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The Effect of Inorganic Ions on Respiration in *Pasteurella tularensis* and *Escherichia coli*

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SUMMARY: The effect of the ionic composition of the medium on the respiration of resting-cell suspensions of two Gram-negative micro-organisms has been investigated. Potassium or rubidium salts with Cl^- , Br^- or I^- as anion were found to be necessary for the maximal rates of oxidation of glutamate or glucose by *Pasteurella tularensis*, and KCl or K_2SO_4 by *Escherichia coli*. The pH value of the medium and the concentration of the substrate influenced the effect of the electrolytes. Under certain conditions, a synergistic effect of Na^+ ion was observed. The same ion mixtures which supported the respiration caused the maximal swelling of the bacterial cells.

The influence of KCl and NaCl on the respiration of halophilic bacteria was investigated by Baxter & Gibbons (1956). Krebs, Whittam & Hems (1957) showed that the oxidation of various substrates by washed cells of *Alcaligenes faecalis* was greatly accelerated by potassium ions. In the present communication observations are reported on the effect of various electrolytes on the rate of oxidation of glutamate and glucose by *Escherichia coli* and by an osmotically-sensitive micro-organism, *Pasteurella tularensis*.

METHODS

Escherichia coli, strain B, was grown on nutrient agar (Difco). *Pasteurella tularensis*, strain S and D, was grown on a 3% (w/v) peptone (Difco) + cysteine medium (Mager, Kindler & Grossowicz, 1954). The cultures were incubated at 37° for 24 hr. The bacteria were collected in 0.25M-sucrose and, unless otherwise stated, washed three times with this sucrose solution. The final sucrose (0.25M) suspensions contained 50 mg. wet weight organisms/ml. Manometric experiments were carried out by the conventional Warburg technique. Spectrophotometric examinations were performed in the Beckman model DU spectrophotometer, with Corex cells of 1 cm. light path.

RESULTS

Effect of various cations and anions on the oxidation of glutamate

Glutamic acid, neutralized by the addition of tris (buffer), 2-amino-2-hydroxy-methylpropane-1:3-diol, to pH 7.4, was not oxidized by thoroughly washed *Pasteurella tularensis* when other salts were excluded from the reaction medium. When, however, various potassium salts were added to the bacteria suspended in isotonic sucrose, it was found that KBr caused the greatest respiratory stimulation, while KCl and KI were slightly less effective; other potassium salts had only an insignificant effect. Of the chlorides tested other than KCl only the

rubidium salt supported the respiration (Table 1). Since KCl and the other effective electrolytes enhanced the respiration at relatively low concentrations, their effect could not be due to changes in osmotic pressure (Fig. 1).

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Effect of electrolyte concentration. Each flask contained 30 μ mole tris glutamate buffer (pH 7.4), 0.25 M-sucrose, the bacterial suspension (*Pasteurella tularensis*: 15 mg. wet weight; *Escherichia coli*: 9 mg. wet weight) and the electrolyte as indicated. $\bigcirc$ - $\bigcirc$ and $\bullet$ - $\bullet$ = KCl; $\triangle$ - $\triangle$ = KBr; $\times$ - $\times$ = KI; $\blacksquare$ - $\blacksquare$ = K_2SO_4 . Unshaded marks; and $\times$ - $\times$: *Pasteurella tularensis*. Shaded marks: *Escherichia coli*. Volume of reaction mixture 3.0 ml.; centre well, 0.2 ml. of KOH. Temperature, 28°; gas phase, air.

Fig. 2. Effect of concentration of potassium glutamate. Each flask contained 0.25 M-sucrose, the bacterial suspension as in Fig. 1, and potassium glutamate as indicated. $\bigcirc$ - $\bigcirc$ and $\bullet$ - $\bullet$ = *Escherichia coli*; $\square$ - $\square$ and $\blacksquare$ - $\blacksquare$ = *Pasteurella tularensis*. Unshaded marks: no KCl added; shaded marks: 30 μ mole KCl added. Conditions of experiment otherwise as in Fig. 1.

Fig. 3. Effect of KCl on the oxidation of glucose. Each flask contained 60 μ mole tris nitrate buffer (pH 7.4) 0.25 M-sucrose and glucose as indicated. *Escherichia coli* and *Pasteurella tularensis* as in Fig. 1. $\bigcirc$ - $\bigcirc$ and $\bullet$ - $\bullet$ = *Escherichia coli*; $\square$ - $\square$ and $\blacksquare$ - $\blacksquare$ = *Pasteurella tularensis*. Unshaded marks: no KCl added; shaded marks: 30 μ mole KCl added. The conditions of the experiment otherwise as in Fig. 1.

