Mechanisms of Active Transport in Isolated Bacterial Membrane Vesicles

XIII. VALINOMYCIN-INDUCED RUBIDIUM TRANSPORT

(Received for publication, November 10, 1972)

FRANK J. LOMBARDI, JOHN P. REEVES,* AND H. RONALD KABACK

From The Roche Institute of Molecular Biology, Nutley, New Jersey 07110

SUMMARY

Valinomycin-induced uptake of rubidium by membrane vesicles prepared from Escherichia coli, Staphylococcus aureus, and Micrococcus denitrificans is analogous in nearly all respects to the transport of sugars or amino acids, or both, by these membrane vesicles. In E. coli membrane vesicles, concentrative rubidium uptake is stimulated maximally by D-lactate and by the artificial electron donor ascorbate-phenazine methosulfate, to a lesser extent by L-lactate, Dl-α-hydroxybutyrate, succinate, and reduced diphenopyridine nucleotide, and not at all by ATP, phosphoenolpyruvate, or a number of other metabolites. There is no direct relationship between the ability of various electron donors to stimulate rubidium uptake (ascorbate-phenazine methosulfate >> D-lactate > L-lactate > Dl-α-hydroxybutyrate > succinate > reduced diphenopyridine nucleotide) and their rates of oxidation by membrane vesicles (ascorbate-phenazine methosulfate >> reduced diphenopyridine nucleotide > succinate > D-lactate > L-lactate > Dl-α-hydroxybutyrate). In the presence of D-lactate and valinomycin, the initial rate of rubidium uptake versus the rate of D-lactate oxidation yields a ratio of nearly 2:1.

Initial rates of D-lactate-dependent rubidium uptake are saturable with respect to rubidium and valinomycin concentrations and exhibit the same temperature optimum at 50° as that observed for D-lactate oxidation. Steady state levels of rubidium accumulation vary with temperature and can be shifted readily from one steady state level to another by raising or lowering the temperature. Competitive uptake experiments indicate that rubidium and potassium are equivalent substrates for valinomycin-induced uptake.

Rubidium uptake in the presence of valinomycin is markedly inhibited by anaerobiosis, by the electron transfer inhibitors oxamate, amytal, 2-heptyl-4-hydroxyquinoline-N-oxide and cyanide, and by β-chloromercuribenzenesulfonate, but not by arsenate or dicyclohexylcarbodiimide; inhibition by β-chloromercuribenzenesulfonate is reversed by dithiothreitol. Moreover, anaerobiosis, amytal, 2-heptyl-4-hydroxyquinoline-N-oxide, and cyanide produce rapid efflux of rubidium from the vesicles. Oxamate and β-chloromercuribenzenesulfonate, however, cause little or no efflux of rubidium accumulated in the presence of valinomycin, even though β-chloromercuribenzenesulfonate-treated vesicles catalyze exchange of intravesicular rubidium with external potassium at normal rates. These findings indicate that valinomycin does not simply catalyze the passive transfer of rubidium across the vesicle membrane.

Valinomycin-induced active rubidium uptake is accompanied by the rapid efflux of intravesicular sodium against its concentration gradient. The vesicles are passively permeable to the lipophilic dibenzyldimethylammonium ion; however, this cation does not inhibit rubidium uptake or rubidium-induced sodium efflux and does not cause sodium efflux. Moreover, radioactive dibenzyldimethylammonium is not accumulated by membrane vesicles under conditions in which rubidium is actively transported. Valinomycin, in the presence of potassium, has no significant effect on the transient acidification of the medium observed upon addition of D-lactate to the vesicles. In addition, vesicles treated with Tween 40 such that they are rendered devoid of a diffusion barrier exhibit pH changes similar to those of untreated vesicles. These findings indicate that active rubidium uptake is an electrogenic process and that proton or potential gradients are not the primary driving force for respiration-linked active transport in isolated bacterial membrane vesicles.

Recently, Bhattacharyya et al. (1) demonstrated that membranes prepared from Escherichia coli accumulate K⁺ or Rb⁺ ions in the presence of D-lactate and the depsipeptide antibiotic valinomycin. This ionophore markedly increases the permeability of bacterial (2-4), erythrocyte (5-7), and mitochondrial (8) membranes to K⁺ and Rb⁺, but not to Na⁺ and Li⁺ ions (2, 9). Studies with black lipid films indicate that valinomycin forms one-to-one complexes with K⁺ ions (7), which then diffuse across the film. By this means, K⁺ is sheltered from the hydrophobic interior of the film within the cyclic valinomycin molecule (10), the exterior of which is soluble within the film. Conductometric measurements have demonstrated that valinomycin...
induces a bi-ionic potential across black lipid films separating equimolar solutions of KCl and NaCl (11). In mitochondria, valinomycin facilitates the net movement of K+ with concomitant acidification of the external medium (8), which has led to the hypothesis that this ionophore exerts its effect by allowing K+ to accumulate in response to a membrane potential generated by means of a proton pump (12).

