The Mechanism of Propionic Acid Formation by
Veillonella gazogenes

By A. T. JOHNS*

Biochemistry Department and Agricultural Research Council Unit of Animal
Physiology, University of Cambridge

SUMMARY: The mechanism of propionic acid formation from lactate by Veillonella
gazogenes is according to the scheme:

\[
\text{CO}_2 \rightarrow \text{pyruvate} \rightarrow \text{oxaloacetate} \rightarrow \text{malate} \rightarrow \text{fumarate} \rightarrow \text{succinate} \rightarrow \text{propionate} + \text{CO}_2
\]

The evidence for this scheme is: (a) Washed suspensions of the organism grown on
lactate attack, under anaerobic conditions, pyruvate, oxalacetate, L-malate, fumarate
and succinate, but not D-tartrate. (b) Organisms grown on D-tartrate attack it and
all the substances listed in (a) except lactate. (c) Succinic acid is quantitatively
decarboxylated to propionic acid and carbon dioxide. (d) The amount of propionic
acid produced from lactate is influenced by the \text{CO}_2 concentration in the medium.
(e) \text{CO}_2 is fixed in the carboxyl group of propionic acid during fermentation of
lactate.

In a previous paper (Johns, 1951) the isolation of a strictly anaerobic micro-
coccus from the rumen of sheep was described. It was identified as Veillonella
gazogenes and the products of its fermentation of lactate shown to be propionic
and acetic acids, carbon dioxide and hydrogen. Unlike Propionibacterium it
does not ferment sugars or form succinic acid as an end product.

The mechanism of propionic acid formation, which has been studied only in
Propionibacterium has remained uncertain (Werkman & Wood, 1942; Krebs &
Eggleston, 1941). Since lactate and pyruvate are readily converted to pro-
pionate by these bacteria it has been assumed that lactate is an intermediate
in the reduction of pyruvate to propionate as follows:

\[
2\text{H}_2\text{CO.CO.CO}_2 \rightarrow 2\text{H}_2\text{CHOH.CO}_2 \rightarrow 2\text{H}_2\text{CH}_2\text{CO}_2 + \text{H}_2\text{O}
\]

Barker & Lipmann (1944), using dried cells of P. pentosaceum, ruled out this
possibility by showing that, in the presence of sodium fluoride, lactate reduction
could be completely blocked with only 57 % inhibition of pyruvate fermenta-
tion. Moreover, when the lactate reduction was blocked, no accumulation of
lactate occurred when pyruvate was fermented.

The experiments of Carson & Ruben (1940) and of Wood, Werkman,
Hemingway & Nier (1940) with isotopic carbon showed that propionic acid
formed in the fermentation of glycerol by Propionibacterium contained fixed
carbon dioxide. Wood, Werkman, Hemingway & Nier (1941) found this fixed
carbon to be exclusively in the carboxyl group of the propionate, and also in
the carboxyl groups of succinate. The quantitative results indicated that the
most likely mechanism for propionic acid production was by decarboxylation

* Present address: Plant Chemistry Laboratory, Palmerston North, New Zealand.
Propionic acid formation by V. gazogenes

of succinic acid. However, Werkman & Wood (1942) stated 'There is some evidence that propionic acid bacteria can decarboxylate succinate anaerobically but it is questionable whether or not the rate of this reaction is high enough to be of any considerable importance.' A number of workers have been unable to demonstrate any decarboxylation of succinate. The discovery that Veillonella gazogenes produces propionic acid offered an opportunity for the investigation of this problem in another bacterial species.

EXPERIMENTAL

Methods

A strain of V. gazogenes isolated from the rumen of sheep was studied. The methods used in this work were as previously described (Johns, 1951) with the addition that washed suspensions of V. gazogenes were prepared and their metabolism studied by the Warburg technique. Bacteria were grown in litre flasks on the same medium as used for their isolation, with the addition of sodium sulphide to 0.01 % (w/v). Good growth was obtained in 12–18 hr.

