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Effects of Nigericin and Monactin on Cation Permeability of *Streptococcus faecalis* and Metabolic Capacities of Potassium-depleted Cells

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At a concentration of 10^{-6} M, nigericin and monactin inhibited growth of *Streptococcus faecalis*, and the inhibition was reversed by addition of excess K^+. In the presence of certain antibiotics, the cells exhibited increased permeability to certain cations; internal Rb^+ was rapidly lost by exchange with external H^+, K^+ Rb^+, and, more slowly, with Na^+ and Li^+. No effect was observed on the penetration of other small molecules. Cation exchanges induced by nigericin and monactin were metabolically passive and apparently did not involve the energy-dependent K^+ pump. When the cells were washed, the cytoplasmic membrane recovered its original impermeability to cations. By use of monactin, we prepared cells whose K^+ content had been completely replaced by other cations, and the metabolic characteristics of K^+-depleted cells were studied. Cells containing only Na^+ glycolyzed almost as well as did normal ones and, under proper conditions, could accumulate amino acids and orthophosphate. These cells also incorporated ^{14}C-uracil into ribonucleic acid but incorporation of ^{14}C-leucine into protein was strictly dependent upon the addition of K^+. When K^+ or Rb^+ was added to sodium-loaded cells undergoing glycolysis, these ions were accumulated by stoichiometric exchange for Na^+. From concurrent measurements of the rate of glycolysis, it was calculated that one mole-pair of cations was exchanged for each mole of adenosine triphosphate produced.

Nigericin is an antibiotic of unknown structure first described by Harned et al. (15). It inhibits growth of a number of gram-positive bacteria and is specifically antagonized by the addition of K^+ to the medium. Nigericin produces complex effects on mammalian mitochondria, including inhibition of respiration, release of cations, and unmasking of adenosine triphosphatase activity (9, 12, 28; S. Estrada-O, S. N. Graven, and H. L. Lardy, Federation Proc., p. 610, 1967). According to Graven et al. (12), the primary site of action of nigericin may be the cation-translocating mechanism.

Monactin and its homologues are also thought to act at the level of cation transport in mitochondria. The "nactins" are macrocyclic antibiotics of defined structure (5) which also inhibit various gram-positive bacteria (23). The "nactins" induce K^+ uptake and H^+ ejection in mitochondria and uncouple oxidative phosphorylation (13, 14). Their physiological effects are similar to those of the polypeptide antibiotics valinomycin and gramicidin (6, 20, 26).

The present report is concerned with the effects of nigericin and monactin on *Streptococcus faecalis*, an organism which relies entirely on glycolysis for the generation of metabolic energy. We show that the mode of action of nigericin and monactin is similar to that previously described for valinomycin and gramicidin (16). Nigericin and monactin markedly increase the permeability of the cytoplasmic membrane to cations, and K^+ is lost from the cells, resulting in inhibition of growth which can be reversed by addition of excess K^+. However, unlike gramicidin or valinomycin, nigericin and monactin are readily removed by washing the cells, and washed cells recover their original impermeability to cations. This property of the washed cells afforded an opportunity to prepare cells whose K^+ was totally replaced by other cations, and some studies on the metabolic characteristics of such K^+-depleted cells are described.

MATERIALS AND METHODS

Since most of our procedures were described in earlier papers (16-19), a brief summary will suffice here.

Growth media. S. faecalis strain 9790 was usually grown on defined media, and both K^+ and Rb^+ supported growth (19). Because of the technical convenience of using ^{86}Rb as an isotope of Rb^+, cells grown on Rb^+ were used whenever possible. Glucose served as an energy source, and growth was followed by turbidimetry at 600 mμ. In all the experiments described here, the cells were grown in medium NaM (19). This medium was buffered with sodium maleate (200 to 350 mM Na^+), and it contained variable amounts of K^+ or Rb^+.

Cation content. Loading with ^{86}Rb was accomplished by growing or incubating the cells for several hours in medium containing 10 mM ^{86}Rb of known specific activity. Under these conditions, the cells exchanged internal Rb^+ for ^{86}Rb (19); at equilibrium the radioactivity of the cells was a measure of their Rb^+ content. Samples were harvested by filtration on filters (Millipore Corp., Bedford, Mass.) and were washed with water or 2 mM $MgCl_2$. The radioactivity of the samples was determined, and, when desired, K^+, Na^+, and Rb^+ were extracted and estimated by flame photometry.

