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EFFECT OF MEMBRANE CHOLESTEROL ON POTASSIUM TRANSPORT IN *MYCOPLASMA MYCOIDES* VAR. CAPRI (PG3)

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Summary

Relationships between membrane lipid composition and physiological properties, particularly intracellular potassium levels, have been studied at 37°C in *Mycoplasma mycoides* var. Capri (PG3).

Native organisms grown on medium supplemented with either oleic acid plus palmitic acid or elaidic acid have identical growth characteristics, acidification properties and intracellular K content.

On the other hand, when the cholesterol normally present in the membrane (20—25% of total lipids) is reduced to less than 2%, we observe: (1) the intracellular K content decreases (20 μg K/mg cell protein instead of 40) and is independent of the phase of growth; (2) K passive permeability is drastically increased but K distribution remains in equilibrium with the transmembrane potential ($\Delta\Psi$); (3) organisms stop growing at pH 6.5 (instead of 5.2) and acidification is reduced by 40%, suggesting a large increase in H^+ permeability, and (4) intracellular Na contents rise from 3 to 9 μg Na/mg cell protein.

Replenishing cholesterol in membranes of depleted cells results in a recovery of the high intracellular K level (35—40 μg K/mg cell protein) and acidification properties.

It is suggested that cholesterol affects the cation content via the increase in proton permeability which in turn controls the value of the $\Delta\Psi$ responsible for the value of intracellular K equilibrium.

Changes in K passive permeability, although related to the amount of cholesterol present in the plasma membrane, are probably not involved in the control of the intracellular K level.

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Introduction

Increasing amounts of cholesterol decrease, above the lipid phase transition temperature, the non-electrolyte permeability of artificial bilayer model systems [1—3] and biological membranes [4—7]. Such an observation holds true for ionic species in liposomes whose permeability towards charged molecules is very low [8,9] and also for the passive leak of monovalent cations from *Acholeplasma laidlawii* cells [10] or erythrocytes [11,12]. The effects of cholesterol on ionic transport properties of metabolizing cells are not so well documented and remain poorly understood. This largely results from the difficulties encountered in controlling the amount of cholesterol present in the plasma membranes.

PG3 appears to be a unique tool for studies on the role of cholesterol in biomembranes. Although, as in other mycoplasmas, they require cholesterol for growth they appear to adapt and grow with very low amounts of cholesterol in the growth medium [13]. This allows the variation of cholesterol concentration from 1 to 30% of total membrane lipids. Moreover, the fatty acid composition of the membrane can also be controlled [13]. Like other organisms, PG3 cells grown in normal cholesterol medium accumulate K against a large concentration gradient. This accumulation can be accounted for by the existence of a transmembrane potential ($\Delta\Psi$) dependent on the activity of a membrane-bound Mg-ATPase, in accord with the Mitchell chemiosmotic hypothesis [14]. We therefore investigated the effects of large cholesterol variations and fatty acid modifications on the physiological, and more particularly the K transport properties of PG3 organisms. We show that changing the fatty acid composition of the plasma membrane has, if any, only a limited effect on the physiology of PG3 cells. On the other hand, reduction in the plasma membrane cholesterol level induces a large decrease in the intracellular K level which now becomes independent of the phase of growth. Other physiological parameters such as H^+ permeability, intracellular Na concentration, and acidification properties are also modified. It is suggested that the control of intracellular K levels by cholesterol occurs via the effect on H^+ permeability and the related $\Delta\Psi$.

Materials and Methods

Organisms and growth conditions. Native PG3 organisms, kindly provided by Dr. E.A. Freundt (FAO/WHO International Reference Center for Animal Mycoplasma, Aarhus, Denmark) and by Dr. P. Perreau (I.M.V.E.T., France), were grown on the modified Edward medium [15] in which 0.5% fatty acid-poor bovine serum albumin (Miles Laboratories Inc., Kankakee, Ill.) was used to replace the PPLO serum fraction. Na salts of palmitic acid plus oleic acid (5 μg/ml each) or elaidic acid (10 μg/ml) were added as the source of fatty acid together with cholesterol (10 μg/ml). Growth adaptation in a medium free of exogenous cholesterol was achieved by successive serial transfers, as per the method described by Rottem et al. [13]. Elaidic acid (10 μg/ml) was used as the fatty acid source.

