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Proton Motive Force Across the Membrane of *Mycoplasma gallisepticum* and Its Possible Role in Cell Volume Regulation

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A proton motive force ($\Delta\bar{\mu}_{H^+}$) of 70 to 130 mV was measured across the membrane of *Mycoplasma gallisepticum* cells. The membrane potential was measured by utilizing the lipid-soluble cation tetraphenylphosphonium. The method was validated by showing that in the presence of valinomycin the ratio of the concentrations (in/out) of tetraphenylphosphonium agreed well with those for K^+ and Rb^+. The pH gradient was calculated from the measured distribution ratio of benzoic acid. The proton motive force was approximately the same in cells harvested at early exponential, midexponential, and stationary phases of growth. The proportion of pH gradient to membrane potential varied with external pH. In the absence of glucose, cells incubated in an isosmotic NaCl solution showed low adenosine triphosphate and $\Delta\bar{\mu}_{H^+}$ levels and a tendency to swell and lyse compared with cells incubated with added glucose. It is concluded that energy is required for normal cell volume regulation.

The structural and biochemical simplicity of mycoplasmas and their dependence on an external supply of lipids make these organisms uniquely suited for studying changes in composition and mode of organization of their membranes (19). The possibility that ion gradients have an effect on the biochemistry and physiology of mycoplasma membranes has so far received relatively little attention. Such biochemical changes have been suggested recently by Amar et al. (1), who showed that exposure of *Acholeplasma laidlawii* cells to valinomycin (which dissipates the K^+ gradient) or to carbonylcyanide *m*-chlorophenylhydrazone (which causes collapse of the proton gradient) resulted in a decreased availability of iodine-binding sites on the cell surface. The increased availability of *A. laidlawii* membrane phosphatidylglycerol to digestion by phospholipase A_2 accompanying a decrease in the energized state of the cells (2), and the marked effect of ionophores on the translocation of cholesterol from the outer to the inner half of the *Mycoplasma capricolum* cell membrane (3), further support the notion that conformational changes in membrane components may be triggered by variations in membrane potential and proton gradients. A possible role of Na^+ gradients in physiological phenomena such as cell volume regulation has been suggested by studies of Jinks et al. (6).

Investigation of these phenomena, however, has been hampered by the dearth of information available on ion gradients in mycoplasmas. Measurements of cellular K^+ (7-9) and Na^+ (7) have been recorded, and the energy dependence of these ion gradients was suggested (7-9). A few recent studies of the membrane potential in these organisms were performed, using a valinomycin technique (22) and potential-sensitive cyanine dyes (20, 21). In this work the total proton motive force was measured in *Mycoplasma gallisepticum* cells; the distribution of a lipid-soluble cation to determine the membrane potential and the accumulation of a weak acid for the measurement of the pH gradient were studied. The possible roles of H^+ and Na^+ gradients in the regulation of cell volume in this organism are discussed.

MATERIALS AND METHODS

Growth of the organisms. *M. gallisepticum* (strain A5969) was grown in 0.5-liter volumes of Edward medium (15) containing 4% horse serum. The cultures were inoculated with 1% of an overnight culture and incubated vertically at 37°C for 18 to 25 h. Growth was followed by measuring the absorbance at 640 nm (A_{640}) and pH changes of the culture. Most experiments were performed with cultures at the midexponential phase of growth (A_{640} = 0.2 to 0.25; pH = 6.9 to 6.5). Cells were harvested by centrifugation at 12,000 × *g* for 10 min, washed once, and suspended in 0.225 M NaCl containing 0.05 M *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES)-2-*N*-morpholineethanesulfonic acid (MES) buffer mixtures adjusted to various pH values with NaOH.