It was of interest, therefore, when Reeves (13) reported that addition of 3-lactate to E. coli membrane vesicles leads to a transient acidification of the medium. In view of these results, a role for proton or potential gradients (14) in valinomycin-induced rubidium transport by bacterial membrane vesicles was considered.

The present work was undertaken with the expectation that, in contrast to the sugar and amino acid transport systems studied earlier (15-24), valinomycin-induced Rb+ uptake by membrane vesicles would exhibit properties consistent with the chemiosmotic theory (14). The results demonstrate, however, that valinomycin-induced Rb+ uptake is analogous in nearly all respects to the transport of sugars and amino acids described previously. The data indicate, moreover, that valinomycin does not catalyze the passive transfer of Rb+ across the vesicle membrane and that active Rb+ uptake is a cause rather than a result of electrochemical gradients.

**MATERIALS AND METHODS**

**Cells**—E. coli ML 308-225 was grown on Medium A (25) containing 1% sodium succinate (hexahydrate) as described previously (10). Staphylococcus aureus U 71 was grown as described by Short and White (26). Micrococcus denitrificans ATCC 13543 was grown by the method of Scholes and Smith (27).

**Preparation of Membranes**—Membrane vesicles of E. coli and M. denitrificans were prepared by the lysozyme-EDTA procedure described previously (28-30). The membranes were suspended in 0.1 M potassium phosphate (pH 6.6) and stored in liquid nitrogen. S. aureus membrane vesicles were prepared from lysozyme-protoplasts as described previously (31) and were suspended in 50 mM potassium phosphate (pH 7.3) for storage in liquid nitrogen.

**Replacement of Buffers**—For Rb+ uptake studies, membrane suspensions (1 to 2 ml) of E. coli or M. denitrificans containing 3 to 6 mg of protein per ml were removed from storage, thawed quickly at 46°C, diluted with 10 ml of 0.1 M sodium phosphate buffer (pH 6.6), and centrifuged at 20,000 × g for 15 min. The supernatant was discarded and the pellet washed three times with 8-ml aliquots of the same buffer. The final pellet (0.050 to 0.10 ml) was then resuspended to approximately 1 mg of protein per ml in 0.1 M sodium phosphate (pH 6.6). S. aureus membrane vesicles were treated in an identical fashion, except that 20 mM sodium phosphate (pH 7.3) was employed. An analogous procedure was used in transferring E. coli membranes to sodium ascorbate, choline phosphate, and choline succinate buffers (Table IV).

**Transport Studies**—Unless indicated otherwise, transport experiments were carried out by methods identical with those reported previously (16, 17, 30). Counting of 86Rb and 22Na was accomplished by means of a Nuclear Chicago gas-flow counter with counting efficiencies of 21% and 55%, respectively.

**Oxygen Uptake Measurements**—Rates of oxygen uptake were measured with a Clark electrode (YSI model 53 oxygen monitor) as described previously (18).

**Measurement of pH changes**—pH Changes accompanying 

\[ \text{d-lactate oxidation were measured on an expanded scale pH} \]

**Synthesis of [methyl-14C]Dibenzyldimethylammonium Chloride**—One hundred micromoles of [14C]methyl iodide (20 mCi per mmole, New England Nuclear Corp.) and 42 μmol of dibenzylamine (Matheson, Coleman and Bell) were added to 50 μl of methanol containing 10 μmol of NaOH. The reaction mixture was incubated at room temperature in a 1-ml glass-stoppered tube and the pH was tested periodically by transferring 0.1-μl aliquots onto pH paper. When the pH dropped below pH 9, 10 μl of 10 M NaOH (in methanol) were added, and the procedure was repeated three times at approximately 15-min intervals until there was no further change in pH. After an additional 2-hour incubation at room temperature, the solvent was evaporated under a stream of nitrogen, and 100 μl of chloroform were added to the radioactive solids. Following centrifugation, the precipitate (containing NaCl and NaOH) was discarded, and the supernatant was evaporated to dryness under a stream of nitrogen. One hundred microliters of toluene and 100 μl of water were then added, the reaction vessel was centrifuged, and the water layer was carefully aspirated. This solution was passed over a 1-cm column of Dowex 1 (chloride form) in a Pasteur pipette, and the column was eluted with water. Eluates were monitored for dibenzylidimethylammonium chloride by testing aliquots for the formation of a bluish-white precipitate when added to 10 mM sodium tetrathylenetetrazol. After pooling of the appropriate fractions, the radiochemical yield of [methyl-14C]dibenzyldimethylammonium chloride (40 μCi per mmole) was 2.14 μmoles. Based on an extinction coefficient of 830 liters per mole·cm at 262 nm (32), the chemical yield of the radioactive compound was 2.50 μmoles.