After incubation at 37°C, organisms were collected by centrifugation (8000 × *g*, 10 min) and washed once with cold 0.25 M NaCl solution containing

0.01 M $MgCl_2$. When membranes were prepared, $MgCl_2$ was omitted from the washing solution. Except when establishing the relationship between phase of growth and intracellular cation content, cells were collected in the early log phase ($A_{640\,nm} \leqslant 0.25$; pH of the growth medium $\geqslant 6.8$).

Rate of acid production. Production of titratable acidity by the organisms was estimated by resuspending the cell pellet in 0.15 M NaCl containing 0.002 M $MgCl_2$ (0.4—1.0 mg cell protein/ml) in the temperature-controlled cuvet (37°C) of a pH stat (Radiometer, Copenhagen). Reaction was started with the addition of 20 mM glucose. pH was maintained constant at 7.5 by the addition of 0.02 M NaOH.

Intracellular cation content. Intracellular K of washed organisms collected from the growth or the transport assay medium was determined by flame photometry after solubilization of cells by sodium dodecylsulfate (SDS) (0.002—0.02 M). For Na determinations, the cell pellet was washed with 0.25 M choline chloride containing 0.01 M $MgCl_2$ and lysed in distilled water.

Unidirectional K fluxes. Initial rates of active K exchange influx were measured under steady-state conditions in organisms resuspended at 37°C, in a buffer containing 0.1 M phosphate/35 mM NaCl/2 mM $MgCl_2$/1 mM KCl/ 20 mM glucose, pH 7.20. In this medium, cells were able to maintain a constant intracellular K value for hours. After a 5 min preincubation period, the assay was started by adding 2.5 μCi ^{42}K (CIS, CEA, France) per ml of cell suspension (approx. 1 mg cell protein/ml). At various times, from 45 s to 15 min, 0.1-ml samples of this cell suspension were collected, diluted in 1 ml ice-cold NaCl/ Mg solution (0.25 M NaCl/0.01 M $MgCl_2$), filtered through HA 45 Millipore filters under a negative pressure of 60—70 mmHg, and washed with 5 ml of the ice-cold solution. 5 ml H_2O were added to the filters once they were transferred into glass scintillation vials for counting, using Cerenkov radiation. Effectiveness of the steady-state conditions was checked by comparing the intracellular K values before and after the unidirectional flux experiments.

K efflux was measured in cells grown in the presence of 1 mCi/l ^{42}K, washed once in ice-cold hypertonic NaCl/Mg solution, and finally resuspended at 37°C in the phosphate medium containing no glucose, for a final concentration of 100 μg/ml. Samples of 1 ml were taken at 5-min intervals, passed through Millipore filters and treated as for influx experiments.

Cholesterol replenishment. Organisms adapted to grow in the absence of added cholesterol were resuspended at various concentrations (5—500 μg cell protein/ml) in the phosphate buffer solution containing 2.5 mg/ml fatty acid-free bovine serum albumin (Miles Laboratories Inc., Kankakee, Ill.) and 10 μg/ml cholesterol labelled with [^{3}H]cholesterol (10 Ci/mM) or [^{14}C]cholesterol (50—60 mCi/mM) (CIS, CEA, France). After various times (ranging from 5 to 120 min) of incubation at 37°C, cells containing labelled cholesterol were centrifuged at low temperature (8000 × g, 10 min at 5°C), washed once in the hypertonic NaCl/Mg solution and recentrifuged. Depending on the experiment, cell pellet was then either: (1) directly solubilized by SDS and an aliquot taken for counting in instagel solution (Packard®) for the calculation of cholesterol fixation from the known specific activity, the remainder being used for protein and K determinations; or, (2) lysed by using warm distilled H_2O and alternately frozen and thawed. The supernatant obtained after centrifugation (55 000 × g,

30 min) was used for K determinations. The membrane pellet, washed once in distilled H_2O, once in 0.05 M NaCl in 0.01 M phosphate buffer (pH 7.5), was solubilized by SDS and an aliquot was taken for protein and cholesterol determinations.