The preparation of active washed suspensions of the bacteria was best accomplished by centrifuging down the cells and washing them with boiled distilled water containing 0.01 % (w/v) sodium sulphide. Much less active suspensions were obtained when glutathione was used as reducing agent. The $Q_{co_2}$ found with succinate as substrate was at least ten times greater in the presence of 0.01 % Na$_2$S than in its absence.

Pyruvic acid was estimated manometrically by the carboxylase method of Westerkamp (1933) as modified by Krebs & Johnson (1937).

Dry weights of bacteria in suspensions were determined by means of a photo-electric turbidimeter which had been calibrated against suspensions containing known dry weights of bacteria. In expressing the rate of a fermentation the conventional quotient 'Q' for gas production is employed. $Q_{co_2}$ is defined as $\mu$l. CO$_2$/mg. dry-weight bacteria/hr.

Preparation of Na$_2$C$^{13}$O$_3$. KCl$^{13}$N (18.8 atoms % excess) was converted to barium carbonate by combustion in the Pregl microcombustion apparatus and the CO$_2$ absorbed in saturated barium hydroxide. The precipitated barium carbonate was centrifuged down and carefully washed. The labelled carbon dioxide was then released by addition of acid in an evacuated vessel and absorbed in sodium hydroxide, thus yielding sodium carbonate containing isotopic carbon.

Carbon dioxide fixation experiment with C$^{13}$O$_2$. A growth experiment with Veillonella was carried out in a sealed tube. The inorganic medium +1.5 ml. sodium lactate (0.3 g./ml.) +0.4 % Difco yeast extract was adjusted to such a pH value that on the addition of 2 ml. of $\alpha$-Na$_2$CO$_3$ the final pH was 7. The medium was autoclaved in the tube closed by a cotton-wool plug and the enriched C$^{13}$ carbonate autoclaved separately. Before inoculation the labelled sodium carbonate was added to the medium. After inoculation from a 24 hr. culture of Veillonella, the cotton-wool plug was pushed down in the tube which was sealed off above the plug. Thus, as the fermentation proceeded, the gas
produced was under pressure. As it had been shown previously that the
amount of propionic acid formed was dependent on the partial pressure of
\( \text{CO}_2 \), it was thought that, by having the gas under pressure, a greater proportion
of propionic acid would be formed and the fixation of \( \text{CO}_2 \), if any, would be
more convincingly demonstrated. A control fermentation in which no carbonate
was added and the tube left unsealed was inoculated at the same time.

When the fermentation was completed, the volatile acids were distilled off,
separated on a partition chromatogram and finally isolated as barium salts.
To determine the total C\(^{13} \) in the acetic and propionic acids these barium salts
were combusted in the Pregl micro-combustion apparatus and the carbon
dioxide produced absorbed in barium hydroxide. The barium carbonate was
dried for assay.

To determine the C\(^{13} \) concentration in the carboxyl group of the propionic
acid, advantage was taken of the fact that when barium salts of lower fatty
acids are heated at a sufficiently high temperature the corresponding ketone
and carbon dioxide are produced, the carbon dioxide coming solely from the
carboxyl group. In the dry distillation of barium propionate, for example,
diethylketone and carbonate are formed:

\[
(\text{CH}_3\text{CH}_2\text{CO}_2)_{2}\text{Ba} \rightarrow (\text{CH}_3\text{CH}_2)_{2}\text{CO} + \text{BaCO}_3.
\]

According to the accepted mechanism of the reaction 50\% of the carboxyl
carbon should be in the carbonate and the other 50\% in the ketone. This
distribution of carboxyl carbon was confirmed by Wood, Werkman, Heming-
way, Nier & Stuckwisch (1941) who used synthetic propionic acid labelled in the
carboxyl group. They found that the reaction proceeded quantitatively at 460°.

A sample of barium propionate produced from the \( \text{Veillonella} \) fermentation
was heated to 460°, the diethylketone driven off and the carbon dioxide
liberated from the barium carbonate that remained and absorbed in barium
hydroxide. The resulting barium carbonate was washed and dried. The samples
of barium carbonate were assayed with the mass spectrograph for C\(^{13} \) excess
over the normal ratio of C\(^{13}/\)C\(^{12} \) of 0.0109.

