VOLATILE FATTY ACID PRODUCTION IN THE RUMEN OF SHEEP

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Summary

A technique using a constant intraruminal infusion of [U-^{14} C] acetate, [U-^{14} C] propionate and [2-3-T] butyrate has been developed to measure simultaneously the production rates of acetic, propionic and butyric acids in the rumen of sheep. The production rates and the concentrations of individual acids in the rumen were simply related.

I. INTRODUCTION

It is not known whether the concentrations of volatile fatty acids (VFA) in the rumen are simply related to their rates of production. A technique which uses a continuous intraruminal infusion of carbon-14 labelled organic acid [^{14}C] has been developed and permits the measurement of the rate of production of a single acid (acetic, propionic or butyric acid) at one time (Leng and Leonard 1965). In the investigations now reported the technique has been extended so that production rates of all three acids can be measured simultaneously.

II. MATERIALS AND METHODS

(a) Experimental Animals

A total of 12 Merino sheep, each with a permanent rumen cannula, were housed singly in pens. The animals were fed one of the following rations daily for at least 12 weeks prior to infusion experiments:

Ration 1. 800 g lucerne chaff.

Ration 2. 400 g maize plus 200 g lucerne chaff.

Ration 3. 300 g maize plus 300 g lucerne chaff.

Ration 4. 450 g wheaten straw chaff plus 50 g lucerne.

For five days, up to and including the day of the experiment, the animals were given their ration in 12 equal feeds at hourly intervals from 8 a.m. to 7 p.m.

(b) Infusion Experiments

It has been shown by Leng and Leonard (1965) that in the rumen there is very little conversion of acetate or butyrate carbon to propionate carbon and ***vice versa*** and so it is possible to measure the rates of production of propionate and one of the other acids when both are labelled with the same isotope [^{14}C]. With acetate and butyrate, considerable interconversion was observed and therefore

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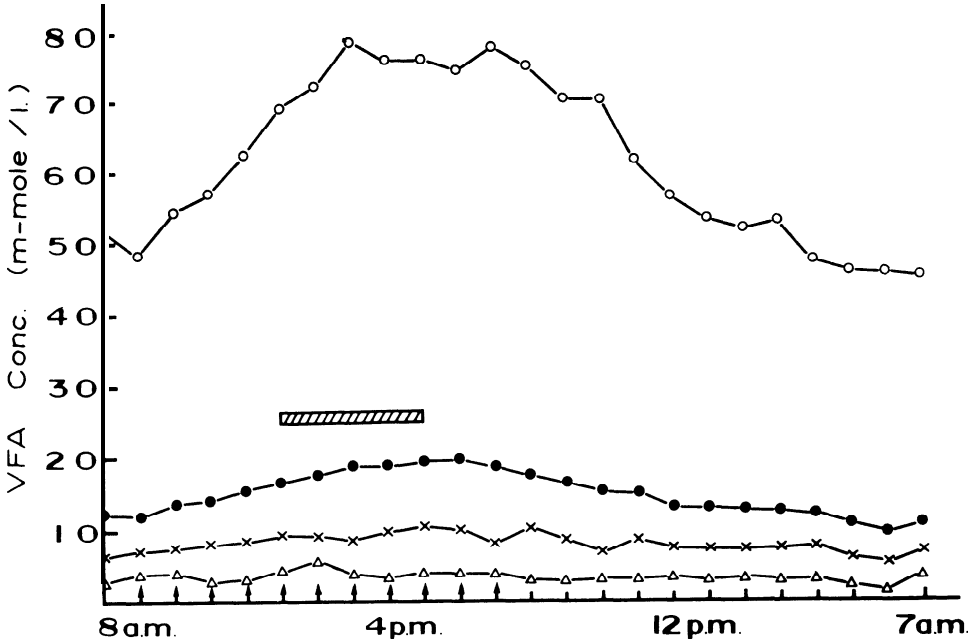

Fig. 1.—Rumen VFA concentrations in sheep fed at hourly intervals from 8 a.m. to 7 p-m.

○, acetic acid; ●, propionic acid; X, butyric acid; △, branch chain and higher acids; ⮝, indicated feeding times; ▨, infusion period.

these acids have to be labelled with different isotopes (Leng and Leonard 1965). It has been demonstrated that essentially the same estimates of production rates were obtained when butyrate labelled with either carbon-14 [^{14}C] or tritium (T) was infused (Leng and Brett 1966). Intraruminal infusions of [U-^{14}C] acetate, [U-^{14}C] propionate and [2-3T] butyrate were therefore used to estimate production rates of these three acids simultaneously. That the rumen samples were fairly representative of the whole rumen contents has been shown previously (Leng and Leonard 1965).

