# Metabolism of Sulfur Amino Acids by Rumen Microorganisms [1]

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**Abstract**

The production of methanethiol and ethanethiol from L-methionine, S-methyl-L-cysteine, ethionine, and S-ethyl-L-cysteine during in vitro fermentation by rumen microorganisms was determined by employing gas-liquid chromatography to analyze the head-space gas produced by the fermentations. Each substrate with rumen fluid was incubated at 39 C in serum bottles equipped to permit syringe sampling of the head-space gas evolved. All substrates were used at a concentration of 1.67 mg/ml rumen fluid. It was found that methanethiol was formed during the in vitro fermentation of S-methyl-L-cysteine and of methionine and that ethanethiol was produced from S-ethyl-L-cysteine and from ethionine. When dimethyl thetin chloride was used as substrate, dimethyl sulfide was formed. S-ethyl-L-cysteine inhibited production of methanethiol from S-methyl-L-cysteine in the early stages of fermentation, but inhibition was largely overcome as fermentation proceeded. Some indication of utilization of methanethiol was obtained and appeared to be related to diet. This is in accord with the production of methanethiol by the reaction: methionine ⟶ S-methyl-L-cysteine ⟶ methanethiol. However, it is possible that both methionine and S-methyl-L-cysteine were directly dethiomethylated.

Plants consumed by dairy cows are known to contain various forms of sulfur-containing compounds. For instance, legumes contain S-methyl cysteine (SMC) (27), alfalfa contains methionine (7), and grass and corn silage contain dimethyl sulfide (19). Upon fermentation of plant material by rumen microorganisms, part of the sulfur-containing compounds (e.g., sulfur amino acids) fulfill some of the requirements of the microorganisms and eventually of the host animal, while another portion of these are released as volatile sulfurous rumen gases (e.g., dimethyl sulfide, methanethiol, hydrogen sulfide, etc.).

Methionine is believed to be an essential amino acid which, if not supplied, limits ruminant growth (9, 10, 12, 16), and it has been shown recently that an appreciable amount of this amino acid is synthesized in the rumen. Conrad et al. (6) have reported that individual cows receiving alfalfa hay and silage synthesized between 31 and 59 mg methionine per kilogram body weight per day. The biosynthesis and degradation of methionine by various microorganisms have been reported. Schlenk and Tillotson (24) found that yeast synthesized methionine from methanethiol (MeSH). Wolff et al. (30) showed that by incubating MeSH plus L-serine with a partially purified enzyme prepared from a yeast extract yielded SMC (the lower homolog of methionine). In view of this finding, Maw (15) suggested that SMC may be a precursor of methionine by a route which excludes cysteine. In addition, Mitsuhashi (17) has obtained an enzyme from soil bacteria that degrades methionine, with a resultant production of MeSH, α-amino butyric acid, and $NH_3$. Similar results have been reported with enzymes from *Escherichia coli* (20, 21), *Clostridium sporogenes* (29), and a *Pseudomonas sp.* (18) obtained from soil. Also, it has been shown that the fungi *Scopulariopsis brevicaulis* (4), *Microsporum gypseum* (26), and a gram-negative bacterium isolated from soil (17) degrade methionine and SMC into MeSH.

Although the synthesis of methionine by rumen microorganisms has been reported (3, 6, 13), little, if any, knowledge is available concerning its biosynthetic route in the rumen. The object of this study was to investigate some possible precursors and intermediates as well as some competitive inhibitors, such as S-ethyl cysteine (SEC) and ethionine, of methionine synthesis by rumen microorganisms in an in vitro system. Since this amino acid is necessary for ruminant growth, knowledge concerning its biosynthetic route in the bovine rumen might permit adjustment of the ration to produce a greater supply of methionine for the animal.

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### Experimental Procedure

Inocula for the laboratory fermentation were obtained from four fistulated bovine females maintained in the University of Delaware herd: two ovariectomized nonlactating animals (Guernsey 111 and Holstein 154), and two intact milking cows (191 and 222, both Holsteins). Unless otherwise indicated, Cows 111 and 154 received a ration of hay (brome grass alfalfa), corn silage, and pasture, while Cows 191 and 222 received hay, corn silage, pasture, and a commercial dairy concentrate.