**RESULTS**

*Succinic acid decarboxylation*

To test whether \( \text{Veillonella} \) decarboxylated succinate, washed suspensions
suspended in 0.1 M phosphate buffer (pH 6.8) were used and the manometers
gassed with nitrogen (oxygen-free) and equilibrated at 37°. On tipping sodium
succinate from the side bulb a rapid evolution of gas took place.

A balance experiment was carried out under the same conditions to identify
the products formed. Gas production was measured with manometers and
a large-scale fermentation was carried out in duplicate in Krebs vessels with
the same proportions of constituents as in the manometers. Carbon dioxide
was identified by using 20\% NaOH in the centre well of the manometer
flasks. In the flasks where the CO\(_2\) was absorbed it was shown that reaction had
taken place by distilling 1 ml. of the liquid in a Markham steam distillation
apparatus, collecting 80 ml. of distillate, and titrating the total volatile acid.
Propionic acid formation by V. gazogenes

The propionic acid from the Krebs vessels was identified by its rate of movement down a silica gel chromatographic column and compared with that of a mixture of known acids. Only one band appeared as the product of the reaction, its identity being confirmed by adding pure propionic acid and observing that with this mixture again only one band appeared on the chromatogram. The results of a typical experiment are shown in Table 1. The theoretical figures given in Table 1 are calculated for the reaction:

\[ \text{HOOC.CH}_2\text{CH}_2\text{COOH} \rightarrow \text{CH}_3\text{CH}_2\text{COOH} + \text{CO}_2. \]

The results are seen to agree very well with this equation.

Table 1. Products of the decarboxylation of sodium succinate by washed suspensions of Veillonella

Gas production measured in Warburg manometers containing 1·1 ml. 0·1 M phosphate buffer (pH 6-8) 0·2 ml. 2 n-H_2SO_4; 1 ml. bacterial suspension (24 mg. dry-wt. bacteria); 0·2 ml. 0·1 M succinate.
Acid formation determined in Krebs vessels using same proportion of constituents. Temp. 37°.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Products (μl. CO_2)</th>
<th>Theoretical*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Gas production</td>
<td>0·2 ml. 0·1 M succinate</td>
<td>445, 440</td>
</tr>
<tr>
<td>(b) Acid production</td>
<td>5·0 ml. 0·1 M succinate</td>
<td>0·505, 0·51</td>
</tr>
</tbody>
</table>

* Calculated for reaction HOOC.CH_2CH_2COOH \rightarrow CH_3CH_2COOH + CO_2.

It was found that washed suspensions of the bacteria did not attack malonate, nor did malonate up to 0·01 M inhibit the decarboxylation of succinic acid.

The pH optimum for the decarboxylation reaction was determined using 0·1 M phosphate buffer at different pH values (checked by the glass electrode). The curve obtained by plotting μl. CO_2 produced in 5 min. against pH is shown in Fig. 1. The pH optimum of the decarboxylation reaction was between 5·9 and 6·0, with a Q_{CO_2} of 280. This is of the same order of activity as Gale’s amino-acid decarboxylases with similar pH optima (Gale, 1946). Attempts to obtain a cell-free extract of the bacteria which should be active in the decarboxylation of succinic acid were unsuccessful.

Fermentation of possible intermediates in propionic acid formation

The action of washed suspensions on other possible intermediates in the formation of propionic acid was tested; these intermediates included malic, fumaric, oxaloacetic and pyruvic acids (Krebs & Eggleston, 1941). The manometric technique was used and the production of hydrogen looked for by the use of CO_2 absorbers in the centre well of the manometer flask. In all cases there was a rapid decomposition of substrate as shown by the production of gas. Only the L-isomer of malic acid was attacked.

