SUMMARY

1. Brain pyrophosphatase is readily inactivated on standing at 0° in slightly acid solution.

2. The fall in activity which takes place when the enzyme is dialysed is not observed when the dialysis is carried out under conditions in which the pH is prevented from falling.

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Potassium Uptake by *Alcaligenes faecalis*

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In experiments on the metabolism of the bacterium *Alcaligenes faecalis* it was noted that the capacity to respire is much reduced, often to almost zero, on washing the cells with distilled water, but is restored on addition of yeast extracts. The essential component of yeast extracts was found to be potassium. This organism differs from many others in that it can lose its intracellular potassium readily to the medium if the potassium concentration of the latter is low. The cells can recover potassium if its concentration in the medium is raised. This recovery is in part due to active transport, depending on respiration, but appreciable amounts of potassium can be taken up against concentration gradients without a supply of energy. This paper is concerned with a study of the potassium distribution between cells and medium under a variety of experimental conditions.

EXPERIMENTAL

Growth conditions. The basal medium contained 1% (w/v) of Difco peptone, 0.5% of Difco yeast extract, 1% (w/v) of casein hydrolysate (Pronutrin or Tryptone), and 1% (w/v) of glucose. In some of the later experiments glucose was omitted when it had been found that it was not oxidized. The pH was adjusted with NaOH to 7.0. The inoculum consisted of a 3–5 ml. liquid subculture of the same medium. Roux bottles containing 100 ml. of medium and 2% of agar were incubated at 30° for about 18 hr. The cells were washed on the centrifuge with three portions of water. After washing, the cells from one Roux bottle were suspended in 15 ml. of water and stored at +2°. This suspension con-

tained generally 11–12 mg. of dry cells/ml. In the later experiments agar was omitted to avoid contamination of cell suspension with pieces of agar gel, and in order to reduce the path of diffusion of oxygen 50 ml. of medium was used/Roux bottle.

Organisms. Three strains of *A. faecalis* were examined. They were: strains 415 and 1347 of the National Collection of Type Cultures; and strain 19 obtained from the Department of Bacteriology, University of Sheffield. The organism is a Gram-negative rod incapable of fermenting sugars. Unless otherwise stated strain 1347 was used.

Ionic distribution. The distribution of potassium and sodium between medium and cells was determined as follows. At the end of the incubation period the suspension was thoroughly shaken and 3 or 5 ml. of the suspension was transferred to a weighed centrifuge tube of 6 ml. capacity. If the conditions of incubation were aerobic the suspension was gassed for 10 min. with oxygen to prevent anaerobic conditions in subsequent manipulations and the suspension was then spun for 10 min. in a refrigerated centrifuge at 20 000 g. As much as possible of the supernatant was removed with pipettes or by decanting and collected for analysis. The amount of cell residue was ascertained by reweighing the centrifuge tubes with the residue. The cell volume was calculated on the assumption (based on measurements) that the specific gravity of the residue was 1.017–27°. The specific gravity of the standard medium was 1.011. Examples of the calculations are contained in Table 3.

For analysis by flame photometry the supernatant was diluted usually to 10 ml., but less if the amounts of potassium or sodium were small. The residue was mixed with 0.05 ml. of conc. HNO₃ and placed for 30 min. in a boiling-water bath. This resulted in a clear solution which was diluted to a known volume, usually 10 ml. The diluting material

Table 1. *Effects of potassium chloride, yeast extract and ashed-yeast extract on the respiration of washed cells of Alcaligenes faecalis, strain 1347*

Cells were stored for 6 days at 2° and incubated at 30°; substrates, 0.005 M; KCl, 0.00125 M. Yeast ash refers to a quantity corresponding to the un-ashed material; sodium phosphate buffer, pH 7.4, 0.04 M; 3 mg. (dry) cells; total volume, 4 ml.; O₂.