Uptake of radioactive substrates. Cells were prepared as described for the individual experiments, and radioactive substrates of known specific activity were added. At intervals, samples were withdrawn, washed with 2 mM $MgCl_2$, and counted. To measure incorporation of ^{14}C into macromolecules, the cells were extracted first with cold 5% perchloric acid and then with 10% perchloric acid at 95 C. Incorporation of ^{14}C-uracil into the fraction insoluble in cold acid was taken as a measure of ribonucleic acid (RNA) synthesis, and incorporation of ^{14}C-leucine into the final residue was taken as a measure of protein synthesis.

Adenosine triphosphate (ATP) content. Samples containing 5 mg of cells were collected by filtration, washed, and extracted with sulfuric acid (10). ATP was assayed by the firefly luminescence method, as modified by Forrest and Walker (10).

Preparation of sodium-loaded cells. S. faecalis was grown on medium NaM containing 10 mM ^{86}Rb. The cells were harvested during exponential growth and were washed and resuspended in 0.1 M sodium maleate, *p*H 8. Monactin (2 μg/ml) was added, and the suspension was incubated at 37 C for 20 min. The cells were then collected by filtration, washed twice with sodium maleate and three times with water, and resuspended as desired. Displacement of Rb^+ by Na^+ was monitored by loss of radioactivity, which routinely attained 99% or better.

Other procedures. Glycolysis was monitored at constant *p*H by automatic titration of the lactic acid produced with a radiometer *p*H-stat. In a few experiments, uptake of H^+ by the cells was likewise followed by automatic addition of standardized HCl at constant *p*H. Metabolism of arginine was followed by automatic titration of NH_3 produced by cells previously grown on arginine (31).

Antibiotics. Nigericin was donated by R. L. Harned, and monactin was a gift from W. Keller-Schierlein. The latter sample contained minor amounts of nonactin and traces of the higher homologues. Since the known effects of these antibiotics are similar to those of monactin, their presence was ignored. The antibiotics were dissolved in ethyl alcohol, and control suspensions always received a volume of ethyl alcohol equal to that of the experimental suspensions (usually 1% by volume).

RESULTS

Inhibition of growth by monactin and nigericin. Addition of the antibiotics at concentrations of 1 to 2 × 10^{-6} M (1 to 2 μg/ml) to cells growing in medium NaM brought about a cessation of growth. The inhibition could be reversed by addition of excess K^+ (Fig. 1). Inhibition of growth was associated with rapid loss of K^+ or Rb^+ from the cells, suggesting that nigericin and monactin interfere with the selective accumulation of these cations.

Induction of cation exchanges by nigericin and monactin. Cells of *S. faecalis* loaded with K^+ or ^{86}Rb retain these cations tenaciously when suspended in solutions of various salts in the absence of a source of energy. However, low concentrations of nigericin or monactin induced rapid loss of ^{86}Rb from the cells by exchange for external cations. As shown in Table 1, the exchanges were quite selective, and K^+, Rb^+, and Cs^+ exchanged more rapidly than did the other cations. The induction of cation exchanges was also strongly dependent upon the temperature. No cation exchanges were induced at 0 C under the conditions listed in Table 1.

Nigericin and monactin also induced a loss of K^+ and ^{86}Rb by exchange for H^+. When antibiotics were added to a suspension of cells in water, the *p*H rose, and considerable amounts

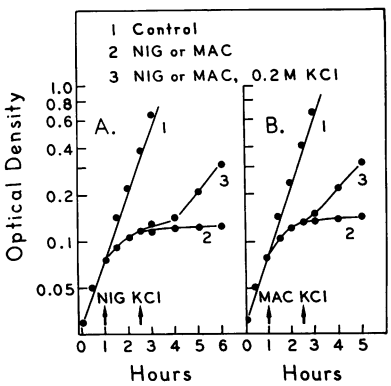

FIG. 1. *Effect of nigericin and monactin on growth of Streptococcus faecalis. (A) An exponentially growing culture in medium NaM was divided (arrow) and the following additions were made: (i) control, ethyl alcohol only; (ii) nigericin (NIG), 1 μg/ml; (iii) nigericin, followed by 200 mM KCl at 2.5 hr. (B) An analogous experiment with monactin (MAC) at a concentration of 2 μg/ml.*