The same procedures were followed when isolated membranes were used instead of whole organisms.

Membrane preparation. Membranes were prepared by osmotic lysis according to Razin and Rottem [16], resuspended in diluted (1 : 20) β-buffer [17] and kept at −45°C until used (generally within two or three days).

Enzyme assay. ATPase activity was measured at 37°C by the release of P_i from the reaction mixture which contained (per ml) 5 μM $MgCl_2$, 50 μM Tris-HCl (pH 8.0), 4.4 μM NaCl, and 150—300 μg membrane protein. The reaction was started by adding 1.25 μM labelled or unlabelled ATP ([γ-^{32}P]ATP, 0.5 μCi/ml, Amersham) [18,19]. When unlabelled ATP was used, the reaction was stopped after 20 min by adding 1 ml 10% trichloroacetic acid to each test tube. After centrifugation (3000 × *g*, 10 min) the liberated P_i in the supernatant was determined according to Chen et al. [20]. With [γ-^{32}P]ATP, 0.15-ml samples were collected at various times (generally 5, 10 and 15 min) and the reaction stopped with 0.3 ml of a solution containing 25% activated charcoal in 5% trichloroacetic acid. After centrifugation (10 000 × *g*, 2 min), part of the supernatant was collected, directly transferred into vials containing Instagel, and counted. Liberated P_i was calculated from the known specific activity.

Freeze-fracture experiments. Native and adapted PG3 organisms were fixed at 37°C by adding glutaraldehyde in isosmotic phosphate solution (final glutaraldehyde concentration 1%). A 20% glycerol solution was then added and the cell suspension centrifuged after 3 h of equilibration. Following resuspension in the same glycerol solution, small droplets were placed on gold holders and frozen in liquid Freon 22® cooled by liquid nitrogen. The preparation was freeze-cleaved without etching in a Balzers BA 301 freeze-etching apparatus, according to the method of Moor and Muhlethaler [21]. Shadowing and replicas (platinum-carbon) were obtained using an electron beam evaporation source (EVM 052 Balzers). Replicas were examined in a Phillips 301 electron microscope after digestion by sulfochromic mixture.

Analytical methods. Lipids were extracted from whole cells or membrane suspensions according to Bligh and Dyer [22]. Neutral lipids were separated from polar lipids by silicic acid chromatography [23]. Thin layer chromatography was done on pre-coated Silica Gel 60 thin layer plates (Merck 5715) using benzene/diethylether/ethanol/acetic acid (50 : 40 : 2 : 0.2, v/v) as developing solvent for neutral lipids, and chloroform/methanol/water/acetic acid (65 : 25 : 4 : 1, v/v) for phospholipids. The cholesterol zone was scraped off after chromatography and the sterol eluted by using chloroform in excess (2 × 5 ml). Cholesterol was determined chemically by following the Wycoff and Parsons method [24]. Comparison with results calculated from labelled cholesterol generally coincided within 5%.

Methyl esters of fatty acids were prepared by heating the lipid samples in methanolic NaOH for 15 min at 65°C and adding Boron trifluoride-methanol for 5 min [25]. Methyl esters were extracted with pentane and, after washing and drying, subjected to gas-liquid chromatography in a Varian apparatus

(Model 1400, column: diethylene glycol succinate, 195°C). Fatty acids were identified by their retention times.

Protein was determined by the Lowry method [26] using bovine serum albumin as standard.

Results

Effects of changes in membrane composition on the general characteristics of PG3 cells. Growing native PG3 organisms in either oleic acid plus palmitic acid or elaidic acid-containing medium, although drastically altering the membrane fatty acid composition (Table I), does not induce any modification in the characteristics (i.e. generation time, yield) of the growth curve. This also holds true when the cholesterol concentration of the growth medium is decreased from 10 to 1.25 μg/ml. Five to ten serial transfers are needed in order for the organisms to adapt to growth in a medium free of exogenous cholesterol. In accordance with the data obtained by Rottem et al. [9] this results in a large drop of the membrane cholesterol (Table I) which decreases from 70—110 to 6—7 μg cholesterol/mg membrane protein. No major changes in the fatty acid composition of polar lipids were observed during the adaptation process.

