APPENDIX

A Simple Filter Outfit for the Short-wave Ultraviolet

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A simple but efficient filter for transmitting the short-wave ultraviolet light from a mercury arc lamp may be made from two 25 ml. fused silica round-bottomed flasks (Vitreosil T/A4/407). One contains the cobalt-nickel solution ($\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ 100 g. + $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ 350 g./l.) and is placed with its centre about 7.5 cm. from the mercury lamp and acts as a lens, forming an image of the lamp about 7.5–8 cm. on the other side. It is stopped down to about 30 mm. diameter by two diaphragms which may be cut in a sheet of metal which is then bent into a U-shape, and the flask pushed in so that the holes grip it. The second flask, which has a glass stopper ground in, is filled with dry chlorine gas. The stopper is greased with vaseline and may with advantage be made hollow so that it can hold some anhydrous calcium chloride held in place with a little glass wool. This flask is so placed that the image of the lamp is formed in its centre, in which position the light passes through

virtually undeviated and appears to come from the second flask. The two flasks are connected by a tube of black paper so that no light can emerge without passing through filters. A very powerful and uniform cone of light is produced. Using an 80 W. Mazda MB/V lamp with the glass removed (and choke MRG 508) the area illuminated at a distance of 4 ft. is about 18×24 in., the exposure with Kodak reflex document paper and Whatman no. 1 filter paper being about 30 sec.

In making the final adjustment of the positions of the flasks it is important to note that, as the system is not achromatic, the focal length of the flask containing the liquid filter solution is rather shorter at 265 m μ . The field covered by the filter system may, however, be visualized by putting a suitable fluorescent substance such as eosin in ethanol in spots on a sheet of paper and then adjusting the position of the lamp to get maximum evenness of illumination.

The Decarboxylation of Malic Acid by *Lactobacillus arabinosus*

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Korkes & Ochoa (1948) have shown that *Lactobacillus arabinosus* Strain 17-5 when grown on a medium containing L-malic acid converts L-malic acid into lactic acid and carbon dioxide. D-Malic acid is not attacked, nor does it affect the decarboxylation of the L-isomer. The enzyme system responsible for this reaction was found to include cozymase and manganese ions. The authors state that the enzyme can be used as a specific reagent for the manometric determination of malic acid. In order to use this method it was necessary to collect further information on the general properties of the malic decarboxylase. The present paper reports results of a study of this enzyme system.

Since the completion of this work Ochoa, Salles & Ortiz (1950) and Blanchard, Korkes, del Campillo & Ochoa (1950) have published further information concerning the *Lactobacillus* enzyme and its use in the estimation of L-malic acid.

METHODS

Preparation of cell suspensions. Subcultures of the organism were maintained on a medium containing 5 g. peptone, 20 ml. yeast extract (obtained by boiling baker's yeast with an equal weight of water and filtering), 5 g. glucose and 0.5 g. KH_2PO_4 in 250 ml.; the pH was adjusted to 6.8 with 2N-NaOH.

The medium used for growing bulk supplies contained, unless otherwise stated, 10 g. Pronutrin (Enzymic casein digest marketed by Herts Pharmaceuticals Ltd., Welwyn Garden City, Herts.), 1 g. Difco Yeast Extract, 10 g. sodium acetate trihydrate, 0.5 g. KH_2PO_4 and 0.5 g. anhydrous MgSO_4 in 400 ml.; the initial pH was 7.0. To this were added 50 ml. M-sodium DL-malate solution and 50 ml. 20% glucose. The medium was inoculated with 5–10 ml. of a 24 hr. subculture. After 20 hr. incubation at 38°, the cells were centrifuged, washed successively with 50 and 25 ml. distilled water and finally suspended in 30 ml. water. The yields varied between 2.5 and 3.5 g. wet wt. (up to 650 mg. dry wt.). The suspension was stored in the refrigerator.

