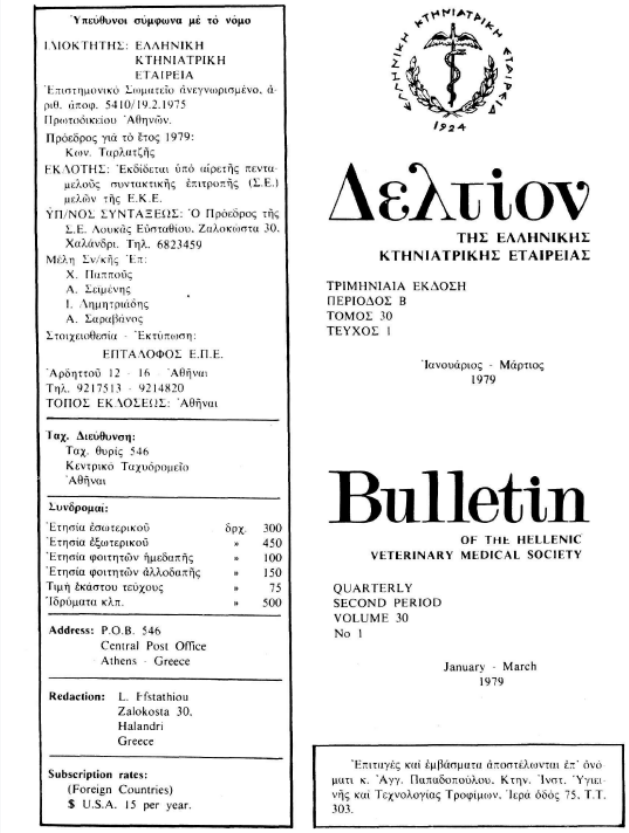
Journal of the Hellenic Veterinary Medical Society

Vol 30, No 1 (1979)



Υπεύθυνοι σύμφωνα με το νόμο

ΙΔΙΟΚΤΗΤΗΣ: ΕΛΛΗΝΙΚΗ ΚΤΗΝΙΑΤΡΙΚΗ ΕΤΑΙΡΕΙΑ
Επιστημονικό Σωματείο άνεγνωρισμένο, αριθ. άποφ. 5410/19.2.1975 Πρωτοδικείου Αθηνών.
Πρόεδρος γιά τό έτος 1979: Κων. Ταρλατζής
ΕΚΔΟΤΗΣ: Εκδίδεται ύπό αίρετής πενταμελούς συντακτικής έπιτροπής (Σ.Ε.) μελών της Ε.Κ.Ε.
ΥΠ/ΝΟΣ ΣΥΝΤΑΞΕΩΣ: Ό Πρόεδρος της Σ.Ε. Λουκάς Εύσταθίου, Ζαλοκώστα 30, Χαλάνδρι. Τηλ. 6823459
Μέλη Συν/κής Έπ:
Χ. Παππούς
Α. Σαμένης
Ι. Δημητριάδης
Α. Σαραβάνος
Στοιχειοθεσία - Έκτύπωση:
ΕΠΤΑΛΟΦΟΣ Ε.Π.Ε.
Άρδηττού 12 - 16 - Άθήναι
Τηλ. 9217513 - 9214820
ΤΟΠΟΣ ΕΚΔΟΣΕΩΣ: Άθήναι

Ταχ. Διεύθυνση:
Ταχ. θυρίς 546
Κεντρικό Ταχυδρομείο
Άθήναι

Συνδρομαί:

Έτησία έσωτερικού	δρχ.	300
Έτησία έξωτερικού	»	450
Έτησία φοιτητών ήμεδαπής	»	100
Έτησία φοιτητών άλλοδαπής	»	150
Τιμή έκάστου τεύχους	»	75
Τόμοι κλπ.	»	500

Address: P.O.B. 546
Central Post Office
Athens - Greece

Redaction: L. Efstathiou
Zalokosta 30,
Halandri
Greece

Subscription rates:
(Foreign Countries)
$ U.S.A. 15 per year.

ΕΛΛΗΝΙΚΗ ΚΤΗΝΙΑΤΡΙΚΗ ΕΤΑΙΡΕΙΑ 1924

Δελτίον
ΤΗΣ ΕΛΛΗΝΙΚΗΣ ΚΤΗΝΙΑΤΡΙΚΗΣ ΕΤΑΙΡΕΙΑΣ

ΤΡΙΜΗΝΙΑΙΑ ΕΚΔΟΣΗ
ΠΕΡΙΟΔΟΣ Β
ΤΟΜΟΣ 30
ΤΕΥΧΟΣ 1

Ίανουάριος - Μάρτιος
1979

Bulletin
OF THE HELLENIC VETERINARY MEDICAL SOCIETY

QUARTERLY
SECOND PERIOD
VOLUME 30
No 1

January - March
1979

Έπιταγές καί έμβάσματα άποστέλλονται έπ' όνόματι κ. Άγγ. Παπαδοπούλου, Κτην. Ίνστ. Ύγιεινής καί Τεχνολογίας Τροφίμων, Ίερά όδός 75, Τ.Τ. 303.

