Biochimica et Biophysica Acta, 554 (1979) 156—167

BBA 78387

ACTIVE K^+ TRANSPORT IN *MYCOPLASMA MYCOIDES* VAR. CAPRI

NET AND UNIDIRECTIONAL K^+ MOVEMENTS

G. LEBLANC * and C. LE GRIMELLEC **

Department of Biology, C.E.N. Saclay, Gif-sur-Yvette 91190 (France)

(Received January 5th, 1979)

Key words: Cell cation composition; K^+ transport; K^+-K^+ exchange; Dicyclohexylcarbodiimide; (Mycoplasma)

Summary

Analysis of the cation composition of growing *Mycoplasma mycoides* var. Capri indicates that these organisms have a high intracellular K^+ concentration (K_i: 200—300 mM) which greatly exceeds that of the growth medium, and a low Na^+ concentration (Na_i^+: 20 mM). Unlike Na_i^+, K_i^+ varies with cell aging.

The K^+ transport properties studied in washed organisms resuspended in buffered saline solution show that cells maintain a steady and large K^+ concentration gradient across their membrane at the expense of metabolic energy mainly derived from glycolysis. In starved cells, K_i^+ decreases and is partially compensated by a gain in Na^+. This substitution completely reverses when metabolic substrate is added (K^+ reaccumulation process). Kinetic analysis of K^+ movement in cells with steady K^+ level shows that most of K^+ influx is mediated by an autologous K^+-K^+ exchange mechanism. On the other hand, during K^+ reaccumulation by K^+-depleted cells, a different mechanism (a K^+ uptake mechanism) with higher transport capacity and affinity drives the net K^+ influx. Both mechanisms are energy-dependent.

Ouabain and anoxia have no effect on K^+ transport mechanisms; in contrast, both processes are completely blocked by dicyclohexylcarbodiimide, an inhibitor of the Mg^{2+}-dependent ATPase activity.

Abbreviation: DCCD, dicyclohexylcarbodiimide.

* To whom correspondence should be addressed.

** Present address: Department of Medicine, Research Center, Maisonneuve-Rosemont Hospital, Montreal H1T 2M4, PQ, Canada.

Introduction

It has already been stressed [1,2] that the absence of a cell wall combined with the possibility of controlling cholesterol content and phospholipid acyl moiety in mycoplasma membranes, make these organisms particularly suitable for studying the physical properties and functions of biological membranes and their interrelationship. This advantage should, in particular, render possible the examination of the relationship between cation transport systems and membrane lipid composition which are less abundantly treated in literature in comparison to the relationships governing the transport of organic solutes [3,4].

The *Mycoplasma mycoides* var. Capri strain (PG3) seems especially well suited to such studies. Thus, Rottem et al. [5] reported that this strain, which requires cholesterol for optimal growth (native strain), could be adapted to grow in media in which the amount of added sterol is gradually reduced. Because these organisms are unable to synthesize cholesterol, the cholesterol content of the adapted strain drops by as much as 90%. In addition, the PG3 strain is auxotroph for both saturated and unsaturated fatty acids, thus permitting extensive control of the composition of the membrane lipid hydrophobic tails [5,6].

The aim of this paper is to characterize the K^+ transport mechanism in the native PG3 strain about which nothing is known. Such a study will not only provide information in relation to the physiological mechanisms regulating the cell cation composition but will also constitute the indispensable basis for any study of the relationship between cation transport and membrane lipid composition.

The results reported here define the cell ionic composition of organisms in their growth medium, or, once harvested, resuspended in saline solution. Analysis of net and unidirectional K^+ fluxes was done on resuspended organisms, with special emphasis on cell metabolism dependence. Some of these results were the subject of a short communication [8].

Materials and Methods

Organisms and growth conditions

Mycoplasma mycoides var. Capri (PG3 strain) was a gift from Professor Perreaux (I.M.V.E.T., France) and *Acholeplasma laidlawii* (PG8 strain) was obtained from Professor Freund (FAO/WHO International Reference Center for animal mycoplasma, Aarhus, Denmark). Identity of the strains was repeatedly checked by growth inhibition tests on agar plates as described by Clyde [9].

Organisms were grown in a medium similar to that used by Rottem et al. [5]. The basal modified Edwards medium, in which potassium phosphate salt was replaced by sodium salt, was supplemented with a 0.5% fatty acid poor serum albumin (fraction V, Pentex), cholesterol (10 μg/ml), oleic and palmitic acids (5 μg/ml each). Under these conditions, the final K^+ concentration in the medium was reduced to 9 mM. In some cases, the basic medium was supplemented with 2% PPLO serum fraction (Difco). The growth rate was estimated by measuring absorbance at 640 nm and culture medium pH.