Balance experiments were carried out with L-malate, fumarate and pyruvate as substrates. Table 2 shows the results obtained. The redox index was calculated by the method of Johnson, Peterson & Fred (1931). With pyruvate under
Table 2. Products obtained from various possible intermediates in propionic acid formation by Veillonella, using washed suspensions

Gas production measured in Warburg manometers and acid formation in Krebs vessels using the same proportion of constituents. Temp. 37°. Results expressed as mmol./100 mmol. substrate.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Propionic acid</th>
<th>Acetic acid</th>
<th>CO₂</th>
<th>H₂</th>
<th>Carbon recovery (%)</th>
<th>Redox index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>5.0</td>
<td>97</td>
<td>94</td>
<td>94</td>
<td>106</td>
<td>1.00</td>
</tr>
<tr>
<td>l-malate</td>
<td>62</td>
<td>32</td>
<td>150</td>
<td>32</td>
<td>100</td>
<td>1.02</td>
</tr>
<tr>
<td>Fumarate</td>
<td>66</td>
<td>31</td>
<td>152</td>
<td>31</td>
<td>103</td>
<td>1.03</td>
</tr>
<tr>
<td>Succinate</td>
<td>101</td>
<td>—</td>
<td>98</td>
<td>—</td>
<td>100</td>
<td>1.06</td>
</tr>
</tbody>
</table>

the conditions used the reaction was almost entirely a dissimilation of pyruvate into acetic acid, carbon dioxide and hydrogen, with only a small amount of propionic acid. As malate and fumarate are at the same level of oxidation it is to be expected that they would produce the products of fermentation in the same relative proportions. It was found that organisms grown on lactate as substrate did not attack d-tartrate in washed suspension. However, when Veillonella was grown on d-tartrate the washed suspension attacked d-tartric acid with great vigour, the Q₉₀ being 400. This is illustrated in Fig. 2.

Fig. 1. The decarboxylation of succinic acid by Veillonella gazogenes of various pH values. V. gazogenes 10 mg. dry-wt./vessel; 0.1 M phosphate buffer, 0.2 ml.; 2N-H₂SO₄, 0.2 ml.; 0.1 M succinate. Temp. 36°. Duplicate manometers, in one, acid tip at beginning; in the other, acid tip after 5 min.

Fig. 2. Action on d-tartrate of washed suspensions of V. gazogenes grown (A) in presence of d-tartrate, (B) in presence of lactate. Equal dry-weight/ml. of bacterial suspension used; 0.2 ml. 0.1 M substrate; 0.1 M phosphate buffer (pH 5.8); temperature 37°.

It was also found that organisms grown on d-tartrate quantitatively decarboxylated succinic acid and dissimilated pyruvate, oxaloacetate, l-malic and fumaric acids, but not lactate. The enzymes responsible for the fermentation of d-tartaric acid were thus adaptive, whereas those responsible for the dissimilation of oxaloacetic, l-malic, fumaric and succinic were constitutive.
Propionic acid formation by V. gazogenes

Effect of an atmosphere of hydrogen on gas production

Cardon & Barker (1947) isolated an organism, Diplococcus glycinophilus, which formed acetic acid, carbon dioxide and hydrogen from glycine. They found that the amount of hydrogen produced depended on the partial pressure of hydrogen in the gas phase; in an atmosphere containing more than 25 % hydrogen, no hydrogen accumulated. Also Kubowitz (1934) found that the production of acetic acid, butyric acid, carbon dioxide and hydrogen in glucose fermentation by Clostridium butylicum was inhibited by hydrogen, lactic acid being formed instead.

To see whether an atmosphere of hydrogen made any difference to the amount of gas produced from pyruvate by Veillonella an experiment was run in which one set of manometers was filled with nitrogen and a duplicate set filled with hydrogen. The hydrogen production was determined with 20 % NaOH in the centre well of the manometer cups. Though hydrogen production was slightly less in an atmosphere of hydrogen the difference was hardly significant when compared with the major effects noted by Kubowitz (1934), and by Cardon & Barker (1947). The washed suspensions of Veillonella were inactive against sodium formate, indicating that hydrogen production was not via formic acid.