Escherichia coli likewise was incapable of metabolizing glutamate neutralized by tris (buffer); addition of KCl or K_2SO_4 was necessary for oxidation of the substrate, but rubidium could not replace the potassium (Table 1, Fig. 1). In order to elucidate the part played by the cation, the rate of oxidation of glutamate in various counter-ion combinations was measured. Sodium glutamate was not oxidized by either of the two micro-organisms. In *Pasteurella tularensis*, the rate of oxidation of potassium glutamate increased with the concentration of the substrate, and the highest rate of respiration was attained only when KCl was added. In suspensions of *Escherichia coli*, KCl accelerated the oxidation of potassium glutamate only when the concentration of the substrate was suboptimal (Fig. 2).

Effect of KCl on the oxidation of glucose

Acceleration of the rate of glucose oxidation by the addition of KCl was also demonstrated. In *Pasteurella tularensis*, the effect was observed at all concentrations of substrate, while in *Escherichia coli* it became apparent only at low glucose concentrations (Fig. 3).

Effect of pH value

The effect of the pH value on the oxidation of glutamate and glucose was studied in *Pasteurella tularensis*. As shown in Fig. 4, the rate of oxidation of both substrates diminished with decreasing acidity. The stimulation caused by KCl became most prominent at slightly alkaline pH values.

Table 1. *The effect of electrolytes on the oxidation of tris glutamate buffer by Escherichia coli and Pasteurella tularensis*

Each flask contained 30 μ mole of tris glutamate buffer (pH 7.4), 0.25 M-sucrose. The final concentration of the electrolytes was 30 μ equiv./cup when one salt was added and 15 μ equiv./cup each salt when a mixture of two was used. Volume of reaction mixture 3.0 ml.

Anion	Cation							Organism	
	K ⁺	Na ⁺	Li ⁺	Rb ⁺	NH ₄ ⁺	Ca ⁺⁺	Mg ⁺⁺	<i>E. coli</i>	<i>P. tularensis</i>
								Oxygen uptake (μ l./hr.)	
Chloride	+	.	.	.	.	.	.	186	120
	.	+	.	.	.	.	.	60	17
	.	.	+	.	.	.	.	0	45
	.	.	.	+	.	.	.	0	142
	.	.	.	.	+	.	.	0	0
	.	.	.	.	.	+	.	40	0
Bromide	.	.	.	.	.	.	+	0	42
	+	.	.	.	.	.	.	10	154
	.	+	.	.	.	.	.	0	.
Iodide	+	+	.	.	.	.	.	138	.
	+	.	.	.	.	.	.	15	106
Nitrate	+	.	.	.	.	.	.	0	0
	.	+	.	.	.	.	.	12	0
	.	.	+	.	.	.	.	0	.
	.	.	.	.	.	+	.	0	.
	.	.	.	.	.	.	+	0	.
	+	+	.	.	.	.	.	185	34
	+	.	+	.	.	.	.	0	.
	+	.	.	.	.	.	+	0	.
	+	.	.	.	.	+	.	0	.
Sulphate	+	.	.	.	.	.	.	185	35
	.	+	.	.	.	.	.	0	20
	+	+	.	.	.	.	.	147	20
Phosphate	+	.	.	.	.	.	.	0	47
	.	+	.	.	.	.	.	0	.
	+	+	.	.	.	.	.	153	.
	.	.	.	.	.	.	.	0	0

Controls containing each electrolyte but no glutamate showed no oxygen uptake.

The synergistic effect of Na⁺ ions

The suppression of the oxidation of glutamate in *Escherichia coli* in the presence of potassium salts other than chloride and sulphate (i.e. NO₃⁻, HPO₄⁻, etc.) can be counteracted by the addition of Na⁺ ions, irrespective of the anionic moiety (Table 1). Li⁺, Rb⁺, Mg⁺⁺ or Ca⁺⁺ could not replace Na⁺ in this respect.

Effect of electrolytes on changes in the state of swelling of bacterial cells

When a suspension of *Pasteurella tularensis* was added to a medium containing KCl alone, or glutamate neutralized with tris (buffer), only slow swelling was observed. In a mixture of the substrate + the electrolyte, however, the optical

Fig. 4

Fig. 5

Fig. 4. Effect of pH value. Each flask contained: 60 μmole tris phosphate buffer of the respective pH value; 30 μmole substrate; 0.25 M-sucrose; *Pasteurella tularensis* as in Fig. 1. ○—○ and ●—● = glutamate; □—□ and ■—■ = glucose. Unshaded marks: no KCl added; shaded marks 30 μmole KCl added. Conditions of the experiment otherwise as in Fig. 1.