Measurement of malic decarboxylase activity. The decarboxylase activity was determined by manometric measurement of the rate of CO_2 evolution. The pH of the solutions used was generally adjusted to 5.0, and in addition 0.025 M (final concentration) acetate buffer, pH 5.0, was present. As a rule, cell suspension, buffer and any other additions except substrates were placed into the main compartment, and the substrate solution into the side arm. The total volume of fluid in the manometer cups was usually 4.0 ml.; the gas space contained air; the temperature of the water bath was 25° , unless otherwise stated.

RESULTS

Effect of yeast extract and glucose. Preliminary experiments showed that addition of glucose and yeast extract increased the activity of malic decarboxylase; their effects were additive (Table 1). Attempts to replace yeast extract by cozymase and a mixture of B vitamins (aneurin, biotin, folic acid, guanine, nicotinamide, *p*-aminobenzoic acid, pantothenic acid, pyridoxine, pimelic acid, riboflavin and xanthine) gave negative results. Among various inorganic substances tested, only Mn^{++} ions and KH_2PO_4 increased the rate of decarboxylation. The effects of Mn^{++} , KH_2PO_4 and glucose were additive (Table 1). In the presence of KH_2PO_4 , Mn^{++} and glucose, yeast extract caused no further increase. As yeast extract is known to contain these three substances, its effect is accounted for.

Lag period. When malate was added to a suspension of freshly harvested *Lactobacilli* there was a lag period of up to 20 min. before the maximum rate of CO_2 evolution was attained (Fig. 1). The lag period was diminished when glucose was added at the same time as malate, and virtually disappeared when the cells were pre-incubated with glucose for 20 min. prior to the addition of malate. The effect of glucose described in the preceding paragraph is not solely due to the abolition of the lag period, because glucose also increases the maximum rate of decarboxylation, especially in thoroughly washed cells. In two typical experiments, the maximum rates of CO_2 evolution

without glucose were 25 and $33\ \mu\text{l.}/5\ \text{min.}$, and with glucose, 33 and $48\ \mu\text{l.}/5\ \text{min.}$ respectively. Glucose has thus two effects which may have a common basis. Pre-incubation with KH_2PO_4 and MnCl_2 did not affect the lag period.

Fig. 1. Effect of glucose on the decarboxylation of malate. 1 mg. dry wt. cells, $2.5 \times 10^{-3}\text{ M}$ - MnCl_2 , $5 \times 10^{-2}\text{ M}$ - KH_2PO_4 , $2.5 \times 10^{-2}\text{ M}$ -acetate buffer, pH 5.0; $20\ \mu\text{mol.}$ DL-malate. Curve A, no glucose; curve B, glucose in side arm (with malate); curve C, glucose in main compartment (with cells).

Effect of concentration of glucose, manganous chloride, potassium dihydrogen phosphate and buffer. Both the effects of glucose described above were almost independent of the glucose concentration between 5×10^{-2} and $8 \times 10^{-5}\text{ M}$ (Table 2); at $1.6 \times 10^{-5}\text{ M}$ the effects were less marked. The effect of MnCl_2 reached a maximum at a concentration of about $2 \times 10^{-3}\text{ M}$, whilst that of KH_2PO_4 increased with concentration up to the surprisingly high value of about 10^{-1} M (Table 3); above this concentration, there was a slight decrease, perhaps because of interference by osmotic forces. The minimum concentra-

Table 1. Activation of the malic decarboxylase by yeast extract, glucose, KH_2PO_4 and MnCl_2

(0.5 ml. freshly harvested cell suspension (10 mg. dry wt.), $5 \times 10^{-2}\text{ M}$ -acetate buffer, pH 5.0, $20\ \mu\text{mol.}$ DL-malic acid. The yeast extract was made by placing the carboxylase preparation of Krebs & Johnson (1937) into a boiling-water bath for 5 min. and filtering.)