Substrate	Period of observation (min.)	-Q _{O₂}			
		No addition	KCl	0.4 ml. of Difco yeast 1% (w/v)	0.4 ml. of yeast ash
L-Glutamate	40-60	10	78	148	80
	100-120	21	93	222	88
Pyruvate	40-60	9	26	124	26
	100-120	8	27	190	28

Table 2. *Effect of K⁺ ions on the respiration of strain 1347 in the presence of various substrates*

Substrate concentration, 0.0125 M. Q_{O₂} calculated for 40-60 min. period; 2.85 mg. of (dry) cells was present in a total volume of 4 ml. Sodium phosphate buffer, pH 7.4, 0.04 M. Cells were 48 hr. old. Acidic substances were added as sodium salts.

Substrate added	-Q _{O₂}	
	Without added KCl	With 0.008 M-KCl
L-Glutamate	43	132
Acetate	8	74
DL-Lactate	24	153
Succinate	10	101
Pyruvate	24	127
Fumarate	9	58
Glycerol	3	22

included in both cases sodium and potassium for internal compensation.

Sodium and potassium were determined by flame photometry. In the earlier experiments the apparatus of Domingo & Klyne (1949) was used, in the later ones a Beckman spectrophotometer with the flame-photometry attachment 9200 and the photo-multiplier 4300. Chloride was determined potentiometrically (Sanderson, 1952).

The cell volume was ascertained by centrifuging a suitable amount (1 ml.) of suspension, the volume depending on the cell concentration, in a 'haematocrit', the capillary part of which had a diameter of about 1 mm. Centrifuging was continued at 20000 g until the volume was constant (usually about 30 min.).

Incubations. Washed suspensions were shaken in conical Warburg vessels under the conditions stated in the tables. The centre well usually contained 0.2 ml. of 2 N-NaOH. The oxygen consumption was measured in most aerobic experiments.

RESULTS

General metabolic properties of the organism

Washed suspensions of this organism showed a very low oxygen uptake on addition of amino acids, lactate, pyruvate, acetate or some intermediates of the tricarboxylic acid cycle [Q_{O_2} (O₂ used/mg. dry wt./hr.) = -10 to -20 at 30°] but respiration is

very rapid (Q_{O₂} up to -250) when complex media such as yeast extracts or casein hydrolysate are added. Ashed-yeast extract, added together with glutamic or pyruvic acid, also increased the rate of oxidation but less effectively than un-ashed-yeast extract. Potassium chloride or other potassium salts have the same effect as the ash, which shows that potassium is an active principle of yeast ash. The effects of potassium chloride, yeast extract and ashed-yeast extract are shown in Table 1. The increase due to potassium was three- to eight-fold; yeast extract caused a further large rise which was much more marked with pyruvate than with glutamate.

The effect of potassium in the presence of various substrates is shown in Table 2. Every oxidizable substrate tested showed the effect though its magnitude varied from substrate to substrate. The effect of potassium chloride was larger and the effect of yeast extract smaller in fresh cells than in old cells, indicating that the factors which limit respiration change in the course of ageing.

Repeated washing on the centrifuge had no major effect on the effects of potassium chloride and yeast extract, one washing being almost as effective as three washings in producing cells which respond to the addition of potassium chloride and yeast extract. The yield of cells was slightly (20%) increased when the medium was supplemented by 0.5% potassium chloride. Cells grown in the presence of 0.5% potassium chloride showed the same response of respiration to potassium chloride after washing.

The same effect was shown by potassium sulphate, potassium chloride, and potassium phosphate when added in equivalent quantities. The effect reached a maximum when the concentration of K⁺ ions in the medium was about 0.002 M. Salts of lithium, sodium, ammonium, magnesium, calcium and barium had no effects. A commercial sample of rubidium chloride (possibly containing potassium chloride) was almost as active as potassium chloride. Caesium chloride (analytical reagent) had a slight effect.

The pH optimum of the respiration with glutamate as substrate was between pH 7.4 and 7.7. At pH 6.2 the respiration was about 50% of the maximum value.

Washed suspensions did not oxidize glucose, ethanol, citrate or α -oxoglutarate. When glutamate was the substrate α -oxoglutarate did not appear in the medium.

As shown in Table 1, yeast extracts stimulate the respiration more than the equivalent quantity of yeast ash or potassium chloride. Many substances known to be present in yeast extracts were examined for their stimulating effect on respiration, but no pure substance or combination of pure substances was found to replace these extracts fully. Substances tested included a mixture of pantothenate, pyridoxine, riboflavin, biotin, nicotinic acid, thiamine, cocarboxylase and inositol, together with mixtures of synthetic amino acids, glucose, pyruvate and other oxidizable substances. Lipoic acid and glutathione were also inactive.