The radioactive product and authentic dibenzylidimethylammonium chloride (Baker) were chromatographed on Silica Gel G thin layer plates (Mann) employing chloroform-methanol-0.1 N aqueous acetic acid (60:70:26, v/v/v). The radioactive and nonradioactive compounds were localized by autoradiography and charring with concentrated sulfuric acid, respectively. The chromatographic mobility as well as the ultraviolet absorption spectrum (32) of the radioactive product were identical with those of authentic dibenzylidimethylammonium chloride. The purity of the synthetic compound was confirmed by mass spectrometry. The mass spectrum of the radioactive material was identical with that of the authentic compound with the exception that peaks at m/e = 58, 135, and 211 (corresponding to the thermal breakdown products trimethylamine, benzyldimethylamine, and dibenzylmethylamine, respectively) were accompanied in the spectrum of the radioactive compound by additional small peaks at m/e = 60, 137, and 213 as a result of the 14C label in the methyl carbon atoms. The localization of 14C was further demonstrated by the absence in both spectra of a peak 2 mass units above a prominent ion at m/e = 128, arising from benzyl [14C]chloride.

A stock solution of [methyl-14C]dibenzyldimethylammonium chloride (6.0 μCi per mmole, 50 nCi) was prepared for transport studies by lyophilizing the Dowex 1 eluate described above and redissolving in water containing an appropriate concentration of the unlabeled compound.

**Materials**—Rubidium chloride (50 to 150 mCi per mmole) and sodium chloride (carrier free) were obtained from New England Nuclear Corp. as aqueous solutions in 0.5 N HCl. Stock solutions of 86RbCl (5 to 15 mCi per mmole, 100 μCi) and 22NaCl (5.05 μCi per mmole, 200 μCi) were prepared by neutralizing the commercial products with RbOH and NaOH,
respectively, and diluting the specific activity with appropriate additions of RbCl and NaCl.

Valinomycin was obtained from Calbiochem. Tetraphenyl-
sonium chloride, triphenylmethylphosphonium bromide, and sodium tetrathenylboron were products of K & K Laboratories.

Nonactin, monactin, dinactin, and trimactin, and nigericin and monomycin were generous gifts of Dr. Simon Silver, Washington
University, St. Louis, Missouri, and Dr. Franklin M. Harold,
National Jewish Hospital and Research Center, Denver, Colo.,
respectively. Gramicidin was obtained from Hoffmann-La Roche,
Inc. Open-chain valinomycin, retrovalinomycin, and prolino-
mycin were generously contributed by Dr. B. F. Gisin, Rockefeller University, New York.

All other materials used in these studies were reagent grade
obtained from commercial sources.

RESULTS

Valinomycin-induced Rb" Uptake by Membrane Vesicles
Prepared from E. coli, S. aureus, and M. denitrificans—As shown
in Fig. 1, the artificial electron donor ascorbate-phenazine
methosulfate markedly stimulates both the rate and extent of
Rb" uptake by E. coli, S. aureus, and M. denitrificans membrane vesicles in the presence of valinomycin. n-Lactate also produces
a pronounced stimulation of Rb" uptake in membranes prepared
from E. coli and M. denitrificans (Fig. 1, A and C), while in S.
aureus membrane vesicles, where L-α-glycerol-P replaces n-
lactate as the primary physiological electron donor (23), Rb" transport is markedly stimulated by L-α-glycerol-P (Fig. 1B).
As shown previously by Bhattacharyya et al. (1), active Rb" uptake requires the presence of both an electron donor and
valinomycin; ascorbate-phenazine methosulfate, n-lactate, and
L-α-glycerol-P at valinomycin, when tested individually, produce little or no stimulation of Rb" transport (Fig. 1).

