Oxidation of Intra-Arterially Administered Carbon^{14}-Labelled Methane In Sheep. (31949)

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In the ruminant, methane production accounts for a large fraction of the gas arising from anaerobic processes in the forestomach. Calculations from data of Hoernicke *et al*(1) indicate that methane production, under certain dietary conditions, can account for 10% of the total carbon turnover of the animal.

Methane is eliminated principally through the process of eructation(2). In addition, some methane diffuses through the ruminal walls to the blood stream and is expired *via* the lungs. Analysis of expired air by gas chromatography indicates the presence of methane under both feeding and fasting conditions. Some of the methane, which is transported *via* the blood to the lungs, may have been absorved from the G.I. tract. Dougherty *et al*(3) have shown that a significant amount of methane can reach the circulation through absorption of ruminal gas in the lungs during and immediately following eructation. The metabolic fate of methane in the ruminant has not been explored, but generally it is believed that animal tissues are incapable of metabolizing methane although a number of reports of methane oxidation by bacteria have appeared(4-6).

Methane is an important constituent of rumen gas. It has been demonstrated that over one-half of the eructated gas is forced and inspired into the respiratory tract during normal eructation(3). It has been demonstrated that some of the gases(2,3) as well as other volatile constituents are absorbed as the blood passes through the pulmonary circulation(12). Because of the variety of pathways involved in the metabolism of other single carbon compounds, and the paucity of evidence in the case of methane, it was decided to explore methane metabolism in sheep using $C^{14}H_4$ and constant infusion techniques.

Experimental procedure. Six sheep were surgically prepared with cannulas placed in the femoral artery and vein on one side and the femoral artery on the other side. Using a previously described technique(7), tracheal cannulas were provided in the sheep to prevent rebreathing expired air and to confine exhaled air to a system which constantly metered portions of the stream to a C^{14} gas monitoring system† and a carbon dioxide trapping manifold. A schematic diagram of the equipment is shown in Fig. 1. Samples of $BaC^{14}O_3$ obtained from the $C^{14}O_2$ traps were counted as described by Cluley(8). Sheep plasma equilibrated with radioactive methane was infused into the abdominal

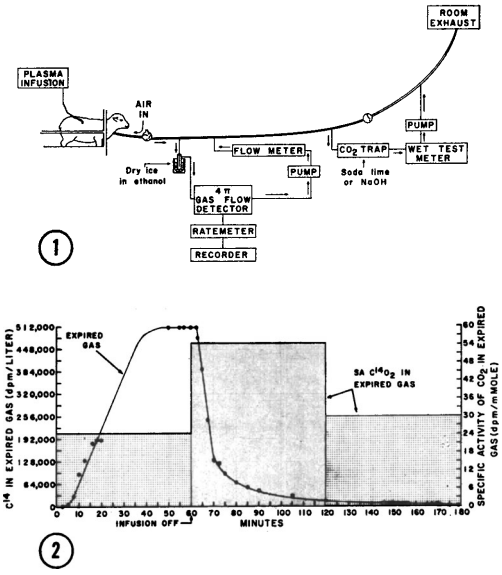

FIG. 1. Scheme for monitoring radioactivity in respiratory gas.

FIG. 2. The curve represents specific activity in expired air going through the counter. The bar graph represents specific activity of $C^{14}O_2$ from the 3 soda lime samples through which a metered part of the expired air was passed.

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† 4 Pi GM gas counter, Nuclear Data, Inc., Palatine, Ill. Model No. 1620BS Analytical Ratemeter and Model R1000A Rectilinear Chart Recorder, Nuclear Chicago Corp., Des Plaines, Ill.

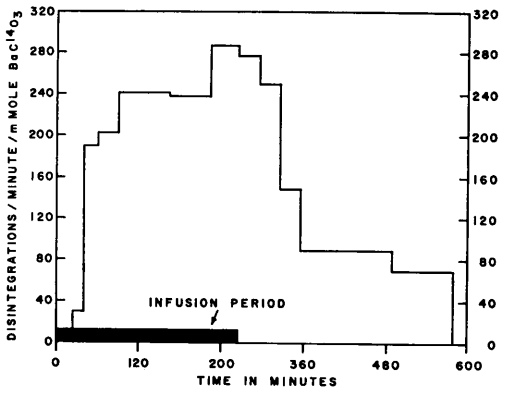

FIG. 3. Specific activity of expired $C^{14}O_2$ during and following C^{14}-methane infusion.

aorta *via* a cannula inserted into the right femoral artery. The infusion rate was 1.5 ml/min; however, in certain cases this rate was preceded by a priming rate of 5 ml/min for a 10-minute period. In the two studies described in Fig. 2 and 3, the infusing plasma contained 0.4×10^6 disintegrations per minute (dpm) per ml and 0.73×10^6 dpm/ml, respectively.

Blood samples were obtained from the left femoral vein and artery, which contained dissolved radioactive methane. These samples were counted in a liquid scintillation counter‡ by layering 1.5 ml under toluene phosphor solution in glass vials and waiting until the radioactive methane diffused into the toluene phase. This process was observed to be complete in 6 or 7 hours.

At the end of each experiment the animals were sacrificed and heart, liver, kidney, and striated muscle tissue samples were taken, immediately frozen with dry ice, and stored for chemical analyses.

Results and discussion. The specific activity of expired $C^{14}O_2$ from sheep during and following the infusion of plasma containing $C^{14}H_4$ is given in Fig. 2 and 3.