Cation content and intracellular volume of growing organisms

At different stages of growth, 10 ml culture medium was centrifuged (10 000 × *g* for 10 min) and washed with hypertonic saline solution (250 mM NaCl, 20 mM $MgCl_2$). Pellets which resulted from centrifugation of the washed cells were dissolved in sodium dodecyl sulfate (SDS) (2 mM). Aliquots were taken to determine proteins according to Lowry et al. [10] using serum albumin as standard, and K^+ was measured by flame photometry. Where Na^+ content was also measured, cells were washed with a choline chloride solution, resuspended in a small volume of distilled water, and sonicated.

Determinations of intracellular water volume were performed as described by Rottem and Razin [11]. The washed organisms were resuspended in a slightly buffered medium (5 mg/ml). Then, either tritiated water (5 μCi/ml) and [^{14}C]inulin (1 μCi/ml), or [^{14}C]urea (1 μCi/ml) and [^{3}H]inulin (5 μCi/ml) were added, inulin being used as an extracellular space marker. After 30 min equilibration, the thick cell suspension was centrifuged and the pellet dissolved in SDS. Radioactivity was measured in a Packard Tricarb liquid scintillation spectrometer using instagel (Packard) as scintillating fluid. Calculations were done according to Rottem et al. [11].

Transport properties in non growing organisms

All experiments were conducted on organisms collected during the early logarithmic phase of growth ($A_{640} \approx 0.18$–0.25; medium pH ≈6.8–6.7). To estimate changes in internal K content, washed cells (0.2 mg cell protein/ml) were resuspended in an isotonic and highly buffered solution (PO_4 buffer) containing per liter: 100 mM sodium phosphate (mono and diacid mixture); 2 mM $MgCl_2$; 75 mM NaCl; pH 7.2. After variable incubation periods at 37°C, cell suspension aliquots were diluted five-fold in cold washing solution, centrifuged and washed again. The final pellet was dissolved in 2 mM SDS, and K^+ and protein were estimated as described above.

When K^+ influx was determined under steady state conditions, i.e., constant internal K^+, cells (1 mg/ml) were incubated at 37°C in PO_4 buffer containing the desired concentrations of glucose and K^+. After equilibration, K^+ uptake measurements were started by the addition of $^{42}K^+$ (5 μCi/ml). Aliquots were taken every 45 s or 1 min, diluted in a cold washing solution, and filtered on 0.45 μm millipore filters using a controlled negative pressure of 60 mmHg. Radioactivity was measured by Cerenkov counting.

For K^+ efflux estimates, cells were loaded with $^{42}K^+$ either during growth by adding the isotope to the growth medium (2 μCi/ml) or after cell harvesting by equilibration in PO_4 buffer containing $^{42}K^+$. The $^{42}K^+$ loaded cells were then diluted in the unlabelled PO_4 buffer (0.1 mg cell protein/ml), and 1 ml aliquots were filtered as described above.

Other processes

Acid production by organisms (1–1.5 mg/ml) resuspended in saline solution (NaCl 150 mM, $MgCl_2$ 2 mM) was monitored at constant pH by automatic titration using a Radiometer pH-stat. In some experiments, the contribution of lactic acid to total acid production was measured in the supernatant using a

Calbiochem rapid lactate stat pack. Variation of cell volume was estimated as the relative change in absorbance measured with an ACTA IV spectrophotometer.

Results and Discussion

Intracellular K and Na levels in growing organisms

Fig. 1 illustrates the typical values and variations of the K^+ content of organisms harvested at different stages of growth in media supplemented with cholesterol and oleic plus palmitic acids. Culture medium pH at time of collection was considered a reliable index of the stage of growth [1].

During the early logarithmic phase ($7.8 > pH > 7.2$), organisms have a K^+ content of up to 60 μg K/mg cell protein. The K^+ content then declines steadily as growth takes place, and cells collected during the stationary phase (pH 5.9) have a K^+ content of 30—40 μg K^+/mg cell protein. On the other hand, values for cell Na^+ were much lower (2.4 μg/mg cell protein), regardless of the stage of growth *.