Exp. no.	Further additions	CO_2 evolved in first 30 min. ($\mu\text{l.}$)
1	None	39
	0.5 ml. boiled yeast extract	105
	Glucose ($5 \times 10^{-3}\text{ M}$)	88
	KH_2PO_4 ($2.5 \times 10^{-3}\text{ M}$)	76
	0.5 ml. boiled yeast extract; glucose ($5 \times 10^{-3}\text{ M}$)	172
	KH_2PO_4 ($2.5 \times 10^{-3}\text{ M}$); glucose ($5 \times 10^{-3}\text{ M}$)	179
2	None	8
	Glucose ($5 \times 10^{-3}\text{ M}$)	43
	KH_2PO_4 ($2.5 \times 10^{-2}\text{ M}$); glucose ($5 \times 10^{-3}\text{ M}$)	106
	MnCl_2 ($2.5 \times 10^{-4}\text{ M}$); glucose ($5 \times 10^{-3}\text{ M}$)	160
	KH_2PO_4 ($2.5 \times 10^{-2}\text{ M}$); MnCl_2 ($2.5 \times 10^{-4}\text{ M}$); glucose ($5 \times 10^{-3}\text{ M}$)	227

Table 2. *Effect of glucose concentration on the lag period and maximum rate of decarboxylation*

(2 mg. dry wt. cells. 2.5×10^{-3} M-MnCl₂, 10^{-1} M-KH₂PO₄, 20 μ mol. DL-malate. Substrate in one side arm, all other solutions in main compartment.)

Location of cells	Glucose added (M)	Rate of CO ₂ evolution (μ l.) over the first four 10 min. intervals, after tipping			
		Interval 1	Interval 2	Interval 3	Interval 4
Side arm	None	11	31	44	51
	1×10^{-2}	23	60	58	48
	4×10^{-4}	23	61	56	44
	1.6×10^{-5}	16	49	47	44
Main compartment	1×10^{-2}	65	73	46	35
	4×10^{-4}	64	70	47	41
	1.6×10^{-5}	43	55	47	48

Table 3. *Effect of dilution on the action of MnCl₂ and KH₂PO₄*

(1.0 mg. dry wt. cell suspension, 2.5×10^{-3} M-glucose; 20 μ mol. DL-malate. 5×10^{-2} M-KH₂PO₄ in Exp. 1; 2.5×10^{-3} M-MnCl₂ in Exp. 2.)

Exp. no.	Further addition	CO ₂ evolved (μ l.)	
		First 15 min.	Second 15 min.
1	None	36	38
	MnCl ₂ (10^{-2} M)	78	68
	MnCl ₂ (4×10^{-4} M)	71	63
	MnCl ₂ (1.6×10^{-5} M)	54	55
2	None	18	18
	KH ₂ PO ₄ (10^{-1} M)	102	74
	KH ₂ PO ₄ (4×10^{-3} M)	77	75
	KH ₂ PO ₄ (1.6×10^{-4} M)	30	35
	KH ₂ PO ₄ (3.2×10^{-5} M)	21	18

Table 4. *Effect of small quantities of glucose, KH₂PO₄ and MnCl₂*

(1.2 mg. dry wt. cells, 20 μ mol. DL-malate. Cell suspension in side bulbs of the cups.)

Glucose (M)	KH ₂ PO ₄ (M)	MnCl ₂ (M)	CO ₂ evolved in first 30 min. (μ l.)
None	5×10^{-2}	2.5×10^{-3}	53
3.2×10^{-6}	5×10^{-2}	2.5×10^{-3}	71
1.6×10^{-5}	5×10^{-2}	2.5×10^{-3}	86
2.5×10^{-3}	None	2.5×10^{-3}	14
2.5×10^{-3}	6.4×10^{-6}	2.5×10^{-3}	16
2.5×10^{-3}	3.2×10^{-5}	2.5×10^{-3}	18
2.5×10^{-3}	5×10^{-2}	None	31
2.5×10^{-3}	5×10^{-2}	3.2×10^{-6}	35
2.5×10^{-3}	5×10^{-2}	1.6×10^{-5}	45

tions at which the three activators exert definite effects are shown in Table 4. In this experiment 1 mol. of glucose, MnCl₂ and KH₂PO₄ caused additional decarboxylation of 62 (1st half hour), 24 and 9 (1st hr.) mol. of malate respectively. All three substances thus act catalytically.