Methionine Metabolism in the Rumen of Sheep

M. HIDIROGLOU, C. G. Zarkadas

doi: 10.12681/jhvms.21373

ΔΕΛΤΙΟΝ ΕΛΛ. ΚΤΗΝ. ΕΤΑΙΡΕΙΑΣ, 1979, Τ. 30. τ. 1

METHIONINE METABOLISM IN THE RUMEN OF SHEEP

By

M. HIDIROGLOU* and C.G. ZARKADAS**

INTRODUCTION

Dietary methionine is essential in ruminant nutrition for its utilization in protein synthesis and as a major source of methyl groups in biological transmethylation. It has been suggested that rumen microflora synthesize most of their required amino acids **de novo,** utilizing ammonia and simple carbon or sulfur precursors for protein synthesis (Allison, 1969), and that one of the major roles of rumen proteins is to provide ruminants with the necessary amino acids for their growth and development (Purser, 1970). Nutritional evidence has indicated that dietary methionine and its analogues or derivatives are extensively degraded to sulfides prior to resynthesis by the microflora in ruminants (Zikakis and Salsbury, 1969; Downes et al., 1970; Salsbury et al., 1971; Belasco, 1972; Amos et al., 1974), and because of this methionine is now regarded as the first limiting amino acid for growing sheep (Nimrick et al., 1970; Schelling et al., 1973). However, direct assimilation of dietary methionine by rumen microflora is suggested by the **in vitro** studies of Nader and Walker (1970) who have indicated that up to 11% of exogenous methionine can be utilized directly for microbial protein synthesis and the remainder enters the free sulfide metabolic pool after degradation. In contrast, Landis (1963) reported that 75 to 83% of the [^{35}S]—L— methionine administered to goats was directly incorporated into rumen bacterial proteins.

The first reaction in the degradation of methionine which occurs in animal and microbial systems is the enzymic synthesis of S—adenosylmethionine (Cantoni, 1952; 1953). This compound is metabolically important as a key methyl donor in many types of transmethylation reactions (Mudd and Cantoni, 1964; Lombardini and Talalay, 1971), as the primary sulfur donor for cysteine biosynthesis, and as a regulator or effector in many enzymatic reactions including its own synthesis (Chou and Talalay, 1972; Finkelstein et al., 1974). In mam-

*Animal Research Institute

**Contribution No. 376 Food Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario, Canada, K1A OC6.

malian tissue, methionine is demethylated sequentially to homocysteine which may be condensed irreversibly with serine to form cystathionine for non-dietary cysteine biosynthesis via the transsulfuration pathway, or it may be remethylated to yield methionine for protein synthesis (Finkelstein and Mudd, 1967; Finkelstein, 1974). The terminal reaction in methionine biosynthesis is a unidirectional cycle which involves a methyl group being transferred **in toto** to homocysteine to form methionine (Du Vigneaud and Rachelle, 1965).

Although mammals are unable to synthesize the 4—carbon chain of homocysteine and depend on dietary methionine for their supply, genetic and nutritional data suggest that in microorganisms different pathways are utilized for cysteine biosynthesis **de novo** from sulfite or thiosulfate as the sulfur donor (Kredish and Tomkins, 1966; Kredish et al., 1969; Paszewski and Grabski, 1974). There is also evidence to indicate that the branched «aspartate family» biosynthetic pathway is responsible following the condensation of O-succinylhomoserine with cysteine, and with aspartyl-β-semialdehyde as the key 4—carbon precursor (Datta et al., 1973; Yen and Gest, 1974; Sauer et al., 1975). Alternative pathways are also known to exist in microorganisms to catalyze the terminal transfer of the methyl group to homocysteine to form methionine (Taylor and Weisbach, 1973). Ruminal microorganisms may also utilize these pathways, however much concerning the metabolic fate and mechanism of methionine biosynthesis form dietary sources and its incorporation into microbial protein in the rumen of sheep is still unclear.

To obtain information pertinent to this question the **in vivo** experiments described in this paper were designed to study the metabolic fate and interrelationship between dietary methionine and its utilization for methyl group neogenesis and protein synthesis in ruminants by rumen microflora. The time-dependent incorporation of labeled methionine into rumen bacterial proteins of one year old wethers and growing lambs was compared following a single intraruminal administration of the radioisotopes. The extent to which the incorporation of radioisotopically labeled methionine was distributed into omasal, abomasal and duodenal protein contents of the digesta of lambs fed a constant protein to energy levels die' 's also documented.

MATERIALS AND METHODS

Animals and Diet

Shropshire sheep from the ins. ite flock were used. At two months of age the sheep were randomly selected, kept indoors, and fed **ad libitum** a diet of equal parts of chopped alfalfa and timothy hay. In addition each sheep rec. ed a daily supplement of 250 g. of a 50:50 mixture of cracked corn and whole oats. Growing lambs were fed this diet for 4 weeks only in metabolism cages whereas the adult sheep after ten months on this diet were placed in metabolism cages in a room maintained at 17±1°C, and weighed once weekly prior to feeding. After two weeks in this environment the following experiments were carried out.

Experimental Design and Preparation of Samples for Analysis.

Experiment 1. This experiment was designed to investigate the metabolic fate of labeled dietary methionine in mature sheep fed a normal diet. The five one year old wethers used in this series (mean body weight 41.0 kg), were each dosed intraruminally by stomach tube with 6.0 μ of methyl—[^{14}C]—L—methionine (54mCi/mmol) in water per Kg body weight (B.W.). Blood samples (10 ml) were withdrawn from the jugular vein at 1 hr intervals for 8 hr and then once more 24 hr after dosing. The blood samples were transferred quantitatively into centrifuge tubes containing heparin (0.05 ml 1%; w/v in saline), and then deproteinized by the addition of an equal volume (10 ml) of 20% trichloroacetic acid solution (TCA) with continuous stirring. The tubes were then centrifuged at 2.000 x g for 20 min. at 2°C, the supernatant and sediment were separated and the amounts of radioactivity present in each fraction determined.

