LI. THE INTERMEDIATE METABOLISM OF THE PROPIONIC ACID BACTERIA¹

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INVESTIGATION of the dissimilation of glucose by the propionic acid bacteria by Wood & Werkman [1936, 2] showed that previous schemes of dissimilation [Van Niel, 1928; Virtanen & Karström, 1931] do not fully meet the requirements of their data. Furthermore, the recent isolation of phosphoglyceric acid from the fermentation of glucose and hexosediphosphate by Stone & Werkman [1936, 1] necessitates reconsideration of previously proposed schemes of bacterial dissimilation. The present investigation is a continuation of the studies by Werkman and co-workers on the intermediate dissimilation of glucose by the propionic acid bacteria. Evidence is presented supporting the intermediary role of phosphoglyceric, pyruvic, lactic, acetic and succinic acids. Results are correlated in a scheme of dissimilation and considered in relation to the role of phosphoglyceric acid and methylglyoxal.

PHOSPHOGLYCERIC ACID

The isolation of a three-carbon monophosphate from yeast fermentation by Nilsson [1929] stimulated research on intermediate compounds containing phosphate groups. Extensive investigations on lactic acid formation in muscle by Embden *et al.* [1933], and Meyerhof & Kiessling [1935, 1, 2] have shown that phosphoglyceric acid plays an important intermediary role in the dissimilation of glucose by muscle and yeast.

Stone & Werkman [1936, 1, 2] have isolated phosphoglyceric acid from glucose dissimilation by the propionic acid and other bacteria in the presence of toluene and NaF, using a modification of the method described by Neuberg & Kobel [1933]. The Ba salt of phosphoglyceric acid was obtained from mixtures of glucose, hexosediphosphate and acetaldehyde, and from glucose and acetaldehyde, without addition of hexosediphosphate. In the latter case it is assumed that hexosediphosphate was formed by phosphorylation of the glucose leading to the same reaction as when the hexose ester was added directly:

This is the reaction proposed by Meyerhof for alcoholic fermentation during the so-called stationary phase: hexosediphosphate is necessary as a catalyst. In the presence of a suitable concentration of NaF, the phosphoglyceric acid is protected from further breakdown and collects as a final product.

In the propionic fermentation, acetaldehyde has not been isolated and evidence indicates that it is not an intermediate product. Hence it may be

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objected that the production of phosphoglyceric acid by a reaction in which acetaldehyde is reduced to ethyl alcohol is not valid for the propionic fermentation. However, it is probable that the acetaldehyde functions here merely as a hydrogen acceptor for oxidation of an intermediate triose ester. In this case other hydrogen acceptors should be able to function so long as they are activated. In fact we should expect some phosphoglyceric acid to be formed with no added acceptor present, by means of the reaction proposed by Meyerhof to take place in the first stage of alcoholic breakdown of sugar (equation (2)):

To determine the effect of various hydrogen acceptors, the following experiment was carried out, based on the method of preparation of phosphoglyceric acid from yeast by Vercellone & Neuberg [1935]. Bacterial cells were centrifuged from 3-day glucose-yeast extract broth cultures. In each of several small flasks were placed: 7 g. bacterial paste, 7 ml. 0.66 *M* phosphate buffer (*pH* 6.8), 6 ml. 20% glucose, 5 ml. 2.5% Na hexosediphosphate, 1.5 ml. 0.2 *M* NaF, 0.1 ml. 1% MgCl_2 , 1 ml. toluene and 8 ml. hydrogen acceptor (or H_2O in the case of the control). The hydrogen acceptors used were made up so that they were approximately equivalent in dehydrogenating capacity: acetaldehyde 2%, propaldehyde 2.6%, acetylmethylcarbinol 4%, Na lactate 5%, Na pyruvate 5%. These concentrations are based on the assumption that the aldehydes are reduced to alcohols, acetylmethylcarbinol to butyleneglycol, lactic acid to propionic acid and pyruvic acid to lactic acid. The mixtures were incubated at 37° for 6 hours with frequent shaking. The flasks were then chilled and stored overnight at 5°. The cells were centrifuged from the solution and the inorganic phosphate removed by precipitation from ammoniacal solution with 4 ml. 20% $\text{Mg}(\text{OAc})_2$. The resulting precipitate was removed by centrifuging and 4 ml. of glacial acetic acid and 1.6 ml. of 50% $\text{Ba}(\text{OAc})_2$ were added to the liquid. The solutions were filtered and the filtrates stored at 5°. After 6–24 hours, the Ba salt of phosphoglyceric acid began to crystallize out. The salt was purified by dissolving in 70 times its weight of 0.05 *N* HCl and reprecipitating by adding twice the volume of alcohol; tiny white leaflets formed: $[\alpha]_D^{20} - 13^\circ$. The results are shown in Table I. Both species of *Propionibacterium* yielded some phosphoglyceric acid in the controls, probably arising according to equation (2). With