Increasing concentrations of acetate buffer progressively reduced the rate of decarboxylation. Thus in one experiment the rates with 0.01, 0.025, 0.05 and 0.075 M-acetate buffer were 127, 109, 92 and 70 μ l./5 min. respectively.

Effects of other substances. As Korkes & Ochoa (1948) have shown that cozymase is required for the decarboxylation of L-malic acid by acetone-powder extracts of Lactobacilli, it was thought that the effect of glucose might be related to the maintenance of the cozymase level in the cells. Experiments were therefore carried out to test whether cozymase could replace glucose. Sufficient MnCl₂ and KH₂PO₄ were added to make their concentrations optimal. However, no acceleration by added cozymase (up to 0.25 mg./ml.) was found.

Glucose was found to be replaceable by fructose, mannose, maltose and galactose (in decreasing order of effectiveness), whereas D-arabinose, L-arabinose, D-xylose, D-ribose, sucrose, lactose and glycogen were inactive (Table 5).

Table 5. *Replacement of glucose by other sugars*

(1 and 2 mg. dry wt. cells respectively, for Exps. 1 and 2, 10^{-1} M-KCl, 2.5×10^{-3} M-MnCl₂, 2.5×10^{-2} M-acetate buffer, pH 5.0, 30 μ mol. DL-malate. Sugars to be tested 2.5×10^{-3} M.)

Exp. no.	Addition	Rate of CO ₂ evolution (μ l.)			
		First 5 min.	Second 5 min.	Third 5 min.	Fourth 5 min.
1	None	19	43	48	46
	Glucose	59	68	56	49
	Fructose	52	60	54	49
	Galactose	28	48	50	45
	Mannose	55	65	58	49
	Sucrose	18	38	48	51
	Lactose	13	36	48	46
	Maltose	35	52	58	48
	D-Xylose	17	40	46	46
	None	22	48	52	—
2	Glucose	59	58	53	—
	L-Arabinose	15	41	49	—
	D-Arabinose	17	43	48	—
	D-Ribose	25	48	48	—
	Glycogen	19	43	52	—

No acid production (at pH 6.0) was found with sugars which had no effect on the decarboxylation, whilst those which accelerated the decarboxylation were fermented, with the exception of galactose,

which always caused a slight stimulation of decarboxylation, but sometimes failed to show any acid production (Table 6). Fructose was generally fermented at the highest rate, and showed no lag period. The fermentation of glucose showed a lag period of up to 20 min. Mannose was fermented less rapidly than glucose and the lag period exceeded that of glucose. Therefore, there seems to be no quantitative correlation between the rates at which various hexoses are fermented by the cells and the extent to which they accelerate the decarboxylation of malate.

Out of a series of inorganic ions tested only Mg^{++} gave an effect similar to that of Mn^{++} ions. The maximum effect of Mg^{++} was attained at a concentration of $4 \times 10^{-2} M$ (sixteen times the optimum concentration of Mn^{++}) and was even then less than the effect of Mn^{++} (Table 8). Adenosinetriphosphate did not replace KH_2PO_4 , either in the absence or presence of added glucose. Sodium pyrophosphate and freshly prepared sodium metaphosphate were inactive. NaH_2PO_4 also failed to replace KH_2PO_4 , indicating that potassium was the active principle of

Table 6. *Comparison between the fermentability of hexoses and their effect on the decarboxylation of malate*

(Decarboxylation and fermentation were measured in solutions containing $2.5 \times 10^{-3} M$ - $MnCl_2$ and $10^{-1} M$ - KCl ; for decarboxylation, $2.5 \times 10^{-2} M$ -acetate buffer, pH 5.0, and 40 μ mol. DL-malate were also present; for fermentation, the solution also contained $1.32 \times 10^{-2} M$ -bicarbonate in a gas phase of 100% CO_2 ; this, at 30°, gives a pH of 6.0. Sugars were added to a final concentration of $5 \times 10^{-3} M$. Decarboxylation and acid production were measured simultaneously using the same cell suspension.)