TABLE 1. *Release of ^{86}Rb from Streptococcus faecalis treated with nigericin or monactin*[a]

Medium	Percentage of ^{86}Rb retained		
	Nigericin	Monactin	Ethyl alcohol only
Water	100	96	100
LiCl, 10 mM	93	69	94
NaCl, 10 mM	92	42	99
KCl, 10 mM	29	8	98
RbCl, 10 mM	29	8	96
CsCl, 10 mM	82	32	93
$MgCl_2$, 10 mM	76	70	98
NH_4Cl, 10 mM	21	22	99

[a] *S. faecalis* cells were loaded with ^{86}Rb, as described in the text. The cells were washed and resuspended in the various solutions at 0.6 mg/ml (*p*H 8). Nigericin (1 μg/ml final concentration), monactin (2 μg/ml final concentration), or ethyl alcohol alone (1% final concentration) were added to these suspensions. After 5 min at room temperature, 1-ml samples of the mixture were filtered and washed three times with alkaline water. The original cells washed with water contained 4,350 counts per min per mg of dry cells. This is taken as 100%, and retention of ^{86}Rb is expressed as the percentage of this substance remaining in the washed cells after exposure to certain antibiotics.

of acid were required to maintain the *p*H at 6. Under these conditions, H^+ entered the cells by stoichiometric exchange for cellular K^+ or ^{86}Rb. Because of the entry of H^+ into the cells, the antibiotics strongly inhibited glycolysis at *p*H 6 but had little effect at *p*H 8. Nigericin, a monocarboxylic acid (15), was considerably more potent at *p*H 6 than at *p*H 8.

Thus, nigericin and monactin increased the passive cation-permeability of the cytoplasmic membrane but apparently did not affect the energy-dependent transport system which mediates cation exchanges in *S. faecalis* (17, 19). This conclusion is based on the finding that the antibiotics neither inhibited nor stimulated energy-dependent uptake of K^+ from water. These experiments, which were entirely analogous to those described in detail earlier (16), were carried out at *p*H 8 on the pH-stat so as to minimize passive loss of K^+ from the cells by exchange for either H^+ or Na^+. As far as we know, the antibiotics at the concentrations employed here did not affect membrane permeability to other cellular constituents. The antibiotics did not induce leakage of amino acids or phosphate from the cells, nor did they cause lysis of protoplasts stabilized osmotically by high concentrations of disaccharides or amino acids. The ready reversal of growth inhibition by K^+ also indicated that the induction of cation permeability is the sole effect of the antibiotics at the concentrations employed.

Restoration of cation impermeability by washing. Thus far, the effects of nigericin and monactin closely resembled those described in detail (16) for gramicidin and valinomycin. However, unlike the latter antibiotics, nigericin and monactin could be readily removed from the cells by washing with buffers or with water. Washed cells recovered their original impermeability to cations (Fig. 2). If the washed cells were placed in medium of low K^+ content, growth resumed after a brief lag. At this point, it became evident that monactin and nigericin could be used to replace K^+ completely with other cations, and our interest shifted to the physiology of such K^+-depleted cells.

Metabolic capacities of K^+-depleted cells: glycolysis. By use of the procedure described in Materials and Methods, over 99% of the cellular ^{86}Rb was replaced with K^+, Na^+, Li^+, Cs^+, or NH_4^+. The cells were washed, resuspended in 10 mM solutions of the same salt (chloride), and glycolysis was followed on the pH-stat. It can be seen from Table 2 that, at *p*H 8, Na^+ and NH_4^+ supported glycolysis almost as well as did K^+, whereas Li^+ and especially Cs^+ were less effective. In most cases, glycolysis was stimulated by the addition of K^+. The rate of glycolysis at a *p*H below 7 was rather variable but was usually considerably lower than the rate of glycolysis at an alkaline *p*H. This may be due to residual traces of monactin, which render the cells somewhat more permeable to H^+ than are normal cells.