Potassium and sodium content of strain 1347. Data on the distribution of potassium and sodium between medium and cells harvested after 16 hr. are shown in Table 3. The standard medium without agar was used. For the calculation of the intracellular sodium and potassium it had to be borne in mind that the centrifuged cells were 'contaminated' with medium, partly due to the impossibility of decanting the supernatant quantitatively and partly due to incomplete 'packing' of the cells. No correction was made for incomplete packing. The contamination with medium was corrected for on the basis of the haematocrit value (which supplied the volume of packed cells) and the analytical data for the medium. As seen in Table 3, the ratio intracellular potassium/extracellular potassium was about 7, the ratio intracellular sodium/extra-

cellular sodium about 0.7. These figures are based on the assumption that potassium and sodium are evenly distributed throughout the cell. The actual potassium and sodium spaces are likely to be smaller than the cell volume and the real concentrations of potassium and sodium in the intracellular fluid may therefore be greater than calculated. This would increase the above ratios and make that for sodium nearer to 1.

Potassium content of washed cells after incubation. Cells of the culture used for the preceding experiment were washed twice with water on the centrifuge. The sediment was suspended in 1 ml. of water and divided into equal parts. One 0.5 ml. portion (containing 18.65 mg. of dry bacteria) was incubated at 15° with 3.5 ml. of fresh culture medium, the other was analysed for potassium. Incubation was for 1 hr. in Warburg flasks shaken in 100% oxygen. The suspension absorbed about 600 μ l. of oxygen in 60 min. The potassium analysis at the end of the incubation gave the following value: potassium in medium, 157 μ g./ml.; potassium in cells, 1530 μ g./ml. The corresponding values before incubation, i.e. on the medium and on the second 0.5 ml. portion of cells, were: potassium in medium, 202 μ g./ml.; potassium in cells, 145 μ g./ml. The data indicate that cells lose potassium on washing with water, but recover it on aerobic incubation in the nutrient medium. The level attained was of the same order as that found in freshly harvested cells (see Table 3).

Effect of external potassium level on the intracellular potassium concentration. Cells were washed twice with water and suspended in a small volume of water. Warburg cups contained 0.5 ml. of this cell suspension (16.4 mg. dry wt. of cells, 152 μ l. volume wet), 1 ml. of 0.5 M-triethanolamine buffer, pH 7.4 (prepared by mixing 100 ml. of M-triethanolamine,

Table 3. *Distribution of potassium and sodium in cells of strain 1347 after growth for 16 hr. at 30°*

Duplicate analyses; for experimental conditions see text.

	Potassium		Sodium	
	5.0	5.0	5.0	5.0
Volume of cells and medium centrifuged (ml.)	4.7	4.8	4.8	4.8
Volume of supernatant collected (ml.)	81.5	65.2	81.4	105.1
Weight of residue (mg.)	80.7	64.5	80.5	104.1
Volume of residue (μ l.)	30.9	30.9	30.9	30.9
Volume of packed cells in residue (μ l.)				
Analyses				
K or Na/ml. of supernatant (μ g.)	220	216	317	318
K or Na in total residue (μ g.)	58.2	54.8	23.2	29.0
K or Na in medium of residue (calculated) (μ g.)	11.0	7.26	15.7	23.1
K or Na in cells of residue (μ g.)	47.2	47.5	7.5	5.9
K or Na/ml. of cells (μ g.)	1525	1540	243	191

Determination of cell volume in suspension

1.012 ml. of suspension gave a haematocrit value of $\left\{ \begin{array}{l} 6.21 \mu\text{l.} \\ 6.16 \mu\text{l.} \end{array} \right\}$ av. 6.185 μ l.

Ratio dry wt./wet wt.; 5 ml. of suspension, spun and washed once, left 3.35 mg. dry wt., 30.9 mg. wet wt.