Exp. no.	Sugar added	Amount of cells (mg. dry wt.)	Lag period before reaching maximum rate of acid production (min.)	Acid production (μ l./hr.)	Maximum acceleration of decarboxylation (% increase over the rate without added sugar)
1	Glucose	1.4	20	243	140
	Fructose	1.4	0-5	289	72
	Mannose	1.4	30	39	75
	Galactose	1.4	—	0	39
2	Glucose	2.1	5	367	53
	Fructose	2.1	5	386	49
	Galactose	2.1	—	0	29
3	Glucose	2.3	10	320	85
	Mannose	2.3	20	287	85
	Galactose	2.3	—	32	37

Lactate was found inactive, but pyruvate accelerated the decarboxylation (Table 7), although much less markedly than equivalent concentrations of glucose. Pyruvate, in contrast to glucose, had no effect at concentrations below $2.5 \times 10^{-4} M$. Above a concentration of $5 \times 10^{-3} M$, pyruvate itself was found to undergo decarboxylation after an initial

KH_2PO_4 . Table 9 shows that KCl had in fact a similar effect and that, in the presence of KCl , NaH_2PO_4 was still inactive. KCl was slightly more

Table 8. *Comparison of the effectiveness of Mn^{++} and Mg^{++}*

(4 mg. dry wt. cells, $2.5 \times 10^{-3} M$ -glucose, $10^{-1} M$ - KCl , 40 μ mol. DL-malate.)

Metal ion added	Final concn. (M)	Increase in activity (compared to the rate without added metal) (%)
Mn^{++}	2.5×10^{-3}	100
Mg^{++}	2.5×10^{-3}	57
Mg^{++}	1×10^{-2}	68
Mg^{++}	4×10^{-2}	70

effective than equivalent amounts of KH_2PO_4 between concentrations of 10^{-1} and $3.2 \times 10^{-5} M$. Since $NaCl$ was inactive, it may be concluded that the observed acceleration of malate decarboxylation by KH_2PO_4 is entirely due to potassium. Amounts of sodium ions, added as $NaCl$, up to 40 times the excess of potassium present, did not reduce the activity appreciably, and $0.1 M$ - Li^+ and NH_4^+ failed to

Table 7. *Replacement of glucose by pyruvate*

(1.9 mg. dry wt. cells, $2.5 \times 10^{-3} M$ - $MnCl_2$, $5 \times 10^{-2} M$ - KH_2PO_4 , 20 μ mol. DL-malate.)

Further additions	CO_2 evolved in first 15 min. (μ l.)
None	67
Glucose ($2.5 \times 10^{-3} M$)	137
Pyruvate ($2.5 \times 10^{-2} M$)	121
Pyruvate ($2.5 \times 10^{-3} M$)	102
Pyruvate ($2.5 \times 10^{-5} M$)	65

lag period, a reaction which will be described in a separate communication. The increased CO_2 output observed when both malate and pyruvate were present was not due to decomposition of pyruvate but to an acceleration of the decarboxylation of malate.

Table 9. *Effect of KCl, and failure of Na₂HPO₄ to increase activity*

(2.4 mg. dry wt. cells, 2.5×10^{-3} M-MnCl₂, 2.5×10^{-3} M-glucose, 40 μ mol. DL-malic acid. The Na₂HPO₄ used was adjusted to pH 5.)