Adapted cells show an increase in their generation time (from 1 h 30 min to 2 h 45 min) and, according to the batch of medium used, stop their growth when the medium pH values range from 6.9 to 6.3 (Fig. 1). These changes in the ability to lower the pH of the medium are associated with a decrease in the titratable acidity production (0.23 μequiv against 0.37 μequiv/mg cell protein per min in native cells) by adapted cells resuspended at 37°C in a NaCl/glucose solution (pH 7.5). Another characteristic of low-cholesterol cells is their increase in mechanical and osmotic fragility. This implies that when physiological experiments have to be performed, cells must be harvested in the early logarithmic phase (pH approx. 7.0) when they show the highest resistance to mechanical stress.

TABLE I

CHOLESTEROL CONTENT AND FATTY ACID COMPOSITION OF POLAR LIPIDS FROM CELLS GROWN WITH OR WITHOUT ADDED CHOLESTEROL AND VARIOUS FATTY ACIDS

Cells were grown in a modified Edward's medium containing either 10 μg (cholesterol (+)) or no (cholesterol (—)) added cholesterol. Fatty acids (oleic plus palmitic acid, 5 μg/ml each or elaidic acid, 10 μg/ml) were given as sodium salts. Cells were harvested during the early logarithmic phase of growth.

	Cholesterol (μg/mg membrane protein)	Fatty acids (mole%)					Non-identified
		14 : 0	16 : 0	18 : 0	18 : 1	18 : 2	
Cholesterol (+), oleic + palmitic	111	0.4	41.7	9.7	42.4	5.8	—
Cholesterol (+), elaidic	74	3.1	12.4	14.5	64.6 *	5.4	—
Cholesterol (—), elaidic	7.2	0.7	15.6	9.9	67.7 *	4.6	1.5

* Determinations of specific activity using [^{14}C]elaidic acid (CIS, France) show that more than 95% of the 18 : 1 can be attributed to 18 : 1_t in elaidate experiments.

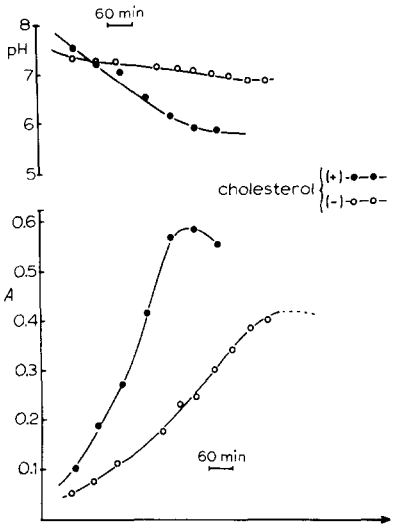

Fig. 1. Growth curves and acidification of the growth medium by native and adapted cells.

One of the reasons for this increased sensitivity of low-cholesterol organisms is apparent from Fig. 2, where platinum-carbon replicas obtained from freeze-fracture experiments done on organisms fixed at 37°C by glutaraldehyde are represented. One can observe that, in contrast with the native organism which shows a distribution at random of particules at the fracture face, areas devoid of particules are present in low cholesterol cells. Differential scanning calorimetry experiments have recently (Le Grimellec, C. and Léblanc, G., in preparation) confirmed that a large part of the acyl chains of adapted organisms must be in the gel state at 37°C. Such a coexistence of lipid domains in the gel and liquid states would indeed affect the mechanical resistance properties of the membrane.