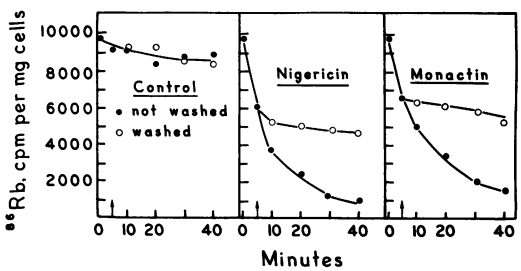

FIG. 2. *Reversal of the effects of nigericin and monactin by washing. Streptococcus faecalis was grown on medium NaM supplemented with ^{86}Rb and was harvested and resuspended at 0.6 mg/ml in 10 mM NaCl. The suspension was divided into three portions which received ethyl alcohol only, nigericin (1 μg/ml), or monactin (1 μg/ml), respectively. After 5 min, a sample of each portion was taken; the cells were filtered, washed with 10 mM NaCl, and returned to 10 mM NaCl at the original cell density. Incubation was conducted at room temperature. Samples (1 ml) of all suspensions were filtered at intervals, washed, and counted.*

TABLE 2. *Rates of glycolysis by Streptococcus faecalis loaded with various cations*[a]

Cells	Cation	Glycolysis, μmoles of H^+/g of cells(x min)
Normal	K^+	90–140
Normal	Rb^+	70–120
Monactin-treated	K^+	100–
Monactin-treated	Na^+	70–
Monactin-treated	Li^+	40–
Monactin-treated	Cs^+	4–
Monactin-treated	NH_4^+	75–

[a] Normal cells were harvested during the exponential phase of growth on medium NaM supplemented with Rb^+ or K^+. These cells contained Rb^+ or K^+ as well as small amounts of Na^+ (17, 20). Monactin-treated cells were loaded with cations, as described in Materials and Methods. The cells were washed and resuspended in 10 mM solutions of the cation present intracellularly (chloride salt). Glycolysis was followed on the *p*H-stat at room temperature (*p*H 8). Cell density was 0.6 mg/ml (dry weight).

Membrane transport. We reported previously (16) that gramicidin and valinomycin inhibit uptake of ^{32}P-P_i (radioactive orthophosphate) and ^{14}C-glutamate by cells suspended in sodium maleate buffer, and we attributed this to replacement of cellular K^+ by Na^+. To determine whether K^+ is required for membrane transport, a series of experiments was carried out with cells fully loaded with Na^+. The cells were suspended in water and the *p*H was maintained by use of the pH-stat. This procedure was adopted after we found that, for reasons which are not understood, tris(hydroxymethyl)aminomethane (Tris) buffer accentuates the K^+ requirement of Na^+-loaded cells. The results may be summarized as follows:

(i) Production of NH_3 from the basic amino acid arginine (at *p*H 6, no glucose) was independent of K^+.

(ii) Uptake of the neutral amino acids, ^{14}C-alanine and ^{14}C-glycine, at *p*H 8 was stimulated by glucose but did not require K^+. In one experiment, sodium-loaded cells were suspended in 5 mM NaCl or 5 mM KCl with 2.5×10^{-5} M ^{14}C-alanine. In both suspensions, uptake occurred to the extent of about 3 μmoles of ^{14}C-alanine per g of cells, and the addition of glucose stimulated the rate and extent of uptake fourfold. Somewhat different results were obtained at *p*H 7. At this *p*H, glucose was more effective in the presence of 5 mM K^+ than in the presence of 5 mM Na^+, and this may be due to the fact that glycolysis was also more rapid in the presence of K^+.

(iii) Accumulation of the acidic substrates, ^{32}P-P_i and ^{14}C-glutamate, required a source of metabolic energy and external cations. In both cases, 10 mM K^+ supported more rapid and extensive uptake than did 10 mM Na^+, but, at *p*H 8 or 9, 100 mM Na^+ could completely substitute for K^+ (Fig. 3). Moreover, it was observed that even cells preloaded with K^+ required either external K^+ or high concentrations of Na^+ for maximal uptake of ^{32}P-P_i. The role of K^+ in membrane transport appears to be indirect and connected with the maintenance of an electrolyte balance.

Macromolecular synthesis. Sodium - loaded cells were washed and were then incubated on the pH-stat in complete growth medium containing Na^+ as the sole cation. In agreement with the findings of Lubin and Ennis (22) with *Escherichia coli*, sodium-loaded cells readily incorporated ^{14}C-uracil into nucleic acids. Incorporation of ^{14}C-leucine into the protein fraction was, by contrast, strictly dependent upon addition of K^+ (Table 3). In the absence of K^+, ^{14}C-leucine accumulated in the acid-soluble pool, and we were unable to find conditions under which Na^+ alone would permit protein synthesis.