To convert the cell K^+ and Na^+ contents into intracellular concentrations (K_i^+ and Na_i^+), cellular water volume was measured. A mean value of 4.8 ± 1 μl/mg cell protein was found, irrespective of harvest time. Consequently, whereas Na_i^+ was measured at about 20 mM water throughout growth, K_i^+ was about 300 mM in cells collected during the early log phase and dropped to 170 mM during the stationary phase of growth. Since K^+ and Na^+ concentrations in the culture medium were respectively 9 and 100 mM, our measurements indicate that the cell K^+ concentration of growing organisms largely exceeds that of the medium, and that the reverse is the case for Na^+.

In spite of the variations in K^+ content noted during growth, K_i^+ values reported for PG3 are definitely higher than those usually measured in PG8 [12,13] a better known strain belonging to this group of microorganisms. Thus, in our experimental conditions, K_i^+ in PG8 was generally between 5 and 12 μg K^+/mg cell protein (mean value: 8 μg K^+/mg cell protein) with no appreciable variation throughout the growth phase for a given culture. These values compare well with previous reports [12,13]. Using the value of 2 μl/mg cell protein as cell water volume for PG8 [11], the intracellular K^+ concentration can be estimated at 100 mM, i.e., at least half the value found in PG3. On the other hand, compared to other microorganisms more extensively studied in the field of K^+ transport, the K_i^+ values reported here for PG3 are similar to those reported for *Escherichia coli* during the logarithmic phase of growth [13]. However, they are consistently lower than those measured in Gram-positive species such as *Streptococcus faecalis* [14,15].

The significant drop in K_i^+ observed in cell aging is not accompanied by cell volume changes, indicating that K^+ are exchanged with other cations. Since no increase in Na_i^+ was noted, another or other cations necessarily contribute to the exchange. In view of the important pH shift of the culture medium that

---

* In experiments where organisms were grown in media supplemented with PPLO serum fraction (PSF), levels and growth dependent variations of K^+ content were found to greatly vary according to the batch of PSF used.

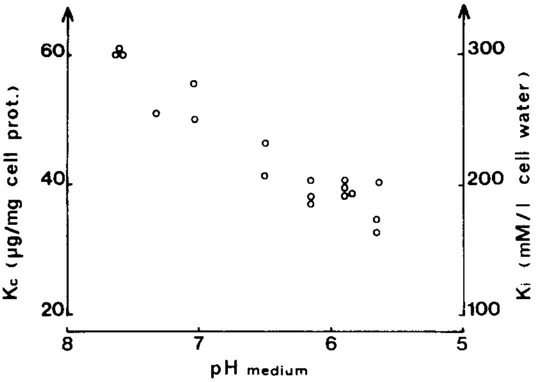

Fig. 1. Intracellular K$^+$ content (Kc) and concentration (K_i) in growing organisms as a function of the age of the culture. Culture medium pH at time of harvest was used as an index of growth age. Each value is the mean of two determinations.

takes place during growth, it is tempting to conclude that protons, influenced by their increasing chemical gradient, move back into the cell in exchange with K$^+$. Such a contribution of protons to cation exchange during growth has been noted in other microorganisms [13,14]. As regards the primary cause for the drop in K_i^+ in growing culture, it should be stressed that this progressive

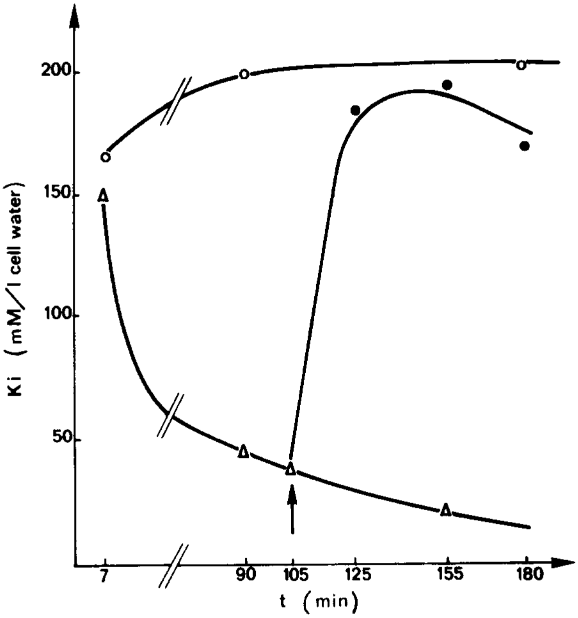

Fig. 2. Effect of glucose addition on K$^+$ concentration K_i of washed organisms. Freshly harvested cells were resuspended (0.2 mg/ml) in saline buffered solution (pH 7.2) containing 1 mM KCl, and the cell suspension divided into three parts. Glucose (20 mM) added at zero time (○) or 105 min after the start of incubation at 37°C (●). The third batch (△) remained in glucose-free medium throughout the experiment. Each value is the mean of three determinations.

inability of aging cells to maintain a high intracellular K^+ concentration is not an isolated phenomenon. Indeed, reduction in the efficiency of other transports and membrane-bound enzymatic activities (for example ATPases) was noted earlier in aging organisms [1].