Further addition	CO ₂ evolved in first 10 min. (μ l.)
None	24
Sodium phosphate (2.5×10^{-3} M)	24
Sodium phosphate (2.5×10^{-3} M); KCl (1.7×10^{-1} M)	82
Sodium phosphate (2.5×10^{-3} M); KCl (1.7×10^{-2} M)	92
Sodium phosphate (2.5×10^{-3} M); KCl (1.7×10^{-3} M)	70
Sodium phosphate (2.5×10^{-3} M); KCl (1.7×10^{-4} M)	58
KCl (1.7×10^{-2} M)	96
KCl (1.7×10^{-2} M); sodium phosphate (5×10^{-2} M)	92
KCl (1.7×10^{-2} M); sodium phosphate (5×10^{-3} M)	101
KCl (1.7×10^{-2} M); sodium phosphate (5×10^{-4} M)	92
KCl (1.7×10^{-2} M); sodium phosphate (5×10^{-5} M)	93

Table 10. *Replacement of potassium by rubidium and caesium*

(2 mg. dry wt. cells, 2.5×10^{-3} M-glucose, 2.5×10^{-3} M-MnCl₂, 40 μ mol. DL-malate.)

Exp. no.	Additions	Maximum rate of CO ₂ evolution (μ l./5 min.)
1	None	20
	KCl (2.5×10^{-2} M)	64
	KCl (1.6×10^{-4} M)	64
	RbCl (2.5×10^{-2} M)	64
	RbCl (1.6×10^{-4} M)	67
	CsCl (2.5×10^{-2} M)	54
	CsCl (1.6×10^{-4} M)	35
2	None	31
	KCl (2.5×10^{-2} M)	71
	RbCl (2.5×10^{-2} M)	76
	CsCl (2.5×10^{-2} M)	62

affect the rate of decarboxylation in the presence of the same concentration of KCl. Potassium could be replaced entirely by rubidium, which was as active at all concentrations tested (Table 10). Caesium was somewhat less effective. The sample of CsCl used was of the 'Analar' quality of British Drug Houses Ltd.; no examination of its potassium and rubidium content was carried out.

The effect of anions. Amongst various salts of potassium tested, the chloride and phosphate were

the most effective. Sulphate, nitrate and acetate inhibited at higher concentrations (Table 11).

Comparison of the decarboxylation in suspensions of intact and impaired cells. Aqueous suspensions of acetone powders (prepared by slowly pouring a concentrated suspension of cells into 10 times its volume

Table 11. *Comparison of the effect of KCl, KNO₃, K₂SO₄ and CH₃COOK*

(1.4 mg. dry wt. cells, 2.5×10^{-3} M-glucose, 2.5×10^{-3} M-MnCl₂, 20 μ mol. DL-malate. Part A: 10^{-2} M-potassium acetate buffer, pH 5.0. Part B: 2.5×10^{-2} M-sodium acetate buffer, pH 5.0.)

Part	Additions	CO ₂ evolved in first 30 min. (μ l.)
A	None	41
	KCl (7.5×10^{-2} M)	130
	KCl (1.5×10^{-1} M)	147
	KCl (3×10^{-1} M)	151
	K ₂ SO ₄ (7.5×10^{-2} M)	79
	K ₂ SO ₄ (1.5×10^{-1} M)	82
	K ₂ SO ₄ (3×10^{-1} M)	97
	KNO ₃ (7.5×10^{-2} M)	89
	KNO ₃ (1.5×10^{-1} M)	77
	KNO ₃ (3×10^{-1} M)	66
B	None	29
	CH ₃ COOK (5×10^{-3} M), pH 5.0	152
	CH ₃ COOK (5×10^{-2} M), pH 5.0	121
	CH ₃ COOK (10^{-1} M), pH 5.0	106

Table 12. *Constituents of the enzyme system in freeze-dried cells*

(0.2 ml. (2 mg. dry wt.) suspension of freeze-dried cells, 4×10^{-2} M-acetate buffer, pH 5.8, 30 μ mol. DL-malate. The complete system further contained 5×10^{-3} M-glucose or pyruvate, 5×10^{-3} M-MnCl₂, 5×10^{-2} M-KCl and 0.2 mg. cozymase.)

