Alkali Metal Ions in Relation to the Growth of *Escherichia coli*: a Comparison with *Staphylococcus aureus*

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SUMMARY

The effects of alkali metal ions on the growth of *Escherichia coli* have been compared with those for *Staphylococcus aureus* (*pyogenes*). Only rubidium could substantially support the growth of both organisms. The efficiency of rubidium as a growth promoter varied with both the potassium and rubidium content of the medium. The uptake of potassium and rubidium during the growth of *E. coli* was examined using radio-isotopes as tracers, and the velocities of uptake, the accumulation and internal concentrations of these cations were calculated. Potassium was taken up rapidly early in growth and this uptake was inhibited by rubidium, which itself was taken up, and in greater amounts than potassium. *Escherichia coli* differed from *S. aureus* in that the former readily leaked both cations when growth slowed down. Rubidium leaked out early in growth only to be taken up again. The two ions appeared to compete for influx and efflux.

INTRODUCTION

Bacteria require potassium for growth and rubidium can usually replace this requirement (Rothstein, 1959). The interactions of the alkali metal ions lithium, potassium, rubidium and caesium on *Staphylococcus aureus* (*pyogenes*) have been examined (Wyatt, 1963*a*) and found to be more complex than those reported for other organisms (e.g. Eddy & Hinshelwood, 1950). In this report we have examined *Escherichia coli* under similar experimental conditions, to see whether it resembles staphylococci.

Wyatt (1963*b*) showed that the uptake of potassium and rubidium during growth of the Gram-positive *Staphylococcus aureus* differed markedly from that of Gram-negative organisms such as *Alcaligenes faecalis* (Krebs, Whittam & Hems, 1957), *Escherichia coli* (Cowie, Roberts & Roberts, 1949) and fungi such as yeast (Conway & Duggan, 1958; Rothstein, 1959) and *Neurospora crassa* (Lester & Hechter, 1958). These differences might have been due to basic differences between Gram-positive and Gram-negative organisms, to different nutritional requirements, or to different experimental conditions and techniques. We have therefore examined the uptake of potassium and rubidium by *E. coli* under experimental conditions similar to those used for *S. aureus*. In the experiments reported here growth was limited by the concentration of potassium present and the potassium and rubidium concentrations were measured using the radio-isotopes ^{42}K and ^{86}Rb , respectively.

METHODS

The general methods and materials were similar to those of Wyatt (1963*a, b*). A medium containing 1 % Lab-lemco, 1 % peptone (Oxoid) and 0.15 % glucose made deficient in cations was used. Cations were removed by passing a 5 % Lab-lemco + 5 % peptone solution down a column of IRC-50 (Na⁺) and a 3 % solution of glucose down IR-120(H⁺). 'Specpure' salts, MgSO₄·7H₂O, CaCl₂, and FeSO₄·7H₂O were added to the medium which had a Na⁺ content of 50 mM.

Sterile ⁴²K and ⁸⁶Rb chlorides were obtained from the Radiochemical Centre, Amer-sham. Suitable dilutions of ⁴²KCl were made and the molarity determined by flame photometry (Wyatt, 1963*b*). The experimental tubes contained 23 μM-K⁺ of which 3 μM was residual K⁺ and the rest ⁴²K + carrier. Rubidium was added at two concentrations, 0.2 and 2.0 mM, of which only 0.2 μM was ⁸⁶Rb plus carrier and the rest 'Specpure' RbCl.