Stoichiometry of cation exchanges by Na^+-loaded cells. When K^+ or ^{86}Rb cations were added to Na^+-loaded cells undergoing glycolysis, these cations were accumulated, whereas stoichiometric amounts of Na^+ were extruded from the

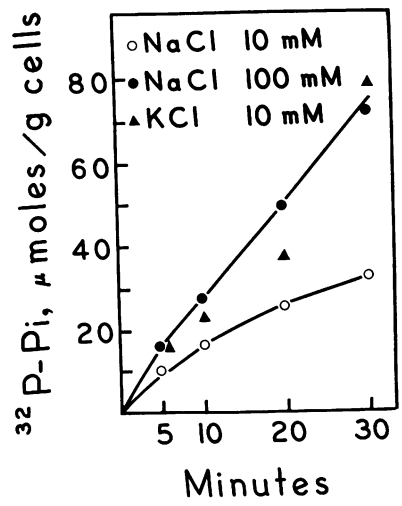

FIG. 3. *Uptake of ^{32}P-P_i by sodium-loaded Streptococcus faecalis. Sodium-loaded cells were prepared (see Materials and Methods) and suspended in water at 0.6 mg/ml. Suspensions were supplemented with KCl or NaCl, as shown, and were allowed to glycolyze at room temperature on the pH-stat at pH 9. After 5 min, ^{32}P-P_i was added to 0.2 mM, and samples were taken at intervals thereafter.*

TABLE 3. *Incorporation of ^{14}C-labeled precursors into macromolecular fractions by sodium-loaded Streptococcus faecalis*[a]

Cation supplement	Count/min per mg of cells in macromolecules	
	^{14}C-uracil into RNA	^{14}C-leucine into protein
K^+, 10 mM	3,200	2,900
Na^+, 10 mM	6,300	70
Na^+, 100 mM	2,550	42

[a] *S. faecalis* was loaded with sodium by use of monactin, as described in Materials and Methods. The cells were suspended at 0.6 mg/ml in a medium containing 5 mM sodium phosphate, 2 mM $MgCl_2$, vitamins, and 0.2 mg/ml of the amino acid mixture required for growth (29). The suspensions were incubated at 37 C, and a *p*H of 8 was maintained by use of the *p*H-stat. ^{14}C-leucine or uracil (2 μc) was added, and, after 20 min, the cells were harvested and fractionated.

cells. The exchange of cations was accompanied by stimulation of glycolysis, but, as cation exchange approached completion, the rate of glycolysis declined and returned to almost its original level. Concurrent measurements of the initial rates of glycolysis and ^{86}Rb uptake are shown in Fig. 4a. The two curves almost coincide, suggesting that approximately one mole-pair of cations was exchanged for each mole of lactic acid produced. Similarly, when arginine served as the energy source, the ratio of moles of arginine metabolized to moles of cation exchanged approached unity.

Sodium-loaded cells also took up ^{22}Na by an exchange for internal Na^+, and again the process required metabolic energy. The stoichiometry was a function of the concentration of external ^{22}Na: at 1 mM, less than 0.2 mole of ^{22}Na was taken up per mole of lactic acid produced, but, with 10 mM ^{22}Na, the ratio rose to 0.5 (Fig. 4b). These findings are consistent with our earlier observations that the apparent K_m for ^{86}Rb uptake under these conditions is about 0.15 mM, whereas the K_m for Na^+ uptake is 15 mM (17).

In view of these findings, it seemed of importance to determine whether K^+ had any obvious effect upon ATP turnover. To this end, Na^+-loaded cells were allowed to metabolize a limited amount of glucose, and ATP levels were determined at intervals. As shown in Fig. 5, cells devoid of K^+ rapidly accumulated ATP; when the glucose was exhausted, the ATP was degraded. In the presence of K^+, the cycle was somewhat shorter, as would be expected from

