The Effects of Glucose and Potassium on the Metabolism of Pyruvate in *Lactobacillus arabinosus*

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During an investigation of the decarboxylation of L-malate by cells of *Lactobacillus arabinosus*, 17-5 (Nossal, 1951*a*) it was observed that pyruvate also yielded carbon dioxide. Like the decarboxylation of malate, the decomposition of pyruvate was found to be most rapid in the presence of glucose, potassium chloride and manganous chloride. Under most conditions the decomposition of pyruvate in *Lb. arabinosus* was found to be in agreement with the equation:

This reaction is known to occur in numerous bacteria, e.g. *Clostridium acetobutylicum* (Wilson, Peterson & Fred, 1927), *Aerobacter aerogenes* (Silverman & Werkman, 1941), *Staphylococcus aureus* (Watt & Krampitz, 1947), *Bacillus subtilis* (Lemoigne, Hooreman & Crosan, 1949), *Micrococcus pyogenes*, var. *aureus* (Watt, 1949), and *Lactobacillus plantarum* (Rowatt, 1949*a, b*). Aerobically, in the absence of glucose, the amount of pyruvate used was not wholly accounted for by the amount of acetoin formed. Part of this work has been communicated to the Biochemical Society (Nossal, 1951*b*).

METHODS

Suspensions of Lactobacillus arabinosus, 17-5. Directions for maintaining subcultures, growing bulk supplies and preparing washed suspensions of *Lb. arabinosus*, 17-5, have been described previously (Nossal, 1951*a*). Addition of 0.1 M-pyruvate to the growth medium did not increase the rate of pyruvate decomposition by the bacteria; malate-adapted cells (Korkes & Ochoa, 1948) were slightly more active than normal cells, and retained their activity better on storage; therefore 0.1 M-DL-malate was usually included in the growth medium. Centrifuged cells were suspended in 5 vol. of 0.1 M-KCl for storage and kept at 2°.

Analytical procedures. Pyruvate was estimated by the carboxylase method of Krebs & Johnson (1937). Preparations of carboxylase lost much of their activity after 1 week's storage at 2°, but if kept at -12° they were still fully active after 10 weeks. Acetoin was estimated by the Voges-Proskauer reaction (see Eggleton, Elsdon & Gough, 1943; Westerfeld, 1945). Since none of the constituents of the experimental mixtures interfered with the estimation or gave

rise to acetoin during the estimation, portions of samples to be estimated were pipetted directly into a mixture of α -naphthol in alkali and saturated aqueous creatine (see Eggleton *et al.* 1943) without preliminary deproteinization, distillation or oxidation. After filtration and allowing 60–90 min. for colour development, the optical densities were measured in a Beckman spectrophotometer at 535 $m\mu$. On this instrument, the optical density was proportional to the concentration of acetoin only up to a level of 50 μg . acetoin (not 100 μg . as in the procedure of Eggleton *et al.* 1943), and samples were accordingly diluted to fall within this range. Standard solutions of acetoin were prepared from the distillate of a commercial sample of acetyl methyl carbinol (Light and Co.) boiling at 140–143° (corr.). These were compared spectrophotometrically with a solution of diacetyl, standardized gravimetrically as the nickel dimethyl glyoxime (Niel, 1927), and were found to be 96–100% pure. Three standards of acetoin (equivalent to 12.5, 25 and 50 μg .) were included in each set of estimations.

Measurement of the rate of pyruvate decomposition. The rate of pyruvate decomposition under varying conditions was followed at acid pH by manometric measurement of the rate of CO_2 evolution in standard Warburg equipment. The bath temperature used throughout was 30° . The gas phase contained air or N_2 ; in anaerobic experiments, sticks of yellow phosphorus were placed into the centre wells of the vessels to ensure complete removal of O_2 ; in aerobic experiments, O_2 uptakes were measured in vessels containing 0.2 ml. 2N-NaOH and fluted filter paper in the centre well. Q values are given as $\mu\text{l. O}_2$ (or CO_2)/mg. dry wt. cells/hr.