Table I. *Effect of various hydrogen acceptors on yield of phosphoglyceric acid*

Organism	Yield of Ba phosphoglycerate in mg.					
	H_2O	Acetaldehyde	Propaldehyde	Acetylmethylcarbinol	Sodium pyruvate	Sodium lactate
<i>P. arabinosum</i> , 34 W	21	133	72	36	227	24
<i>P. pentosaceum</i> , 49 W	17	169	79	22	452	17

acetylmethylcarbinol and lactic acid as acceptors the results are little better. Both acetaldehyde and propaldehyde give considerably increased yields, but Na pyruvate gives much higher quantities of phosphoglyceric acid. This is to be expected if pyruvic acid is an intermediary. With regard to lactic acid as a precursor of propionic acid (Fig. 1), it is probable that enough energy is not available in the somewhat artificial system to bring about the difficult reduction of lactic to propionic acid.

These results show clearly that acetaldehyde may be replaced by other suitable hydrogen acceptors, and that no added acceptor is necessary for the formation of small amounts of phosphoglyceric acid from glucose by the propionic acid bacteria.

Fig. 1. Scheme for the dissimilation of glucose by the propionic acid bacteria.

The isolation of phosphoglyceric acid in the presence of toluene and NaF does not prove its intermediary nature. The presence of the NaF is considered necessary to prevent breakdown of phosphoglyceric acid. The toluene probably serves as a plasmolysing agent to aid in releasing cell enzymes. The following experiment was conducted to determine the necessity of NaF and toluene for the formation of phosphoglyceric acid. Suspensions of freshly centrifuged organisms were prepared as before. The same quantities of reactants were used, with the exception that NaF and toluene were added as shown in Table II, and with acetaldehyde as the hydrogen acceptor throughout. Time, temperature and treatment were the same as in the previous experiment. The yields of Ba phosphoglycerate are shown in column 5, and the approximate phosphorus uptake, estimated from the Mg precipitate, is given in the last column. In the presence of toluene a yield of 101 mg. was obtained; substituting chloroform gave (flask 2) a slight increase. With toluene omitted (flasks, 3, 4 and 5) a considerable amount of phosphoglyceric acid is formed; however, the yield

Table II. *The effect of toluene and sodium fluoride on the yield of phosphoglyceric acid by P. arabinosum*

Flask No.	Toluene ml.	CHCl ₃ ml.	NaF 0.2 M ml.	Ba phosphoglycerate mg.	Approx. P uptake mg.
1	1	—	2.0	101	55
2	—	1	2.0	120	65
3	—	—	2.0	35	45
4	—	—	4.0	15	45
5	—	—	1.0	6	45
6	1	—	—	2	40
7	—	—	—	0	—*

* Phosphate uptake not determined.

is much less than in the presence of both toluene and fluoride. Flask 6 shows that a small quantity of phosphoglyceric acid was obtained with toluene but without NaF. With neither toluene nor NaF there was no phosphoglyceric acid detected. It is noteworthy that the phosphorus uptake correlates roughly with the yield of phosphoglyceric acid, also that there is much more phosphate esterified, especially in flasks 3, 4, 5 and 6, than can be accounted for by the yield of intermediate product isolated. This phosphate is probably bound in a hexose or 3-carbon ester, presumably a precursor of phosphoglyceric acid.