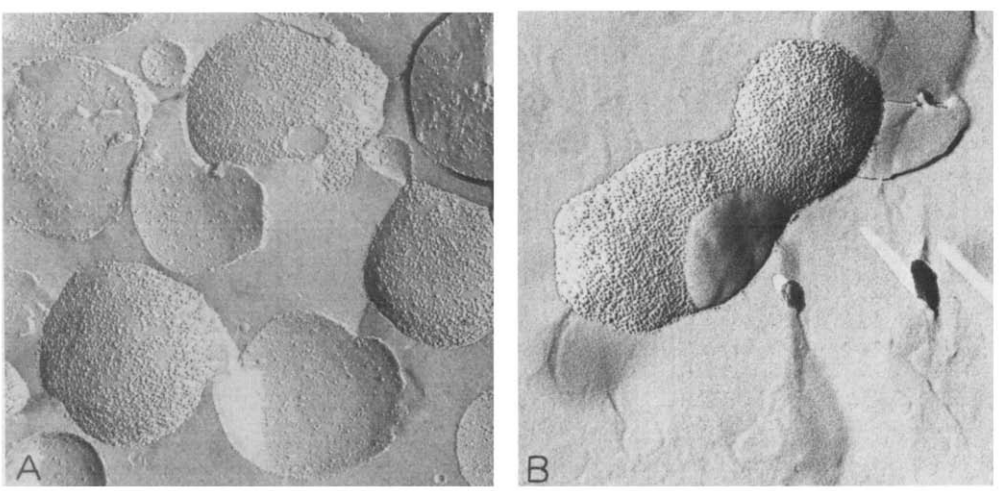

Fig. 2. Freeze-fracture photograph of *Mycoplasma* PG3 fixed at 37°C by glutaraldehyde. (A), native cells (×42 500); (B), adapted cells (×54 000).

Effects on intracellular cation contents and unidirectional K fluxes. Intracellular K contents of organisms grown in the presence of elaidic acid or oleic acid plus palmitic acids comprise between 50 and 60 μg K/mg cell protein at the early phase of growth (Fig. 3). They decrease continuously during growth to attain values as low as 25—30 μg K/mg cell protein at the end of the logarithmic phase.

Lowering the cholesterol concentration of the medium to 0.5 μg/ml results in a decrease of the intracellular K value of young cells (40 μg K/mg cell protein). Once adaptation is achieved, intracellular K levels are low (16—28 μg, mean value 23 μg K/mg cell protein) and are independent of the phase of growth. This is associated with a 3-fold increase in the intracellular Na content (9 μg Na/mg cell protein as compared to 3 μg in the native strain).

In contrast with non-energized cells, valinomycin ($5 \cdot 10^{-7}$ M) added to cells resuspended in the phosphate buffer containing glucose and 1 mM K does not change the intracellular K level (Fig. 4), suggesting that, as in native organisms [14,27], the intracellular K level of these adapted organisms is in equilibrium with a $\Delta\Psi$. Taking into account an intracellular water space of 4.8 μl/mg cell protein (Leblanc, G. and Le Grimellec, C., in preparation) it can then be calculated by using the Nernst equation that growth in the absence of exogenous cholesterol must result in a decrease of $\Delta\Psi$ from 140 to 120 mV (negative inside).

For native and adapted organisms, active unidirectional K influx assayed during steady-state conditions was inhibited by $5 \cdot 10^{-5}$ M *N,N'*-dicyclohexylcarbodiimide (DCCD) (inhibition $\geqslant$80%). Mean values for cells grown with cholesterol and either elaidic or oleic plus palmitic acids were, respectively, 0.42 $\pm$ 0.05 (n = 4) and 0.65 $\pm$ 0.13 (n = 19) μg K/mg cell protein per min as