K^+ transport properties in non growing organisms

Fig. 2 shows that when organisms are resuspended in isosmotic and buffered saline solution containing 1 mM KCl and glucose, their cellular K^+ content remains constant for 3 or 4 h, or even increases (upper curve). Using the steady K^+ content value of 1 μequiv. K^+/mg cell protein and a cell volume of 4.8 μl H_2O/mg cell protein, the intracellular K_i^+ concentration amounts to 210 mM. Depending on the batch of cells used, K_i^+ was found to vary from 190 to 250 mM, i.e., 190 to 250 times the external concentration. These cells will be referred to as K^+-rich cells.

On the other hand, if sugar is omitted from the suspension, the organisms lose their internal K^+ at a rate less than 1% per min ($t_{1/2}$ loss ranging from 50–75 min), leading to K^+ depleted organisms (Fig. 2, open triangles). The cell K^+ drop is reversible since even cells which have lost up to 80% of their internal K^+ can reaccumulate K^+ as soon as glucose is added to the suspension (closed symbols). K^+ reaccumulation is complete within 30 min, and the cell K^+ level recovered is almost comparable to that measured in cells immediately incubated with the sugar.

As estimated from the relative change in absorbance, organisms losing their cell K^+, or K^+-depleted cells reaccumulating K, did not show a significant associated change in cell volume ($\Delta A/A < 2\%$). In order to maintain a constant intracellular osmotic balance, the changes in cell K^+ must therefore, under both conditions, be compensated by a change in the cellular level of one or several cations. The experiment illustrated on Fig. 3 shows that the loss of K^+ in

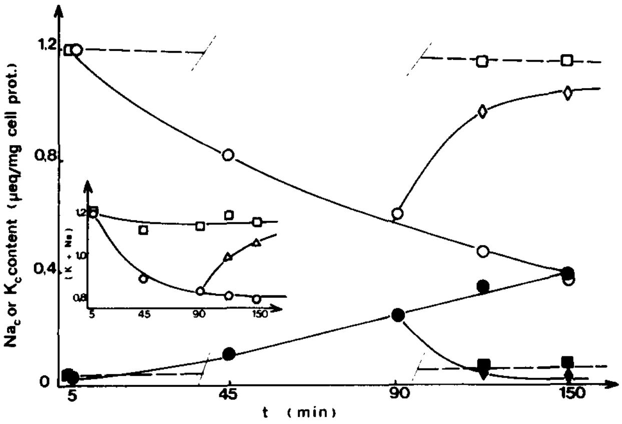

Fig. 3. Effects of glucose on K^+ and Na^+ cell contents. Experimental conditions as in Fig. 2. K^+ level (open symbols) and Na^+ level (closed symbols) were determined in cells resuspended in the presence of 20 mM glucose (□, ■), without glucose (○, ●), or in cells resuspended for 90 min in a glucose-free medium followed by sugar addition (◇, ◆). Inset: Total (K_C + Na_C) content in cells incubated with glucose (□), in the case of late sugar addition (△) or without sugar (○).

