# CONVERSION OF GLUCOSE-$C^{14}$ TO PROPIONATE BY THE RUMEN MICROBIOTA[1]

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ABSTRACT

BALDWIN, R. L. (Michigan State University, East Lansing), W. A. WOOD, AND R. S. EMERY. Conversion of glucose-$C^{14}$ to propionate by the rumen microbiota. J. Bacteriol **85**:1346–1349. 1963.—Rumen microbiota enriched on three different diets calculated to present different levels of available carbohydrate were incubated with glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$ to determine the contribution of the randomizing (succinate) and nonrandomizing (acrylate) routes to propionate. The propionate was labeled as though 70 to 100% was formed via the randomizing route and 0 to 30% via the nonrandomizing route. The contribution of the acrylate pathway increased with higher carbohydrate availability of the diet. These results are discussed with respect to earlier data using lactate-2-$C^{14}$ and lactate-3-$C^{14}$, and a unifying concept for both sets of data is presented.

The routes of lactate utilization in the bovine rumen have been investigated by Jayasuriya and Hungate (1959), Bruno and Moore (1962), and Baldwin, Wood, and Emery (1962). Baldwin et al. employed position-labeled lactate and degradation techniques to determine the contributions of the randomizing (succinate) pathway and the nonrandomizing (acrylate) route in the conversion of lactate to propionate. They reported that nonsymmetrical intermediates, presumably involving the coenzyme A esters of lactate, acrylate and propionate, performed a major role and that the contribution of this route increased with increasing carbohydrate availability of the ration. The well-known pathway involving succinate was a minor contributor in all experiments. There was a lack of agreement between the molar ratios of acetate to propionate present at zero time and the ratio of these acids formed from lactate-$C^{14}$. This discrepancy and calculations based upon the rate of lactate utilization by rumen microbiota, as observed by Jayasuriya and Hungate (1959), indicate that lactate may not be a major intermediate in propionate formation from hexose. Thus, to determine the contribution of the two pathways of propionate formation from hexose, glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$ were fermented by ruminal fluid, and the propionate was isolated and degraded to determine its labeling pattern.

## MATERIALS AND METHODS

*Bacteriological.* Three fistulated cows, each maintained on a different diet as indicated in Table 1, were used as sources of ruminal fluid. For the incubations with glucose-$C^{14}$, 20-ml samples of strained ruminal fluid, removed 3 hr after feeding, were added to 5 ml of 0.05 M sodium phosphate buffer (pH 7.0) containing 1.0 $\mu$c of glucose-1-$C^{14}$, glucose-2-$C^{14}$, or glucose-6-$C^{14}$ and sufficient unlabeled glucose to bring the final concentration to 0.5%. A 3-ml sample was removed at zero time, and the remainder incubated for 45 min at 39 C under a nitrogen atmosphere. The fermentation was stopped with $H_2SO_4$. Glucose utilization was virtually complete in all cases.

*Radiochemical.* Radioactivity determinations and degradation procedures were carried out as described previously (Baldwin et al., 1962), with some minor modifications as follows. Carbon dioxide derived from $C_1$ of the fatty acid was collected in 0.25 M NaOH (10 ml), and 1-ml samples were counted in a gel scintillation system made up on 5 g of 2,5-diphenyloxazole (PPO),

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50 mg of α-naphthylphenyloxazole (α-NPO), 80 g of naphthalene, and 40 g of Cab-O-Sil (Cabot Corp., Boston, Mass.) dissolved in 385 ml each of dioxane and xylene and 230 ml of ethanol (Gordon and Wolfe, 1960). Samples (0.5 ml) of methylamine·HCl and ethylamine·HCl derived from the degradation were also counted in the gel scintillation system. Glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$ were purchased from New England Nuclear Corp.

## Results

The diets indicated in Table 1 were selected so that, relative to each other, microbial activity would produce different levels of available carbohydrate in the rumen. Previous experience (Annison and Lewis, 1959) indicated that increased availability would cause a decrease in the molar ratio of acetate to propionate in the rumen. The molar proportions of acetate to propionate observed at time of sampling are presented in the first column of Table 2. The amounts of these acids produced from glucose-$C^{14}$, as determined by the amount of radioactivity isolated in the acetate and propionate, appear in the second column. The latter data are expressed as total counts in acetate divided by the total counts in propionate.