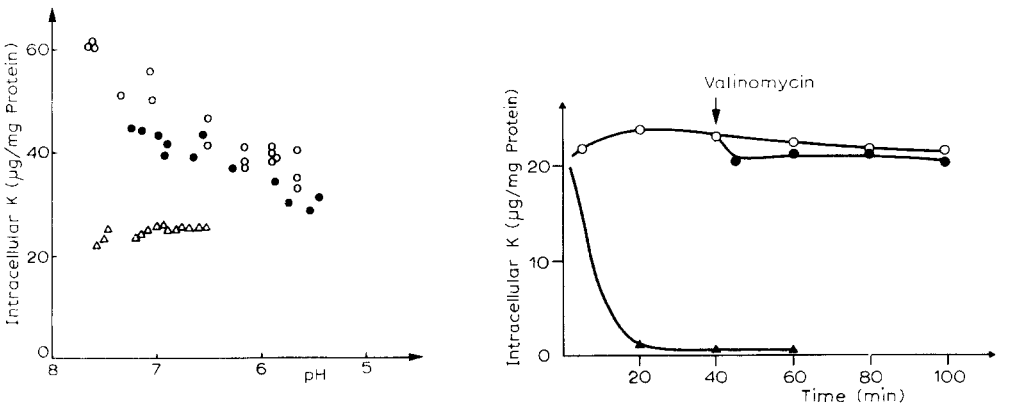

Fig. 3. Intracellular potassium content as function of the phase of growth. Age of culture was estimated from the pH of the medium. Native cells grown with oleic acid plus palmitic acid (5 μg/ml each) (○———○) or elaidic acid (●———●) (10 μg/ml), △, adapted cells grown with 10 μg/ml elaidic acid in the absence of cholesterol.

Fig. 4. Effect of valinomycin on intracellular potassium content of adapted PG3 cells. After a control period, valinomycin ($5 \cdot 10^{-7}$ M) was added to the suspension (100—200 μg cell protein/ml) of metabolizing cells. Potassium concentration of the phosphate buffer was 1 mM/l. ○———○, control; ●———●, valinomycin-treated; ▲———▲, valinomycin added at t = 0, to the same suspension but containing no glucose.

compared to 0.69 ± 0.13 (n = 18) μg K/mg cell protein per min for elaidate-adapted organisms.

The passive K permeability was drastically increased by depletion of cholesterol from the growth medium: leakage half-times dropped from 68 ± 6 min (n = 4, oleic plus palmitic) and 53 ± 12 min (n = 3, elaidic) to 18 ± 3 min (n = 4, elaidate) when cholesterol was omitted.

The Mg-ATPase activity of isolated membrane preparations was found to be extremely variable according to the membrane batch (values ranging from 0.72 to 1.51 μM P_i/mg membrane protein per 5 min). No great or consistent difference in this ATPase activity was noted with the three different membrane lipid compositions studied.

Effects of cholesterol replenishment. Dilution of growing cells adapted to low cholesterol by a medium containing 10 μg/ml cholesterol (final concentration 5 μg/ml) results in an immediate increase in the titratable acidity production. Their ability to continue to grow when the pH of the medium is less than 6.0 is also recovered (Fig. 6).

The effect of cholesterol replenishment on intracellular K content was tested on cells resuspended in the phosphate buffer medium (see Materials and Methods). Cholesterol uptake by adapted PG3 whole cells is an energy-independent process which allows incorporation of up to 200 μg cholesterol/mg membrane protein. This is achieved without any change in cellular volume, as demonstrated by the absence of concomitant change in the absorbance of the suspension. The morphology of the cells is also unchanged when examined under electron microscopy (data not shown). As illustrated in Fig. 7, an increase in the intracellular K level is associated with the uptake of cholesterol by cells. Intracellular K values obtained after recovery (35—40 μg K/mg cell protein) are comparable to those of native organisms at the end of the early log phase.

Uptake of cholesterol on isolated membranes to a level comparable to that of native cells (80 μg cholesterol/mg membrane protein) does not induce a change in the membrane-bound Mg-ATPase activity (activity ratio of cholesterol-repleted membranes to control membranes = 0.97).

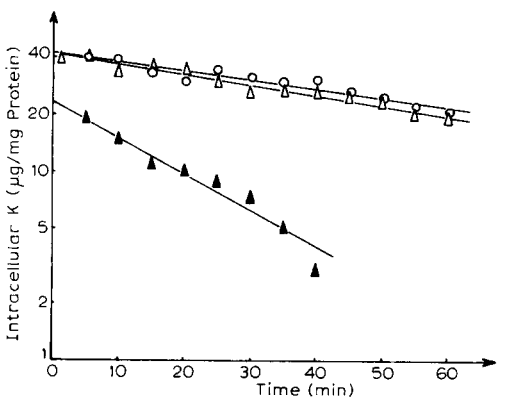

Fig. 5. Leakage of $^{42}K^+$. Cells were grown in labelled medium (1 mCi ^{42}K/l) and after harvesting and washing, resuspended in the phosphate buffer solution containing no glucose. Open symbols, native cells grown with oleic acid plus palmitic acid (○———○) or elaidic acid (△———△). Closed symbols, adapted elaidate cells.