Measurement of cell water. Cells at a final con-

centration of about 2 mg of cell protein per ml were incubated in 2.3 ml of a solution containing NaCl (225 mM), TES buffer (50 mM; pH 7), ^{3}H-labeled water (4.3 μCi/ml), and [^{14}C]inulin (0.4 μCi/ml; 100 μg/ml). Glucose (5 to 10 mM) was added unless otherwise indicated. After incubation at 37°C (for various time intervals), 1-ml samples in duplicate were pipetted onto the surface of silicone oil (0.5 ml) in 1.5-ml plastic Microfuge tubes and centrifuged at 12,800 × *g* for 2 min. Under these conditions, the cells pass through the silicone oil and form a pellet at the bottom of the tube. The aqueous phase remains above the oil. Samples of the supernatant fluid were taken for analysis. Both the aqueous phase and oil were removed by suction, and the tip of the plastic centrifuge tube (containing the cell pellet) was cut off with a razor blade and placed in a vial containing 10 ml of scintillation fluid (13). With vigorous mixing on a Vortex vibrator, the cell pellet was freed from the tip of the centrifuge tube and broken into small fragments. ^{3}H-labeled water was used to measure total pellet water. It was assumed that inulin (molecular weight, 5,000) did not penetrate the cell and could be used to measure extracellular fluid in the cell pellet. The inulin space was approximately 20% of the total pellet water. The water space minus the inulin space was taken as the intracellular water space.

Measurement of ΔpH. Cells at a final concentration of about 2 mg of cell protein per ml (final volume, 2.3 ml) were incubated in a solution containing NaCl (225 mM), TES buffer (50 mM; pH 7), and ^{14}C-labeled benzoic acid (0.04 μCi/ml; 0.4 μg/ml). After incubation at 37°C for various time periods, 1-ml samples in duplicate were pipetted onto the surface of silicone oil in 1.5-ml plastic Microfuge tubes and treated as described above. Intracellular and extracellular concentrations of benzoic acid were determined, and the pH gradient was calculated (12).

Measurement of Δψ. For the measurement of membrane potential, cells were exposed to radioactive tetraphenylphosphonium (TPP^+), a lipid-soluble cation that distributes across the cell membrane according to the electrical potential difference. Three separate measurements were made. In the first tube, cells were incubated in 0.225 M NaCl–0.05 M TES buffer (pH 7) plus TPP^+ for various time periods at 37°C. Cells were separated from the medium by centrifugation through silicone oil as described above, and the TPP^+ content was measured. The values for cell uptake were corrected for binding by two additional measurements. In a second tube, the TPP^+ uptake was measured in cells incubated in the same medium containing KCl plus valinomycin. The actual membrane potential obtained in the presence of KCl and valinomycin was determined in a third tube containing buffer, KCl, and valinomycin plus $^{86}Rb^+$. The rubidium ratio (in the presence of valinomycin) was taken as a measure of membrane potential, assuming that the Nernst equation was applicable. Consistent with this view was the finding that under these conditions K^+(in)/K^+(out) equaled Rb^+(in)/Rb^+(out). In most experiments, this ratio was approximately 2 (18 mV) in the absence of glucose and 3 (29 mV) in the presence of glucose.

In a typical experiment, cells at a final concentration of 2 mg of cell protein per ml were exposed to [^{3}H]-TPP^+ (0.03 μCi/ml; 87 μM), glucose (5 mM), NaCl (225 mM), and TES buffer (50 mM), pH 7. A second tube contained the same as the first tube plus KCl (117 mM) and valinomycin (2 μM). A third tube contained the same as the second tube minus TPP^+ but with added $^{86}Rb^+$ (1 μCi/ml; 22 μM). The $^{86}Rb^+$ in/out ratio was used to calculate the membrane potential in the presence of valinomycin (usually 29 mV). This value was used to evaluate the fraction of TPP^+ bound and free in the cells exposed to this lipid-soluble cation in the presence of valinomycin. Finally, the value for bound TPP^+ was subtracted from the total TPP^+ in the pellet of the first incubation to give the concentration of free TPP^+, and the membrane potential was calculated from the Nernst equation. Validation of this method is shown in Fig. 1.

ATP measurement. A sample of cells (0.4 ml) was placed in a test tube containing 0.1 ml of 3 M perchloric acid. After 10 min at 0°C, 0.3 ml of 1 M KOH was added, and the potassium perchlorate was allowed to settle. Samples were taken, and the ATP level was determined by the luciferase method of Cole et al. (4).