(c) Chemical Methods

Total VFA concentrations were estimated by steam distillation and acid proportions were determined by gas liquid chromatography (Leng and Leonard 1965). Individual VFA were isolated by using silicagel chromatography with butanol-hexane as eluting solvent. The acids were assayed for radioactivity as previously described (Leng and Leonard 1965).

III. RESULTS

(a) Effects of feeding regime on the concentration of volatile fatty acids in the rumen of sheep

The pattern of fermentation in the rumen of sheep fed hourly is shown in Figure 1. VFA concentration in the rumen (Figure 1) rose for five hours after the first hourly feed and then became approximately constant giving the steady state

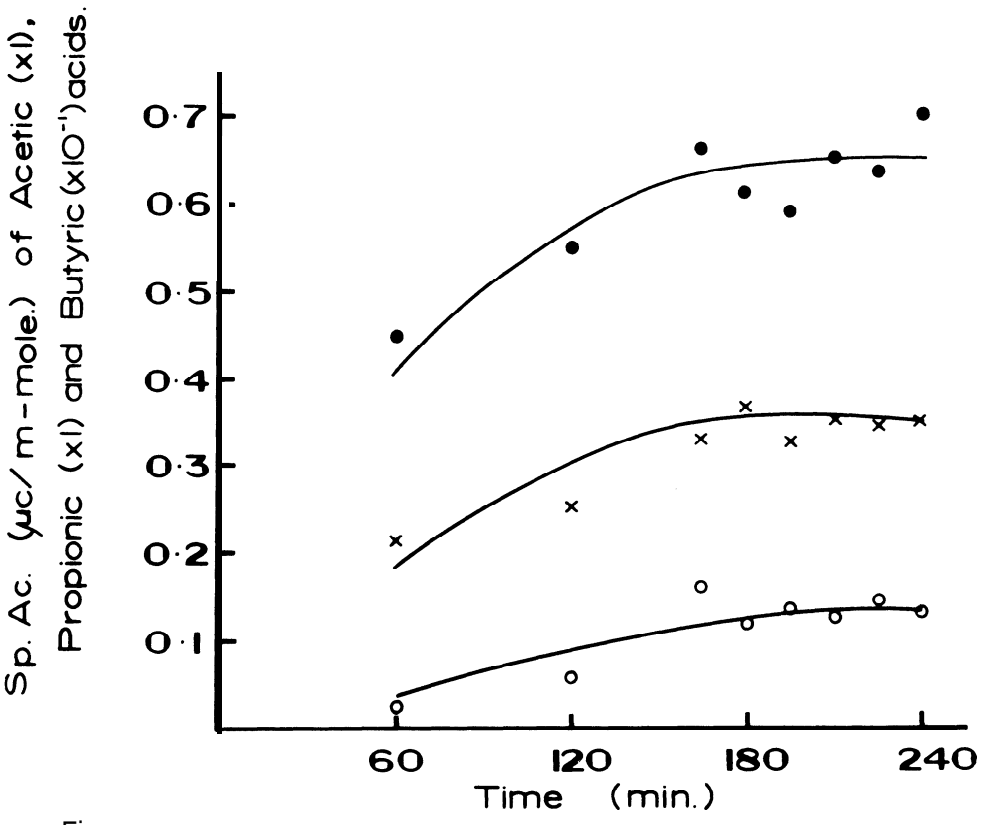

Fig. 2.—Specific activities (sp. act.) of volatile fatty acids in the rumen of sheep during simultaneous infusion of [U-^{14}C] acetate, [U-^{14}C] propionate and [2-3T] butyrate.

The sheep was given a total of 450 g wheaten straw chaff plus 50 g lucerne. Infusion was at a rate per min of 0.2μc for [U-^{14}C] acetate and [U-^{14}C] propionate and 0.5μc for [2-3T] butyrate. ○, sp. act. acetic acid; • sp. act. proprionic. X, sp. act. butyric acid.

conditions required for the application of isotope dilution techniques. Infusions were made over this period as indicated by the hatched area in Figure 1.

(*b*) *Calculation* of *production rate*

Figure 2 shows the specific activities of ruminal VFA when [U-^{14}C] propionate, [U^{-14}C] acetate and [2-3T] butyrate were infused simultaneously into the rumen of a sheep fed Ration 4. These results were typical of most experiments. Production rates in the rumen (m-mole/min) were calculated by dividing the infusion rate (mμc/min) by the mean specific gravity (mμc/m-mole of each acid during the steady state period (between 180 and 240 minutes).