The isolation of phosphoglyceric acid has been accomplished under varied conditions. It has been shown that the formation of this intermediate ester using glucose plus hexosediphosphate as substrate involves phosphorylation and occurs both in the presence and absence of toluene. To give further evidence that phosphoglyceric acid is an intermediate, it must be shown to undergo breakdown to normal products of fermentation. The dissimilation of phosphoglyceric acid is being investigated and will be dealt with in a later paper.

These results show clearly that this phosphate ester may be an intermediary in bacterial dissimilation. The extensive studies of Meyerhof and others have placed in doubt the importance of methylglyoxal in the breakdown of sugar by yeast and muscle. Although our investigations offer no information which excludes methylglyoxal as an intermediary in the propionic fermentation, proof is given that bacteria have enzymic equipment capable of forming phosphoglyceric acid. Accordingly application of the essentials of the Embden-Meyerhof scheme to the bacterial dissimilation of glucose should be given serious consideration.

PYRUVIC ACID

Wood & Werkman [1934, 1] isolated pyruvic acid from the fermentation of glucose by sulphite fixation using *P. arabinosum* (34 W). They supported Van Niel's suggestion of the hydration of pyruvic acid at the α -carbon with subsequent dehydrogenation and splitting into acetic acid and CO_2 . This reaction does not involve acetaldehyde as an intermediate (Fig. 1). The evidence supporting this conclusion has been extended by experiments using *P. pentosaceum*, 49 W; *P. petersonii*, 11 W; *P. shermanii*, 52 W and 48 W. The cultures were identified by Werkman & Brown [1933] and Wood & Werkman [1936, 1]. The experimental procedure was that described by Wood & Werkman [1934, 1]. The bacteria were grown in a medium consisting of yeast extract (Difco) 0.5%, glucose 2.0%, calcium carbonate 2.0% and calcium sulphite 1.0%. The cultures were incubated at 30° in cotton-stoppered 500 ml. flasks, containing 300 ml. of medium. Analyses were made after 4–18 days' incubation. Cultures 34 W, 49 W and 11 W produced vigorous fermentations but 48 W and 52 W failed to grow. The last two cultures produced H_2S which apparently inhibited growth. The medium was tested for aldehydes and ketones by treating an aliquot part with 2:4-dinitrophenylhydrazine dissolved in 2*N* HCl. An abundant yellow precipitate was obtained immediately in the cases of 34 W, 49 W and 11 W. It was completely soluble in 20% Na_2CO_3 solution and after recrystallization from alcohol was identified as the 2:4-dinitrophenylhydrazone of pyruvic acid. Melting-points of the unknown and known dinitrophenylhydrazones were identical with a mixed melting-point, 213°. In no case was acetaldehyde detected.

The pyruvic acid was determined quantitatively by the iodoform reaction [Wendel, 1932]. The free pyruvic acid was obtained by acidifying a portion of the medium with H_2SO_4 to Congo red and extracting the solution continuously

for 48 hours with ether. Considerable quantities of pyruvic acid were formed; 100 mMol. of fermented glucose gave 27.4 mMol. with 34 W, 52.5 mMol. with 11 W and 28.5 mMol. with 49 W. More complete analyses of two fermentations are shown in Table III. The methods used in these and subsequent experiments

Table III. *Fixation of pyruvic acid in the dissimilation of glucose by culture 11 W*

Medium: glucose 3.0%, CaCO_3 2.0%, CaSO_3 0.75% at 30°

Yeast extract (Difco) %	Glucose fermented %	Products per 100 mMol. of fermented glucose			
		Propionic acid mMol.	Acetic acid mMol.	Lactic acid mMol.	Pyruvic acid mMol.
0.5	56.5	66.1	23.3	14.4	59.4
0.14	69.0	98.2	25.4	24.5	54.8

are those described by Wood & Werkman [1936, 1, 2]. One modification was made in that the volatile acid distillate was redistilled to eliminate pyruvic acid. The results show that some acetic and propionic acids were formed in the presence of a fixative. It is probable that some free pyruvic acid occurs in presence of the sulphite and is dissimilated. The significance of the lactic acid occurring in these fermentations will be discussed later.