Osmotic stability measurements. The swelling of *M. gallisepticum* cells was determined spectrophotometrically. The washed-cell suspensions were diluted in the various suspending media to A_{500} = 1.0 to 2.5. The cell suspensions were then incubated at 37°C for up to 4 h, and cell swelling was followed by measuring the A_{500}. Results were expressed as the change in the percentage of initial absorbance with time. More sensitive determinations of volume changes were made

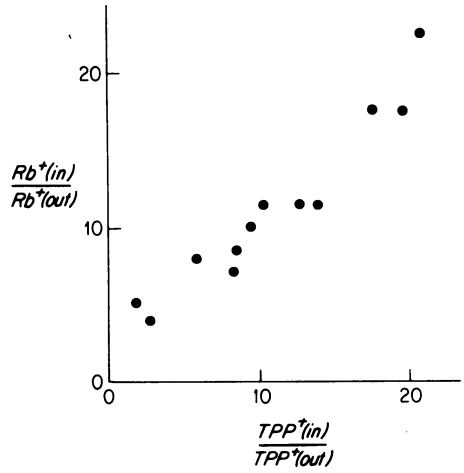

FIG. 1. *Comparison of Rb^+ and TPP^+ ratios at different membrane potentials. Each tube contained cells (2 mg of protein per ml) suspended in 224 mM TES buffer (neutralized to pH 7.1 with 134 mM tetramethylammonium hydroxide) plus 11 mM glucose, 2 μM valinomycin, and different concentrations of K^+ (2 to 100 mM). Two tubes were incubated for each K^+ concentration; one contained the above solution plus [^{3}H]TPP^+ (0.03 μCi/ml; 87 μM), and the other contained the above solution plus $^{86}Rb^+$ (1 μCi/ml; 22 μM). Incubation was for 10 min at 37°C. The data given were from three separate experiments.*

by measuring intracellular water by the Microfuge method previously described.

Chemicals. [^{3}H]TPP$^+$ was a generous gift from Ronald Kaback. ^{3}H-labeled water, [^{14}C]inulin, and ^{86}Rb$^+$ were from New England Nuclear Corp. Inulin was purified by either paper chromatography or dialysis to remove traces of fructose.

RESULTS

The first series of experiments was designed to determine whether TPP$^+$ could be used to measure membrane potential in *M. gallisepticum*. As Libermann and Skulachev (10) have demonstrated, several lipid-soluble cations may be utilized to measure membrane potential in bacterial and animal cells. They showed that such lipid-soluble cations diffuse across the membrane and accumulate within the cell to an extent expected from the Nernst relationship. In this study, a comparison was made between the accumulation ratio of TPP$^+$ and Rb$^+$ in the presence of valinomycin. Since valinomycin makes the membrane extremely permeable to Rb$^+$ (and K$^+$), the membrane potential ($\Delta\psi$) may be readily measured by applying the Nernst equation to the experimentally determined Rb$^+$ (in)/Rb$^+$ (out) ratio. The $\Delta\psi$ was varied by exposing cells to various concentrations of external K$^+$ in the presence of valinomycin. The distribution ratio for TPP$^+$ agreed well with that for Rb$^+$ (correlation coefficient, 0.95) (Fig. 1). In addition, the K$^+$ ratios (determined by flame photometry) agreed well with the Rb$^+$ ratios (data not shown). It was concluded that the TPP$^+$ method was satisfactory for the measurement of membrane potential in these cells.

Membrane potential and pH gradient were measured across the membranes of cells at different phases of growth. Cells in the early (16 h, A_{640} = 0.10), mid- (20 h, A_{640} = 0.22), and late (24 h, A_{640} = 0.34) exponential phases exhibited very similar values for $\Delta\psi$ (47 to 50 mV), ΔpH (30 to 36 mV), and $\Delta\bar{\mu}_{H^+}$ (77 to 84 mV) at pH 6.5 (three experiments).

The effect of external pH on the proton motive force is shown in Fig. 2. The ΔpH was greatest at pH 5.5 and least at pH 8. The total proton motive force showed a maximum value of about 130 mV at pH 6.5.

In the absence of added glucose, the intracellular ATP concentration fell within 15 min to less than 5% that of cells incubated with glucose at 37°C. During this period the pH gradient fell to zero, whereas the electrical gradient was maintained (Table 1). Longer incubation periods of up to 4 h (data not shown) without glucose resulted in a progressive fall in $\Delta\bar{\mu}_{H^+}$ and ATP levels and a residual $\Delta\psi$ of approximately 15 mV. This residual membrane potential is probably