Omissions or substitutions	CO ₂ evolved in 1 hr. (μ l.)
Complete system (with glucose)	184
Complete system (with pyruvate)	189
Complete system (with glucose and pyruvate)	179
Complete system (substituting KH ₂ PO ₄ for KCl)	161
No glucose or pyruvate	146
No cozymase	43
No MnCl ₂	70
No KCl	7

Table 13. *The effect of pH and buffer composition on enzymic activity*

(0.2 ml. enzyme suspension (2 mg. dry wt.), 2.5×10^{-3} M-glucose, 2.5×10^{-3} M-MnCl₂, 10^{-1} M-KCl, 40 μ mol. DL-malate. 0.2 mg. cozymase in Exp. 3. Buffer concentration, 5×10^{-2} M throughout.)

Exp. no.	System	pH of buffer	Composition of buffer	pH at conclusion of the experiment (glass electrode)	Location of enzyme	CO ₂ evolved (μl.)		
						First 10 min.	Second 10 min.	Third 10 min.
1	Intact cells	3.6	Acetate	4.0	Main compartment	45	58	59
		4.0	Acetate	4.2	Main compartment	70	102	85
		4.5	Acetate	4.6	Main compartment	111	113	83
		4.7	Acetate	4.7	Main compartment	112	105	80
		5.0	Acetate	4.9	Main compartment	129	109	81
		5.4	Acetate	5.4	Main compartment	77	74	58
2	Intact cells	3.6	Acetate	4.0	Side arm	54	84	98
		4.0	Acetate	4.2	Side arm	61	111	101
		4.5	Acetate	4.6	Side arm	62	109	96
		4.7	Acetate	4.7	Side arm	53	106	86
		5.0	Acetate	4.9	Side arm	47	109	89
		5.4	Acetate	5.4	Side arm	28	67	57
3	Suspension of freeze-dried cells	4.7	Acetate	4.7	Main compartment	15	13	11
		5.0	Acetate	4.9	Main compartment	23	19	20
		5.4	Acetate	5.5	Main compartment	54	46	41
		5.8	Acetate	5.7	Main compartment	60	48	44
		6.1	Acetate	5.9	Main compartment	61	46	44
		5.8	Phosphate	5.8	Main compartment	57	43	42
		6.1	Phosphate	6.0	Main compartment	55	45	40
		6.5	Phosphate	6.4	Main compartment	45	36	35
		6.9	Phosphate	6.8	Main compartment	23	22	20
						First 15 min.		
4	Intact cells	4.9	KH ₂ PO ₄ only	—	Main compartment		205	
		5.0	Acetate	—	Main compartment		175	
		5.0	Citrate	—	Main compartment		183	
		5.1	Phthalate	—	Main compartment		68	

of ice-cold acetone) were inactive, even when 0.5 mg. cozymase as well as glucose, MnCl₂ and KH₂PO₄ were added. Aqueous suspensions of cells frozen at -12° and then dried *in vacuo* over P₂O₅ showed up to half the activity of the original intact cells in the presence of Mn⁺⁺, K⁺ (KH₂PO₄ could still be replaced by KCl), glucose (or pyruvate) and cozymase (Table 12). Omission of glucose or pyruvate from this system caused much less reduction in activity than omission of cozymase.

After extraction up to 14 hr. of freeze-dried cells at 25° with sufficient water to give the same cell concentration as the original suspension, 90 % of the activity still remained in the non-soluble residue, and 10 % was in the supernatant. Extraction at higher temperatures caused inactivation.

Intact and freeze-dried cells differed in respect to their pH optima of malate decarboxylation and their activities in various buffers (Table 13). The pH optimum of the enzyme system in intact cells was 4.5–5.0. Suspensions of freeze-dried cells showed a pH optimum around 6.0. Intact cells were more active in phosphate than in acetate buffer, whereas freeze-dried cells were at least as active

in acetate buffer as in phosphate buffer of the same pH.

The half maximum rate of decarboxylation was attained at a substrate concentration of 1.8×10^{-3} M in intact cells, and 8×10^{-3} M in freeze-dried cells.