Semicarbazide also was used as a fixative. The medium consisted of glucose 2.0%, yeast extract (Difco) 0.5%, CaCO_3 1.5% and semicarbazide hydrochloride 0.1%. The semicarbazide was added after vigorous fermentation had started. The medium was tested for aldehydes and ketones with 2:4-dinitrophenylhydrazine. The 2:4-dinitrophenylhydrazone of pyruvic acid was isolated and in no case were other aldehydes or ketones detected. Culture 49 W proved most adaptable to the method. Other cultures were sensitive to semicarbazide and, when more dilute solutions of the reagent were used to facilitate growth, no fixation was observed.

These identifications substantiate the occurrence of pyruvic acid as an intermediate. The formation of pyruvic acid without subsequent production of acetaldehyde apparently results from the inability of the propionic acid bacteria to decarboxylate the acid. The fixed pyruvic acid is not broken down into acetic acid and CO_2 because it is not subject to hydration and subsequent dehydrogenation. Since the free acid is readily fermented by propionic acid bacteria and large quantities may be fixed in glucose dissimilation, all the evidence supports its occurrence as an intermediate.

Support of the proposed mechanism of breakdown of pyruvic acid has been obtained from the results of its fermentation in the presence of dimedon (dimethylidihydroresorcinol). Dimedon is a specific reagent for aldehydes and if acetaldehyde is an intermediate it should be fixed. Fermentation of the calcium and sodium salts of pyruvic acid in the presence of dimedon showed no fixation. Apparently acetaldehyde is not an intermediate.

Pyruvic acid has also been identified in the fermentation of a number of other substrates by sulphite fixation (lactic acid, glycerol and mannitol). Erb *et al.* [1936] found that a large amount of pyruvic acid was produced in the aerobic dissimilation of lactic acid even in the absence of fixatives.

LACTIC ACID

Foote *et al.* [1930] observed that two of their cultures of propionic acid bacteria (11 and 12) produced lactic acid from arabinose and xylose. Culture 11 also produced this acid from glucose when NaOH was used for neutralization. Lactic acid was not produced from glucose, however, when calcium carbonate was used as the buffer. Fromageot & Tatum [1933] found lactic acid in starch fermentations of culture 12. Although lactic acid has been found as a product of fermentation, conditions resulting in its accumulation have not been determined. The investigations of Foote *et al.* might indicate that the pH and concentration of the sodium ion are influential. They made the medium alkaline to bromocresol purple and the pH was undoubtedly higher than with calcium carbonate. It seemed probable that with sodium bicarbonate buffer the production of lactic acid could be increased since the pH remains at 7.2 or above, even during active fermentation. On the other hand fermentations neutralized with NaOH become acid during intervals between neutralizations.

Culture 49 W (transplant of culture 11 received from Dr E. B. Fred) and cultures 34 W and 48 W were tested (Table IV). The CO₂ was not determined in