68 ml. of $N-HCl$ and 32 ml. of water), 0.4 ml. of 0.1M-sodium acetate, 0.1 ml. of 0.02M-sodium fumarate and 2 ml. of potassium chloride solution of varying strength. Triethanolamine buffer, rather than a buffer containing a metallic cation, was used in order to keep the potassium or sodium concentration low. The temperature of incubation was 15°. This lower temperature was chosen because considerable quantities of cells were needed for the determination of intracellular potassium, and oxygenation of these quantities is difficult at higher temperatures. The gas phase was 100% oxygen. The data on the concentrations of external and internal potassium are given in Table 4. For comparison, data on the effect of Difco yeast extract are also given. The intracellular potassium level rose on incubation from 143 $\mu g./ml.$ to about 1000 $\mu g./ml.$ The intracellular level reached was independent of the external potassium concentration within the range tested. Difco yeast extract increased the oxygen uptake, but decreased the intracellular potassium level.

Another experiment at lower external potassium levels is shown in Table 5. The conditions were the same as in the previous experiment except that glucose was omitted from the growth medium. The intracellular potassium level reached in this experiment was lower than in the previous one. Whether this was connected with the absence of glucose from the growth medium remains to be investigated. The intracellular level was inde-

pendent of the external level until the latter fell below 10 $\mu g./ml.$ The ratio intracellular potassium/extracellular potassium rose as the external potassium concentration fell and was in the region of 100 at the lowest external concentration.

As shown in the last column of Table 5, potassium was also taken up anaerobically, but the internal level reached was about 30% lower than under the same conditions aerobically.

Further data on the anaerobic uptake of potassium at varying potassium concentrations in the medium are given in Table 6. The cells (grown in the absence of glucose) were added to the remainder of the solution from the side arm of the manometer cup after the air had been replaced by nitrogen and any remaining traces of oxygen had been absorbed by the yellow phosphorus. Considerable amounts of potassium were again taken up anaerobically against concentration gradients, but less than aerobically. The last two horizontal rows show the recovery of the potassium originally present and represent a check on the reliability of the procedure.

Effect of the constituents of suspending medium on the concentration gradient of potassium. Table 7 shows that at comparable external potassium concentrations much higher intracellular concentrations were obtained in the presence of the growth medium than in a simple medium containing oxidizable substrates. The concentration gradients were roughly twice as great in the presence of growth medium, aerobically as well as anaerobically. In order to

Table 4. *Effect of varying potassium concentrations in the medium on the level of intracellular potassium*

Dry cells (16.4 mg.) were present in a total volume of 4 ml. Incubation was for 1 hr. at 15°. For full details see text. The data refer to concentrations after incubation; the initial intracellular potassium concentration was 143 $\mu g./ml.$

Cup no.	...	...	1	2	3	4
KCl included in 4 ml.			2 ml. of 0.115%	2 ml. of 0.330%	2 ml. of 0.66%	2 ml. of 0.115% 2 mg. of Difco yeast
K in supernatant ($\mu g./ml.$)			48.5	109.2	236	65.2
K in cells ($\mu g./ml.$)			1060	928	1195	765
Ratio: $\frac{\text{intracellular K}}{\text{extracellular K}}$			22	8.5	5.1	11.7
O ₂ uptake in 50 min. ($\mu l.$)			479	481	463	630

Table 5. *Effect of varying potassium concentrations in the medium on the level of intracellular potassium*

Dry cells (19.76 mg.) were present in 4 ml. of suspension. Incubation for 1 hr. at 15°. Initial intracellular potassium concentration, 120.1 $\mu g./ml.$

Cup no.	...	...	1	2	3	4	5
KCl included in 4 ml.			2 ml. of 0.115%	2 ml. of 0.0575%	2 ml. of 0.029%	2 ml. of 0.00143%	2 ml. of 0.0575%
Gas			O ₂	O ₂	O ₂	O ₂	N ₂
K in supernatant ($\mu g./ml.$)			57.4	26.5	12.3	4.55	29.9
K in cells ($\mu g./ml.$)			567	590	553	459	429
Ratio: $\frac{\text{intracellular K}}{\text{extracellular K}}$			9.9	22.2	45.0	101	14.8
O ₂ uptake in 50 min. ($\mu l.$)			356	351	343	342	0

examine the effects of the constituents of the growth medium, cells were incubated with modified media (Table 8). Omission of glucose or peptone or casein hydrolysate had no major effect on the concentration gradient.