RESULTS

Identification of acetoin as a product of pyruvate decomposition

After bacterial suspensions had acted on pyruvate under optimum conditions (see below), the distillate from the reaction mixture gave the Voges-Proskauer test for diacetyl and acetoin. Some tests distinguishing between acetoin and diacetyl are given in Table I, from which it can be seen that the product of pyruvate decomposition behaved like acetoin; after oxidation with ferric chloride-ferrous sulphate mixture (Westerfeld, 1945), which is known to convert acetoin to diacetyl, the product of pyruvate decomposition gave the reactions of diacetyl. The osazones prepared from acetoin, diacetyl and the reaction product were identical (melting points and mixed melting points all falling within the range 237–242°, corrected).

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Table 1. *Differentiation between acetoin, diacetyl and the product of pyruvate decarboxylation*

(The contents of several Warburg vessels were pooled after completion of an experiment (complete system as in Table 2) and distilled almost to dryness to remove the volatile product from residual glucose and pyruvate. This distillate is the 'product' referred to below. A portion of the distillate was oxidized according to Westerfeld (1945) and the mixture distilled again. This second distillate is referred to as the 'oxidized product'.)

	Voges-Proskauer reaction		Ni dimethylglyoxime	Formation of		
	Spectrum peak (m μ .)	Time for development of maximum colour (min.)		Semicarbazide	Phenylhydrazone	2:4-Dinitrophenylhydrazone
Product } Acetoin }	520	60	-	Only after concentrating reaction mixture	Begins to form at 70°	Begins to form at 70°
Oxidized product } Diacetyl }	530	7-10	+	Forms readily at room temperature	Forms immediately at room temperature	Forms immediately at room temperature

Effect of glucose, potassium chloride and manganese chloride, on the anaerobic decomposition of pyruvate. Maximal rates of CO₂ evolution with pyruvate as the substrate were observed anaerobically in the presence of added glucose, KCl and MnCl₂ (Table 2); the effect of KCl is less pronounced

Table 2. *Effect of glucose, KCl and MnCl₂ on the anaerobic decomposition of pyruvate*

(Complete system contains 6 mg. dry wt. freshly harvested cells, 0.025 M-MnCl₂, 0.1 M-KCl, 0.01 M-glucose, 0.05 M-citrate-phosphate buffer pH 4.0, 0.01 M pyruvate. Gas: N₂. Data in μ mol. Experimental period: 60 min.)

Notes	Pyruvate utilized	CO ₂ evolved	Acetoin formed
Complete system	41	42	21
No glucose	16	14	8
No KCl	37	35	18
No MnCl ₂	25	24	11

than in the decarboxylation of L-malate (Nossal, 1951a); unless the cells are freshly harvested, the anaerobic decomposition of pyruvate is very slow in the absence of glucose (see Table 7). The amount of pyruvate decomposed is almost totally accounted for by the amounts of acetoin and CO₂ formed.

Variations in the behaviour of cell suspensions towards pyruvate in the presence of oxygen. Although grown under apparently identical conditions, some washed suspensions of *Lb. arabinosus* showed a considerable oxygen uptake with pyruvate as substrate (Q_{O_2} about 50), whereas others had a lower Q_{O_2} (about 10 to 20), but the Q_{CO_2} values in air or nitrogen did not differ appreciably; the variations occurred, not at random, but in cycles, both variants being approximately equally distributed. The magnitude of the effect of glucose on the rate of pyruvate decomposition in the presence of oxygen varied inversely as the Q_{O_2} . In suspensions having a high Q_{O_2} value neither glucose nor KCl increased the rate of aerobic CO₂ evolution from pyruvate (Table 3), but in cells having a low Q_{O_2} , the same effects of glucose and KCl were observed as those found when pyruvate decomposition was tested anaerobically.

It seemed possible that variations in growth conditions and the composition of the medium were responsible for the variability of the oxygen uptake. However, it will be seen from Table 4 that partial deficiencies of pantothenic acid, nicotinic acid and biotin did not cause variations in either Q_{O_2} or Q_{CO_2} ; the growth period, age of the medium, and inclusion

Table 3. *Aerobic conversion of pyruvate to acetoin*

(The complete system contains 4 mg. dry wt. cells, 0.0025 M-glucose, 0.025 M-MnCl₂, 0.05 M-KCl, 0.05 M-citrate-phosphate buffers, 0.01 M-pyruvate. Experimental period: 60 min. Gas: air. Data in μ mol. $Q_{O_2} = \mu$ l. O₂/mg. dry wt. cells/hr.)