Table IV. *Dissimilation of glucose with NaHCO₃ as buffer*

Culture no.	Incubation temperature °C.	Incubation period days	Glucose in medium %	NaHCO ₃ in medium %	Products per 100 mMol. of fermented glucose				Calculated CO ₂ per 100 mMol. of fermented glucose	Ratio CO ₂ /acetic acid	Ratio propionic/acetic acid	C recovery %
					Propionic acid mMol.	Acetic acid mMol.	Succinic acid mMol.	Non-reducing material† mMol.				
34 W	37	14	2.5	2.0	130.8	33.7	9.1	7.8	60.7	1.80	3.88	100.5
34 W*	30	12	2.0	1.5	121.1	50.2	5.2	—	57.7	1.15	2.42	—
49 W	37	8	1.8	1.8	129.8	43.3	7.6	3.8	60.9	1.14	3.00	98.6
49 W	37	14	2.5	2.0	128.7	38.5	7.4	8.5	60.8	1.58	3.34	100.6
49 W	30	10	1.6	1.5	117.2	43.9	8.6	—	54.5	1.24	2.68	—
48 W	30	19	1.6	1.5	101.0	39.2	7.0	16.4	47.0	1.20	2.58	92.4

* 97.2 % of the glucose fermented; utilization complete in other experiments.

† Calculated as glucose.

these fermentations but has been calculated on the basis of the oxidation-reduction balance. The CO₂ is calculated from the equation, 2CO₂ + succinic acid = propionic acid, which expresses the oxidation-reduction balance of the fermentation. The compounds on the left side of the equation are oxidized with respect to glucose, those on the right reduced. The quantity of each is expressed in mMol. and multiplied by its respective oxidation or reduction value. The oxidation or reduction value of a compound is the difference between the sum of the hydrogen and oxygen atoms when the hydrogen atom is assigned a value of one-half and the oxygen atom 1. Compounds formed in the fermentation and not occurring in the equation are neutral. In this calculation it is assumed that CO₂ is the only undetermined product. This assumption is checked by calculating carbon balances using the calculated CO₂ value. If the carbon balance is acceptable it is evidence that the calculated CO₂ is the true value. The carbon recovery shown in Table IV is satisfactory, indicating correctness of the calculated values of CO₂.

The fermentations were also analysed at intervals throughout the dissimilation but in no case was there a significant formation of lactic acid. Although we have not succeeded in obtaining lactic acid by this method the acid has been observed. Culture 11 W has been found to produce lactic acid consistently in media containing a low concentration of yeast extract (0.1–0.2 %). With higher

Table V. *Dissimilation of glucose by culture 11 W*30°, 3.0 % glucose, 2.0 % CaCO₃

Nitrogen source %	Period of incubation days	Oxygen tension	Glucose fermented %	Products per 100 mMol. fermented glucose					Calculated CO ₂ per 100 mMol. of fermented glucose mMol.	C recovery %
				Pro-pionic acid mMol.	Acetic acid mMol.	Lactic acid mMol.	Suc-cinic acid mMol.	Non-reducing material† mMol.		
1.0 yeast water*	12	Anaerobic	98.0	76.4	16.8	43.7	5.1	14.4	35.6	89.4
10.0 yeast water*	12	Anaerobic	100.0	114.9	54.0	1.5	11.8	7.1	51.6	99.8
0.1 yeast extract	28	Aerobic†	72.2	74.3	27.2	53.4	10.0	—	32.1	—
0.2 yeast extract	51	Aerobic†	63.0	12.2	4.6	126.0	3.8	32.3	4.2	105.5

* Water extract of an equivalent quantity of dry commercial yeast.

† Flask stoppered with cotton plug.

‡ Calculated as glucose.

concentrations occurrence of lactic acid is not consistent (Table V). A number of other cultures (49 W, 52 W, 34 W, *P. shermanii*, 3 W; *P. freudenreichii*, 1 W; *P. rubrum*, 38 W) have been tested under similar conditions but in no case has lactic acid been observed. Particular care was taken to establish the purity of culture 11 W. Isolations were made from cultures in which lactic acid occurred. Colonies picked were found to be identical; all were catalase-positive and yielded fermentations similar to those of the original culture.

The fermentations are also of interest because of the rather large quantity of non-reducing material. This is determined by measuring the increase in reduction of Fehling's solution after acid hydrolysis as described by Wood & Werkman [1934, 3].

