The Influence of NaCl Concentration of the Medium on the Potassium Content of *Aerobacter aerogenes* and on the Inter-relationships between Potassium, Magnesium and Ribonucleic Acid in the Growing Bacteria

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SUMMARY

Irrespective of the nature of the growth-limiting substance, the potassium content of *Aerobacter aerogenes* (growing in a chemostat at a fixed dilution rate) increased progressively from about 1.3 % to 4.2 % of the bacterial dry weight with changes in culture NaCl concentration from 0 to 40 g./l. Similarly, the potassium content of glycerol-limited *A. aerogenes* organisms, growing in a low ionic strength medium, varied with growth rate (from 0.9 % to 1.6 % of the bacterial dry weight with changes in dilution rate from 0.05 to 0.7 hr⁻¹); but this was not related to the small changes in culture NaCl content that occurred simultaneously. These observations are discussed with reference to conflicting views on the particular role of potassium in growing bacteria that accounts for its presence intracellularly in high concentration.

INTRODUCTION

Potassium is present in growing bacteria in concentrations that are substantially higher than those of other cations (Rouf, 1964; Tempest & Dicks, 1967; Tempest, Dicks & Ellwood, 1968) but the reason for a high intracellular potassium requirement has not been established unequivocally. Since the potassium concentration in growing bacteria usually is greater than 0.1 M (see the above references and Christian & Waltho, 1964), it seems unlikely to be required solely for purposes of enzyme activation. Dicks & Tempest (1966) concluded that the potassium content of *Aerobacter aerogenes* was determined largely by the bacterial RNA content and probably was required for maintenance of a functional ribosomal configuration, whereas Epstein & Schultz (1965) concluded that the bulk of the intracellular potassium was needed for osmoregulation since the potassium content of *Escherichia coli* varied with medium osmolarity. However, Epstein & Schultz did not equalize the growth rates of organisms in cultures of different salinity so that consequent differences in bacterial RNA and ribosome contents may have contributed to the observed differences in bacterial potassium content. On the other hand, the experiments reported by Dicks & Tempest (1966) took no account of the total salts concentration of the medium which was not controlled and probably varied; therefore, the parallel changes in cellular potassium and RNA contents that were observed when the growth rate was varied may have been fortuitous. To resolve these doubts it was necessary to establish whether the NaCl

concentration of the medium had an effect on the potassium content of bacteria growing at a fixed rate (in a chemostat) and, if so, whether changes in total salts concentration could have accounted for the observed changes in bacterial potassium content associated with changes in the growth rate.

METHODS

Organism. *Aerobacter aerogenes* NCTC 418 was maintained and subcultured monthly on tryptic meat-digest agar slopes.

Growth conditions. The organisms were grown in 0.5 l. Porton-type chemostats with automatic pH control (Herbert, Phipps & Tempest, 1965) in low ionic strength media of the following basal composition: Na_2HPO_4 , 0.5 mM; $\text{NH}_4\text{H}_2\text{PO}_4$, 4.5 mM; $(\text{NH}_4)_2\text{SO}_4$, 2.5 mM; citric acid, 0.5 mM; MgCl_2 , 0.75 mM; K_2SO_4 , 3 mM and trace amounts of Fe^{3+} , Ca^{2+} , Mn^{2+} , Zn^{2+} , Cu^{2+} , Co^{2+} , Na_2MoO_4 . For carbon-limitation, glycerol was added to a final concentration of 10 g./l.; for K^+ -limitation, the glycerol concentration was increased to 30 g./l. and the K_2SO_4 concentration lowered to 0.5 mM; for Mg^{2+} -limitation, the glycerol concentration was 30 g./l. and the MgCl_2 concentration lowered to 0.25 mM. Each of these media contained barely sufficient NH_4^+ to provide the bacterial nitrogen requirement, but excess nitrogen was made available by using an ammonia solution (about 2 M) as the titrant for pH control. NaCl was added to the above 'low ionic strength' media as indicated in the Results section; however, some NaCl always was present in the culture since the reference electrode was connected to the culture through a 'bridge' tube containing a saturated NaCl solution. The flow rate of saturated NaCl into the growth vessel never exceeded 3 ml./day so that its concentration in the culture was insignificant at all but the lowest dilution rates (Table 2).

Analytical procedure. Culture bacterial dry weights and bacterial potassium, magnesium, phosphorus and RNA contents were determined by previously described methods (Tempest, Hunter & Sykes, 1965; Tempest, Dicks & Hunter, 1966).