(*c*) *Relationship between VFA concentration and production rate in the rumen*

Production rates in the rumen were measured for acetic, propionic and butyric acids in sheep given different rations and 15 results for each acid have been obtained. Figure 3 shows the relationship between production rates of these acids

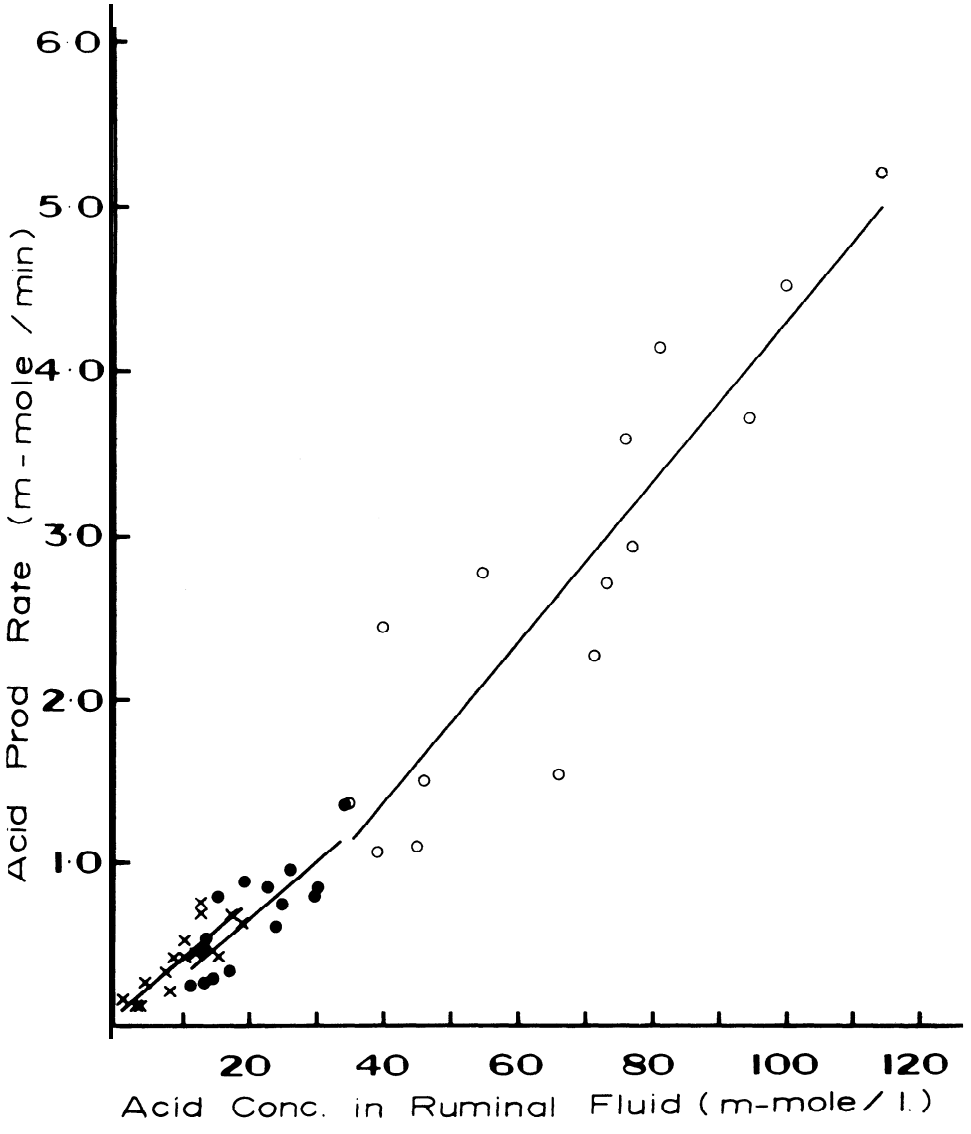

Fig. 3.—Volatile fatty acid production in relation to concentration in the rumen. ○, acetic acid; ●, propionic acid; ×, butyric acid.

and their relative concentrations in the rumen. The regression equations for the prediction of production rates from a knowledge of the concentration of acids in the rumen were as follows:

(1) $Yac = 0.048Xac - 0.558$ S.E. of b $= \pm 0.007$
(2) $Ypr = 0.035Xpr - 0.059$ S.E. of b $= \pm 0.006$
(3) $Ybu = 0.035Xbu + 0.055$ S.E. of b $= \pm 0.006$

where Yac, Ypr and Ybu are the production rates of acetic, propionic and butyric acids respectively, and Xac, Xpr and Xbu the concentrations of these acids in rumen fluid.

Since production rates of the three acids were measured simultaneously, it was possible to obtain a total production rate by summing the three individual production rates. The correlation of this with the total concentration of VFA in the rumen is shown in Figure 4. The graph was best described by the regression equation:

$$Yt = 0.0476Xt - 1.540$$

where Yt and Xt are the total VFA production rate and concentration respectively.