Samples of rumen contents (150 ml) were collected by means of a stomach tube at 2, 4, 6, 8, and 24 hr after methionine administration and were immediately strained through four layers of cheese cloth to remove coarse particles for subsequent fractionation. The bacterial and cell free fractions were prepared from clarified portions of the rumen filtrate, previously freed from protozoa and plant materials by centrifugation at 600 x g for 2 min. These fractions were then further purified by repeated centrifugation (20,000 x g; 4°C; 30 min) and washed according to the procedure described by Wright and Hungate (1967). Washed bacterial suspensions consisting of 1 part wet weight and 4 parts water were subjected to ultrasonication in a 20 Kc/sec sonicator (Model W-185G Sonifier Cell Disruptor, Harvey Instruments, N. Tunawanda, New York). The ultrasonically-disrupted bacterial cells were then treated with an equal volume of 20% TCA and the precipitated bacterial proteins were freed of TCA by repeated ether extractions (Hidiroglou et al., 1974). The bacterial and cell-free fractions were then frozen and stored at −20°C for subsequent analyses.

Experiment 2. The purpose of this experiment was to study the distribution of intraruminally administered labeled methionine in the ruminal, omasal, abomasal and duodenal contents of growing lambs fed an identical diet. For this purpose the metabolic fate of methionine radioisotopically labeled in the carbon or hydrogen atoms of the methyl group or in the sulfur atom was studied in four lambs (mean body weight 25 Kg.). Two lambs were each given intraruminally 40μ Ci/Kg B.W. methyl-[^{14}C]-L-methionine (45.7mCi/mmol). Two hours later the animals were sacrificed, and the entire alimentary tract was taken out with as little disturbance as possible after ligating it at different levels. Samples from the ruminal contents were taken and the bacterial and cell free fractions were prepared as described previously. Samples (50 ml) from the omasal, abomasal and duodenal digesta were taken and transferred into separate centrifuge tubes, an equal volume of 20% trichloroacetic acid solution was added and the TCA-insoluble materials were saparated by centrifugation (2000 x g; 10 min), and freed of TCA as before. The total radioactivity, nitrogen and dry matter were determined in each of the digesta and TCA-insoluble materials and stored at −20°C for analysis.

A third lamb was given intraruminally 45.7 mCi/Kg B.W. of methyl -[^{3}H]-L-methionine while a fourth sheep received 5.5. μCi/Kg B.W. of [^{35}S]-L- methionine in water. Both animals were sacrificed two hours later, samples of rumen contents (100 ml) were taken and the bacterial and cell free fractions were prepared as described previously.

Analytical Methods

Amino acid analyses

Amino acid analyses were carried out either on a Beckman Spinco model 120B or on a Jeol model JLC-5AH amino acid analyzers equipped with an LKB Ultrorac fraction collector (LKB-Produkter AB, Bromma, Sweden). Triplicate samples of blood plasma and the various fractions from rumen liquor and digesta of the alimentary tract, equivalent to 1.0 to 3.0 mg. of nitrogen, were hydrolyzed under vacuum in constant boiling HCl at 110°C for 24,48 and 72 hr, re-

spectively, with the usual precautions described by Moore and Stein (1963). Identical samples were submitted to the oxidation procedure of Moore (1963) for 18 hr and then hydrolyzed as before for 24 hr. Diluted samples (equivalent to 0.1-0.3 mg of N) were analyzed on the amino acid analyzer, and the eluant was collected in 2.0 ml fractions at a flow rate of 105 ml/hr. Fractions were monitored for cysteic acid and methionine sulfone by radioactive counting of 2.0 ml. samples and by absorbancy measurements at 570 nm and 440 nm in the amino acid analyzer. The recovery of cyst(e)ine as cysteic acid and of methionine as methionine sulfone was calculated relative to tne yields obtained by the performic acid treatment of their standards.

RESULTS AND DISCUSSION

The present studies show that most of the methyl-[^{14}C] label incorporated into rumen bacterial proteins 2 hr after dosing was found to be in methionine and with time, up to the limits tested, the label from the bacterial proteins began to disappear rapidly. The data reported in Tables 1 and 2 shows that the disappearance of the label from the bacterial proteins corresponded with the label uptakes by the protozoa and the label that entered the circulation of the wethers. Although plasma radioactivity accounted for only a small proportion of the administered dose, the results obtained with the increasing incorporation

TABLE 1

Experiment 1 Distribution of radioactivity in blood plasma of mature wethers following a single administration of methyl-[^{14}C]-L-methionine (6 μ Ci/Kg B.W.).

Time after dosing (hr)	Quantitative recoveries of specific activity (d.p.m./ml plasma)[a]	Percentage distribution of methyl-[^{14}C] in the TCA soluble fraction
1	4486±1216	33.76±12.56
2	5697± 500	22.73± 7.43
3	5843± 650	16.10± 1.94
4	6447±2325	12.49± 2.62
5	6530±2325	12.61± 2.15
6	6877±1235	13.39± 4.17
7	7721±1143	8.20± 2.52
8	8040±1417	7.95± 2.73
24	6230±1014	8.05± 4.49

[a]The values are expressed as disintegrations per minute per ml of plasma.

TABLE 2

Experiment 1 Distribution of radioactivity in the various rumen liquor fractions from five mature wethers given a single administration of methyl-[^{14}C]-L-methionine (6 μ Ci/Kg B.W.)