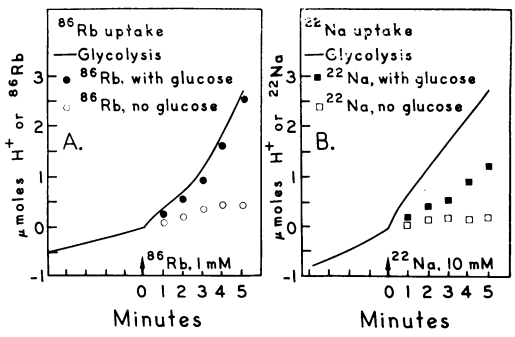

FIG. 4. *Stoichiometric relationship between glycolysis and the uptake of ^{86}Rb or ^{22}Na by sodium-loaded Streptococcus faecalis. (A) Sodium-loaded cells were prepared (see Materials and Methods), suspended in water (0.6 mg/ml), and allowed to glycolyze at room temperature on the pH-stat at pH 7.5. At 0 min, $^{86}RbCl$ of known specific activity was added (final concentration, 1 mM), and samples were taken at 1-min intervals. Glycolysis, from the trace of the pH-stat, is expressed as μmoles of H^+ produced per 20 ml of suspension; Rb^+ uptake is expressed as μmoles of ^{86}Rb per 20 ml of suspension in the cells. A control was set up in the same way with the exception that it received no glucose. (B) A parallel experiment except that 10 mM $^{22}NaCl$ was added at 0 min.*

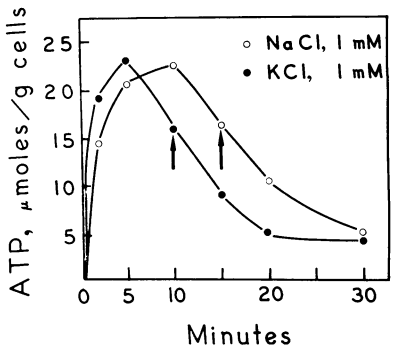

FIG. 5. *Synthesis and degradation of adenosine triphosphate (ATP) by sodium-loaded Streptococcus faecalis. Sodium-loaded cells were resuspended in water at 1 mg/ml. To 40 ml of this suspension, NaCl or KCl (to 1 mM final concentration) and 15 μmoles of glucose were added. Glycolysis was allowed to proceed at room temperature (pH 8) and was monitored by use of the pH-stat (the arrows indicate the points at which glucose was exhausted). Samples were taken at intervals and the ATP content of the cells was determined.*

the stimulation of glycolysis by K^+, but it was evident that K^+ is not essential either for the synthesis or the degradation of ATP.

DISCUSSION

The mode of action of nigericin and monactin on *S. faecalis* is very similar to the mode of action

described earlier for gramicidin and valinomycin (16). Nigericin and monactin interact with the cytoplasmic membrane and markedly increase its passive permeability to univalent cations. Consequently, in the presence of these antibiotics, internal ^{86}Rb readily exchanges with cations from the medium. Under the conditions used, the order of exchange preference in the presence of monactin is K^+, $Rb^+ > Cs^+ > Na^+ > Li^+$. In the presence of nigericin, exchange with K^+ and Rb^+ is also much more rapid than is exchange with Cs^+, Na^+, and Li^+ (Table 1). The selectivities of the cation exchanges are thus intermediate between those induced by valinomycin and by gramicidin (16). It may be noted that monactin and nigericin facilitate entry of K^+ and Rb^+ more than entry of Cs^+, even though Cs^+ has a smaller hydrated ionic radius. Mueller and Rudin (25) have considered steric factors which may account for this.

The molecular mechanisms by which certain macrocyclic antibiotics increase the cation permeability of membranes were explored by Mueller and Rudin (25) by use of artificial lipid bilayers. Gramicidin and antibiotics of the valinomycin, "nactin," and enniatin groups increased membrane conductance by many orders of magnitude. On the basis of molecular models, these investigators proposed that the antibiotics adsorb to the membrane to form artificial pores or carriers which mediate cation exchanges. The findings reported here and in our previous paper (16) are in full agreement with the model proposed by Mueller and Rudin. We would stress particularly that monactin, nigericin, valinomycin, and gramicidin increase membrane permeability to cations but not to anions. Further, the cation exchanges are metabolically passive, and no evidence could be obtained to indicate any effect on the cation pump of *S. faecalis*. The physiological effects of the antibiotics thus arise from the loss of K^+ by passive exchange for Na^+ and H^+, and these effects are reversed by addition of excess K^+ (Fig. 1).