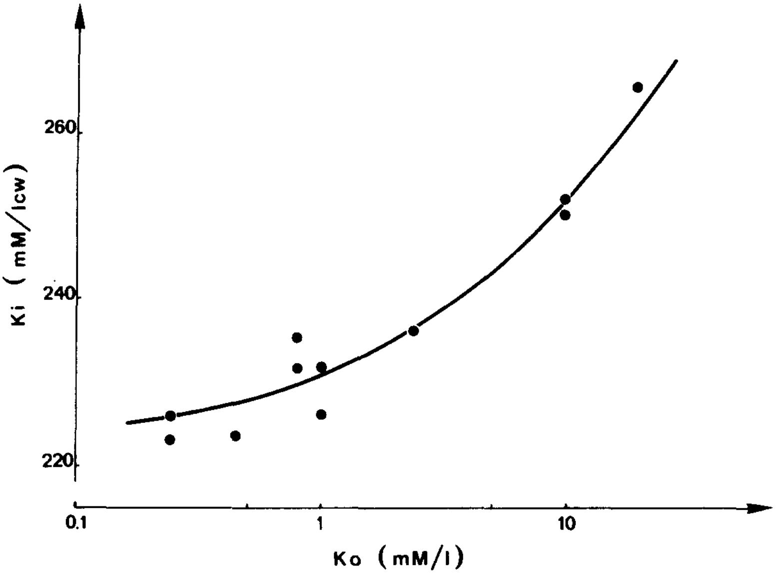

Fig. 4. Equilibrium of intracellular K^+ content levels in freshly harvested cells as a function of K^+ concentration in the suspending medium. Experimental conditions as in Fig. 2. Each batch of cells received glucose (20 mM).

starved cells is associated with a gain of Na ions. Conversely, during K^+ reaccumulation by K^+-depleted systems, the cell Na^+ concentration returns to its original level. As shown in the inset of Fig. 3, substitution of Na^+ for K^+ during depletion, or K^+ for Na^+ during reaccumulation is not at a ratio 1 : 1 since the sum of Na^+ and K^+ is about 30% lower in K^+-depleted cells than that calculated in K^+-rich cells or after complete recovery of cell K^+. This suggests that other cationic species participate in the exchange. Previous reports in *E. coli* [13] and *Streptococcus faecalis* [14] have shown that protons contribute

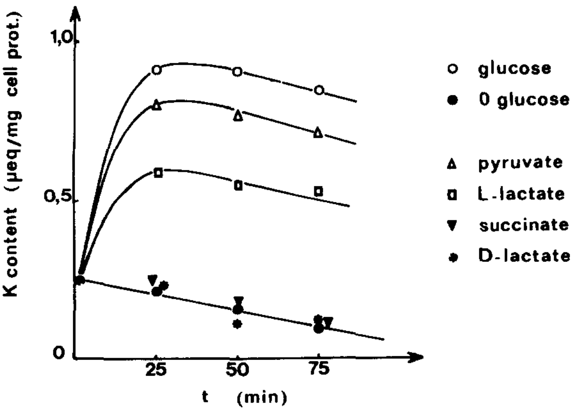

Fig. 5. Effects of different substrates on K^+ reaccumulation by K^+-depleted cells. Organisms were first preincubated for 100 min in glucose-free medium. Then (at $T = 0$), either glucose (○), pyruvate (△), L-lactate (□), D-lactate (*) or succinate (▲) was added (20 mM) to control glucose-free conditions (●). Values re the mean of three determinations.

TABLE I

RATE OF ACID PRODUCTION BY ORGANISMS RESUSPENDED IN THE PRESENCE OF VARIOUS SUBSTRATES

Substrates were added to give 20 mM final concentration. Steady state cells refer to freshly harvested organisms; K^+-depleted cells correspond to organisms whose K^+-content has been lowered by incubation in glucose-free medium for 2 h at 37° C.

Substrate	Rate of acid production (μequiv. H^+/mg protein/min)	
	Steady state cells	K^+-depleted cells
None	0.028	0.002
Glucose	0.350	0.191
Pyruvate	0.180	0.147
L-Lactate	0.082	0.093
D-Lactate	0.020	0.002
Succinate	0.015	0.003
Acetate	0.006	0.010

to the cation exchange. It is possible that H^+ play a similar role in mycoplasma.

Finally, Fig. 4 shows that the K^+ level in K^+-rich cell metabolizing glucose increases with extracellular K^+ concentration. The increase is nevertheless limited since a hundred-fold variation of the external K^+ concentration only produced a 20% variation in the cell K^+ content.

All these experiments confirm that PG3 accumulate K^+ against a concentration gradient, even when resuspended in saline solution: at the lowest external K^+ concentration (0.2 mM), the K^+ concentration gradient value is higher than one thousand-fold.

Construction and maintenance of the chemical K^+ gradient across the membrane rely on glucose utilization by the organisms. Thus, both processes are associated with a high increase in acid production rate of which 30% is accounted for by lactic acid production. It is to be noted that K^+-rich cells produce 30% more acid than K^+-depleted organisms (Table I).