Fig. 5. Effect of glutamate and electrolytes on turbidity changes. Each Beckman cell contained: 0.25 M-sucrose and tris glutamate buffer and/or electrolytes as indicated. ○—○ = no addition; ●—● = 30 μmole tris glutamate buffer; □—□ = 30 μmole KCl; △—△ = 30 μmole KNO₃; ■—■ = 30 μmole glutamate + KCl; ▲—▲ = 30 μmole glutamate + KNO₃. Total volume 3.0 ml. At zero time 0.05 ml. of a dense suspension of *Pasteurella tularensis* added. Control cell, water. Room temperature. Optical density measured at wavelength 500 mμ.

density of the suspension decreased rapidly and exponentially until a new state of equilibrium was reached. Like KCl, KBr and KI accelerated swelling but other potassium salts had no effect (Fig. 5). In an experiment with a suspension of *Escherichia coli*, KCl, K₂SO₄ or a mixture of KNO₃ + NaNO₃ was found to promote swelling.

DISCUSSION

The effect of electrolytes on the respiration of a variety of biological systems has been described before. Acceleration of the respiration of brain cortex slices by chlorides, bromides or iodides of either potassium, rubidium or caesium has been demonstrated (Ashford & Dixon, 1935; Dickens & Greville, 1935). The stimulation of glucose oxidation in rat-liver homogenates by K⁺, Rb⁺, Cs⁺, Mg⁺⁺ was reported by Haugaard & Itzkovitz (1956); on addition of NaCl, LiCl or CaCl₂ to a system activated by potassium ions, glucose oxidation was inhibited. In *Alcaligenes faecalis* washed with distilled water, Krebs *et al.* (1957) demonstrated an acceleration of the rate of oxidation of various substrates by the addition of K⁺ or Rb⁺ ions. These authors attributed the activity of Rb⁺ to contamination with potassium salts. In the present work, the same

batch of rubidium chloride which replaced K^+ in *Pasteurella tularensis* proved to be quite ineffective on the oxidation of glutamate in *Escherichia coli*. In this case, therefore, the action of Rb^+ cannot be attributed to small amounts of K^+ present as impurity. In *Alcaligenes faecalis*, accumulation of K^+ ions was shown to occur during the incubation and could be accounted for by exchange diffusion. The penetration of K^+ was not accompanied by a similar movement of the anion, and moreover the nature of the anion was without influence on the rate of acceleration of the respiration. In contrast to these findings, K^+ ion was effective only in certain counter-ion combinations in *Escherichia coli* and *Pasteurella tularensis*. Exchange diffusion seems, therefore, to play a smaller part in this case. It might be relevant to this point that, in the case of *Escherichia coli*, potassium salts which alone caused no stimulation of the respiration, could be activated by the addition of any sodium salt which by itself was similarly ineffective. A promoting effect on the exchange diffusion of potassium by sodium ions, overcoming thereby the retardation due to the anion, might be involved in this phenomenon. The control of the influx of K^+ ions by the Na^+ concentration in the medium into erythrocytes and nerve fibres has repeatedly been demonstrated (Conway, 1954). Krebs *et al.* (1957) have also shown that Na^+ ions facilitate the accumulation of K^+ in the bacterial cell.

As potassium glutamate is oxidized by *Escherichia coli* at maximum rate without the addition of other electrolytes, the anion has probably only an indirect effect. The anion might exert a modifying action on the permeability of the cation, due to the different diffusion potentials created (Davson, 1940). The effectiveness of the halogen salts of potassium and rubidium in *Pasteurella tularensis* is in accordance with this view, as biological membranes are known to be permeable to Cl^- , Br^- and I^- ions (Conway, 1954). The penetration of the cation used as its halogen salt will thus not be hindered by its counter-ion. The ready penetration of the sulphate ion in *Escherichia coli*, on the other hand, could not be anticipated.

Correlation between the swelling of ascites cells suspended in media containing various substrates and the accumulation of the metabolites in the cell interior has been demonstrated before (Christensen, Riggs, Fisher & Palatine, 1952). The finding that the same ion combination, which accelerated the oxidation of substrates in the micro-organisms studied, was also the most effective in eliciting the swelling of the bacteria (Mager, Kuczynski, Schatzberg & Avi-Dor, 1956; Avi-Dor, Kuczynski, Schatzberg & Mager, 1956) could possibly be interpreted as the effect of the ionic environment on the rate of penetration of the substrate. The latter might become the rate limiting step under certain experimental conditions (higher pH values, low substrate concentration). Hence, the increased dependence of the respiration on the addition of K^+ ions under such conditions.

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