The results in column one are not in strict accordance with the general rule (i.e., decrease in ratio with increase in carbohydrate) in that the ratio observed on diet C is somewhat higher than expected. This result is not without precedent (Jacobson et al., 1958), however, since the cows had been fed the experimental diets for only 2 weeks before sampling. The ratios expressing relative acetate and propionate production from glucose (column two) are somewhat lower than the values obtained at zero time. However, these two values agree much more closely than those reported for lactate-$C^{14}$ (Baldwin et al., 1962).

The acetate and propionate isolated from the incubation mixtures after 45 min were purified and degraded. The distribution of radioactivity within these acids is presented in Tables 3 and 4. The acetate formed from glucose-1-$C^{14}$ and glucose-6-$C^{14}$ was predominantly methyl-labeled, and the acetate formed from glucose-2-$C^{14}$ was carboxyl-labeled (Table 3), as should be expected from fermentation of glucose via the Embden-Meyerhof pathway. These values also show that the degree of randomization between carbon atoms 2 and 3 of the several three-carbon intermediates arising from glucose is not large, usually less than 5%. Table 4 shows the radioactivity appearing in the individual carbons of propionate formed from glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$. Calculations of the contribution of the nonrandomizing (acrylate) pathway shown in Table 4 were based on the following equation for propionate formed from glucose-1-$C^{14}$ and glucose-6-$C^{14}$: nonrandomizing pathway (%) = [(specific activity of carbon 3) − (specific activity of carbon 2)/(specific activity of carbon 3) + (specific activity of carbon 2)] × 100. Similar calculations were employed for glucose-2-$C^{14}$. No

TABLE 1. *Diets of cows used as sources of ruminal fluid*

| Cow | Diet | Amt |
|---|---|---|
| | | *lb/day* |
| A | Alfalfa hay | 20 |
| B | Alfalfa hay | 4 |
| | Grain | 18 |
| C | Alfalfa hay | 2 |
| | Grain | 18 |

TABLE 2. *Ratios of acetate to propionate in ruminal fluid*

| Diet | Acetate/propionate ratio | |
|---|---|---|
| | At zero time | After 45 min |
| | *μmole/μmole* | *dpm/dpm** |
| A | 4.0 | 3.1 |
| B | 2.6 | 1.4 |
| C | 3.2 | 1.8 |

* Incubation with labeled glucose. Average of values obtained with glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$; dpm = disintegrations per min.

TABLE 3. *Distribution of label in acetate from glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$*

| Diet | Glucose-1-$C^{14}$ | | Glucose-2-$C^{14}$ | | Glucose-6-$C^{14}$ | |
|---|---|---|---|---|---|---|
| | $C_1$ | $C_2$ | $C_1$ | $C_2$ | $C_1$ | $C_2$ |
| A | 25* | 720 | 800 | 73 | 56 | 625 |
| B | 25 | 500 | 760 | 11 | 30 | 600 |
| C | 7 | 1,000 | 990 | 17 | 6 | 845 |

* Values expressed as disintegrations per min per μmole.

TABLE 4. *Distribution of label in propionate from glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$*

| Diet | Substrate | Specific activity* | | | | Acrylate pathway (%) |
|---|---|---|---|---|---|---|
| | | Total | $C_1$ | $C_2$ | $C_3$ | |
| A | Glucose-1-$C^{14}$ | 87.0† | 2.7 | 42.0 | 44.5 | 2.9 |
| B | | 86.0 | 2.8 | 33.8 | 52.0 | 21.2 |
| C | | 42.0 | 0.7 | 14.7 | 26.3 | 28 |
| A | Glucose-2-$C^{14}$ | 100.5 | 17.0 | 42.5 | 43.5 | 0 |
| B | | 115.5 | 6.8 | 64.5 | 41.5 | 21.9 |
| C | | 138.0 | 1.7 | 84.0 | 50.5 | 24.8 |
| A | Glucose-6-$C^{14}$ | 77.5 | 1.7 | 33.2 | 37.2 | 5.7 |
| B | | 114.0 | 1.7 | 39.5 | 68.0 | 26.5 |
| C | | 32.0 | 1.2 | 11.2 | 18.4 | 24.3 |

* Results expressed as disintegrations per min per $\mu$mole.

† After addition of carrier (1 mmole).

corrections were applied to offset the randomization of label as reflected in the acetate values. The data show that the contribution of the acrylate pathway varied between 0 and 30% depending on the diet, with an increased contribution with diets of higher carbohydrate availability. By difference, the contribution of the randomizing (succinate) pathway was 70 to 100% on the same diets. The results obtained with glucose-1-$C^{14}$, glucose-2-$C^{14}$, and glucose-6-$C^{14}$ were consistent.