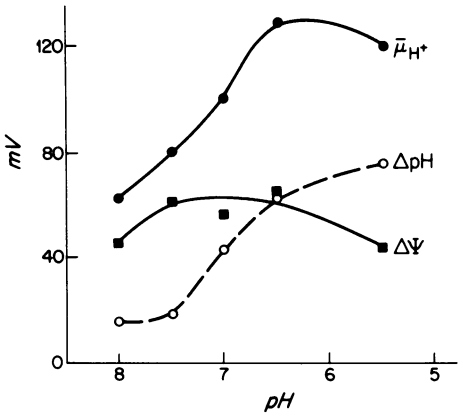

FIG. 2. *Effect of external pH on the proton motive force of M. gallisepticum. Cells (2 mg of cell protein per ml) were suspended in 0.25 M NaCl containing 0.05 M TES–0.05 M MES buffer mixtures adjusted to various pH values with NaOH. The determinations of ΔpH and $\Delta\psi$ were carried out by the procedure described in the text. The total proton motive force ($\Delta\bar{\mu}_{H^+}$) is the sum of $\Delta\psi$ and 59 ΔpH. The data given represent the mean of two experiments.*

due primarily to the Donnan equilibrium. Associated with these changes was a progressive increase in cell volume from 1.76 to 1.93 μl of cell water per mg of protein (Table 1). A total of 75 min after the addition of glucose, the ATP and $\Delta\bar{\mu}_{H^+}$ fell, and the cells increased in volume from 1.55 to 1.67 μl of cell water per mg of protein. Incubation of cells in the presence of glucose plus DCCD (200 μM) resulted in low levels of $\Delta\bar{\mu}_{H^+}$ but high ATP levels (data not shown). Measurements of these cells were quite variable due to the tendency of cells to lyse (see below).

Volume changes of cells. Marked swelling and lysis was observed when cells were suspended for several hours in 250 mM NaCl, KCl, choline chloride, or tetraethylammonium chloride in the absence of glucose at 37°C. The tendency to swell in NaCl was the greatest with stationary-phase cells and the least in early-log-phase cells (Fig. 3). The swelling of cells in NaCl was reversed by the addition of glucose (Fig. 4). In the absence of glucose, cells exposed to 500 mM sucrose (plus 10 mM NaCl) showed no swelling during 5 h of incubation (data not shown). Cells exposed to 240 mM sucrose and 130 mM NaCl swelled at a significantly slower rate than those exposed to 250 mM NaCl (data not shown). Addition of DCCD (0.1 to 1.0 mM) in the presence of NaCl caused a very rapid swelling and lysis of the cells even in the presence of glucose (Fig. 4).

Various solutes were tested in an attempt to prevent the DCCD-induced lysis of the cells.

TABLE 1. *Correlation of ATP level, proton motive force, and cell volume in M. gallisepticum*[a]

Glucose (mM)	Incubation time (min)	No. of expt	Cell water per mg of protein (μl)	Δψ (mV)	ΔpH (mV)	$\Delta\bar{\mu}_{H^+}$ (mV)	ATP (mM)
0	15	7	1.76	35	0	35	0.13
0	75	4	1.93	15	0	15	0.03
5	15	7	1.55	42	28	70	4.03
5	75	4	1.67	32	16	48	0.93

[a] Cells were grown in Edward medium (15) containing 5% horse serum to the midexponential phase of growth. Cells were incubated in 0.225 M NaCl–0.05 M TES buffer (pH 7.1) for the indicated times at 37°C. The total proton motive force ($\Delta\bar{\mu}_{H^+}$) is the sum of ΔpH and Δψ. The ATP level and cell volume were determined as described in the text.

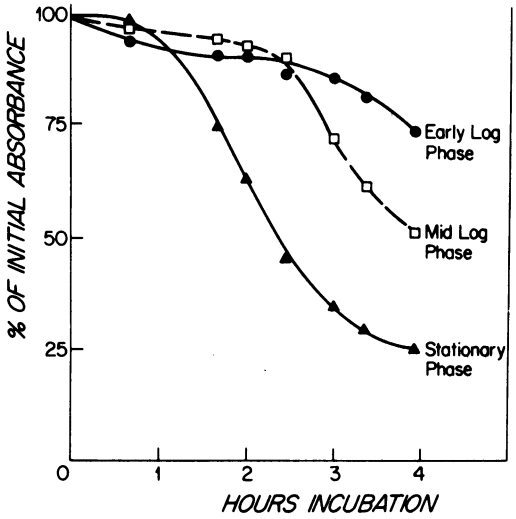

FIG. 3. *Swelling of M. gallisepticum harvested at various growth phases. Cells were grown in Edward medium (15) for 18 h, early exponential-phase cells (A_{640} = 0.08; final pH of medium, 7.6); 22 h, midexponential-phase cells (A_{640} = 0.23; pH, 6.9); and 26 h, stationary-phase cells (A_{640} = 0.38; pH, 5.7). The cells were harvested, washed once, and suspended in 0.25 M NaCl–10 mM TES buffer (pH 7.1) to a final A_{500} of about 1.0. The cells were then incubated at 37°C, and the change in absorbance of the cell suspension was monitored.*