As it is difficult to measure ⁴²K and ⁸⁶Rb together duplicate series of tubes were used containing either ⁴²K or ⁸⁶Rb, but the same total amounts of K⁺ and of Rb⁺. The extent and rate of growth were almost identical in the duplicate series. After incubation, 5 ml. portions were removed, cooled to 0° and centrifuged at 1000*g* for 20 min. Samples (4 ml.) of the supernatant fluid were then diluted to 10 ml. for counting in a liquid GM counter, type M 6 H. Counting times gave a statistical accuracy of better than 1 % for ⁸⁶Rb and 3 % or better for ⁴²K, except for three samples with extremely low counts. The activities were corrected for decay (⁴²K) and for lost counts (⁸⁶Rb). A Spekker reading of 0.1 (with 1 cm. cells and H 508 filter) corresponded to a dry weight of 97 ± 7 mg./l. and gave 77 × 10⁶ colonies per ml. suspension. Accumulations (internal concentration/external concentration) were calculated using the value of 3.2 ml. per g. dry wt., i.e. 4 ml./g. dry wt. less 26 % inter-space volume using the data of Roberts (1959) and Roberts *et al.* (1955).

Escherichia coli NCTC 8007 was used throughout.

RESULTS

Cation requirements for growth

The broth with 3 μM residual K⁺ did not support the growth of either *Escherichia coli* or *Staphylococcus aureus*. We found no evidence of an arithmetic response by *E. coli* in low K⁺ broth (see Roberts & Roberts, 1950). Growth of *E. coli* was proportional to the logarithm of the K⁺ concentration up to about 0.1 mM-K⁺ and addition of Rb⁺ at different K⁺ levels increased the cell yield (Fig. 1). The growth due to the K⁺ in the broth was subtracted from the total growth, i.e. that due to K⁺ plus Rb⁺, and the remaining growth was assumed to be due to Rb⁺. From the K⁺ growth response curve (see for example Fig. 1 of Wyatt, 1963*a*) the additional K⁺ concentration required to give the same growth as that due to Rb⁺ was calculated. A series of Rb⁺-K⁺ equivalent curves similar to that shown by Wyatt (1963*a*, Fig. 3) was obtained. For Rb⁺ concentrations up to 0.2 mM the relationship was $[Rb^+] = m[K^+]^n$, where $[K^+]$ is the equivalent concentration of K⁺ and m and n are constants. From the graphs the calculated constants were $\log[Rb^+] = 1.43\log[K^+] + 10.3 - 0.2[K^+]*$ (*E. coli*). This is similar to the expression for *Staphylococcus aureus*:

$$\log[Rb^+] = 1.25\log[K^+] + 1.36 + 0.5[K^+]*$$

(Wyatt, 1963*a*), where $[K^+]*$ is the concentration of K⁺ in the medium.

The efficiency of Rb^+ as a growth promoter decreased with increasing Rb^+ concentration, but at low Rb^+ concentrations this efficiency was increased by raising the K^+ content of the broth (Fig. 2).

The addition of from 1 to 10 mM- Li^+ did not alter the cell mass obtained either with or without K^+ present, but Cs^+ gave increased growth with all levels of K^+ used—the efficiency of Cs^+ relative to K^+ varied from 0.2 to 5.0% (over a 1–10 mM- Cs^+ range). As with Rb^+ , the efficiency of Cs^+ increased as the K^+ concentration was raised.

Fig. 1

Fig. 2

Fig. 1. Effect of rubidium on the growth of *Escherichia coli*. $\times$, 0.003 mM- K^+ ; $\circ$, 0.013 mM- K^+ ; $\bullet$, 0.023 mM- K^+ . All tubes contained 0.2 mM- Mg^{2+} and 0.1 mM of both Ca^{2+} and Fe^{3+} . Growth was measured after incubation for 24 hr at 37°.

Fig. 2. Efficiency of rubidium as a growth promoter for *Escherichia coli*. For details see Fig. 1 and text.

The uptake and retention of potassium

There appeared to be logarithmic uptake of K^+ during the logarithmic period of growth (Fig. 3). From the plotted growth curves and the cumulative K^+ uptake the K^+ accumulation (Fig. 4) and rates of uptake were calculated. The rate of K^+ uptake decreased with decreasing extra-cellular K^+ (at 17.3 and 1.6 μM - K^+ the rates of K^+ uptake were 22.5 and 1.3 $\mu moles/100$ mg. dry-wt/hr respectively). As the rates of uptake decreased during growth so the K^+ concentration per cell also decreased (Table 5). The cells leaked K^+ when growth ceased (Figs. 3, 4).