The formula used for these calculations is similar to one used for calculating pathway distributions with lactate as substrate (Baldwin et al., 1962), but has been modified to eliminate an error in the previous formula. Accordingly, the values for the per cent nonrandomizing pathway for the conversion of lactate to propionate (70 to 90%) were too high and should be, after recalculation, in the range of 54 to 88%. This error does not alter the qualitative conclusions reached, but does lower the per cent contribution of the nonrandomizing pathway and elevate the randomizing pathway by 5 to 10%.

## DISCUSSION

The isotope experiments establish the fact that two mechanisms of propionate formation exist in the natural ecological system of bovine rumen. It is interesting to note that the lesser-known nonrandomizing route under some circumstances performs a role in glucose utilization, as well as in lactate metabolism as reported earlier (Baldwin et al., 1962). These experiments show that opposite situations prevail in the fermentation of lactate and glucose, in that the succinate route plays a minor role in lactate fermentation and a major role in glucose metabolism, whereas the acrylate route performs a major role in lactate fermentation and a minor role in glucose fermentation.

The feeding regimens were selected to present varied amounts of available carbohydrate in an effort to achieve several different relative rates of acetate and propionate production. The data clearly show that the contribution of the nonrandomizing pathway is highest when large quantities of readily available carbohydrate are present in the diet. The contribution of the nonrandomizing pathway was negligible on the all-hay diet, but increased to approximately 30% on the diets containing large quantities of grain. The conclusion that the contribution of the nonrandomizing route is closely correlated with carbohydrate availability is supported by the studies carried out earlier with lactate-$C^{14}$ (Baldwin et al., 1962), and by the results presented herein.

The contrast between the glucose-$C^{14}$ experiments, wherein the molar and isotope ratios of acetate to propionate agree, and the lactate-$C^{14}$ experiments, where there is a sharp discrepancy between the molar and isotope ratios, suggests that lactate does not equilibrate with the three-carbon intermediates arising during hexose fermentation. Consideration of this observation and the fact that the pathways of propionate forma-

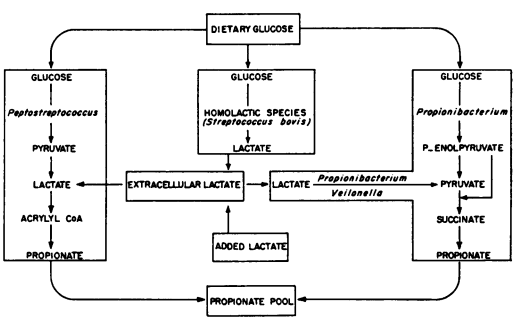


FIG. 1. *Interrelationships among bacterial fermentations producing propionate.*

tion differ quantitatively with respect to the two substrates led to the concept presented in Fig. 1. Two routes of glucose fermentation are indicated: the first involves a direct conversion of glucose to propionate and acetate by a single organism such as a propionibacterium; the second route requires the participation of two types of organism (a lactate former such as *Streptococcus bovis* and a lactate utilizer such as *Peptostreptococcus elsdenii*, Gutierrez et al., 1959). If diets high in starch promote the growth of homolactic species such as *S. bovis*, and the increased lactate formed by these organisms in turn selectively increases the population of lactate fermentors [a supposition amply supported by direct evidence (Annison and Lewis, 1959; Bryant, 1959; Bryant and Robinson, 1961)], the apparent result would be a relative increase in the participation of the two-step sequence. Further, if the preferentially selected lactate utilizers convert lactate to propionate via acrylate, the expected result would be an increase in the contribution of the acrylate pathway. These effects have been clearly demonstrated and are consistent with the postulate presented in Fig. 1. Lack of knowledge of the many types of organisms isolated from lactate enrichment cultures precludes critical examination of the suggestion that the bulk of these organisms convert lactate to propionate via the nonrandomizing pathway. However, under the conditions employed in the experiments with uniquely labeled lactate, the acrylate route was predominant (Baldwin et al., 1962).

The measurements employed in these experiments allow study of the net result of dietary selections only. Subtle changes in the functional roles of the various species such as those indicated in Fig. 1 for *Peptostreptococcus* and *Propionibacterium* can only be implied. For this reason, alterations in the substrate patterns of specific species which may accompany changes in diet were not considered in the foregoing discussion.

Also, it should be recognized that the experimental conditions employed were not strictly parallel to the conditions existing in the rumen, and the degree to which these differences influence the distribution of pathways is not known. However, changes in the gaseous atmosphere and slight changes in pH would not be expected to produce very drastic alterations in the metabolism of the microbiota, nor should the relative numbers of the various organisms change in the short time interval employed.

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