Hydrogenase. Experiments with washed suspensions in Thunberg tubes showed that methylene blue was rapidly decolorized in an atmosphere of hydrogen, indicating the presence of hydrogenase in the bacteria.

Pyruvate dissimilation

The optimum pH for the fermentation of sodium pyruvate was determined in Warburg manometers by measuring the hydrogen production at various pH values in 0·1 m phosphate buffer, CO₂ being absorbed by 20 % NaOH. As will be seen from Fig. 3 the pH optimum was about 6·2 and the $Q_H = 114$. This pH optimum is considerably higher than that found for the fermentation of pyruvate by Propionibacterium (Barker & Lipmann, 1944). Woods & Clifton (1937) found a $Q_H$ of 25–30 for hydrogen production by Clostridium tetanomorphum from pyruvate.

Fermentation of lactate by washed suspensions

Washed suspensions prepared by the method described above were often only feebly active against lactate. In a typical experiment 512 µl. of gas were formed from 0·2 ml. of 0·06 m pyruvate in 60 min. compared with 5 µl. from 0·2 ml. of 0·1 m lactate in the same period.

Generally it was found that the fermentation of lactate proceeded much better in bicarbonate buffer and an atmosphere of CO₂ than in phosphate buffer at the same pH and an atmosphere of nitrogen. On several occasions when there was no activity against lactate with washed suspensions in phosphate buffer, the reaction proceeded in bicarbonate. A typical result is shown in Fig. 4.
Fumarate was added in catalytic amounts (100 μg./vessel) to see whether it would act as a hydrogen acceptor and initiate the reaction. In flasks in which CO₂ was absorbed there was only a slight evolution of hydrogen which soon ceased; the reaction proceeded satisfactorily, however, in flasks which had no NaOH in the centre well and therefore CO₂ in the gas phase.

![Graph](image)

**Fig. 3.** The rate of pyruvate dissimilation by washed suspensions of *Veillonella* as measured by H₂ production, at different pH values.

**Fig. 4.** The effect of the presence and absence of CO₂ on the rate of dissimilation of lactate by washed suspensions of *Veillonella*. The manometers gassed with nitrogen contained phosphate buffer at the same pH as the bicarbonate, pH 6·8.

### Effect of CO₂ concentration on fermentation of lactate

As Elsden (1938) had shown with *Escherichia coli* that the amount of succinic acid formed in fermentation of pyruvate, glucose and galactose was altered by the carbon dioxide concentration in the medium, it was thought that if propionic acid were formed via decarboxylation of succinic acid, the CO₂ concentration should influence the amount of propionic acid formed.

To test this three Krebs vessels were shaken in a water-bath at 37°; each vessel contained the same volume of liquid, sodium lactate solution, washed suspension of bacteria, and were all at pH 6·8. Vessel 1 contained 0·1 M phosphate buffer and nitrogen (CO₂ and oxygen free) was bubbled through the medium continuously during incubation to remove CO₂ as it was formed. Vessel 2 contained 0·1 M phosphate buffer and was gassed with nitrogen at the start of the experiment but not during it, so that any CO₂ produced could accumulate. Vessel 3 contained bicarbonate buffer at pH 6·8 through which CO₂ (oxygen-free) was bubbled continuously during the fermentation. The vessels were thoroughly gassed with their respective gases before the substrate was added. The result was that while no lactate in vessel 1 disappeared, in vessels 2 and 3 all the lactate was utilized and the following propionic:acetic acid ratios were obtained, namely in vessel 2, 1·21:1 and in vessel 3, 2:1. From these results it will be seen that the manometric experiments with lactate in which CO₂ was absorbed were verified, i.e. fermentation did not take place in the absence of CO₂, and that the amount of propionic acid formed was dependent on the carbon CO₂ concentration in the medium. The experiment was repeated several times and in no case did fermentation occur when CO₂-
free nitrogen was bubbled vigorously through the fermentation liquid. The results indicated fairly strongly that formation of propionic acid follows the scheme proposed by Carson, Foster, Ruben & Barker (1941) and by Krebs & Eggleston (1941) for the formation of succinic acid, with the addition that propionic acid is formed by decarboxylation of succinic acid (see Fig. 5).