Age of cell suspension (days)	Q_{O_2}	pH	Notes	Pyruvate utilized	CO ₂ evolved	Acetoin formed
5.5	48	4.0	Complete system	41	42	20
			No glucose	41	41	15
			No KCl	42	41	18
			No MnCl ₂	37	38	17
			Complete system	28	29	13
1	15	5.0	No glucose	15	17	4
			No KCl	19	19	6
			No MnCl ₂	22	22	10
			Complete system	22	22	10

Table 4. *Effects of variations in growth conditions on the aerobic utilization of pyruvate by Lb. arabinosus*

(Normal growth conditions given under Methods; all cell suspensions tested in the complete system as described in Table 3. Q_{O_2} (or Q_{CO_2}) = μ l. O_2 (or μ l. CO_2)/mg. dry wt. cells/hr.)

Exp.	Yield of cells (mg. dry wt./ 100 ml. medium)	Growth temperature (°)	Medium	Q_{O_2}	Q_{CO_2}
1	183	30°	Normal	46	210
	155	38	Normal	19	90
	177	38	Glucose added to medium before sterilization	34	185
2	198	30	Added 10^{-6} M-pantothenic acid	39	214
	206	30	Sterilized at pH 4.5 (to partly remove pantothenic acid)	47	208
	205	30	As above, added 10^{-6} M-pantothenic acid	48	189
3	250	30	Normal; fresh	37	224
	280	30	Normal; 6 weeks old	49	218
4	—	38	Nicotinic acid deficient*	60	252
5	—	38	Biotin deficient†	40	205
6	—	30	Normal; growth period: 12 hr.	11	116
			growth period: 16 hr.	24	172
			growth period: 20 hr.	30	200
			growth period: 24 hr.	37	200
			growth period: 36 hr.	21	147

* Hughes & Williamson (1949).

† Nicotinic acid assay medium (Barton-Wright, 1946) with an old solution of biotin added; poor growth. Normal growth could be restored by addition of 0.4 μ g. biotin.

of glucose in the medium during sterilization (instead of sterilizing the glucose separately and adding it at the start of the experiment) also caused no marked differences in the ratio Q_{O_2}/Q_{CO_2} ; when grown at 38° instead of 30°, both Q_{O_2} and Q_{CO_2} values were reduced to approximately the same extent. The reason for the observed variations in the aerobic behaviour of different cell suspensions therefore remains obscure. Bacteriological tests on different cell suspensions showed that the variations were not due to contamination by other bacteria.

Table 5. *Effect of glucose concentration on the rate of CO_2 evolution from pyruvate.*

(6 mg. dry wt. cells (age of suspension 2 days); 0.025M- $MnCl_2$, 0.1M-KCl, 0.05M-citrate-phosphate buffer pH 4.0; 0.01M-pyruvate. Gas: air.)

Glucose (final concn. M)	Maximum rate of CO_2 evolution (μ l./5 min.)	Total CO_2 evolved in first hr. (μ l.)
—	35	336
4×10^{-5}	60	500
1.6×10^{-4}	116	619
6.2×10^{-4}	159	706
2.5×10^{-3}	182	791
10^{-2}	167	790

Catalytic effect of glucose. Both aerobically and anaerobically, the effect of glucose on the rate of CO_2 evolution from pyruvate is catalytic. In the experiment shown in Table 5, addition of 0.16 μ mol.

glucose (the lowest concentration tested) increased CO_2 evolution by 8 μ mol. in the first hour. No CO_2 evolution occurred when glucose was the only substrate.

Glucose depressed the oxygen uptake with pyruvate as the substrate. Aerobically, the yield of acetoin from pyruvate as the only substrate was not quantitative, but as low as 50% of the expected value (see Table 3, Exp. 2). The yield became quantitative in the presence of glucose. The second aerobic pathway of pyruvate decomposition has not been examined.

Effects of various substances on pyruvate decomposition and acetoin formation. Fructose accelerated the rate of CO_2 evolution from pyruvate, and increased the amount of acetoin formed, about half as effectively as equivalent amounts of glucose. Galactose and DL-glyceraldehyde had a slight effect. At pH 5.0, $MnCl_2$ accelerated the rate of CO_2 evolution twice as effectively as $MgCl_2$, but at pH 4.0 both were equally effective. RbCl was as effective as KCl, and CsCl was slightly less effective. Similar observations have been reported earlier for the decarboxylation of L-malate (Nossal, 1951a).