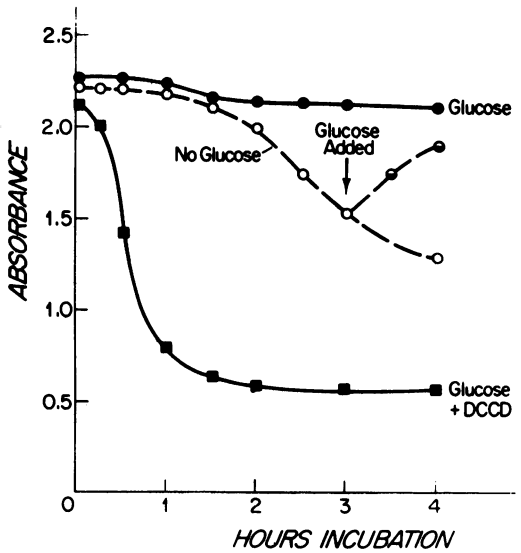

FIG. 4. *The effect of glucose and DCCD on the swelling of M. gallisepticum. Washed midexponential-phase cells (A_{640} = 0.27) were incubated at 37°C in 0.25 M NaCl solution containing 10 mM TES buffer (pH 7.0) with (●) or without (○) 10 mM glucose or 1 mM DCCD plus 10 mM glucose (■). At various time intervals the absorbance of the cell suspension was monitored at 500 nm. Glucose was added (◒) to half of the glucose-free suspension, and incubation was continued for 1 h. The remaining glucose-free suspension (○) was kept in incubation for 1 h.*

Substitution of most of the NaCl by KCl, choline chloride, taurine, or tetraethylammonium chloride failed to prevent swelling. Slight protection was provided by TES buffer. The most striking protection was provided by sucrose (Fig. 5). *M. gallisepticum* cells suspended in 500 mM sucrose (plus 10 mM NaCl) and incubated at 37°C showed no significant decrease in absorbance for several hours. In the presence of sucrose, DCCD had no effect on swelling during the first 30 min, and subsequent swelling was less than that observed in cells suspended in NaCl plus DCCD. This experiment is consistent with the view that DCCD does not significantly alter the permeability barrier per se.

DISCUSSION

All mycoplasmas tested so far, including *M. gallisepticum*, possess a membrane-bound ATPase (14, 16 ,17), suggesting an important role in cell function. Although most of the bacterial ATPases resemble the proton-translocating ATPases of mitochondria and chloroplasts (5), ATPase of *A. laidlawii* is quite different. Jinks et al. (6) have found that this enzyme is stimulated fourfold by Na^+ but unaffected by K^+ or by ouabain. It cannot be removed from the membrane by low ionic buffers or EDTA and is less sensitive to DCCD than bacterial ATPases.

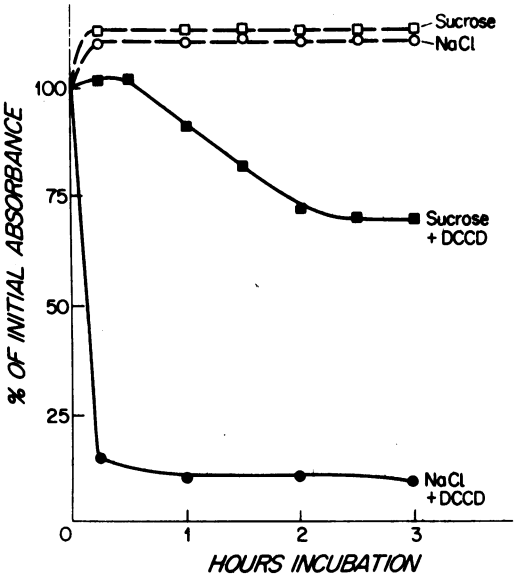

FIG. 5. *The effect of sucrose on the swelling of M. gallisepticum cells induced by DCCD. Washed mid-log-phase cells (A_{640} = 0.20) were incubated at 37°C in 0.25 M NaCl or 0.5 M sucrose solutions (plus 10 mM NaCl) with or without 0.2 mM DCCD. All solutions contained glucose (10 mM) and TES buffer (10 mM; pH 7.1). The initial densities of the cell suspensions were A_{500} = 0.9 for the sucrose suspensions and A_{500} = 1.2 for the NaCl suspensions. The absorbance of the cell suspensions was monitored at 500 nm.*