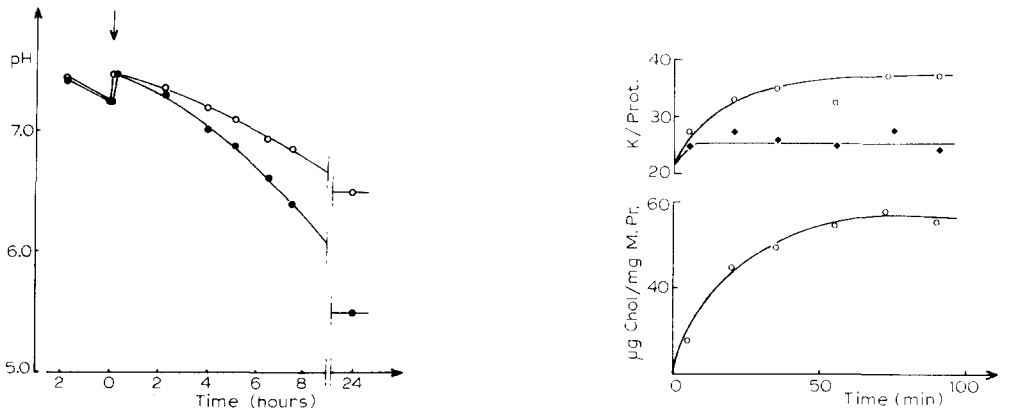

Fig.6. Recovery of acidification properties during cholesterol replenishment. At $t = 0$ (arrow) adapted growing cells were diluted by a cholesterol-containing medium (final concentration 5 µg/ml). pH of the medium (ordinate) was then assessed as a function of time in control (○———○) and cholesterol-repleted (●———●) cells.

Fig. 7. Relationship between cholesterol uptake by the membrane of adapted PG3 cells and their intracellular potassium content. Cholesterol uptake (µg/mg membrane protein, lower curve) and intracellular potassium content (µg/mg cell protein, upper curve) were measured at 37°C during replenishment experiments (see Materials and Methods). Recovery solution was constituted of the usual phosphate buffer containing fatty acid-free bovine serum albumin (2.5 g/l) plus 10 µg/ml cholesterol (○———○). ◆, control with bovine serum albumin but without cholesterol. Chol, cholesterol; M.Pr., membrane protein.

Discussion

The general characteristics of adaptation of PG3 strain to low cholesterol levels concord with those previously described by Rottem et al. [13]. In the present experiment, however, we did not observe an increase in the ratio of saturated to unsaturated fatty acids during adaptation. This is likely due to the fact that our adapted cells were harvested at the early logarithmic phase of growth. As shown by Rottem et al. [13], the value of the saturated/unsaturated ratio in young culture of adapted cells is comparable to that of native organisms grown in the presence of cholesterol (Tables V and VIII of ref. 13) for which the ratio is less variable as a function of aging.

In mycoplasmas, Arrhenius plots of the Mg-ATPase activity show a single break whose temperature, dependent on the membrane lipid composition, corresponds to the beginning of the gel → liquid crystalline phase transition of those lipids [28—30]. Above this temperature, however, neither the activation energy nor the basic activity at a given temperature seems to be affected by the lipid composition of the membrane [28—30]. Our experiments performed at 37°C, i.e., about 15°C above the onstart of the lipid-phase transition (Le Grimellec, C. and Leblanc, G., in preparation) of adapted cells, the lack of modification in the Mg-ATPase activity for the three membrane lipid compositions studied concurs with those previously reported by other groups. K accumulation and exchange by PG3 organisms is an energy-dependent process linked to the activity of a membrane-bound Mg-ATPase [14,27]. In steady-state conditions, K distribution can be accounted for by the existence of a $\Delta\Psi$ (negative inside) and most of the experimental observations can be explained in terms of

a chemiosmotic hypothesis. Present data show that intracellular K values of native strains grown with either elaidic acid or oleic plus palmitic acid are identical and follow exactly the same evolution in function of the age of culture. Thus, although some differences of the initial rate of K exchange may exist, the fatty acid composition of the membrane does not modulate the intracellular K content of native PG3 cells. None of the other physiologically controlled parameters (i.e., titratable acidity production, ability to continue to grow when pH of the medium is lowered to 5.5, generation time, etc.) are modified when changing the fatty acid composition of the growth medium.