Glucose, fructose, galactose, glyceraldehyde, lactate and acetaldehyde did not form acetoin in the absence of pyruvate; lactate and acetaldehyde did not increase acetoin formation from pyruvate.

Inorganic phosphate (0.05M) and ATP (0.0025M) did not affect either the rate of CO_2 evolution or acetoin formation.

Effect of potassium ions on the rate of fermentation of glucose

Because of the connexion between the effects of glucose and KCl in the decomposition of pyruvate, the effect of KCl on the rate of acid production from glucose was tested. Acid production was measured manometrically in 0.013M-sodium bicarbonate and 100% CO₂, giving a pH of 6.0. It was found that addition of 0.1M-KCl increased the rate of acid production from glucose by 60–100% and reduced the lag period from 20 to 25 min. to 5 min. Thus potassium ions have a similar effect on acid production from glucose in *Lb. arabinosus* as on glucose fermentation by yeasts (Farmer & Jones, 1942).

Effect of buffer composition and of pH. Pyruvate decomposition was more rapid in 0.05M-citrate-phosphate buffers than in 0.05M-acetate buffers of the same pH: at pH 5.0, the Q_{CO_2} values were 164 in citrate-phosphate buffer and 66 in acetate buffer; at pH 4.0, Q_{CO_2} values were 390 in citrate-phosphate buffer and 300 in acetate buffer; virtually no decomposition occurred in 0.05M-phthalate buffers.

Utilization of pyruvate proceeded at a high rate only within the pH limits 3.4–5.5. Aerobically the pH optimum was 4.6, anaerobically it was 3.7 (Table 6).

Table 6. *Effect of pH on the rate of decarboxylation of pyruvate*

(Complete system in all vessels as in Tables 2 and 3.)

Buffer pH	Gas	Lag period (min.)	Max. rate of CO ₂ evolution (μl./5 min.)
	N ₂		
3.2		30	8
3.5		20	112
3.7		10	148
4.0		10	139
4.2		10	110
4.5		10	89
4.9		15	49
	Air		
3.4		25	36
3.8		15	66
4.0		10	120
4.5		10	180
4.7		10	180
5.1		15	112
5.5		20	50

The pH is liable to change in the course of the experiments. At the high rates of decarboxylation observed around pH 4, the pH rises on account of the base released by decarboxylation, and at pH 5 glucose is so rapidly converted to lactic acid that the pH drops. The data of Table 6 are taken from initial rates of decarboxylation, during which the pH of the experimental mixture was near that of the buffers added. At all pH levels the amount of

pyruvate used was quantitatively accounted for by acetoin formation (as in the complete systems of Tables 2 and 3) in the presence of added glucose, but, aerobically, not in its absence.

Loss of activity on storage. Rowatt (1950) observed that cells of *Lb. plantarum* rapidly lost the ability to metabolize pyruvate anaerobically on storage at 4°. This is also true for *Lb. arabinosus*, but only when the activity was tested anaerobically without added glucose (Table 7); therefore the cells

Table 7. *Loss of activity on storage*

(Complete system as in Tables 2 and 3, omitting glucose. 4 mg. dry wt. cells. Experimental period 60 min. Cells in Exp. 1 malate-adapted, in Exp. 2 grown in absence of malate.)

Exp.	Duration of storage (days)	Glucose added (0.005M)	Cells stored in	Gas phase	Total CO ₂ evolved (μl.)
1	0	+	—	N ₂	790
	0	—	—	N ₂	356
	0	+	—	Air	842
	0	—	—	Air	740
	5	+	H ₂ O	N ₂	654
	5	—	H ₂ O	N ₂	50
	5	+	0.1M-KCl	N ₂	794
	5	—	0.1M-KCl	N ₂	37
	5	+	0.1M-KCl	Air	884
	5	—	0.1M-KCl	Air	825
2	0	+	—	N ₂	753
	0	—	—	N ₂	327
	0	+	—	Air	788
	0	—	—	Air	715
	2	+	H ₂ O	N ₂	740
	2	—	H ₂ O	N ₂	70
	2	+	H ₂ O	Air	800
	2	—	H ₂ O	Air	720
	4	+	H ₂ O	N ₂	462
	4	—	H ₂ O	N ₂	20

did not lose the ability to metabolize pyruvate, but rather to metabolize it in the absence of glucose or O₂. The ability to use pyruvate in the presence of glucose or O₂ was retained longer in 0.1M-KCl than in water.