Among other substrates tested for their capacity to promote K^+ reaccumulation, pyruvate appeared almost as good as glucose (Fig. 5). This figure also shows that L-lactate but not D-lactate allowed partial recovery to the initial K^+ level. Similar differences were noted in the capacity of these substrates to maintain a steady K^+ level in K^+-rich cells: thus, while the level of K^+ in organism metabolizing glucose or pyruvate was high and similar, a new lower steady state K^+ level (about 40% lower) was reached within the following 30 min in the presence of L-lactate. In contrast to pyruvate and L-lactate, neither acetate, succinate or 2-ketoglutarate activated K^+ transport. Also, no K^+ transport was observed after addition of energy-rich compounds such as ATP, acetyl-phosphate or the electron donor system phenazine methosulfate/ascorbate mixture. Finally, it was noted that the increase in acid production correlates with the ability to drive K^+ transport across the membrane for each substrate tested (Table I).

Kinetic parameters of K^+ transport

The experiments presented on Figs. 2 and 3 show that kinetic analysis of the

TABLE II

EFFECTS OF GLUCOSE AND EXTERNAL K^+ CONCENTRATION ON $^{42}K^+$ EFFLUX AND INFLUX IN STEADY STATE CELLS

None: cells resuspended in glucose-free medium; 20 mM: cells incubated in 20 mM glucose; Δ efflux: difference between columns 1 and 2, i.e., in the absence or presence of glucose.

External K^+ concn. (mM)	$^{42}K^+$ fluxes (μg K^+/mg cells protein/min			
	Glucose		Δ efflux	Influx 20 mM glucose
	None	20 mM		
0	0.37	0.23	—	—
1.5	0.36	0.67	0.31	0.58
20	0.35	0.93	0.58	0.88

K^+ influx across the mycoplasma membrane can be studied under two different and well defined sets of conditions: (a) in K^+-rich cells when cellular K^+ remains constant, i.e., in a steady state system, and (b) during the K^+ reaccumulation which follows K^+ depletion.

Under both conditions, initial rates of K^+ influx were found to follow saturation kinetics and were thus analyzed by the Lineweaver and Burk procedure. In the case of steady state cells, $^{42}K^+$ influx has a K_m of 1.5 mM and a V of approximately 14 nequiv./mg cell protein/min with extreme values of 10 and 20 nequiv./mg. On the other hand, the reaccumulation process has a higher affinity for K^+ ($K_m = 0.3$ mM) and allows much greater influxes to occur ($V =$ 80 nequiv./mg cell protein/min). On the basis of these kinetic data it is suggested that two different K^+ transport systems may be present in the membrane of PG3.

A further insight into the mechanism responsible for K^+ movement across the membrane under steady state conditions may be obtained by examining the properties of K^+ efflux, particularly its dependence on an energy supply and external K^+ concentration (K_o^+). Table II shows that the $^{42}K^+$ efflux from cells resuspended in a glucose-free medium is independent of K_o^+. In contrast, the $^{42}K^+$ efflux from metabolizing glucose increases with K_o^+. It is unlikely that in the presence of energy, the increase in K^+ efflux by external K^+ is secondary to an increase in intracellular K^+ concentration since K_i^+ only increased by 10% in the external K^+ concentration range used (Fig. 4). These observations rather imply that the energy dependent K^+ efflux, or more precisely the difference between the $^{42}K^+$ effluxes from metabolizing and starved cells (Δ efflux), is coupled with the energy-dependent K^+ influx. Such a K^+-K^+ exchange is similar to the autologous K^+-K^+ exchange described by Harold in *Streptococcus faecalis* [16,17]. Assuming a coupling ratio of one for the exchange process, it follows that a large fraction of the K^+ influx is driven by the autologous K^+-K^+ exchange at higher external K^+ concentration.

Harold's terminology can also be used to define the K^+ influx leading to K^+ reaccumulation. This energy-dependent K^+ influx corresponds to a net influx of K^+ and is associated with a concomitant efflux of Na^+ and possibly H^+ as discussed above. Accordingly, it can be considered as a net uptake mechanism. In conclusion, kinetic analysis of K^+ movement across the membrane in the two different experimental conditions, steady state and reaccumulation, renders possible the distinction of two different K^+ transport processes in PG3 *. Under physiological conditions, i.e., in the culture medium that contained KCl and glucose, the organisms maintained a steady cell K^+ level. In these conditions, the autologous K^+-K^+ exchange probably accounts for much of the K movement across the membrane.