Time after dosing (hr)	Rumen Liquor (d.p.m./ml)[a]	Percentage distribution of methyl-[^{14}C] label			
		Cell Free fraction	Bacteria	Protozoa and plant material	Total
2	9195±3442	78.8±9.01	21.6±8.31	15.9± 9.06	116.3
4	7159±3518	68.5±3.32	6.8±0.51	26.2± 2.09	101.5
6	4704±2871	71.6±2.24	6.3±3.09	28.8± 6.09	106.7
8	4879±2338	69.6±7.69	5.6±3.82	31.5±12.52	106.7
24	3864±1718	59.4±4.86	7.4±4.73	38.5±16.54	105.3

[a]The values are expressed as disintegrations per minute per ml of the original rumen liquor (d.p.m./ml)

of methyl - [^{14}C]-L-methionine into plasma proteins is in reasonable agreement with those reported by Edwards et al. (1960) for rats. As may be seen in Tables 2 and 3, a large amount (78.8%) of the administered radioactivity to wethers was found in the cell-free fraction 2 hr after dosing, and a substantial amount (28.23%) of the total radioactivity could be traced by both amino acid and radioactivity measurements of cell-free fraction hydrolysates to coelute as methionine sulfone. Bird and Moir (1972) from their nutritional observations on sheep, suggested that ruminal infusions of methionine could merely substitute for ingested organic or inorganic sulfur and concluded that dietary methionine is essential for the growth of certain ruminal microbes has come from the work of Pittman and Bryant (1964) who indicated that methionine is required for the growth of **Bacteroides ruminicola,** and from the finding that exogenous methionine effectively increased the **in vitro** rate of ruminal protein synthesis by 1.7 times (Gil et al., 1973). Patton et al. (1968) also reported that methionine affects microbial lipid biosynthesis. Bucholtz and Bergen (1973) estimated that 25% or more of the total ruminal synthesis **in vitro** may be involved in turnover and concluded that such rates of turnover of microbial protein represents the synthesis of a 30 g digestible protein to Mcal of digestible energy ratio, an amount which is considered nutritionally adequate for growing lambs and calfs.

From the experimental evidence described and the results presented in Ta-

TABLE 3

Experiment 1 Incorporation of radioactivity into methionine and cyst(e)ne of the rumen liquor bacterial and cell free fractions from five mature sheep (mean B.W. 41.0 Kg) 2 hr afer a single intraruminal administration of methyl-[^{14}C]-L-methionine

Amino Acid	Quantitative recoveries of specific activity (d.p.m./μ mole a.a./mg N)[a]	Percentage distribution[b] of radioactivity
Bacteria Protein Fraction		
Cysteic acid	679± 226[c]	0.75±0.44
Methionine sulfone	12947±7434	19.84±8.39
Cell Free Fraction		
Cysteic acid	12172±10455	31.60±12.89
Methionine sulfone	10355± 5103	28.23± 5.39

[a]The data are mean values of five triplicate determinations and are expressed as disintegrations per minute per μ mole amino acid per mg of Nitrogen (d.p.m./μ mole a.a./mg N).
[b]The values are expressed as percentage distribution of radioactivity of the original digesta.
[c](±) Standard deviation.

bles 1 to 4, it can be concluded that a substantial proportion of the administered methyl-[^{14}C] group of methionine is incorporated into bacterial protein methionine of sheep 2 hr after dosing. The present data also indicates that incorporation of dietary methionine into rumen bacterial proteins by direct assimilation can occur in the rumen of three month old sheep and that the ability to use dietary methionine for the stimulation of bacterial protein synthesis is retained through the adult life of sheep. The total **in vivo** incorporation of methyl-[^{14}C]-L-methionine into the rumen bacterial protein fraction was much higher in mature wethers (19.84%) than in growing sheep (10.70%) fed an identical diet. Higher levels of radioactivity were also noted in the rumen liquor cell-free fractions of mature wethers than those found in growing lambs. One possible

TABLE 4

Experiment 2 Incorporation of radioactivity into methionine and cysteine of the rumen liquor bacterial and cell free fractions from three months old sheep (mean B.W. 25 Kg) 2 hr after a single intraruminal administration of either methyl-[^{14}C]-L-methionine (40μ Ci/Kg B.W.), or methyl-L-[^{3}H]-methionine (45.7 m Ci/Kg B.W.), or 5.5 μ Ci/ Kg B.W. of [^{35}S]-L-methionine was given

Amino Acid	Quantitative recoveries of specific activity (d.p.m./μ mole a.a.)[a] L-methionine labeled at			Percentage distribution of radioactivity		
	methyl-[^{14}C]	methyl-[^{3}H]	[^{35}S]	methyl-[^{14}C]	methyl-[^{3}H]	[^{35}S]
Bacterial Protein Fraction						
Cysteic acid	5158	1776	128356	0.15	0.18	4.50
Methionine sulfone	93970	30257	254010	10.70	6.90	7.30
Cell Free Fraction						
Cysteic acid	136389	46775	160468	19.000	7.9	5.4
Methionine fulfone	86918	93360	120084	11.2	31.8	4.2

[a]The values are expressed as disintegrations per minute per μmol amino acid.

interpretation of these results is that these differences may be due to the age of growing lambs, for neither their rumen nor its microbial population would have been fully developed. An alternative explanation may be that this difference is due to a faster rate of utilization of the available labeled methionine to meet their increased metabolic requirements. There also appears to be small sifferences between the levels of radioactivity incorporated into bacterial protein methionine of lambs which received methyl-[^{3}H]-, of [^{35}S]-L-methionine and those administered methyl-[^{14}C]-L-methionine (Table 4). Although at the present time, there is no definite explanation for these small differences, in general this data is consistent with the **in vitro** data obtained by Nader and Walker (1970) who have estimated that 11% of exogenous methionine was directly incorporated into ruminal bacterial proteins.