The further evidence which seemed desirable to justify this hypothesis appeared to be the demonstration of CO₂ fixation, and the location of the fixed carbon in the carboxyl group of the propionic acid formed. It was felt that the most satisfactory way to show CO₂ fixation in propionic acid would be by a growth experiment in the presence of Na₂C¹³O₃ with sodium lactate as substrate. The results are given in Table 3. The 0.01 atom percentage excess in the acetic acid is not regarded as significant. The chromatographic method does not give complete separation of the bands (Barker & Wikén, 1948).

![Proposed mechanism of propionic acid formation from pyruvate.](image)

There is definite evidence that CO₂ was fixed in the propionic acid formed. If the tracer carbon were located solely in the carboxyl group, the C¹³ excess in the carboxyl should be three times that in the whole molecule; in fact it was not quite three times. This can be explained by a slight mistake in the manipulation of material in the preparation of the barium carbonate; unfortunately the author did not have the opportunity to repeat the experiment. Despite these inaccuracies the results indicate that no CO₂ was fixed in the acetic acid and that it was fixed in the carboxyl group of propionic acid.

The ratio propionic acid:acetic acid in the fermentation carried out under

<table>
<thead>
<tr>
<th>Sample</th>
<th>Atoms % C¹³</th>
<th>Atoms % excess C¹³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder CO₂</td>
<td>1.10</td>
<td>—</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>1.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Cylinder CO₂</td>
<td>1.10</td>
<td>—</td>
</tr>
<tr>
<td>Total propionic acid</td>
<td>1.78</td>
<td>0.68</td>
</tr>
<tr>
<td>Cylinder CO₂</td>
<td>1.10</td>
<td>—</td>
</tr>
<tr>
<td>Carboxyl of propionic acid</td>
<td>2.75</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Table 3. Results of C¹³ assay with mass spectrograph of acetic and propionic acids produced in Veillonella fermentation of lactate in the presence of C¹³ enriched sodium carbonate
pressure with sodium carbonate added was 1.9:1, whereas the control tube without carbonate and not under pressure was 1.19:1. All the lactate was fermented in each case. This confirms, in a growth experiment, the results already obtained with washed suspensions, namely, that the amount of propionic acid formed is dependent on the amount of CO₂ present.

DISCUSSION

The scheme set out in Fig. 5 appears to be supported for Veillonella by the following evidence: (a) During the fermentation of lactic acid, CO₂ is fixed in the carboxyl group of propionic acid. (b) All the postulated intermediates are attacked by the bacteria. (c) The occurrence of the reversible reactions from pyruvic acid to succinic acid are well established in other micro-organisms (Krebs, 1943) and in animal tissues (Utter & Wood, 1945). (d) Fermentation of lactate by washed suspensions of Veillonella did not take place in the absence of CO₂. (e) The amount of propionic acid formed depended on the CO₂ concentration in the medium. From the nature of the experimental conditions it was not possible to state whether all the propionic acid arose from CO₂ fixation. (f) Only bacteria which were grown on D-tartrate attacked this compound in washed suspension. The dicarboxylic acids of very similar structure, oxaloacetate, L-malate, fumarate and succinate, were fermented by bacteria grown on lactate or D-tartrate. This indicated that the enzymes which attack the postulated intermediates in the formation of propionic acid are truly constitutive, whereas those responsible for the primary breakdown of D-tartrate are adaptive. The fact that bacteria grown on D-tartrate did not attack lactate, shows that no propionate is formed by the direct reduction of lactate, confirming the findings of Barker & Lipmann (1944) in sodium fluoride inhibition studies with Propionibacterium pentosaceum.

Two mechanisms of hydrogen formation have been established with micro-organisms: (a) bacteria of the coli-aerogenes group form hydrogen by the decomposition of formate (Stephenson & Stickland, 1932, 1933; Kalnitsky & Werkman, 1943); (b) Koepsell, Johnson & Meek (1944) have shown that Clostridium butylicum will convert pyruvate anaerobically to acetic acid, CO₂ and H₂, formic acid not being an intermediate in this reaction.