Lactic acid has also been observed when a mixture of magnesium oxide and phosphoric acid was used as a buffer. The preparation was made by mixing the indicated proportions of acid and oxide in distilled water with bromothymol blue as an indicator and autoclaving at 20 lb. for half an hour. Following sterilization, the pH of the supernatant liquid was 6.5–7.0 while the precipitated phosphate was alkaline to the indicator.

Lactic acid has not been observed consistently in cultures buffered with the magnesium phosphate mixture. Even with the same culture and medium the lactic acid varies from quantities such as are shown in Table VI to small or

Table VI. *Propionic acid fermentations with MgO and H₃PO₄ buffer*

30°, 3.0 % substrate, 0.5 % yeast extract (Difco)

Culture no.	Incubation period days	Oxygen tension	Substrate	Volume of medium fermented ml.	Buffer mixture		Products per litre of medium			
					H ₃ PO ₄ ml. 4 M	MgO g.	Propionic acid mMol.	Acetic acid mMol.	Lactic acid mMol.	Succinic acid mMol.
34 W	10	Anaerobic	Glucose	500	35	10	69.6	18.7	52.3	1.5
49 W	17	Aerobic*	Glucose	800	38	8	124.3	15.7	24.4	23.4
34 W	40	Aerobic*	Glycerol	800	62	15	214.2	5.4	7.9	5.8

* Flask stoppered with cotton plug.

negligible amounts. The cause of this variation is not known. The pH changes are very similar to those in fermentations containing calcium carbonate, starting at approximately 7.0 and dropping to about 5.0 at the conclusion of the fermentation.

These results show, however, that lactic acid occurs in the propionic acid fermentation of glucose. Since this acid is readily fermented by all species of propionic acid bacteria it is logical to assume that it functions as an intermediate.

ACETIC AND SUCCINIC ACIDS

Stone *et al.* [1936, 1, 2] showed that acetic acid is activated by propionic acid bacteria and supported Wood & Werkman's [1936, 2] suggestion that succinic acid is formed by synthesis from acetic acid and is subsequently attacked. The latter authors showed that quantitative data were adaptable to a scheme involving these proposals but indicated that schemes based solely on quantitative data were not conclusive. In order to show that a compound may be an intermediary in a fermentation, it is necessary to isolate the compound and demonstrate that it is fermented as rapidly as its precursor. A direct proof of the fermentability of succinic and acetic acids under the conditions under which they are proposed to act as intermediates, i.e. in the fermentation of glucose, was undertaken. Propionic acid fermentations were analysed throughout the course of dissimilation to determine the quantities of propionic, acetic and succinic acids. The results are shown in Table VII. In Exps. 1 and 2 there was

Table VII. *Dissimilation of glucose*

30°, 3.0% glucose, 0.4% yeast extract (Difco), 1.4% CaCO₃, 6 litres of medium, anaerobic under nitrogen

Incubation period days	Glucose fermented %	Glucose fermented per litre mMol.	Products per litre medium			Ratio: propionic/ acetic
			Propionic acid mMol.	Acetic acid mMol.	Succinic acid mMol.	
Culture 49 W, Exp. 1						
3	26.2	46.3	48.6	16.2	1.7	3.00
5	—	—	91.6	21.8	18.3	4.20
7	85.5	151.1	127.8	31.0	50.4	4.12
9	95.2	168.3	161.0	28.7	68.7	5.61
14	97.4	172.0	183.2	34.3	60.5	5.34
32	99.5	175.8	194.0	39.7	57.5	4.98
Culture 34 W, Exp. 2						
5	30.3	49.4	49.1	17.2	4.1	2.85
7	45.9	74.7	78.4	26.2	5.0	3.00
9	51.4	90.3	101.7	27.1	9.5	3.76
12	64.7	105.2	123.5	27.1	6.9	4.56
15	70.6	114.9	140.4	26.8	7.3	5.25
19	76.0	123.5	164.3	23.5	5.6	7.00
22	79.6	129.4	176.7	20.3	3.7	8.71
Culture 34 W, Exp. 3						
3	16.4	29.2	36.1	14.1	0.3	2.56
5	23.2	41.4	57.7	23.4	0.4	2.47
9	47.9	85.3	91.6	37.1	0.4	2.47
14	63.0	112.1	121.1	48.1	5.6	2.52
32	97.8	174.2	188.0	80.5	14.3	2.34