Effects of ouabain, anoxia and metabolic inhibitors

In all the experiments to be described below, glucose was added as an energy source to activate K^+ transport.

Because ouabain has been recognized as a specific inhibitor of Na^+-K^+-activated ATPase [18], we used it as a tool to investigate if this ATPase is involved in the mechanism of K^+ regulation in PG3. Even when added to a final concentration of 10^{-3} M, ouabain had no effect on K^+ level, on autologous K^+-K^+ exchange or on the net K^+ uptake mechanisms. This enzyme does not therefore contribute to the regulation of cations in the PG3 strain. Since a similar conclusion has already been reached where PG8's K^+ transport mechanism is involved [12], it appears that Na^+-K^+-activated ATPase does not play a role in cation transport in molecules.

All our attempts at establishing a close relationship between K^+ transport and repiratory activity were unsuccessful. First, anoxia reduced K^+ level equilibrium in K^+-rich cells by only 20% after 2 h. On the other hand, K^+ movement remained insensitive to cyanide (2 mM), antimycin ($2 \cdot 10^{-5}$ M), rotenone ($2 \cdot 10^{-6}$ M) and was only reduced by 25% in the presence of amytal (10 mM).

A note of caution should be exercised in the interpretation of the inhibitor's effect because it has been claimed that many *Mycoplasma* strains do not possess the cytochrome components of the respiratory chain [19,20]. Unfortunately, no direct information is available on the PG3 strain under study here. In any case, and particularly if the respiratory chain was complete in this strain, our experiments indicate that the metabolic energy used to accumulate K^+ is not exclusively derived from respiration.

Effect of dicyclohexylcarbodiimide

Recent reports point out that in bacterial systems [17,22], the membrane-bound Mg^{2+}-dependent ATPase plays a crucial role in the transduction of the metabolic energy into a form of energy directly available in the different transport processes. Since such enzyme activity operates in the membrane of *Mycoplasma* [1,5,12], we have investigated its possible contribution to the mechanism of K^+ transport in the PG3 strain by studying the effect of DCCD, a specific inhibitor of this ATPase [15,17,23] on K^+ influx.

Fig. 6 shows that the K^+ influx which takes place in freshly harvested cells metabolizing glucose is drastically inhibited by low concentrations of DCCD ($5 \cdot 10^{-5}$ M). Since most of this K^+ influx is thought to represent the operation of an autologous K^+-K^+ exchange, it can be concluded that such a mechanism is directly or indirectly related to the ATP activity.

---

* One cannot exclude the possibility that the two K^+ influx systems correspond to a different behaviour of a single system whose properties are modulated by the intracellular K^+ level.

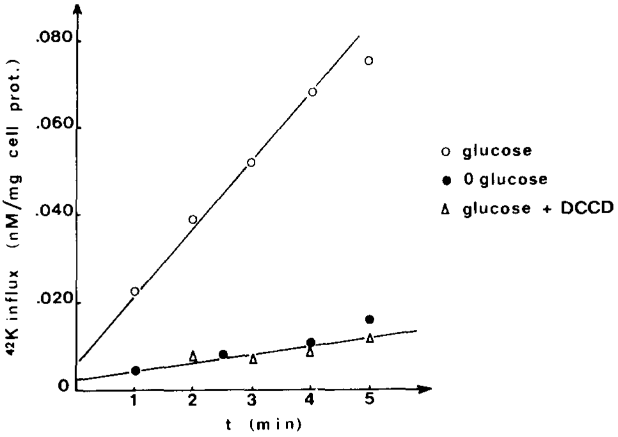

Fig. 6. Effect of DCCD ($5 \cdot 10^{-5}$ M) on ^{42}K influx in steady state cells. $^{42}K^+$ influx was measured in cells resuspended without glucose (●), with glucose (○) or with glucose and DCCD (△). Glucose and DCCD were respectively added 10 min and 5 min prior to $^{42}K^+$ influx determination.

Fig. 7 demonstrates that the energy-dependent K^+ influx that allows recovery of a high K_i^+ in K^+-depleted cells is also severely depressed by $5 \cdot 10^{-5}$ M of carbodiimide derivative. This net K^+ influx is therefore also linked to the ATPase activity.

This observation emphasizes that the net K^+ uptake mechanism in PG3 shares many properties in common with the net K^+ uptake mechanism which operates in the membrane of *Streptococcus faecalis*. In contrast, the autologous K^+-K^+ exchange differs in both organisms since it is inhibited by DCCD in the mycoplasma strain but remains insensitive to this agent in *Streptococcus faecalis* [16,17].