Surprisingly, the cyst(e)ine content of both the rumen bacterial protein and cell-free fractions were found to contain radioactivity which must have originated from the methyl-[^{14}C] group of the andministered methionine as shown in Tables 3 and 4. This raises the question of how this cyst(e)ine became labeled during rumen fermentation and whether the methyl group was being transferred **in toto** or whether it was being utilized by anaerobic bacterial oxidation and subsequent reduction through an intermediate such as carbon dioxide, formaldehyde or formate. To resolve this question additional **in vivo** experiments were carried out in which methyl-[^{14}C]- and methyl-[^{3}H]-L- methionine, and [^{35}S]-L-methionine were given intraruminally to growing lambs. The rationale was that, in the event that formaldehyde or formate were the obligatory intermediates for anaerobic cysteine biosynthesis in the rumen, the cysteine content of both the bacterial and cell-free fractions must be tritiated. In the event that the methyl group was oxidized to carbon dioxide, it would be expected that both methionine and cysteine would not be tritiated. Thus the concentration of radioactivity actually found should provide an experimental chemical basis for preferring one of these two hypotheses.

From the results presented in Table 4 and the fact that both cysteine and methionine of the bacterial and cell-free fractions were tritiated, it may be concluded that the methyl group of dietary methionine is transferred as a one-carbon unit at the oxidation levels of formate or formaldehyde for the **de novo** biosynthesis of the methylene group of cysteine. The high concentration of tritiated cysteine in the cell-free fraction, and the finding by Kredich and Becker (1971) that in microorganisms L-cysteine is synthesized from L-serine, acetyl-CoA, and sulfide strongly suggests that formate or formaldehyde may be combined with tetrahydrofolate to form 5,10 methylenetetrahydrofolate which is in equilibrium with either serine or methionine (Blakley, 1959). This is especially interesting since it is known that the reversible transfer of a one-carbon group between glycine and serine is catalyzed by serine hydroxymethylase in the presence of pyridoxal phosphate and Mn^{++} with 5,10 methylenetetrahydrofolate as the active substrate of the reaction (Blakley, 1960, 1969; Rader and Huenne-

kens, 1973). The high [^{14}C] and tritium content found in the methylene group of cysteine (Tables 3 and 4) of the bacterial and cell-free fractions seems to be a reflection of this exchange. Thus, the methyl-[^{14}C]-, and methyl-[^{3}H]-groups of the administered methionine may enter the matabolic pool of serine as the immediate precursor of the methylene group of cysteine by the following reactions:

Nonenzymic interaction

Tetrahydrofolate+formaldehyde ⇌ 5,10-methylenetetrahydrofolate (1)

L-Serine: tetrahydrofolate 5,10-methylenetransferase [EC 2.1.2.1]

5,10-Methylenetetrahydrofolate + glycine + H_2O ⇌ L-serine + tetrahydrofolate (2)

Serine transacetylase

L-Serine + acetyl-CoA → O-acetyl-L-serine + CoA (3)

O-Acetylserine sulfhydrylase

O-Acetyl-L-serine + sulfide → L-cysteine + acetate (4)

according to Blakley (1959), Kredich et al. (1969) and Becker et al. (1969). Although the individual steps have not yet been demonstrated directly in rumen microorganisms nor all of the reactants and products fully characterized, the present results are all consistent with this pathway since this sequence appears to be the most likely explanation of the labeling.

The question may be raised whether the serine hydroxymethylase transfer reaction is the only mechanism of single-carbon unit formation or whether rumen microorganisms may oxidize the methyl group of dietary methionine to carbon dioxide. If carbon dioxide was the obligatory intermediate for methyl group neogenesis by rumen microorganisms, as has been suggested for certain anaerobic microorganisms (Ljungdahl and Wood, 1965; Jungerman et al. 1968), then both bacterial protein cysteine and methionine would be labeled with [^{14}C] only. This is expected since upon regeneration of the methyl group from formaldehyde only the oxygen of the original methyl group would remain attached to the carbon. Since bacterial protein and cell-free fraction cysteine and methionine were labeled with both tritium and [^{14}C], the present data appear to exclude such a possibility. Furthermore, it has recently been shown that the **in vitro** reductive carboxylation of labeled carbon dioxide to form pyruvate from acetate for the synthesis of serine by rumen microorganisms, almost all its activity was found in the carboxyl rather than the methylene group of serine (Sauer et al., 1975). The labelling pattern of cysteine in the bacterial and cell-free fraction (Table 4) with [^{35}S] however, is again consistent with reactions (3) and (4) and with the reductive carboxylation of acetate to pyruvate pathway proposed by Sauer et al. (1975) for the synthesis of serine. These authors have also suggested that some serine may also be synthesized in the rumen by reductive carboxylation to form 3-hydroxypyruvate which could then be transaminated to give serine. However, at present, there is no reliable evidence for such a possibility.