Samples of the fluid portion of rumen ingesta were obtained using the procedure of Salsbury et al. (22) at 8 to 9 AM on the day of experiment. No attempt was made to restrict the feeding habits of the animals to obtain a more uniform inoculum.

Gas-liquid chromatography (GLC) was used to determine the amount of methane, MeSH, ethanethiol (EtSH), and dimethyl sulfide in the head-space gas evolved during fermentation of the various substrates by rumen fluid. The analysis of organic compounds in the head-space gas by GLC has been studied by several investigators (1, 2, 14, 28). This method was employed recently by Field and Gilbert (8) to measure the amount of MeSH in urine. Their method was modified to adapt it to the conditions of our experiments.

The method used in this study was as follows: 15 ml of rumen fluid plus known amounts of various substrates were placed in 60-ml serum bottles and sealed with channel rubber stoppers to permit syringe sampling of the head-space gas. One bottle without substrate served as a control. S-methyl cysteine, L-methionine, SEC, DL-ethionine, and dimethyl thetin chloride were used as substrates[2] at a concentration of 1.67 mg/ml rumen fluid. The serum bottles were incubated at 39 C in a water bath. At intervals, a sample of the head-space gas was obtained from each serum bottle by using a gas-tight Hamilton syringe, and injected directly into the chromatograph. Gas injections ranged from 50 to 1,000 $\mu$liters, but the most satisfactory volume was found to be 250 $\mu$liters. The gas-chromatographic response was measured as integrator units times attenuation factor.

A dual hydrogen flame gas-liquid chromatograph (F & M Scientific, Model 810) equipped with a 6.4-mm by 3.7-m stainless steel column with 10% silicone oil DC-200 on 60 to 80 mesh Diatoport S was used for analysis of the head-space gas. The flows of flame gases were: hydrogen at 62.5 ml/min and air at 460 ml/min. The carrier gas was helium and used at a flow rate of 47.2 ml/min. Ranges and attenuations most frequently employed were 10 by 64 to 10 by 4 at a chart speed of 2.54 cm/min. All analyses were performed isothermally with the detector temperature maintained at 300 ± 5 C, the injection port at 285 ± 5 C, and the column at 60 ± 2 C.

[2] Reagents used were obtained from Nutritional Biochemicals Corp., 21010 Miles Ave., Cleveland, Ohio.

### Results

Head-space gases were tentatively identified by comparing retention times on the gas chromatographic column with those of known compounds (Table 1). Also the chromatographic peaks obtained with head-space gases were augmented by adding to the samples with known

TABLE 1. Comparison of retention times of known compounds with those obtained from the in vitro fermentation of rumen inoculum.[a]

| Peak number | Retention time | | Known compounds |
|---|---|---|---|
| | Unknown (min) | Known (min) | |
| 1 | 1.4 | 1.4 | $CH_4$ (methane) |
| 2 | 3.0 | 3.0 | $CH_3SH$ (methanethiol) |
| 3 | 4.6 | 4.6 | $CH_3CH_2$-SH (ethanethiol) |
| 4 | 5.2 | 5.2 | $CH_3$-S-$CH_3$ (dimethyl sulfide) |

[a] Each value is the average of eight determinations.

TABLE 2. Production of methanethiol from S-methyl cysteine and from L-methionine.

| Treatment[a] | Incubation time | Methanethiol[b] |
|---|---|---|
| | (min) | (Relative production) |
| Rumen fluid | 40 | 0 |
| | 454 | 100 |
| | 1,430 | 0 |
| Rumen fluid + S-methyl cysteine | 54 | 320 |
| | 590 | 5,800 |
| | 1,547 | 6,400 |
| Rumen fluid + L-methionine | 110 | 160 |
| | 598 | 680 |
| | 1,540 | 1,640 |

[a] Source of rumen fluid: Cow 154.

[b] Each value is the average of two experiments.