Fig. 2, a representative experiment, relates the course and magnitude of total radioactivity to carbon dioxide radioactivity in the animals' expired gas. Although the expired air was counted in the gas phase and recorded as dpm/l, this value approximates dpm/mmole by the following calculation Analysis of expired air in the sheep indicated CO_2 concentrations of 2.5 to 3.5%, or 25 to 35 ml of CO_2/l. Since one mmole of any gas is 22.4 ml, the relationship of dpm/l to dpm/mmole $BaCO_3$ is of the same order of magnitude.

It is apparent from Fig. 2 that $C^{14}O_2$ in the expired gas is only a minor fraction of the total radioactivity and that most of the $C^{14}H_4$ is expired unchanged. The very rapid fall in the expired radioactivity upon the cessation of infusion indicates a rapid clearance of methane.

The persistence of $C^{14}O_2$ in expired air after the $C^{14}H_4$ infusion is ended suggests a delay consistent with a metabolic process converting a fraction of the labelled methane to carbon dioxide. This is particularly evident in the experiment shown in Fig. 3 where carbonate samples were prepared at more frequent intervals during a longer infusion period.

The fraction of infused methane that was converted to carbon dioxide during the period of observation can be estimated from the data of the experiment shown in Fig. 3. The mean $C^{14}O_2$ specific activity of the collected $C^{14}O_2$ during the 9 hours and 40 minutes of observation was 160 dpm/mmole. If the average sheep excretes 10 mmole CO_2/min(9), total $C^{14}O_2$ excretion during the experiment approximated 160 dpm/mmole $\times$ 10 mmole/min $\times$ 580 minutes $=$ 928,000 dpm. The infusion contained 756,000 dpm/ml and an infused volume of 369 ml $= 0.756 \times 10^6 \times 369 = 280 \times 10^6$ dpm. The percentage of conversion during 9 hours and 40 minutes of observation was $0.928 \times 10^6 \div 280 \times 10^6 = 0.33\%$.

The thesis was considered that diffusion of $C^{14}H_4$ occurred from the arterial blood through the ruminal walls and into the rumen contents thus exposing labelled methane to microbial action. This possibility was explored by obtaining rumen ingesta *via* a fistula from a sheep receiving a 30-minute infusion of plasma containing $C^{14}H_4$. Although the ingesta did contain small amounts of radioactive methane, the ingesta carbonate had a specific activity of only 5 dpm/mmole compared to 201 dpm/mmole for expired CO_2.

‡ Model 314 EX, Packard Instrument Co., Downers Grove, Ill.

A methane-oxidizing organism has been isolated from an enrichment culture from the rumen of a cow(10). Since the active rumen microflora is strictly anaerobic, methane-oxidizing bacteria obtained from the rumen may not have been functional but merely casual passengers brought in with the food(11). In this work, it was found that $C^{14}H_4$ was transported from the blood to ruminal contents but since the specific activity of carbonate in the rumen was much less than the specific activity of carbonate from expired air, the oxidation of CH_4 in the rumen seemed unlikely.

With only a small fraction of the total C^{14} recovered as CO_2, it is important that the $C^{14}H_4$ infused did not contain $C^{14}O_2$ or other labelled compounds. The NaOH washes of the $C^{14}H_4$ infused were radioactive, but the radioactivity was probably dissolved CH_4 as it disappeared from the NaOH solution after bubbling with cold carrier methane and did not precipitate as $BaCO_3$ when carrier carbonate was added.

Under the conditions of the experiments, the calculations from these experiments suggest that the quantity of methane that is oxidized by ruminants is too small to be considered as an important factor in energy metabolism. The evidence for the oxidation of CH_4 by animal tissue, however, is of considerable interest from the viewpoint of comparative biochemistry. Information concerning the size of the methane pool in body tissues, its turnover rate, and intermediary metabolic products will be presented in a later report.

Summary. Oxidation of methane was demonstrated in intact mammals by trapping radioactive respiratory carbon dioxide following arterial infusion of C^{14}-labelled methane ($C^{14}H_4$). Only a small amount of the infused $C^{14}H_4$ was oxidized.

1. Hoernicke, H., Williams, W. F., Waldo, D. R., Flatt, W. P., Proc. 3rd Symposium, Energy Metabolism, Troon, Scotland, 1964, London, Academic Press, 1965, 165-178.

2. Dougherty, R. W., Stewart, W. E., Nold, M. M., Lindahl, I. L., Mullenax, C. H., Leek, B. F., Am. J. Vet. Res., 1962, v23, 205.

3. Dougherty R. W., Allison, M. J., Mullenax, C. H., Am. J. Physiol., 1964, v207, 1181.

4. Sohngen, N. L., Z. Bakterial., Parasitenk Abt. II, 1906, v15, 513.

5. Strawinski, R. J., Brown, L. R., Bact. Proc., 1957, 18.

6. Foster, J. W., Davis, R. H., J. Bact., 1966, v91, 1924.

7. Cresswell, E., Thesis, Massey Agric. College, Univ. of New Zealand, 1958.

8. Cluley, H. J., Analyst, 1962, v87, 170.

9. Johnson, B. Conner, Sabine, J. R., Mayfield, E. D., Huber, T. L., Essig H. W., Sheppard, A. J., in International Atomic Energy Agency, Radioisotopes in Animal Nutrition and Physiology, (IAEA/FAO Symposium, Prague, Nov. 1964), Vienna, 1965, 111.

10. Stocks, P. K., McCleskey, C. S., J. Bact., 1964, v88, 1065.

11. Bryant, M. P., Bact. Rev., 1959, v23, 125.

12. Dougherty, R. W., Shipe, W. F., Gudnason, G. V., Ledford, R. A., Peterson, R. D., Scarpellino, R., J. Dairy Sci., 1962, v45 472.

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