IV. DISCUSSION

There appears to be a close correlation between production rates and concentration of the individual acids in the rumen. This is surprising since estimates of the fluid fill of the rumen using Cr^{51} EDTA (Downes and McDonald 1964) showed that the fluid volume in the rumen of sheep used in these experiments varied between three and six litres and there was also considerable variation in rumen fluid pH. For instance, in sheep given chopped wheaten straw diets pH was around 7, whilst in sheep given high maize diets the pH was around 6.

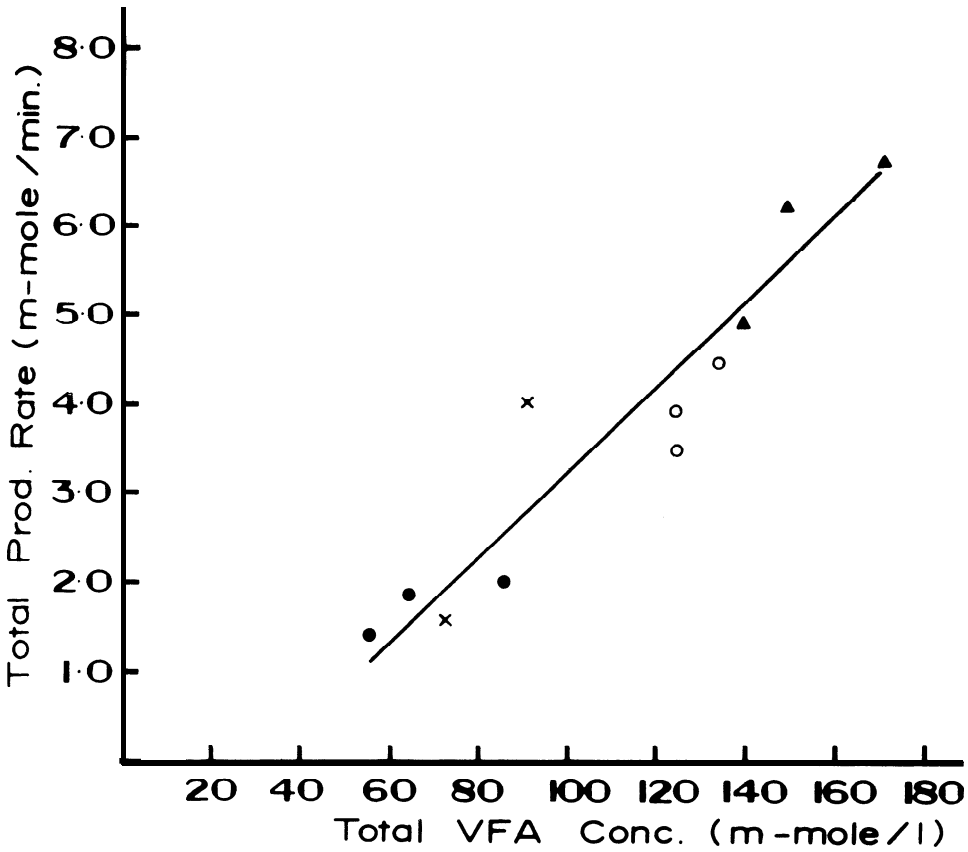

Fig. 4.—Sum production rates of acetic, propionic and butyric acids (mmole/min) in relation to the total concentration of volatile fatty acids in the rumen.
▲, Sheep given 800 g lucerne; ○, 400 g maize, 200 g lucerne; ×, 300 g maize, 300 g lucerne; ●, 450 g wheaten straw chaff, 50 g lucerne.

The concentration of VFA in the rumen must be a resultant of the equilibrium between the rates of production and of absorption, the movement of rumen contents along the intestinal tract and the rate of uptake of VFA by rumen micro-organisms. The concentration of VFA finally attained in the rumen may be primarily due to a balance between the rates of production and absorption. Absorption is apparently a passive uptake due to a concentration gradient (Dobson 1961) and may depend on the amount of rumen epithelium actually in contact with the ruminal liquor. It may be that the amount of tissue actually in contact with the liquor would not be changed appreciably by the volume of materials in the rumen because of the ability of the rumen wall to become more convoluted. The apparent lack of effect of pH is more difficult to explain. If it is assumed that the rate of fatty acid absorption is determined by the pH of the medium, then it may be that the fluid immediately in contact with the papillae of the rumen wall has its own micro-environment which is maintained by the movement of ions from the blood, and this allows the uptake of the acids at a constant rate which is dependent on the concentration of VFA in the rumen.

The regression equations obtained in these investigations may be useful tools for extending field observations on VFA concentrations and it may be possible to assess the nutritive value of fodders for livestock from the total concentrations and pattern of VFA in the rumen. However, further work is required to relate production of individual organic acids in the rumen with animal production and to assess the contribution of VFA to total energy metabolism of the animal.

V. REFERENCES

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