The experiments presented in this study demonstrate that PG3 actively accumulates K^+ against a concentration gradient. The effects of DCCD suggest

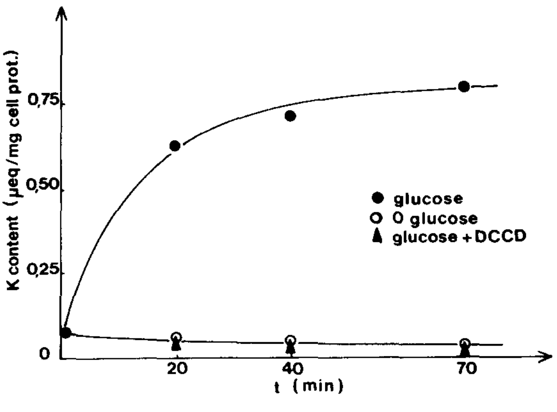

Fig. 7. Effect of DCCD ($5 \cdot 10^{-5}$ M) on K^+ reaccumulation by K^+-depleted organisms. Experimental condition leading to K^+-depleted cells as in Fig. 5. (●) glucose (20 mM) and K^+ (1 mM) were added at $t = 0$; (▲) 10 min before glucose and K addition, cells were poisoned with DCCD; (○) only K added.

that the membrane-bound Mg^{2+}-dependent ATPase might be involved in the mechanism of active K^+ transport. The role of this enzyme in the mechanism of K^+ regulation will be analyzed in greater detail in a subsequent paper.

References

1 Razin, S. (1973) in: Advances in Microbial Physiology, (Rose, A.H. and Tempest, D.W., eds.), Vol. 10, pp. 1—80, Academic Press, New York

2 Razin, S. (1974) in: Progress in Surface and Membrane Science (Cadenhead, D.A., Danielli, J.F. and Rosenberg, M.D., eds.), Vol. 9, pp. 257—312, Academic Press, New York

3 Fox, C.F. (1972) in: Membrane Molecular Biology (Fox, C.F. and Keith, A., eds.), pp. 345—385, Sinauer, Stanford, CN

4 Overath, P., Thilo, L. and Trauble, H.V. (1976) Trends Biochem. 1, 186—189

5 Rottem, S., Yashouv, J., Ne'Eman, Z. and Razin, S. (1973) Biochim. Biophys. Acta 323, 495—508

6 Rottem, S., Cirillo, V.P., De Kruyff, B., Shinitzky, M. and Razin, S. (1973) Biochim. Biophys. Acta 323, 509—519

7 Leblanc, G. and Le Grimellec, C. (1979) Biochim. Biophys. Acta 554, 168—169

8 Leblanc, G. and Le Grimellec, C. (1976) 77th meeting of the Society for General Microbiology, Glasgow. Abstract, p. 172

9 Clyde, S. (1964) J. Immunol. 92, 958—965

10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275

11 Rottem, S. and Razin, S. (1969) J. Bacteriol. 97, 787—792

12 Rottem, S. and Razin, S. (1969) J. Bacteriol. 92, 714—722

13 Schultz, S.G. and Solomon, A.K. (1961) J. Gen. Physiol. 45, 355—369

14 Zarlengo, M.H. and Schultz, S.G. (1966) Biochim. Biophys. Acta 126, 308—320

15 Harold, F.M., Baarda, J.R., Baron, C. and Abrams, A. (1969) J. Biol. Chem. 244, 2261—2268

16 Harold, F.M., Harold, R.L., Baarda, J.R. and Abrams, A. (1967) Biochemistry 6, 1777—1782

17 Harold, F.M. and Altendorf, K.H. (1974) Curr. Topics Membrane Transp. 5, 5—20

18 Glynn, I.M. (1964) Pharmacol. Rev. 16, 381—420

19 Rodwell, A.W. (1954) Austr. J. Biol. Sci. 7, 18

20 Rodwell, A.W. (1967) Ann. N.Y. Acad. Sci. 143, 88—109

21 Vandemark, P.J. (1969) in: Mycoplasmatales and L. Phase of Bacteria (Hayflick, L., ed.), pp. 491—501, Appleton Century Crofts, New York

22 Boos, W. (1974) Annu. Rev. Biochem. 43, 123—144

23 Evans, D.J. Jr. (1970) J. Bacteriol. 104, 1203—1212