The discovery that monactin and nigericin are readily removed by washing the cells (Fig. 2) led us to explore the metabolic capacities of *S. faecalis* cells whose K^+ was completely (99%) replaced by other cations, especially Na^+. Membrane transport was of particular interest since it has long been known that K^+ stimulates the uptake of amino acids (11, 24), sugars (1, 2), and phosphate (18, 27) in streptococci and other organisms. Our experiments were conducted at an alkaline *p*H on the pH-stat to avoid complications due to the buffer. Under these conditions, cells fully loaded with Na^+ or NH_4^+ glycolyzed nearly as well as did K^+-loaded cells (Table 2). Similar findings were previously reported by Abrams (1) for protoplasts of *S. faecalis* 9790 and by Conway and his associates for yeast (7, 8). The uptake of arginine, glycine, alanine, leucine, and uracil was not stimulated by K^+. [Mora and Snell (24) reported that K^+ stimulates the uptake of glycine and alanine by protoplasts of *S. faecalis*. Their experiments were conducted at *p*H 7.2, some of them in Tris buffer. Under these conditions, we also found that K^+ stimulates the uptake of alanine and glycine by Na^+-loaded cells. Swelling and lysis of sodium-loaded protoplasts suspended in glycylglycine buffer did not require K^+, but, even at a high *p*H, swelling of such protoplasts in sucrose was strongly stimulated by K^+ (1, 2). The role of K^+ in this phenomenon remains obscure.] Potassium did stimulate the uptake of phosphate and glutamate, but even here high concentrations of sodium could replace K^+. Thus, we conclude that K^+ does not play an *obligatory* role in membrane transport. Instead, the function of K^+ appears to be the maintenance of electrolyte balance (27, 30). The characteristics of the cation transport system of *S. faecalis* are such that K^+ is transported into the cells in preference to Na^+, whereas Na^+ and H^+ are preferentially extruded (17, 19). Thus, K^+ uptake is favored, but Na^+ can be accumulated by the cells at a high *p*H (17) and it will then replace K^+ as a counterion for phosphate and glutamate.

According to the findings of Lubin and Ennis (21, 22), the synthesis of protein uniquely requires K^+ rather than Na^+. In agreement with their observations, we found that sodium-loaded *S. faecalis* incorporated ^{14}C-uracil into RNA, but, under all conditions tried, incorporation of ^{14}C-leucine into protein was strictly dependent upon the addition of K^+ (Table 3).

When K^+ or Rb^+ cations were added to sodium-loaded cells, the normal ionic composition was rapidly restored by an energy-dependent stoichiometric exchange of Rb^+ for Na^+. To estimate the energy cost of cation exchange, we assumed that ATP is the immediate energy donor and that glycolysis yields 2 moles of ATP per mole of glucose, whereas arginine degradation yields only 1 mole of ATP. From concurrent measurements of the rates of ^{86}Rb uptake and metabolism of the energy source, it therefore appeared that up to 1 mole of cations was exchanged per mole of ATP produced (Fig. 4). These results confirm and extend the conclusion drawn by Zarlengo and Schultz (31) in relation to *S. faecalis* cells partly loaded with Na^+ and H^+.

The finding that not more than 1 mole of cations is exchanged per mole of ATP available to

the cells is open to several interpretations. It is conceivable that several moles of cations are exchanged per mole of ATP, but that only a fraction of the ATP produced by the cells is channeled toward cation transport. According to this view, it is fortuitous that the overall ratio approaches unity. However, it appears more instructive to postulate that 1 mole of ATP is required to exchange 1 mole of cations, and that Na^+-loaded cells may expend virtually all the ATP they produce on this process. (The cells studied here were not growing and presumably had only minimal energy demands for processes other than cation transport.) At the same time, let us note that ATP turnover can proceed in the absence of cation transport (Fig. 5). We can thus propose that, in the absence of external cations, ATP degradation is not coupled to transport. When external Rb^+ or Na^+ cations are added, a fraction of the ATP turnover is coupled to cation exchange, and this fraction approaches unity if the concentration of external cations is sufficient to saturate the transport system. The degradation of ATP is probably catalyzed by the adenosine triphosphatase which is localized in the cell membrane (3, 4). However, it remains to be determined whether this adenosine triphosphatase, like the membrane-bound adenosine triphosphatase found in mammalian cells, is involved in cation transport.

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