RESULTS

When bacteria are limited in their growth by the availability of a particular element they may be expected to contain the minimum concentration of that element necessary to satisfy the structural and metabolic needs for growth at the imposed rate. Therefore, by growing organisms at a fixed rate in a series of K^+ -limited environments, each containing graded amounts of NaCl, it should be possible to establish unequivocally whether there is a dependence of bacterial potassium content on NaCl concentration. Since in a K^+ -limited culture nearly all the potassium is contained within the organisms, little being present in the extracellular fluid, any change in bacterial potassium content must produce a change in the steady-state concentration of bacteria in the culture. Clearly this occurred with K^+ -limited chemostat cultures of *Aerobacter aerogenes* (growing at a fixed rate) when the medium NaCl concentration was increased progressively from almost zero to 40 g. NaCl/l. (Table 1). Analysis of the organisms separated from each culture confirmed that the steady-state bacterial potassium content had increased with NaCl concentration, but the bacterial magnesium, phosphorus and RNA contents did not vary proportionately (Table 1). Thus, whereas the

ratios of cell-bound magnesium:RNA were maintained constant the potassium:RNA ratios increased progressively with medium NaCl concentration (Fig. 1). Although the potassium content of K^+ -limited *A. aerogenes* varied with medium NaCl concentration, the presence of 20 g. NaCl/l. in the growth environment did not annul the effect of growth rate on the bacterial potassium, magnesium, RNA and phosphate contents; each changed proportionately when the dilution rate was lowered from 0.3 to 0.1 hr^{-1} (Table 1). At the lower growth rate the concentration of potassium in *A. aerogenes* organisms was less than one-half that in organisms growing at the

Fig. 1. Steady-state ratios of potassium/RNA (circles) and magnesium/RNA (triangles) in chemostat cultures of *Aerobacter aerogenes* which were K^+ -limited (blocked symbols) and glycerol-limited (open symbols) and in which the medium NaCl content was progressively increased.

higher rate, even though 20 g. NaCl/l. was present in the culture throughout. Thus, changing the growth rate modified the bacterial requirement for potassium and, presumably, altered the osmotic differential between the extracellular and intracellular environments.

With chemostat cultures of *Aerobacter aerogenes* in which K^+ was present in excess of requirement and growth limited by the availability of another component of the medium, changes in the culture NaCl content again caused changes in bacterial potassium content (Table 1) similar to those detailed above. When the carbon and energy source (glycerol) limited growth, the steady-state culture bacterial concentration again varied with medium NaCl content indicating either a decreased efficiency of glycerol metabolism or, more likely, an increased maintenance energy requirement with the presence of NaCl in the culture. With Mg^{2+} -limited cultures of *A. aerogenes* addition of more than 10 g. NaCl/l. to the medium affected magnesium assimilation;

when the NaCl concentration was increased to 40 g./l. steady-state growth conditions no longer could be maintained.

The fact that medium NaCl content did affect the potassium content of bacteria growing in a chemostat raised the question of whether the changes in bacterial potassium content with dilution rate, reported by Tempest *et al.* (1966), resulted from associated changes in culture salts content rather than accompanying changes in bacterial RNA content. This possibility was difficult to check with cultures in which the carbon source was present in excess of requirement, since excreted metabolic end-products (e.g. acetate) accumulated in the environment and added to the osmolarity of the culture extracellular fluids. With glycerol-limited cultures of *Aerobacter aerogenes* no significant

Table 1. *The effect of NaCl on the magnesium, potassium, phosphorus and RNA contents of Aerobacter aerogenes, growing in a chemostat (35°, pH 6.5)*

The figures quoted in this table are average values from at least two samples collected, processed and analysed on separate days.

Diln. rate (hr ⁻¹)	NaCl concn. (g./l.)	Dry wt of organisms (g./l.)	g./100 g. dry bacteria			
			Magnesium	Potassium	Phosphorus	RNA
Potassium-limited organisms						
0.3	Nil	3.09	0.194	1.30	2.14	15.5
0.3	10	2.13	0.202	1.88	2.35	—
0.3	20	1.62	0.210	2.49	2.44	15.2
0.3	40	0.82	0.225	4.20	2.50	15.7
0.1	20	2.54	0.124	1.52	1.50	9.5
Glycerol-limited organisms						
0.3	Nil	5.16	0.203	1.30	2.37	13.3
0.3	10	4.57	0.204	2.14	2.34	13.0
0.3	20	4.20	0.200	3.28	2.33	13.3
0.3	40	3.08	0.208	4.15	2.68	15.3
Magnesium-limited organisms						
0.3	Nil	2.96	0.196	1.35	2.13	—
0.3	10	2.94	0.194	2.38	2.01	—
0.3	20	2.32	0.205	3.25	2.20	—
0.3	40	Steady state not attained				

accumulation of extracellular metabolic end-products could be detected at growth rates less than 0.8 hr⁻¹ (35°, pH 6.5) and because the glycerol concentration in the cultures also was extremely low, the medium osmolarity could be assessed approximately from the weight of total salts present in the extracellular fluids after drying (104°, 16 hr.) accurately measured volumes (usually 10.0 ml.). The results of an experiment in which the dilution rate was increased progressively from 0.05 to 0.7 hr⁻¹ are shown in Table 2. Clearly, the extracellular salt concentration varied with dilution rate, but the cellular potassium content varied differently. [As the dilution rate was raised progressively the bacterial potassium content increased, but this obviously was not due to changes in culture salinity which actually *decreased*. As reported previously with K⁺-, Mg²⁺- and PO₄³⁻-limited cultures of *A. aerogenes* (Tempest *et al.* 1966; Dicks & Tempest, 1966), the bacterial magnesium, phosphorus and RNA contents of glycerol-limited *A. aerogenes* varied with dilution rate in a manner that paralleled the changes

in bacterial potassium content, and the molar proportions of magnesium:potassium:RNA:phosphorus were maintained constant at about 1:4:5:9 (Table 2).