On the other hand, reduction of cholesterol contents of the membrane to less than 2% of total lipids is associated with a large decrease in the intracellular K content whose value becomes independent of the phase of growth. This decrease appears to be compensated for by a concomitant increase in the intracellular Na content. Simultaneously, cells lose the ability to grow when the pH of the medium is lowered to 6.5 and the production of titratable acidity is reduced.

The proposal that cholesterol is really responsible for these effects is clearly substantiated by cholesterol-recovery experiments: replenishing cholesterol to adapted cells resuspended in a buffered medium results in an immediate increase of the intracellular K content. This increase occurs in the absence of any cellular volume change and correlates well with the uptake of cholesterol by the plasma membrane. Similarly, acidification properties of native cells are recovered when cholesterol is re-added to the growth medium of adapted cells. In the light of these results, we can now reconsider the decrease in intracellular K content observed during aging native PG3 organisms. Although the hypothesis of a direct effect at the level of active K transport mechanisms cannot be ruled out, it seems possible to explain the decrease of intracellular K by the concomitant decrease of the membrane cholesterol which is known to occur in aging native PG3 cells [13]. Efflux experiments show that cholesterol modulates the K leak from PG3 organisms. However, valinomycin experiments demonstrate that the effect of cholesterol on K passive permeability cannot account for the differences between intracellular K level of native and adapted cells. Thus, K conductance drastically increased by valinomycin does not induce significant changes in intracellular K level of either native [14,27] or adapted metabolizing cells. On the other hand, non-metabolizing cells rapidly lose their intracellular K in the presence of such doses of valinomycin. This observation strongly suggests that K distribution is in accordance with $\Delta\Psi$. Consequently, this $\Delta\Psi$, which is dependent on the activity of the membrane-bound Mg-ATPase (DCCD experiments and ref. 27), is slightly depressed in adapted organisms (-120 against -140 mV in native cells). In other words, a 10% decrease in $\Delta\Psi$ can account for the 50% reduction of intracellular K level. The effects of cholesterol on K must, therefore, be a result of an effect on $\Delta\Psi$. Comparison of the Mg-ATPase activities of native and adapted cells suggests that the decrease in $\Delta\Psi$ is not due to a decrease in ATPase activity. Furthermore, replenishment experiments performed on whole cells or isolated membranes indicate that an increase in intracellular K level can occur without change in the ATPase activity. In fact, the ATPase activity is only a measure of the amount of ATP split per minute. The ability to generate and maintain a proton gradient across a

membrane is also strongly dependent on the permeability of this membrane towards protons.

Several factors point to such an increase in this proton permeability as the explanation for the decrease in $\Delta\Psi$. First, cells grown in the presence of cholesterol are able to maintain a large transmembrane pH gradient, as shown by the pH value when growth stops. When cholesterol is omitted, growth stops within the pH range of 6.9—6.3, suggesting that the membrane is more permeable to protons. Secondly, the decrease in titratable acidity production by adapted cells may also be explained by an increase in the proton backflux. The third argument comes from the observation that following the addition of cholesterol to adapted cells, the acidification properties of native organisms rapidly recover. It is also conceivable that the observed increase in passive permeability of low-cholesterol cells is the expression of a more general increase in cations and, thus, H^+ permeability. Such an effect of cholesterol on the ionic permeability properties of protein-containing liposomes has already been documented [31]. Finally, Haslam and Fellows [32] recently demonstrated that the proton permeability of yeast mitochondria is a function of the membrane-lipid compositions. We therefore suggest that following the decrease in plasma membrane cholesterol, an increase in proton permeability results in and induces a 10—20% reduction of $\Delta\Psi$ via a simple partial shunt mechanism. This in turn modifies the intracellular K level.

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