an initial increase in succinic acid which was followed by a decrease. Likewise in Exp. 2 there was an increase and decrease in the acetic acid. These results show that there was a dissimilation of the succinic and acetic acids. The quantity of acetic and succinic acids fermented may of course have been much larger than that shown. A decrease will be observed only when the compounds are dissimilated more rapidly than they are formed from the glucose, a condition which occurred only in the latter part of the fermentation. The fact that acetic acid was utilized throughout the fermentation is indicated by the increase in the ratio of propionic acid to acetic acid. If glucose were dissimilated to propionic

acid, acetic acid and CO_2 with no subsequent conversion of these products, the ratio of propionic acid to acetic acid should remain 2:1 and that of CO_2 to acetic acid 1:1 as shown in equation (3):

Some propionic acid fermentations yield these ratios as several investigators have observed. In our experience this is more nearly the case with sodium bicarbonate as a buffer (Table IV). Wood & Werkman [1934, 3] found the ratio with sodium bicarbonate to be practically constant throughout the fermentation. With calcium carbonate as a buffer the ratio varies widely as does the yield of succinic acid. Wood & Werkman [1936, 2] found the ratio of propionic to acetic acid to vary from 2.13 to 14.72, that of CO_2 to acetic acid from 0.92 to 6.29 and the amount of succinic acid from 7.9 to 26.1 mMol. per 100 mMol. fermented glucose. It is suggested that this variation occurs because of the more rapid dissimilation of succinic and acetic acids at the low pH which occurs when calcium carbonate is the buffer. At the higher pH of sodium bicarbonate these acids apparently are more stable.

The low initial ratios of propionic to acetic acid (Table VII, Exps. 1 and 2) indicate that the first breakdown of glucose occurs according to equation (3) with little subsequent conversion of acetic acid. As the acidity increases it seems probable that an increased dissimilation of acetic acid occurs which causes the observed increase in the ratio of propionic to acetic acid. In the fermentation with 34 W, Exp. 2, the succinic acid probably was dissimilated almost as rapidly as formed, whilst with 49 W the dissimilation was less vigorous and the succinic acid accumulated.

Exp. 3, Table VII, shows the results of a fermentation in which there was little utilization of acetic acid. The ratio of propionic to acetic acid was practically constant at 2.5 and the acetic acid increased throughout the dissimilation. Exps. 2 and 3 illustrate the diverse dissimilations which may occur with the same culture under similar conditions. Comparison of the two experiments gives an indication of the quantity of acetic acid which may have been utilized.

It should be mentioned that calculation of CO_2 from the oxidation-reduction balance shows that a considerable quantity of glucose is unaccounted for in the products. The non-reducing material was not usually determined but it did not account for the remainder of the glucose. Apparently some compound seldom encountered in propionic acid fermentation was produced. Foote *et al.* [1930] likewise observed the formation of an unidentified compound.

In summary, the data presented in Table VII indicate that the propionic acid bacteria are able to dissimilate acetic and succinic acids under anaerobic conditions during the fermentation of glucose. Positive proof that the acetic acid is converted into succinic acid is not yet available; however, all present evidence supports this suggestion. The evidence that succinic acid is dissimilated by direct decarboxylation as proposed in Fig. 1 will be presented in a separate report.