The effect of rubidium on growth

With added K^+ there was logarithmic growth for about 11 divisions with a mean generation time (M.G.T.) of 26 min. whether or not Rb^+ was added. When Rb^+ was added there then followed a further short logarithmic phase of increased M.G.T. With low K^+ (3 μM) no growth occurred unless Rb^+ was present and again the M.G.T. increased to 77 min. after 11 divisions.

The effect of rubidium on the uptake and leakage of potassium

The concentration of K^+ in the culture fluid was reduced to $1.6 \mu M$ from $23 \mu M$ in 7 hr in the absence of Rb^+ , but when Rb^+ was present the rate of K^+ uptake was less and so also was the K^+ concentration per unit cell wt. When the initial $Rb^+ : K^+$ ratio of the medium was increased by a factor of 10 to 88:1 there was no uptake of K^+ for the first 5 hr. Although the rate of K^+ uptake was less, the maximum K^+ uptake from the medium was greater, but as expected the time taken was longer (Table 1); although the K^+ concentration per cell was lower at this stage, the accumulation was greater (Fig. 4). Greater $Rb : K$ ratios did not reduce the maximum K^+ uptake.

Fig. 3. Uptake of potassium during growth by *Escherichia coli* and *Staphylococcus aureus*. (*E. coli*: with 0.023 mM-K^+ , 0.2 mM-Mg^{2+} and 0.1 mM of both Ca^{2+} and Fe^{2+} ; $\times$, growth; $\bullet$, K^+ uptake. *S. aureus* (data taken from Wyatt, 1963b): with 0.021 mM-K^+ , 1 mM-Mg^{2+} , 2.5 mM arginine and 0.1 mM of both Ca^{2+} and Fe^{2+} ; $+$, growth; $\circ$, K^+ uptake.

When the growth rate decreased there was a substantial net gain of K^+ by the medium but in the presence of Rb^+ this efflux was greatly reduced (Table 2) and consequently the final K^+ content per cell and the accumulation were greater (Table 3).

The uptake of rubidium

More Rb^+ than K^+ was taken up but this was due to the higher ratio of $Rb^+ : K^+$ external concentrations. The maximum Rb^+ uptake was affected by the external concentrations of both K^+ and Rb^+ (Table 4). Initially Rb^+ was taken up more rapidly than K^+ . With 0.2 mM-Rb^+ and 0.023 mM-K^+ the velocities of Rb^+ and K^+ uptake were 105 and $35 \mu\text{moles}/100 \text{ mg. dry wt.}/\text{hr}$ respectively during the first 5 hr; with 2 mM-Rb^+ the corresponding values were 460 and 0 respectively. The pattern of Rb^+ uptake was more complicated than that shown by staphylococci (Wyatt, 1963b). In the four series studied, after the initial uptake between 0 and 5 hr, the cells lost some Rb^+ . More Rb^+ was then taken up until, near the end of growth, it leaked out together with K^+ . With low K^+ , the Rb^+ concentration per cell wt dropped suddenly near the end of growth but with higher K^+ this was not so. This suggests that K^+ inside the cell was reducing the efflux of Rb^+ just as Rb^+ affected K^+ efflux.

The net rates of uptake of potassium and rubidium

When 0.023 mM- K^+ and 0.2 mM- Rb^+ were present together, the initial velocities of K^+ and Rb^+ uptake were lower than when either was present alone. The rate of K^+ uptake remained constant but that of Rb^+ fell. The ratio of the concentrations of Rb^+ to K^+ in the supernatant ($[S]_{Rb}:[S]_K$) increased until at 40:1 the rates of uptake of K^+ (decreasing) and Rb^+ (increasing) were equal. The ratio continued to increase until