In other experiments the effect of Difco yeast extract at a final concentration of 0.05 % was tested, with 0.01 M-sodium acetate and 0.0005 M-sodium fumarate as substrates. Aerobically the yeast extract increased the rate of oxygen consumption by about 25 % but there was no effect on the concentration gradient of potassium aerobically or anaerobically. Addition of sodium chloride (0.066 M) to the

medium, on the other hand, caused a substantial uptake of potassium by the cells and a slight uptake of sodium (Table 9). After incubation in the presence of sodium chloride the potassium gradient was 1:47 in favour of the cells, whereas the sodium gradient was 1:18.5 in favour of the external medium.

Movements of other ions. The uptake of potassium must be accompanied by other movements of ions to maintain electroneutrality. Either an equivalent cation must migrate in the opposite direction, or an equivalent of an anion must move together with potassium. Experiments were set up in which the

Table 6. *Effect of potassium concentration of external medium on internal potassium level under anaerobic conditions*

The side arm of each cup contained 1 ml. of washed cells in water (23.8 mg. dry wt.); main compartment contained 1 ml. of 0.5 M-triethanolamine buffer, pH 7.4; 0.4 ml. of 0.1 M-sodium acetate; 0.1 ml. of 0.02 M-sodium fumarate and varying quantities of 0.23 % KCl solution. Total volume, 4 ml., made up with water. Incubation for 1 hr. at 15°.

Cup no.	1	2	3	4	5
0.23 % KCl added (ml.)	0.1	0	0.025	0.1	0.4
Gas	O ₂	N ₂	N ₂	N ₂	N ₂
O ₂ used (μl.)	510	0	0	0	0
K in supernatant (μg./ml.)	22.7	3.76	10.5	31.3	123
K in cells (μg./ml.)	515	176	214	306	472
Total K added (cells + KCl) (μg.)	159	37.9	68.1	158	518
K recovered (μg.)	172	41.9	73.8	166	550

Table 7. *Effect of oxygen on distribution of potassium in the presence and absence of growth medium*

Each cup contained 0.5 ml. of cell suspension (19.1 mg. dry wt.) in the side arm; main compartment contained 0.4 ml. of 0.1 M-sodium acetate; 0.1 ml. of 0.02 M-sodium fumarate and items shown below. Incubation for 1 hr. at 15°.

Cup no.	1	2	3	4
Main compartment ...	0.12 ml. of KCl 1.15 % (w/v) 2.9 ml. of H ₂ O	3 ml. of growth medium	0.131 ml. of KCl 1.15 % (w/v) 2.9 ml. of H ₂ O	3 ml. of growth medium
Gas	O ₂	O ₂	N ₂	N ₂
O ₂ used in 50 min. (μl.)	341	633	0	0
K in supernatant (μg./ml.)	160	157	193	183
K in cells (μg./ml.)	1050	1800	633	976
Cell volume (μl.)	99.5	82.3	99.3	79.8
Ratio: $\frac{\text{intracellular K}}{\text{extracellular K}}$	6.6	11.5	3.27	5.3
K added (μg.)	754	769	820	769
K recovered (μg.)	730	765	823	788

Table 8. *Distribution of potassium between cells and medium under varying conditions*

Growth medium: 1 % of casein hydrolysate; 1 % of Difco peptone; 1 % of glucose; 0.5 % of Difco yeast extract; pH was adjusted to 7.0 with NaOH. Cells, 0.5 ml.; 3.5 ml. of medium. Incubation for 60 min. at 15° in O₂.