Table 2. *The effect of dilution rate on the magnesium, potassium, phosphorus and ribonucleic acid contents of Aerobacter aerogenes, growing in a chemostat (35°, pH 6.5)*

The figures quoted in this table are average values from at least two samples that were collected and analysed on separate days. The weights of total salts in the extracellular fluids were obtained by drying known volumes of culture that had been freed from bacteria by centrifugation (3000 g, 10 min.)

Diln. rate (hr ⁻¹)	Extra- cellular salt concn. (g./l.)	Dry wt of organisms (g./l.)	g./100 g. dry bacteria				Molar ratios		
			Mag- nesium	Potas- sium	Phos- phate	RNA	K/Mg.	P/Mg	RNA/Mg
0.05	3.1	4.90	0.124	0.92	1.50	7.9	4.5	9.4	4.5
0.1	2.0	5.00	0.168	1.07	1.95	10.9	3.9	9.0	4.6
0.3	1.4	5.15	0.206	1.37	2.43	14.3	4.1	9.1	4.9
0.7	1.0	5.05	0.260	1.62	2.90	17.2	3.9	8.6	4.7

DISCUSSION

The experiments reported in this paper show that both the rate at which *Aerobacter aerogenes* organisms were grown and the NaCl content of the medium in which they were grown influenced markedly their intracellular potassium content. But whether these two effects were related in a common cellular function (e.g. maintenance of ribosomal activity, osmoregulation, stimulation of respiration, etc.) cannot be decided from the available evidence. It seems unlikely that all the potassium contained in organisms growing in the presence of, say, 40 g. NaCl/l. was associated with the ribosomes, since the ratio of potassium to RNA-phosphate would then have been greater than 2, whereas it is likely always to be less than 1 since the net charge on the RNA-phosphate is unity. On the other hand, if the potassium content of *A. aerogenes* organisms was totally unrelated to their RNA content, then it is difficult to understand why both varied with growth rate (Table 2) and growth temperature (Dicks & Tempest, 1966) in a parallel manner and quite independent of small changes in culture osmolarity.

Bacterial respiration rate has been shown to vary with growth rate (Herbert, 1958; Tempest, *et al.* 1965) and possibly this could have accounted for the growth-rate-linked variation in potassium content if the two were related (as suggested by Eddy & Hinshelwood, 1951). In support of this hypothesis was the observation that addition of 20 g. NaCl/l. to the medium in which either glycerol-limited or K⁺-limited *A. aerogenes* was being grown caused the steady-state respiration rate to be increased by about 25 %. However, both glycerol-limited and K⁺-limited *A. aerogenes* organisms contained the same amount of potassium when grown at identical rates in media of low ionic strength (Table 1), yet their steady-state respiration rates were markedly different (e.g. Q_{O_2} -values of 114 *versus* 383 when grown at a dilution rate of 0.3 hr⁻¹, 35° pH 6.5, in glycerol-limited and K⁺-limited environments, respectively). Hence there can be no simple quantitative relationship between bacterial potassium content and respiration rate.

The simplest hypothesis that can be proposed to accommodate the effects of both

growth rate and medium NaCl content on the potassium content of bacteria assumes that they are not related to a single cellular function. Thus, we propose that the potassium content of an organism basically is prescribed by its intracellular polyanion content (largely RNA in the case of the above *A. aerogenes* organisms) and this concentration of potassium, together with that of other low molecular weight compounds, is sufficient to provide an adequate internal hydrostatic pressure when the organisms are suspended in environments of moderately low ionic strength (i.e. water activity > 0.99). However, when exposed to environments of water activity ≤ 0.99 , plasmolysis can be prevented only by a further uptake of potassium (plus an equal amount of low molecular weight anion, presumably), and this occurs. But since there is no corresponding change in the magnesium content of the organisms or, more particularly, in the RNA:Mg²⁺ ratio (Fig. 1), this 'extra' potassium must be compartmented in a way such that it does not affect the structural configuration and functioning of the ribosomes.

Although the magnesium content of *Aerobacter aerogenes* was unaffected by the medium NaCl content (Table 1), addition of NaCl to the growth environment affected the efficiency of magnesium uptake. Thus, steady-state conditions could not be obtained with Mg²⁺-limited chemostat cultures of *A. aerogenes* containing 40 g. NaCl/l., and even in the presence of 20 g. NaCl/l. less than 80% of the available magnesium was taken up by the organisms. Sodium can displace magnesium that is adsorbed on the surface of bacteria (Strange & Shon, 1964; Tempest & Strange, 1966) and the adsorption process may be a necessary stage in the assimilation of magnesium by bacteria (Tempest, Dicks & Meers, 1967). Hence, NaCl toxicity in general may be due, at least in part, to interference in the uptake of magnesium by the growing organisms.

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