Jinks et al. (6) suggest that ATPase of *A. laidlawii* is an ion pump for the extrusion of Na^+ and perhaps K^+. This would be consistent with the observation that addition of glucose to starved cells of *Mycoplasma mycoides* leads to extrusion of Na^+ from the cells (7). In addition to the Na^+ and K^+ gradients, there is also an electrochemical potential difference for protons across the membrane of *Mycoplasma* species. In this study, proton motive force values as high as 129 mV were observed at pH 6.5, with a pH gradient of one unit (inside alkaline). The measurements of $\Delta\psi$ performed by using the lipophilic ion TPP^+ agreed well with those obtained by Schummer et al. (20, 21) with a fluorescent dye. Addition of glucose resulted in high intracellular ATP levels and a sustained proton motive force. Incubation of cells in the absence of glucose led to a rapid fall in both ATP and ΔpH levels, but a much slower fall in the $\Delta\psi$ level. Clearly, several energy-dependent ion gradients are present, although the exact mechanism of their formation is not yet clear.

One of the important physiological functions of ion pumping in cells without cell walls is volume regulation. In the absence of such pumps solute (usually NaCl) and water enter the cell due to the colloid osmotic effect of intracellular macromolecules (23). In animal cells, the action of the sodium potassium ATPase extrudes Na^+ electrogenically (with Cl^- passively following), and the cells shrink due to the associated osmotic movement of water (11). In *Mycoplasma* species the ionic pumping events are apparently different since the ouabain-sensitive Na-K ATPase is not present in these organisms. An alternative mechanism for Na^+ extrusion found in many microorganisms is the pumping of protons via the H^+-ATPase and extruding Na^+ by means of the H^+-Na^+ exchange carrier. An additional possibility is the suggestion by Jinks et al. (6) that *Mycoplasma* species possess a ouabain-insensitive Na^+-translocating ATPase.

Our studies represent the first stage of an investigation into the role of cations in volume regulation of *M. gallisepticum*. The swelling of energy-depleted cells when suspended in NaCl is prevented by addition of glucose, which clearly indicates an energy requirement for cell volume regulation, in agreement with the results of Jinks et al. with *A. laidlawii* (6). Stimulation of the rate of swelling by 100 μM DCCD is consistent with blockage of the membrane ATPase by the inhibitor. Whether this ATPase transports protons followed by Na^+-H^+ exchange or whether it transports Na^+ remains to be determined. In either case an energy-dependent process extrudes the NaCl and water which enters the cell by diffusion, thus regulating its cell volume.

The process of aging in *M. gallisepticum* cells is accompanied by an increase in the tendency of the cells to lyse in 250 mM NaCl in the absence of glucose (Fig. 3). This manifestation of aging may be associated with alterations in the composition and physical properties of the cell membrane (18, 19). Accordingly, membrane lipid content was found to decrease most markedly upon aging of *M. gallisepticum* cells, resulting in a significantly higher protein-to-lipid ratio and reduced fluidity (18). Such changes may result in altered ion permeability or decreased transport activity (16, 17). An alternative explanation for the observed effects of aging on swelling is an age-associated decrease in the endogenous energy reserves necessary for maintenance of cell volume.

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LITERATURE CITED

1. **Amar, A., S. Rottem, and S. Razin.** 1978. Disposition of membrane proteins as affected by changes in the

electrochemical gradient across mycoplasma membranes. Biochem. Biophys. Res. Commun. **84**:306–312.