The enzyme in suspensions of intact cells had a temperature optimum at 35° ; with freeze-dried cells the highest initial rates of CO₂ evolution were observed at 40° , but at that temperature there was subsequently a marked inactivation.

DISCUSSION

The main new finding is the accelerating effect of potassium ions on the rate of decarboxylation of malate. Potassium ions are already known to be required for several other enzymic processes, namely the phosphate transfer between pyruvate and adenosinetriphosphate (Lardy & Ziegler, 1945), the oxidative decarboxylation of malate to pyruvate by *Moraxella lwoffii* (Lwoff & Ionesco, 1947) and possibly the conversion of hexosemonophosphate to hexosediphosphate by yeast extracts (Munz, 1947). Two of these are thus directly concerned with phos-

phorylation, and the third, the oxidative decarboxylation, is assumed to involve phosphorylation.

The conversion of L-malic acid into pyruvic acid in *Moraxella* resembles the reaction investigated in this paper in that both involve oxido-reduction; the two reactions probably have some stages in common, but in the case of *Moraxella* molecular oxygen takes part and the product is pyruvate, whereas in *Lactobacillus* the oxido-reduction is internally compensated and the product is lactate. In *Moraxella*, both rubidium and caesium replace potassium; in *Lactobacillus*, rubidium is as effective as potassium; the effect of caesium was less pronounced; although the sample of caesium used was of 'Analar' quality, the possibility cannot be excluded that the observed acceleration of the decarboxylation of malate was due to contamination by potassium.

The main differences between the decarboxylation of malate by intact cells of *Lactobacillus* and by the acetone powder extracts of Korkes & Ochoa (1948), concern the effects of glucose and cozymase. In the intact cells glucose catalytically accelerates decarboxylation whilst added cozymase is inactive; in acetone-powder extracts (which are unlikely to metabolize glucose under the experimental conditions used) glucose has no effect, but added cozymase is required for full activity. The independence of the enzyme in intact cells of added cozymase is presumably due to the fact that they already contain optimal amounts of the coenzyme (Hughes, 1950). The detailed mechanism of the glucose effect remains obscure: there is a qualitative correlation between the fermentability of sugars and their ability to accelerate the decarboxylation of malate. It is perhaps a linked oxido-reduction involving malate and a derivative of glucose; since pyruvate has been shown to accelerate the decarboxylation of malate, the effect of glucose may to some extent be explained by assuming that glucose gives rise to pyruvate; however, the effect of glucose was always more marked than that of pyruvate, and therefore other derivatives may well be concerned in this linked reaction.

SUMMARY

1. The decarboxylation of L-malic acid by washed suspensions of malate-adapted cells of *Lactobacillus arabinosus* is accelerated by glucose and yeast extract, the effects being additive.

2. Yeast extract can be replaced entirely by manganous chloride and potassium dihydrogen phosphate. The action of potassium dihydrogen phosphate is wholly due to potassium. The latter can be replaced by rubidium, and possibly by caesium, but not by lithium, sodium or ammonium.

3. The effectiveness of glucose is increased by pre-incubating it with the cells, and is equal between concentrations of 10^{-4} and 10^{-1} M. The optimum concentrations of manganous chloride and potassium dihydrogen phosphate are 10^{-2} and 10^{-1} M respectively, but all three substances act catalytically.

4. Fructose, mannose, galactose and maltose are, like glucose, fermented and increase the rate of decarboxylation. Unfermentable substances do not. There is no quantitative correlation between the fermentability of hexoses and their effect on decarboxylation.

5. Pyruvate accelerates decarboxylation of malate, its effect being smaller than that of glucose. At higher concentrations (above 5×10^{-3} M final concentration) pyruvate itself undergoes decarboxylation.

6. Suspensions of freeze-dried cells show up to half the activity of the original whole cells. They require cozymase, are affected only slightly by glucose, and differ from suspensions of intact cells in their pH and temperature optima, and their substrate-activity curve.

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