Fig. 4. Accumulation of K^+ and Rb^+ by *Escherichia coli*. For calculation of accumulations see Methods. All tubes contained 0.2 mM- Mg^{2+} and 0.1 mM of both Ca^{2+} and Fe^{2+} . Accumulation of K^+ (0.023 mM- K^+): $\circ$, no Rb^+ added; $\bullet$, 0.2 mM- Rb^+ ; Δ , 2.0 mM- Rb^+ . Accumulation of Rb^+ (0.2 mM- Rb^+): $\times$, 0.003 mM- K^+ ; $\odot$, 0.023 mM- K^+ . (c) Accumulation of Rb^+ (2.0 mM- Rb^+): $+$, 0.003 mM- K^+ ; $\blacktriangle$, 0.023 mM- K^+ .

at 1000:1 K^+ and Rb^+ leaked out. When Rb^+ was increased to 2.0 mM, K^+ uptake was completely inhibited for the first 5 hr during which Rb^+ uptake was rapid. When the external $Rb:K$ ratio was 84:1, K^+ uptake began, and Rb^+ leaked out. At an $[S]_{Rb}:[S]_K$ of 85:1 the rates of K^+ and Rb^+ uptake were equal and almost zero. As with 0.2 mM- Rb^+ the supernatant $Rb^+:K^+$ ratio increased until at 100:1 the rates of uptake were once again equal. Finally K^+ was lost from the cell at a rate approximately one-fifth that of Rb^+ .

Table 1. *Effect of rubidium on the maximum uptake of potassium during growth of Escherichia coli*

	Rb ⁺ present (mm)*		
	0	0.2	2.0
Maximum K ⁺ uptake (μ M) and percentage K ⁺ removed from medium	21.4 (93 %)	22.9 (99.5 %)	22.8 (99.1 %)
Time of maximum uptake (hr)	7	10	10
μ moles K ⁺ /100 mg. dry wt at time of maximum uptake	13.3	4.8	4.8

* All tubes contained 23 μ M-K⁺, 0.2 mm-Mg²⁺ and 0.1 mm of both Ca²⁺ and Fe²⁺.

Table 2. *Net efflux of potassium and rubidium in Escherichia coli*

K ⁺ concn. (mm)	Rb ⁺ present (mm)		
	0	0.2	
	V_K	V_K	V_{Rb}
0.003	—	—	0.7
0.023	1.7	0.12	0.2

Growth medium contained 0.2 mm-Mg²⁺ and 0.1 mm of both Ca²⁺ and Fe²⁺. Velocities of efflux (V_K and V_{Rb}) were measured at 13 hr and are expressed as μ moles/100 mg. dry wt./hr.

Table 3. *Residual potassium after uptake has ceased*

	<i>Staphylococcus aureus</i> (<i>pyogenes</i>) (Wyatt, 1963b)		<i>Escherichia coli</i>	
	Rb ⁺ present (mm)		Rb ⁺ present (mm)	
Time (hr)...	0	0.2	0	0.2
Mean counts/min. ⁴² K, corrected	14 to 24	14 to 24	8½ to 10	9 to 11
	149 ± 8.9	96 ± 3.4	269 ± 30.5	9 ± 6.8
Standard error of means	4.8		16.4	
Difference of means	53, $P < 0.001$		260, $P < 0.001$	
Accumulation	72,000	101,000	8,000	119,000

Media contained: (a) *S. aureus*: 0.021 mm-K⁺, 1.0 mm-Mg²⁺, and 0.1 mm of both Ca²⁺ and Fe²⁺; (b) *E. coli*: 0.023 mm-K⁺, 0.2 mm-Mg²⁺ and 0.1 mm of both Ca²⁺ and Fe²⁺.

Table 4. *Effect of initial potassium and rubidium concentrations on rubidium uptake during growth of Escherichia coli*

Rb ⁺ present (μ M)	Maximum Rb ⁺ uptake (μ M) with K ⁺ (mm)	
	0.003	0.023
200	25	56
2000	134	155

Growth media contained 0.2 mm-Mg²⁺ and 0.1 mm of both Ca²⁺ and Fe²⁺. Maximum Rb⁺ uptake expressed as the change in supernatant concentration.