TABLE 5

Experiment 2 Distribution of radioactivity in the TCA-insoluble fraction of the digesta of the alimentary tract of growing lambs 2 hr after a single intraruminal administration of methyl-[^{14}C]-L-methionine

Percent incorporation of label into TCA-insoluble (d.p.m./μmole a.a.) contents of digesta	Quantitative recoveries of specific activities		Percent distribution of label	
	Cysteic acid	Methionine sulfone	Cysteic acid	Methionine sulfone
Omasum (86.2%)	5053	68046	2.87	82.6
Abomasum (58.1%)	10057	60266	4.28	45.2
Duodenum (23.4%)	3506	51022	1.2	21.8

The specific activity incorporated into bacterial protein methionine (Tables 3 and 4) was equal to or higher than that of the cell-free fraction and in all cases substantial amounts of the total radioactivity could be traced as methionine sulfone. These results are expected since in the **de novo** biosynthesis of the methyl group of bacterial methionine the label can only be derived from a one-carbon unit at the oxidation level of formaldehyde (Blakley, 1959) or from a methyl group being transferred **in toto** to homocysteine to form methionine (Du Vigneaud and Rachele, 1965; Guest et al., 1962; Taylor and Weissbach, 1973). The two principal steps that have been suggested are:

5,10-Methylene-H_4-folate + $FADH_2$ ⇌ 5-methyl-H_4-folate + FAD (5)

5-methyl-H_4-folate (Glu_1, Glu_3, etc.) + Homocysteine $FHDH_2$ → AMe methionine + H_4-folate (Glu_1, Glu_3, etc.) (6)

Reaction (5) is catalyzed by the enzyme 5,10-methylene tetrahydrofolate reductase and reaction (6) by 5-methyl tetrahydrofolate homocysteine transmethylase which depends for its activity on a reducing system, anaerobiosis, AMe and B^{12} or cobalamin. $FADH_2$ may be supplied by enzymatic reduction of FAD by either DPNH or TPHN in the presence of a suitable reductase (Rosenthal and Buchanan, 1965; Buchanan, 1971). The possibility remains that O-acetyl-L-homoserine and methanethiol (CH_3SH) or sulfide (Kerr, 1971) are the obligatory intermediates for anaerobic methionine biosynthesis in the rumen as has been suggested by Bird and Moir (1972), Zikakis and Salsbury (1969)

Experiment 2 Amino acid composition[a] of rumen bacterial protein and cell free fractions, and the omasal, abomasal, and duodenal protein contents of the digesta for three months old sheep

Amono Acid	Rumen Liquor Components		Omasum	Abomasum	Duodenum
	Bacterial Protein	Cell free extracts			
Lysine	3.85±0.98[b]	3.24±1.14	3.53±0.53	4.26±0.12	4.15±0.40
Histidine	0.64±0.09	0.42±0.14	0.56±0.15	–	–
Ammonia	8.08±2.63	14.23±4.99	9.22±1.58	11.05±1.38	15.04±4.35
Arginine	1.43±0.33	0.83±0.15	1.01±0.25	1.27±0.43	1.31±0.59
Cysteic acid	0.57±0.11	0.53±0.11	0.85±0.13	0.43±0.01	1.28±0.72
Methionine sulfone	1.31±0.42	1.21±0.42	2.06±0.52	1.75±0.19	2.39±0.60
Aspartic acid	5.28±1.26	4.58±1.66	4.49±1.34	4.72±0.17	4.38±0.64
Thieonine	2.46±0.56	1.87±0.51	2.32±0.37	2.38±0.11	2.49±0.15
Serine	2.24±0.47	1.72±0.49	2.07±0.35	2.30±0.12	2.80±0.41
Glutamic acid	4.61±0.83	4.61±1.66	4.05±1.71	4.52±0.08	4.90±0.52
Proline	1.65±0.28	1.63±0.48	2.54±0.34	2.02±0.04	2.78±0.32
Glycine	3.91±0.80	3.44±0.94	4.06±0.75	3.56±0.14	5.27±0.98
Alanine	4.33±1.04	3.00±0.76	3.77±0.68	3.81±0.33	3.98±0.70
Valine	3.10±0.75	2.36±0.65	4.20±1.62	3.78±1.82	3.43±0.51
α,ε-diaminopimelic acid	0.87±0.44	–	–	–	–
Isoleucine	2.58±0.53	1.93±0.63	2.45±0.37	2.00±0.01	2.67±0.34
Leucine	3.53±0.72	2.49±0.76	3.67±0.51	2.48±0.03	4.40±0.40
Tyrosine	1.28±0.36	0.82±0.26	1.89±0.37	1.34±0.41	1.69±0.12
Phenylalanine	1.83±0.35	1.19±0.34	1.92±0.24	1.33±0.11	2.28±0.50

[a]The data are mean values of eight determinations and are expressed as μmol/mg Nitrogen.
[b](±) Standard deviation.

and Salsbury et al. (1971). However, there is no reliable evidence for such a possibility since the normal function of O-acetyl-L-homoserine and CH_3SH in the metabolism of methionine is not yet clear (Kerr, 1971). In addition, Sauer et al. (1975) have recently presented evidence to indicate that the major biosynthetic pathway for the **in vitro** synthesis of methionine by rumen microorganisms appears to be the condensation of O-succinylhomoserine with cysteine by way of cystathionine with aspartyl-b-semialdehyde as precursor. Thus, from the experimental evidence described and the results presented in Tables 3 and 4, it appears that the methyl group of dietary methionine may be transferred as a one-carbon unit either via reactions (5) and (6) or in the presence of 5-methyl tetrahydrofolate-homocysteine transmethylase. These results are consistent with those reported for anaerobic microorganisms by others (Taylor and Weissbach, 1973) and the labelling pattern reported for methionine when [^{35}S]-L-methionine was administered to growing sheep.