DISCUSSION

The scheme shown in Fig. 1 embodies our present knowledge of the propionic acid fermentation, but it is not to be considered a rigidly proven mechanism. There is supporting evidence for each reaction proposed; however, other conversions may be involved and the mechanism of dissimilation of the intermediate compounds may vary with conditions. The evidence supporting the scheme is the result of a number of investigations. Virtanen [1925] and Stone & Werkman

[cf. Werkman, 1936] showed that the propionic acid bacteria phosphorylate glucose, and Virtanen & Karström [1931] isolated a hexosemonophosphate. No one as yet has isolated either a hexosediphosphate or triosephosphate from this fermentation but present day evidence is in support of their occurrence. Pett & Wynne [1933] found that hexosediphosphate was converted into methylglyoxal, and Neuberg & Gorr [1925] demonstrated that methylglyoxal is converted into lactic acid by propionic acid bacteria. Wood & Werkman [1936, 2] showed that the scheme is adaptable to the quantitative variations of the end-products. The evidence supporting the occurrence of phosphoglyceric, pyruvic, lactic, acetic and succinic acids has been considered above. The utilization of CO_2 by the propionic acid bacteria was demonstrated by Wood & Werkman [1936, 1] in the fermentation of glycerol and the occurrence of this conversion in glucose dissimilation is suggested by these authors [1936, 2]. If the formation of succinic acid by condensation of acetic acid is accepted, it is necessary to assume a utilization of CO_2 to account for its low yields. As yet little evidence is available relative to the mechanism of CO_2 utilization. Wood & Werkman [1935] isolated formaldehyde from the dissimilation of glucose and suggested that it might originate by the reduction of CO_2 .

The nature of the non-reducing material is not known, but its origin from a 3-carbon compound was suggested since it has been observed in fermentations of glycerol and lactic acid.

Two mechanisms are discussed for the formation of pyruvic acid: one through phosphoglyceric acid and the other through methylglyoxal. Present information indicates that both may function in the fermentation and each must be considered until excluded by further evidence. The isolation of phosphoglyceric acid produced by propionic acid bacteria indicates the strong probability that it occurs as an intermediate. Further investigations of the roles of methylglyoxal and phosphoglyceric acid are being made.

Wood & Werkman [1934, 2] isolated propaldehyde from the fermentation of glycerol with culture 34 W and suggested that this compound was a precursor of propionic acid. The occurrence of propaldehyde in the glycerol fermentation has been established now with cultures 34 W, 49 W, 11 W and 48 W. The aldehyde occurs in significant quantities; for example with 34 W 15.5 mMol. of propaldehyde were formed per litre of medium. The formation of propaldehyde by the phosphoglyceric acid mechanism is not easily accounted for and Wood & Werkman [1936, 1] suggested that propionic acid might originate from glycerol with methylglyoxal and propaldehyde as intermediates. In the fermentation of glucose by the same methods we have failed in every attempt to fix propaldehyde. It seems unlikely that propaldehyde is an intermediate in the fermentation of glucose and from this viewpoint there is no evidence of the occurrence of methylglyoxal in glucose dissimilations.

The fermentations shown in Table III were set up particularly to detect intermediary compounds involved in the formation of lactic acid. Methylglyoxal and α -hydroxypropaldehyde are possible intermediates as well as pyruvic acid. It has frequently been suggested that lactic acid is formed by rearrangement of methylglyoxal hydrate. The occurrence of lactic acid indicates that conditions might be suitable for fixation of methylglyoxal. Pyruvic acid alone was detected and evidence was not obtained by this method of the occurrence of the glyoxal.

SUMMARY

Evidence is presented supporting the intermediate nature of phosphoglyceric, pyruvic, lactic, acetic, and succinic acids in the fermentation of glucose by the propionic acid bacteria. A scheme of fermentation is presented in which the pyruvic acid is oxidized to acetic acid and CO_2 and reduced through lactic acid to propionic acid. Succinic acid is synthesized from acetic acid and then dissimilated to propionic acid and CO_2 . CO_2 is synthesized to an unknown fermentable compound. Phosphoglyceric acid has been isolated from the propionic acid fermentation.

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