Expt. no.	Suspension medium	K (μg./ml.)		Ratio: K in cells K in medium
		Medium	Cells	
1	Growth medium (cells 4 days old)	195	1145	5.9
2	Growth medium (fresh cells)	194	1775	9.2
3	Growth medium (cells 2 days old)	173	1440	8.3
	Same without glucose	136	1020	7.5
	Same without peptone	112	1030	9.2
	Same without glucose and peptone	118	1045	8.9
	Same without casein hydrolysate	128	1010	7.9

movements of potassium, chloride and sodium were measured. Cell suspension (4 ml.) containing 76.8 mg. of dry cells was shaken for 15 min. at 22° with 4 ml. of 0.0575% potassium chloride. No substrate was added. The potassium concentration of cells rose from 2.7 to 13.1 mM but the chloride concentration did not increase; in fact it showed a small decrease from 1.4 to 0.85 mM. There was a small drop in the sodium concentration from 2.87 to 0.70 mM. Thus rather less than 20% of the potassium uptake could be accounted for by an exchange with sodium. The remainder of the potassium uptake was not accounted for by the movements of other ions. Tests in similar experiments showed no change in acidity of the medium, indicating that potassium is not exchanged for H⁺ ions (see Conway & O'Malley, 1946). Nor was there a change in the overall concentrations of nitrogenous substances, which makes an exchange for amino acids unlikely.

Another experiment in which sodium glutamate was added as a substrate aerobically and anaerobically gave similar results (Table 10). Again the increase in the intracellular concentration of potassium was not accompanied by an uptake of chloride or by a loss of sodium. On the contrary sodium was taken up by the cells, presumably together with the glutamate added to the medium.

Exchange of potassium between cell and medium. In order to obtain information on the nature of the forces by which the concentration gradients of potassium between cells and medium are maintained ⁴²K was added to a suspension of cells in which a steady-state concentration gradient had been established. The specific activity of the potassium in cells and medium was measured after further incubation both aerobically and anaerobically. Data obtained from this kind of experiment indicate whether the intracellular potassium is firmly bound or in rapid equilibrium with K⁺ ions; ⁴²K₂CO₃ neutralized with HCl served as a source of ⁴²KCl. The quantity of ⁴²K added (0.43 mg. in 0.5 ml.) did not cause a major change in the potassium concentration of the medium. The main compartments of six conical Warburg vessels each contained 0.5 ml. of a suspension of washed cells in water (9.5 mg. dry wt.) and 3.5 ml. of a solution consisting of 0.4 ml. of M-sodium acetate, 0.1 ml. of 0.2M-sodium fumarate, 2 ml. of 0.2M-potassium chloride and 32.5 ml. of the growth medium (without glucose) described earlier. The side arms of two vessels contained 0.5 ml. of water and those of the four others 0.5 ml. of ⁴²KCl solution. One of each set contained oxygen as gas phase and 2N-NaOH solution in the centre well, the other nitrogen and yellow phosphorus.

Table 9. *Effect of sodium chloride on the distribution of potassium between cells and medium*

Each cup contained 0.5 ml. of cell suspension in water (17.6 mg. of dry cells); 1 ml. of 0.5M-triethanolamine buffer, pH 7.4; 0.1 ml. of 0.23% KCl; 0.1 ml. of 0.1M-sodium acetate; 0.1 ml. of 0.02M-sodium fumarate; 0.2 ml. of 1% Difco yeast extract; 1.5 ml. of water in 1 and 2 and 1.5 ml. of 0.90% NaCl in 3 and 4. The contents of 1 and 3 were used for the determination of potassium, of 2 and 4 for the determination of sodium. Incubation for 60 min. at 15° in O₂.

Cup no.	...	1	2	3	4
		No NaCl		NaCl added	
Supernatant		42.1 µg. of K/ml.	116 µg. of Na/ml.	28.3 µg. of K/ml.	1490 µg. of Na/ml.
Cells		645 µg. of K/ml.	~0 µg. of Na/ml.	1325 µg. of K/ml.	80.2 µg. of Na/ml.

Table 10. *Simultaneous determination of changes in the concentrations of potassium, sodium and chloride in cells incubated in the presence of glutamate*

Cell suspension (4 ml., 58.4 mg. of dry cells); 2 ml. of 0.0287% KCl; 0.16 ml. of 0.25M-sodium glutamate; 1.84 ml. of water. Incubation for 60 min. at 15°. Data 'before incubation' refer to cells suspended in 8 ml. of water (without KCl and glutamate); this suspension was analysed immediately after preparation. The data for the medium are given in order to indicate the magnitude of the concentration gradients between cells and medium.

