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Effect of CCl_4 on CH_4 and Volatile Acid Production in Continuous Cultures of Rumen Organisms and in a Sheep Rumen

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Chlorine-containing analogues of methane were shown to inhibit CH_4 formation by organisms in rumen contents (1). Halogenated methane analogues react with reduced vitamin B_{12} and inhibit cobamide-dependent methyl-transfer reactions involved in CH_4 formation (5). We investigated the effect of CCl_4 on the production of volatile fatty acids (VFA) and gases by use of in vitro continuous cultures of rumen microorganisms. We also attempted to influence the in vivo rumen fermentation by dosing the rumen of a fistulated sheep with CCl_4. The results of these studies are presented in this report.

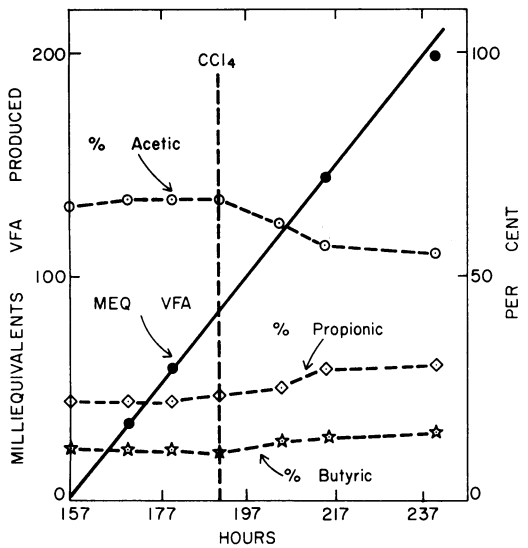

FIG. 1. *Effect of CCl_4 on VFA production in a continuous culture. A 1-mg amount of CCl_4 in aqueous suspension was added to a 500-ml culture at the point indicated by the vertical dashed line (191 hr).*

The in vitro continuous culture studies were carried out as described previously (4). The substrate used to maintain the cultures was 12.1 g of alfalfa hay fed every 12 hr. A single continuous culture was dosed with increasing concentrations of CCl_4 to determine the amount necessary to inhibit CH_4 formation. We found that approximately 13 μM CCl_4 was necessary for inhibition of CH_4 production. Hydrogen production was significant when CH_4 production ceased. These results generally confirm those of Bauchop (1), except for the somewhat higher amounts of CCl_4 necessary to effect inhibition in the continuous cultures.

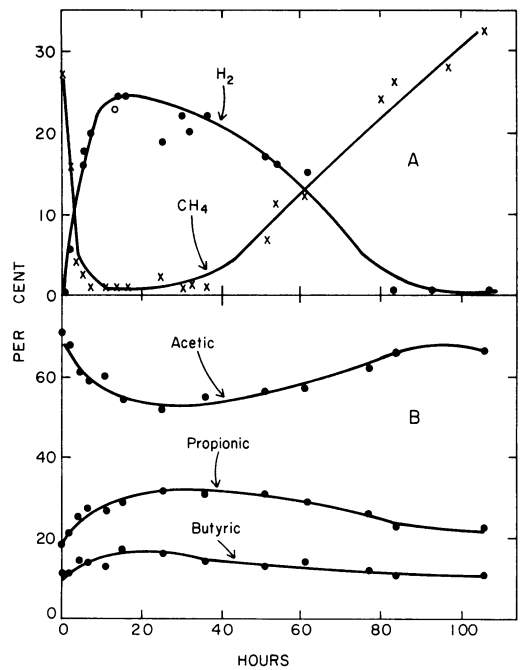

FIG. 2. *Effect of CCl_4 on gases and VFA in a sheep rumen. A mature, fistulated ewe was fed hay ad libitum and 1 lb of a concentrate mixture per day before and during the experiment. A gas collection bladder was fitted to the fistula. A 1-g amount of CCl_4 in water suspension (50 ml) was added to 8 to 10 locations in the rumen with a glass tube at zero-time. Rumen contents were withdrawn at the indicated times for gas chromatographic VFA analyses; the collected gases were also analyzed by gas chromatography. Part A shows the per cent of H_2 and CH_4 in the rumen gas and part B shows the percentage composition of the rumen VFA.*

A single dose of 13 μM CCl_4 was given to a continuous culture after 191 hr of in vitro operation. In 29 hr, no CH_4 was detected in the gas produced, whereas large amounts of H_2 were present. Total VFA production (acetic plus propionic plus butyric) was not disturbed by the CCl_4 treatment, but the proportions of propionic and butyric acids increased and acetic acid decreased after CCl_4 dosage (Fig. 1). Small amounts of CH_4 reappeared at 263 hr.

In vivo effects with a fistulated sheep were only apparent with high levels of CCl_4; i.e., 0.5 to 1.0 g introduced into the rumen. One gram gave a concentration of 13 mM in a 500-liter volume. A rough estimate of the rumen liquid pool volume is 4.5 liters (2). In addition to the high concentrations of CCl_4 necessary to produce in vivo effects, these effects were more transient than the in vitro effects. CH_4 production was almost instantaneously inhibited upon the addition of CCl_4 with concommitant accumulation of H_2 in the gas phase (Fig. 2). Shifts in the proportions of fermentation products paralleled the in vitro patterns, but whether this reflects in vivo changes in production rates cannot be determined from these data. In 85 hr, the gas and VFA picture returned to the values obtained before treatment.

These results generally confirm the results of Bauchop (1), who showed that rumen CH_4 production was inhibited by CCl_4 with accompanying accumulation of H_2. In addition, the results with the continuous cultures showed that the production of VFA is unaffected by CCl_4 concentrations that inhibit CH_4 formation except for the changes in the proportions of the produced VFA. The results with the fistulated sheep were similar to the in vitro results except for the massive amounts of CCl_4 necessary to produce a transient effect; this suggested a rapid removal or detoxification of CCl_4 in vivo. The amount of CCl_4 which showed in vivo inhibition is slightly below the amount recommended for liver fluke therapy in sheep (1.6 g of CCl_4 in oil suspension; 3).

We did not try other halogenated analogues of CH_4 or prolonged dosage with CCl_4 in the continuous culture or in the in vivo system.

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LITERATURE CITED

1. Bauchop, T. 1967. Inhibition of rumen methanogenesis by methane analogues. J. Bacteriol. **94**: 171–175.
2. Hungate, R. E. 1966. The rumen and its microbes. Academic Press, Inc., New York.
3. Merck Sharp and Dohme Research Laboratories. 1961. The Merck veterinary manual, 2nd ed. Merck & Co., Inc., Rahway, N.J.
4. Slyter, L. L., W. O. Nelson, and M. J. Wolin. 1964. Modifications of a device for maintenance of the rumen microbial population in continuous culture. Appl. Microbiol. **12**:374–377.
5. Wood, J. M., F. S. Kennedy, and R. S. Wolfe. 1968. Biochemistry **7**:1707–1713.