Potassium and rubidium contents of Escherichia coli during growth

The potassium and rubidium contents of *Escherichia coli* were measured during growth in the presence of K^+ alone and in the presence of K^+ and Rb^+ (Table 5). Using the values for the efficiency of Rb^+ (in terms of growth, Fig. 2) the corresponding K^+ equivalents of the Rb^+ present in the cell were calculated and added to the existing K^+ content of the cell. The results expressed as K^+ uptake were fairly uniform for the different conditions of growth (Table 5).

Table 5. *Comparison of potassium uptake with potassium and rubidium uptake (expressed as potassium equivalents) during growth of Escherichia coli*

Time (hr)	Rb ⁺ present (mM)		
	0	0.2	2.0
	K ⁺ uptake (μ moles/100 mg. dry-wt)	K ⁺ uptake + K ⁺ equivalents of Rb ⁺ uptake (Rb ⁺ efficiency 53.4 %)	K ⁺ uptake + K ⁺ equivalents of uptake (Rb ⁺ efficiency 6.1 %)
6	30.4	28.5*	14.1
7	13.3	14.0	11.3
8	8.8	10.0	8.3
9	7.7	10.5	6.7
10	7.3	10.0	6.5
11	6.8	10.5	6.4
12	6.4	5.5	6.3

Efficiencies calculated using Fig. 2; 0.023 mM- K^+ present.

* Example: K^+ uptake was 18.50 μ moles/100 mg. dry-wt

Rb⁺ uptake was 18.80 μ moles/100 mg. dry-wt $\equiv \frac{18.8 \times 53.4}{100} \equiv 10.02$ μ moles K^+ /100 mg. dry-wt

K^+ uptake + K^+ equivalent of Rb⁺ uptake = 18.50 + 10.02 $\equiv$ 28.52.

DISCUSSION

The efficiency of the alkali metal ions (excluding sodium) in promoting the growth of *Escherichia coli* and *Staphylococcus aureus* are potassium > rubidium $\gg$ caesium $\gg$ lithium. These results are consistent with those expected from enzyme studies; both rubidium and caesium are commonly alternative activators of potassium-requiring enzymes while lithium is not (Dixon & Webb, 1964). Rubidium competes with potassium for entry but caesium competition cannot be detected: it would be expected that caesium would, however, compete with rubidium for entry and so cause some inhibition of growth (Wright & Wyatt, in preparation). The interactions of potassium and rubidium are complicated as they represent the sum of the effects due to their mutually competitive inhibition of entry (Wyatt, 1963*b*; Wright & Wyatt, in preparation), their probable competition for exit (Wyatt, 1963*b*), their efficiency as enzyme activators and their relative stability constants for each chelate within the cell (Wyatt, 1964). Nevertheless the response of both staphylococcus and *E. coli* to alkali metal ions is very similar. Just as the growth responses and alkali ion requirements of *E. coli* and *S. aureus* are similar, so the uptakes of these ions (potassium and rubidium) have many

features in common. Potassium and rubidium were accumulated by both organisms during growth but while *S. aureus* appeared to have a tight membrane with respect to potassium, *E. coli* lost much of its accumulated potassium when growth slowed down: *S. aureus* maintained a 50,000-fold accumulation, but in *E. coli* the accumulation rapidly dropped to 200-fold.

In both organisms rubidium delayed the uptake of potassium and also reduced the rate of uptake, probably by a competitive inhibitory mechanism (Wright & Wyatt, 1965). Potassium similarly inhibited the entry of rubidium and probably also inhibited its efflux. The amount of potassium in the cell was lower when rubidium was present, and the presence of rubidium also brought about a decrease in the leakage of potassium from both organisms. In the presence of rubidium both organisms showed the same diphasic logarithmic growth, though this was more pronounced in *Staphylococcus aureus*. In conclusion, these results show that, contrary to previous data, *S. aureus* and *E. coli* behave similarly in their requirements for and their uptake of alkali metal ions, when tested under similar conditions.

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