Expt. no.	Gas phase	Ion	Medium		Cells	
			Before incubation (mM)	After incubation (mM)	Before incubation (mM)	After incubation (mM)
1	O ₂	K	0.17	1.26	3.79	10.4
		Cl	~0	1.06	~0	~0
		Na	0.20	4.91	2.41	9.14
2	N ₂	K	0.51	1.06	2.62	5.81
		Cl	~0	0.96	0.52	0.12
		Na	0.46	6.54	1.77	14.8

Table 11. *Distribution of ^{42}K between cells and medium*

For experimental conditions see text.						
Cup no.	1	2	3	4	5	6
Gas phase	O ₂	N ₂	O ₂	N ₂	O ₂	N ₂
Period of incubation (min.)	0	0	5	5	30	30
Weight of residue after removal of supernatant (mg.)	66.8	62.7	64.0	60.5	70.8	67.9
Volume of residue ($\mu\text{l.}$)	65.6	61.6	62.9	59.4	69.6	66.7
Volume of cells in residue ($\mu\text{l.}$)	39.2	36.6	41.2	35.7	44.8	37.9
Volume of supernatant in residue ($\mu\text{l.}$)	26.4	25.0	21.7	23.7	24.8	28.8
K in supernatant ($\mu\text{g./ml.}$)	422	431	484	488	488	511
K in total residue ($\mu\text{g.}$)	93	64	89	64	99	67
K in total supernatant of residue ($\mu\text{g.}$)	9.9	9.6	10.5	11.5	12.1	14.7
K in total cells of residue ($\mu\text{g.}$)	83.1	54.4	78.5	52.5	86.9	52.3
K in 1 ml. of cells ($\mu\text{g.}$)	2120	1485	1905	1470	1940	1380
Ratio: $\frac{\text{K in cells}}{\text{K in medium}}$	4.78	3.45	3.93	3.01	3.96	2.69
Counts in supernatant (counts/min./ml.)	—	—	716 450	797 300	776 350	823 200
Specific activity of K in supernatant (counts/min./ $\mu\text{g.}$ of K)	—	—	1 480	1 635	1 590	1 610
Counts in total residue (counts/min.)	—	—	95 385	75 385	151 494	102 712
Counts in supernatant of residue (counts/min.)	—	—	15 546	18 896	19 253	23 708
Counts in total cells of residue (counts/min.)	—	—	79 840	56 490	132 240	79 005
Counts in 1 ml. of cells (counts/min.)	—	—	1 938 000	1 582 000	2 950 500	2 083 000
Specific activity of K in cells (counts/min./ $\mu\text{g.}$ of K)	—	—	1 015	1 075	1 520	1 510
Ratio: $\frac{\text{specific activity of K in cells}}{\text{specific activity of K in medium}}$	—	—	68.4	65.8	95.6	93.8
O ₂ absorbed ($\mu\text{l./ml.}$ of cells)	—	—	—	—	1 250	—

The cups were first shaken at 30° for 20 min. to establish a steady state between medium and cells. The first pair were then removed for the determination of the potassium values in cells and medium before the addition of ^{42}K . In the others ^{42}K was added from the side arm at this stage and shaking was continued for 5 and 30 min. respectively. The results are shown in Table 11. The concentration gradient between cells and medium was of the order of 3–4 in favour of the cells, and was consistently higher aerobically than anaerobically. Both aerobically and anaerobically extracellular potassium exchanged rapidly with the intracellular potassium. The exchange was slightly more rapid in oxygen than in nitrogen. After 5 min. the specific activity of the intracellular potassium was 68.4% of the extracellular potassium in oxygen, and 65.8% in nitrogen. After 30 min. the corresponding figures were 96.5 and 93.8%. Thus almost all the intracellular potassium exchanged readily with the K⁺ ions of the medium.

Comparison of different strains of Alcaligenes faecalis. The experiments so far discussed were all carried out with strain 1347. Some experiments were repeated with two other strains (19 and 415) but no major differences were found. Cells of these strains also lose most of their intracellular potassium on washing with water and recover it on suspension in a potassium-containing medium.