REFERENCES

1. Allison, M.J. 1969. Biosynthesis of amino acids by ruminal microorganisms. J.Anim. Sci. **29:** 797-807.
2. Amos, H.E., Little, C.O., Digenis, G.A., Schelling, G.T., Tucker, R.E. and Mitchell, Jr. G.E. 1974. Methionine, DL-homocysteine thiolactone and N-acetyl-DL-methionine for ruminants. J. Anim. Sci. **39:** 612-617.
3. Becker, M.A., Kredich, N.M. and Tomkins, G.M. 1969. The purification and characterization of O-acetylserine sulfhydrylase-A from **Salmonella typhimurium.** J. Biol. Chem. **244:**2418-2427.
4. Belasco, I.J. 1972. Stability of methionine hydroxy analog in rumen fluid and its conversation **in vitro** to methionine by calf liver and kidney. J. Dairy Sci. **55:** 353-357.
5. Bird, P.R. and Moir, R.J. 1972. Sulphur metabolism and excretion studies in ruminates. VIII. Methionine degradation and utilization in sheep when infused into the rumen or abomasum. Aus. J. Biol. Sci. **25:** 835-848.
6. Blakley, R.L. 1959. The reaction of tetrahydropteroylglutamic acid and related hydropteridines with formaldehyde. Biochem. J. **72:** 707-715.
7. Blakley, R.L. 1969. The biochemistry of folic acid and related pteridines. **In** Frontiers of Biology. **Edited by** A. Neuberger and E.L. Tatum. North-Holland Publ. Amsterdam. pp 332-
8. Buchanan, J.M. 1971. Methionine Biosynthesis. **In** Methods in enzymology. **Edited by** S.P. Colowick and N.O. Kaplan, and by H. Tabor and C.W. Tabor. **Vol. 27B:** Academic Press Inc., New York. pp 371-378.
9. Bucholtz, H.F. and Bergen, W.G. 1973. Microbial phospholipid synthesis as a marker for microbial protein synthesis in the rumen. Appl. Microbiol. **25:** 504-513.

10. Burril, P.H., Sattelmeyer, P.A. and Lerner, J. 1974. Effect of theophylline and Na^+ on methionine influx in Na^+ - depleted intestine. Biochim. Biophys. Acta **373:** 265-276.
11. Cantoni, G.L. 1952. The nature of the active methyl donor formed enzymatically from L-methionine and adenosinetriphosphate. J.Amer. Che. Soc. **74:** 2942-2943.
12. Cantoni, G.L. 1953. S-Adenosylmethionine; A new intermediate formed enzymatically from L-methionine and adenosinetriphosphate. J.Biol. Chem. **204:** 403-416.
13. Champredon, C., Pion, R. and Prugnaud, J. 1973. Etude du metabolism de la méthionine dans le rumen de la chévre. Ann. Biol. Anim. Biochem. Biophys. **13:** 774-775.
14. Chou, T.C. and Talalay, P. 1972. The mechanism of S-adenosyl-L-methionine synthesis by purified preparations of Baker's Yeast. Biochemistry, **11:** 1065-1073.
15. Datta, P., Dungan, S.M. and Feldberg, R.S. 1973. Regulation of amino acid biosynthesis of the aspartate pathway in different microorganisms. **In** Genetics of industrial microorganisms. **Edited by** Z.Venek, Z. Hostalek, and J. Cudlin. Academia, Publishing House of the Czechoslovak Acad. Sci. Prague, pp. 177-191.
16. Downes, A.M., Reis, R.J., Sharry, L.F. and Tunks, D.A. 1970. Evaluation of modified [^{35}S] methionine and [^{35}S] casein preparations as supplements for sheep. Br. J. Nutr. **24:** 1083-1089.
17. Du Vigneaud, V. and Rachelle, J.R. 1965. The concept of transmethylation in mammalian metabolism and its establishment by isotopic labelling through **in vivo** experimentation. **In** Transmethylation and methionine biosynthesis. **Edited by** S.K. Shapiro and F. Schlenk. The University of Chicago Press, Chicago, pp 1-20.
18. Du Vigneaud, V., Rachelle, J.R. and White, A.M. 1965. A crucial test of transmethylation **in vivo** by intermolecular isotopic labelling. J. Amer. Chem. Soc. **78:** 5131-5132.
19. Finkelstein, J.D. 1974. Methionine metabolism in mammals: The biochemical basis for homocystinuria. Metabolism **23:** 387-398.
20. Finkelstein, J.D., Kyle, W.E.and Harris, B.J. 1974. Methionine metabolism in mammals: Regulatory effects of S-adenosyl-homocysteine. Arch. Biochem. Biophys. **165:** 774-779.
21. Finkelstein, J.D. and Mudd, S.H. 1967. Trans-sulfuration in mammals. The methionine-sparing effect of cystine. J.Biol. Chem. **242:** 873-880.
22. Hidiroglou, M., Jenkins, K.J. and Knipfel, J.E. 1974. Metabolism of selenomethionine in the rumen. Can. J. Anim. Sci. **54:** 325-330.
23. Gil, L.A., Shirley, R.L., Moore, J.E. Easley, J.F. 1973. Effect on rumen bacteria of methionine hydroxy analog and Proc. Soc. Exp. Biol. and Med. **142:** 670-674.