DISCUSSION

Effects of glucose on acetoin formation from pyruvate have been noted by other workers: Watt (1949) showed that *Micrococcus pyogenes* var. *aureus* formed acetoin from pyruvate only when grown on glucose-containing media. Rowatt (1949a, b) described an initial increase in Q_{CO_2} and a reactivation of inactivated cells when glucose was added to *Lb. plantarum* acting on pyruvate. However, the inhibition by glucose of oxygen uptake with pyruvate as the substrate does not seem to have been found previously. The effect of glucose in *Lb. arabinosus* is twofold: it markedly accelerates anaerobic decomposition of pyruvate (the products of which are

solely acetoin and carbon dioxide), and aerobically it increases acetoin formation. Catalytic effects of glucose have been described for other oxido-reduction systems, e.g. the formic hydrogenlyase of *Bacillus coli* (Lascelles, 1948) and the decarboxylation of L-malate in adapted cells of *Lb. arabinosus* (Nossal, 1951a), but the mechanism of these effects remains obscure.

An influence of potassium on acetoin formation has not been described hitherto. In the system studied here, the effects of glucose and potassium are interlinked: potassium accelerates pyruvate decomposition and increases acetoin formation only in experiments where glucose also has an effect (compare Exps. 1 and 2, Table 3). Since potassium increases glucose fermentation in *Lb. arabinosus*, its action could be accounted for by acceleration of glucose metabolism. In this respect the effect differs from that observed in the decarboxylation of malate by the same organism: the increases in activity caused by potassium in the malate system are independent of glucose; furthermore, the potassium effect is also found in cell-free, partially purified extracts of 'malic enzyme' from *Lb. arabinosus* (Korkes, del Campillo & Ochoa, 1950, footnote, p. 898); since these extracts are unlikely to contain or to be able to metabolize glucose, the effect of potassium in the decarboxylation of malate can hardly be related to glucose metabolism.

Potassium has also been found to accelerate the oxidative decarboxylation of malate (Lwoff & Ionesco, 1947) and the decarboxylation of oxaloacetate (Lwoff & Ionesco, 1948) in *Moraxella Lwoffii*, but no effects of glucose are described.

There is no evidence that *Lb. arabinosus* grown on the media used for this work converts pyruvate anaerobically to lactate, acetate and carbon dioxide, as suggested by Blanchard, Korkes, del Campillo & Ochoa (1950). It is possible that growth on the different media employed by Blanchard *et al.* modified the pathway of pyruvate decomposition.

SUMMARY

1. Anaerobically, washed suspensions of *Lb. arabinosus* require glucose, potassium chloride and manganous chloride for maximum rates of pyruvate decomposition. The amount of pyruvate utilized is accounted for by formation of carbon dioxide and acetoin.

2. Aerobically, different batches of cell suspensions differ in their Q_{O_2} values with pyruvate as substrate, the Q_{O_2} varying from 10 to 50. Cells with high Q_{O_2} values require only addition of manganous chloride for the maximum rates of pyruvate utilization; cells with low Q_{O_2} values require glucose, potassium chloride and manganous chloride for full activity. Glucose acts catalytically, depresses oxygen consumption and increases acetoin formation. Potassium chloride is effective only in cells which also require glucose for maximum activity.

3. Fructose and galactose accelerate pyruvate utilization and increase acetoin formation, but less effectively than glucose. No acetoin is formed from sugars, glyceraldehyde, lactate or acetaldehyde; lactate and acetaldehyde do not increase acetoin formation from pyruvate.

4. Pyruvate utilization is more rapid in citrate-phosphate buffers than in acetate buffers of the same pH; phthalate inhibits strongly. Aerobically, the pH optimum of the reaction is about 4.6, anaerobically about 3.7.

5. Cell suspensions rapidly lose their ability to decompose pyruvate anaerobically without added glucose; the ability to decompose pyruvate aerobically, or anaerobically in the presence of glucose, is retained for several days at 2°, especially when the cells are suspended in 0.1 M-potassium chloride.

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