DISCUSSION

The need for potassium in bacterial growth (Rahn, 1935), the role of potassium as a cofactor in enzyme systems (Lardy & Ziegler, 1945; Lwoff & Ionescu, 1947; Nossal, 1951), the accelerating effect of potassium on bacterial respiration (Quastel & Webley, 1942) and the accumulation of potassium by bacterial cells (Cowie, Roberts & Roberts, 1949; Roberts, Roberts & Cowie, 1949; Carroll, Danby, Eddy & Hinshelwood, 1950; Eddy, Carroll, Danby & Hinshelwood, 1951) are all well-established phenomena. The present experiments reveal an organism in which the intracellular potassium is much less firmly bound than in organisms which have formerly been studied, in that most of the intracellular potassium is removed from the cells by washing with distilled water. The fact that the cells can recover potassium added to the suspension fluid in the absence of energy sources shows that the cells contain a material which binds potassium. The 'bound' potassium, as indicated by experiments with ^{42}K , readily exchanges with the K⁺ ions of the medium. In this respect the potassium-binding material behaves like a colloid electrolyte or an ion-exchange resin.

Several authors have discussed the presence of potassium-binding cell constituents with reference to the uneven distribution of potassium between

cells and medium, but the previous search for potassium-binding substances has not proved successful (Roberts *et al.* 1949; Steinbach, 1950). The observations reported in this paper strongly support the view that *Alcaligenes faecalis* contains a potassium-binding constituent, but no statements can as yet be made about the nature of the potassium-binding reaction. Further work on disrupted cells aiming at the isolation and study of the potassium-binding material is required.

A potassium-binding material would account for the concentration gradients under anaerobic conditions and in the absence of oxidizable substrate. Superimposed upon that 'passive' transport of potassium is a mechanism of active transport which increases the concentration gradients in respiring cells.

SUMMARY

1. Cells of *Alcaligenes faecalis* lost most of their intracellular potassium when washed with distilled water. This treatment also removes most of the capacity to respire.

2. Respiration is restored by the addition of potassium (and an oxidizable substrate) to the suspending medium. The restoration is accompanied by an uptake of potassium against a concentration gradient.

3. The distribution of potassium between cells and medium was studied under a variety of conditions. Substantial amounts of potassium (up to 1.8 mg./ml. of cells) are accumulated in the cells against concentration gradients. Accumulation also occurs anaerobically in the absence of substrates but the final gradients reached are lower than in respiring cells.

4. Measurements of the simultaneous move-

ments of potassium, sodium and chloride show that the uptake of potassium is not accompanied by an equivalent movement of chloride or release of sodium. How electroneutrality is maintained when potassium ions move has not been established.

5. The distribution of radioactivity between cells and medium after addition of ^{42}K to the medium shows that the intracellular potassium exchanges readily with the K^+ ions of the medium. The exchange was slightly more rapid aerobically than anaerobically.

6. It is concluded that the organism contains a potassium-binding substance which behaves similarly to a colloid electrolyte or an ion-exchange resin.

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16 α -Hydroxyoestrone in the Urine of Pregnant Women

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Watson & Marrian (1955) detected the presence in extracts of the urine of pregnant women of a ketonic Kober chromogen which was more 'polar' than oestrone, and, on solvent-partition evidence, concluded that it was 16-oxo-oestradiol-17 β (16-oxo-oestra-1:3:5-triene-3:17 β -diol). More recently Marrian, Watson & Panattoni (1957) concentrated this Kober chromogen (KC-5) and showed conclusively that the major component in it must have been either 16 α -hydroxyoestrone (17-

oxo-oestra-1:3:5-triene-3:16 α -diol) or 16 β -hydroxyoestrone (17-oxo-oestra-1:3:5-triene-3:16 β -diol), and not 16-oxo-oestradiol-17 β . On biogenetic grounds the view was favoured that the major component in KC-5 was 16 α -hydroxyoestrone rather than 16 β -hydroxyoestrone, but since reduction with sodium borohydride yielded a mixture of oestriol (oestra-1:3:5-triene-3:16 α :17 β -triol) and 16-epioestriol (oestra-1:3:5-triene-3:16 β :17 β -triol) it was suggested that either 16 β -hydroxyoestrone or arti-