24. Guest, J.R., Friedman, S., Woods, D.D. and Smith, E.L. 1962. A methyl analogue to cobamide coenzyme in relation to methionine synthesis by bacteria. Nature, **195:** 340-342.
25. Jungermann, K., Thauer, R.K. and Decker, K. 1968. The synthesis of one-carbon units from CO_2 in **Clostridium Kluyveri.** Eur. J. Biochem. **3:** 351-359.
26. Kerr, D. 1971. O-Acetylhomoserine sulfhydrylase **(Neurospora) In** Methods in enzymology. **Edited by** S.P. Colowick and N.O. Kaplan, and by H.Tabor and C.W. Tabor. Vol. **27B:** Academic Press Inc., New York, pp 446-450.
27. Kredich, N.M. and Becker, M.A. 1971. Cysteine biosynthesis: Serine transacetylase and O-acetylserine sulfydrylase **(Salmonella typhimurium). In** Methods in enzymology, Vol. **27:** Academic Press, Inc., New York, pp 459-470.
28. Kredich, N.M., Becker, M.A. and Tomkins, G.M. 1969. Purification and characterization of cysteine Synthetase, a bifunctional protein complex, from **Salmonella typhimurium.** J.Biol. Chem. **244:** 2428-2439.
29. Kredich, N.M. and Tomkins, G.M. 1966. The enzymic synthesis of L-cysteine in **Escherichia coli** and **Salmonella typhimurium.** J.Biol. Chem. **241:** 4955-4965.
30. Landis, J. 1963. Untersuchnngen über der schwefelstoffweschel des weinderkäuers. I. Mitteilung: Die verwertung von [^{35}S] sulfat, DL-[^{35}L] methionin und DL-[^{35}S] cystin durch laktierende ziegen. Z. Tierphysiol Tierernähr. Futtermit **18:** 357-370.
31. Ljungdahl, L. and Wood, H.G. 1965. Incorporation of [^{14}C] from carbon dioxide into sugar phosphates, carboxylic acids, and amino acids by **Clostridium thermoacetium.** J. Bacteriol. **89:** 1055-1064.
32. Lombardini, J.B. and Talalay, P. 1971. Formation, functions and regulatory importance of S-adenosyl-L-methionine. **In** Advances in enzyme regulation. **Edited by** G.Weber. Vol. **9:** Pergamon Press, Oxford, pp 349-384.
33. Moore, S. 1963. On the determination of cystine as cysteic acid. J.Biol. Chem. **283:** 235-237.
34. Moore, S. and Stein, W.H. 1963. Chromatographic determination of amino acids by the use of automatic recording equipment. **In** Methods in enzymology. **Edited by** S.P. Colowick and N.D. Kaplan. Vol **6:** Academic Press, Inc., New York, pp 819-831.
35. Mudd, S.H. and Cantoni, G.L. 1964. Biological transmethylation, methyl-group neogenesis and other one-carbon metabolic reactions dependent upon tetrahydrofolic acid. **In** Comprehensive biochemistry. Vol. **15:** Elsevier Publishing Company, Amsterdam, pp 1-47.
36. Nader, C.J. and Walker, D.J. 1970. Metabolic fate of cysteine and methionine in rumen digesta. Appl Microbiol. **20:** 677-681.
37. Nimrick, K., Hatfield, E.E., Kaminski, J. and Owens, F.N. 1970. Qualita-

tive assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. J. Nutrition **100:** 1293-1300.

38. Patton, R.A., McCarthy, R.D. and Griel, Jr. C.C. 1968. Lipid synthesis by rumen microorganisms. I. Stimulation by methionine **in vitro.** J.Dairy Sci. **51:** 1310-
39. Paszewski, A. and Grabski, J. 1974. Regulation of S-amino acid biosynthesis in **Aspergillus nidulans.** Molec. gen. Genet. **132:** 307-320.
40. Pittman, K.A. and Bryant, M.P. 1964. Peptides and other nitrogen sources for growth of **Bacteroides ruminicola.** J.Bacteriol. **88:** 401-410.
41. Purser, D.B. 1970. Nitrogen metabolism in the rumen: microorganisms as a source of protein for the ruminant animal. J. Anim. Sci. **30:** 988-1001.
42. Rader, J.I. and Huennekens, F.M. 1973. Folate coenzyme-mediated transfer of one-carbon groups. **In** the Enzymes. **Edited by** P.D. Boyer. Vol. **9:** Academic Press, Inc., New York, pp 197-223.
43. Rosenthal, S. Smith, L.C. and Buchanan, S.M. 1965. J. Biol. Chem. **240:** 825-836-843.
44. Salsbury, R.L., Marvill, D.K., Woodmansee, C.W. and Haenlein, G.F.W. 1971. Utilization of methionine and methionine hydroxy analog by rumen microorganisms **in vitro.** J. Dairy Sci. **54:** 390-396.
45. Sauer, F.D., Erfle, J.D. and Mahadevan, S. 1975. Amino acid biosynthesis in mixed rumen cultures. Biochem. J. **150:** 357-372.
46. Schelling, G.T., Chandler, J.E. and Scott, G.C. 1973. Postruminal supplemental methionine infusion to sheep fed high quality diets. J. Anim. Science **37:** 1034-1039.
47. Taylor, R.T. and Weissbach, H. 1973. N^5-Methyl-tetrahydrofolate-homocysteine methyltransferases. **In** the enzymes. **Edited by** P.D. Boyer. Vol. **9:** Academic Press, Inc., New York, pp 121-165.
48. Yen, H. and Gest, H. 1974. Regulation of biosynthesis of aspartate family amino acids in the photosynthesis bacterium **Rhodopseudomonas palustris.** Arch. Microbiol. **101:** 187-210.
49. Zikakis, J.P. and Salsbury, R.L. 1969. Metabolism of sulfur amino acids by rumen microorganisms. J.Dairy Sci. **52:** 2014-2019.