2. **Bevers, E. M., G. Leblanc, C. Le Grimellec, J. A. F. Op Den Kamp, and L. L. van Deenen.** 1978. Disposition of phosphatidylglycerol in metabolizing cells of *Acholeplasma laidlawii*. FEBS Lett. **87**:49–51.
3. **Clejan, S., R. Bittman, and S. Rottem.** 1978. Uptake, transbilayer distribution, and movement of cholesterol in growing *Mycoplasma capricolum* cells. Biochemistry **17**:4579–4583.
4. **Cole, H. A., J. W. T. Wimpenny, and D. E. Hughes.** 1967. The ATP pool in *Escherichia coli*. I. Measurement of the pool using a modified luciferase assay. Biochim. Biophys. Acta **143**:445–453.
5. **Downie, J. A., F. Gibson, and G. B. Cox.** 1979. Membrane adenosine triphosphatases of prokaryotic cells. Annu. Rev. Biochem. **48**:103–131.
6. **Jinks, D. C., J. R. Silvius, and R. N. McElhaney.** 1978. Physiological role and membrane lipid modulation of the membrane-bound (Mg^{2+}, Na^+)-adenosine triphosphatase activity in *Acholeplasma laidlawii*. J. Bacteriol. **136**:1027–1036.
7. **Leblanc, G., and C. Le Grimellec.** 1979. Active K^+ transport in *Mycoplasma mycoides var. capri* net and unidirectional K^+ movements. Biochim. Biophys. Acta **554**:156–167.
8. **Leblanc, G., and C. Le Grimellec.** 1979. Active K^+ transport in *Mycoplasma mycoides var. capri*. Relationships between K^+ distribution, electrical potential and ATPase activity. Biochim. Biophys. Acta **554**:168–179.
9. **Le Grimellec, C., and G. Leblanc.** 1978. Effect of membrane cholesterol on potassium transport in *Mycoplasma mycoides var. capri* (PG3). Biochim. Biophys. Acta **514**:152–163.
10. **Liberman, E. A., and V. P. Skulachev.** 1970. Conversion of biomembrane-produced energy into electrical form. IV. General discussion. Biochim. Biophys. Acta **216**:30–42.
11. **Macknight, A. D. C., and A. Leaf.** 1978. Regulation of cellular volume, p. 315–334. *In* T. E. Andreoli, J. F. Hoffman, and D. D. Fanestil (ed.), Physiology of membrane disorders. Plenum Publishing Corp., New York.
12. **Maloney, P. C., E. R. Kashket, and T. H. Wilson.** 1975. Methods for studying transport in bacteria, p. 1–49. *In* E. Korn (ed.), Methods in membrane biology, vol. 5. Plenum Publishing Corp., New York.
13. **Patterson, M. S., and R. C. Greene.** 1965. Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. Anal. Chem. **37**:854–857.
14. **Pollack, J. D., S. Razin, and R. C. Cleverdon.** 1965. Localization of enzymes in mycoplasma. J. Bacteriol. **90**:617–622.
15. **Razin, S., and S. Rottem.** 1976. Techniques for the manipulation of mycoplasma membranes, p. 3–26. *In* A. M. Maddy (ed.), Biochemical analysis of membranes. Chapman and Hall, New York.
16. **Razin, S.** 1978. The mycoplasmas. Microbiol. Rev. **42**: 414–470.
17. **Rottem, S., and S. Razin.** 1966. Adenosine triphosphatase activity of mycoplasma membranes. J. Bacteriol. **92**:714–722.
18. **Rottem, S., G. M. Slutzky, and R. Bittman.** 1978. Cholesterol distribution and movement in the *Mycoplasma gallisepticum* cell membrane. Biochemistry **17**: 2723–2726.
19. **Rottem, S.** 1980. Membrane lipids of mycoplasmas. Biochim. Biophys. Acta **604**:65–90.
20. **Schummer, U., H. G. Schiefer, and U. Gerhardt.** 1978. Mycoplasma membrane potential determined by a fluorescent probe. Hoppe Seyler's Z. Physiol. Chem. **359**: 1023–1025.
21. **Schummer, U., H. G. Schiefer, and U. Gerhardt.** 1979. Mycoplasma membrane potentials determined by potential-sensitive fluorescent dyes. Curr. Microbiol. **2**: 191–194.
22. **Schummer, U., H. G. Schiefer, and U. Gerhardt.** 1980. A novel method for the determination of electrical potentials across cellular membranes. II. Membrane potentials of acholeplasmas, mycoplasmas, streptococci and erythrocytes. Biochim. Biophys. Acta **600**:998–1006.
23. **Wilson, T. H.** 1954. Ionic permeability and osmotic swelling of cells. Science **120**:104–105.