With Veillonella it is thought that hydrogen production takes place by method (b) since it was shown that: (1) the bacteria do not attack formate; (2) in the fermentation of pyruvate, acetic acid, CO₂ and H₂ were formed in approximately equal quantities (Table 2); (3) the fermentation of L-malate and fumarate produced equal amounts of hydrogen and acetic acid. These findings seem to fit well with the idea that CO₂, H₂ and acetic acid arise in equimolar proportions from pyruvate as precursor. However, contrary to the findings of Kubowitz (1934) with Clostridium butylicum and of Cardon & Barker (1946) with Diplococcus glycinosphilus, it was found that an atmosphere of hydrogen had no effect on the amount of hydrogen produced by Veillonella from pyruvate.

In addition to the formation of acetic acid from pyruvic acid it has been claimed that a direct splitting of succinic acid into two molecules of acetic acid can take place (Slade & Werkman, 1948). There seems to be no question of
acetic acid being formed by this method in the present instance, for when
succinic acid was attacked in washed suspensions only propionic acid and
$\text{CO}_2$ were formed, and in the $\text{CO}_2$ fixation experiments significant amounts of
isotopic carbon were not found in the acetic acid formed from the fermentation
of lactate. If acetic acid had arisen from succinic acid, carboxyl-labelled acetic
acid would have been expected to appear.

The fermentation of tartrate by bacteria has been little studied. Barker
(1936) studied the fermentation of tartaric, fumaric and L-malic acids by
*Aerobacter aerogenes* and found that they give rise to the same products as
glucose. The probable course of the reaction is via oxaloacetic acid followed by
decarboxylation to $\text{CO}_2$ and pyruvic acid (Stephenson, 1989). Propionic acid
would be formed via L-malic, fumaric and succinic acids.

The anabolic (biosynthetic) process from pyruvate to carbohydrate has
usually been conceived as the reverse of the catabolic process. This involves the
production of phosphorylated derivatives of glucose, and the question arises,
why is it that glucose is not fermented by some bacteria such as *Veillonella*?
The so-called ‘direct’ fermentation of disaccharides also appears to be related
to this problem. Peleczar & Doetsch (1949), when isolating and identifying
bacteria of the genus *Neisseria* from the nasopharynx of humans, encountered
several strains which fermented maltose with acid production while glucose
was not fermented. It seems that in both cases the enzyme necessary to carry
out the primary phosphorylation of the glucose may be missing.

My thanks are due to Dr S. R. Elsden and Dr R. Scarisbrick, for their interest and
advice throughout this work, and to the late Dr Marjory Stephenson and Dr E. F.
Gale for many useful discussions. My thanks are also due to Dr A. S. Macfarlane
of the National Institute of Medical Research, Mill Hill, for the KCN enriched with
$\text{C}^{13}$ and to Mr Palmer of the Atomic Energy Research Establishment for carrying out
the $\text{C}^{13}$ assays.

REFERENCES

Barker, H. A. (1936). Fermentation of some dibasic $\text{C}_4$ acids by *Aerobacter aerogenes.*

Barker, H. A. & Lipmann, F. (1944). On lactic acid metabolism in propionic acid
bacteria and the problem of oxido-reduction in the system fatty-hydroxy-keto

Barker, H. A. & Wikén, I. (1948). The origin of butyric acid in fermentation of
threonine by *Clostridium propionicum.* *Arch. Biochem.* 17, 165.

Cardon, B. P. & Barker, H. A. (1947). Amino acid fermentations by *Clostridium
propionicum* and *Diplococcus glycinophilus.* *Arch. Biochem.* 12, 165.

Carson, S. F. & Ruben, S. (1940). Carbon dioxide assimilation by propionic acid
26, 422.

carbon as an indicator of carbon dioxide utilization. V. Propionic acid bacteria.


6, 1.*


(Received 4 January 1950)