The capacity of the valinomycin-induced Rb" uptake process
is considerably higher than that observed for any of the individual
sugar and amino acid transport systems (19–21, 23). The initial
rate of Rb" uptake by the E. coli vesicles is about 10-fold greater
than that of the lac transport system, for instance (19). In
E. coli vesicles, which contain approximately 2.2 μl of internal
volume per mg of membrane protein (19), the steady state level
of Rb" accumulation in the presence of n-lactate (Fig. 1A)
corresponds to an intravesicular concentration of approximately
100 μM, representing a 50-fold concentration gradient.

Energy Source Specificity for Valinomycin-induced Rb" Uptake in E. coli Membrane Vesicles—Previous studies demonstra-
ted that the transport of sugars and amino acids by E. coli
ML 308-225 membranes is stimulated maximally by ascorbate-
phenazine methosulfate and n-lactate, and to a much lesser extent by succinate, L-lactate, L-α-hydroxybutyrate, and NADH (18, 21, 31). As shown in Table I, the same electron donors stimulate valinomycin-induced Rb" uptake. Moreover, ascorbate-phenazine methosulfate (Line 2) and n-lactate (Line 5) are considerably more effective in stimulating Rb" transport than are succinate, L-lactate, L-α-hydroxybutyrate, and NADH (Lines 8 to 9). Ascorbate produces much less stimulation in the absence of phenazine methosulfate (Line 3), while phenazine methosulfate alone has no significant effect (Line 4). Membrane vesicles convert n-lactate and L-lactate stoichiometrically to pyruvate and succinate stoichiometrically to fumarate (16); the latter compounds produce no stimulation of Rb" uptake (Table I). Of the 33 additional metabolites and cofactors tested, including ATP, P-enolpyruvate, and NADPH (Table I), all failed to stimulate Rb" transport to any extent whatsoever.

![Fig. 1. Effect of ascorbate-phenazine methosulfate (ASC-PMS) and n-lactate (D-LAC) or α-glycerol-P (α-GP) on the time course of Rb" uptake in the presence or absence of valinomycin (VAL) by membranes prepared from Escherichia coli, Staphy-
lococcus aureus, and Micrococcus denitrificans. Membrane vesicles prepared from E. coli ML 308-225 (A), S. aureus U-71 (B), and
M. denitrificans ATCC 13543 (C) were washed as described under "Methods" and resuspended to 3 ml in 0.1 M sodium phosphate
(pH 7.3) (B) or 0.1 M sodium phosphate (pH 6.6) (C) at membrane protein concentrations of 1.0, 1.1, and 1.45 mg per ml, respectively. Each sample was divided into two equal portions; to one portion was added 1.5 μl of dimethylsulfide and to the other was added 1.5 μl of
1.0 mM valinomycin (in dimethylsulfide). Reaction mixtures were prepared by diluting 50-μl aliquots of control or valinomycin-
treated membranes to a final volume of 100 μl containing, in final concentrations, 20 mM sodium phosphate (pH 6.6) (A) and
10 mM sodium phosphate (pH 7.3) (B) or 0.1 μM sodium phosphate (pH 6.6) (C) at membrane protein concentrations of 1.0, 1.1, and 1.45 mg per ml, respectively. Samples containing control membranes (■, □) or valinomycin-treated membranes (▲, △, ▼) or valinomycin-treated membranes (■, □) or valinomycin-treated membranes (▲, △, ▼) as indicated. Samples labeled NO ADD (●) and VAL (○) contained control membranes and valinomycin-treated membranes, respectively, but received no added energy source. The reactions were initiated immediately thereafter by the addition
of 20 μCi of radiolabeled RbCl (11.5 nCi per mmole) at a final concentration of 2.0 mM. The incubations were continued at 25°, and, at the
ulations indicated, the reactions were terminated and the samples
were assayed by methods described previously (16, 17). Samples
containing ascorbate-phenazine methosulfate were gassed with pure oxygen during the 2-min preincubation and throughout the
incubation period by reported methods (31). Each experimental
point was corrected for an control sample as described previously
(28).
Table I

Effect of various energy sources on valinomycin-induced Rb⁺ uptake
by E. coli ML 308-225 membrane vesicles

<table>
<thead>
<tr>
<th>Energy source</th>
<th>Rb⁺ uptake (μmol/mg membrane protein/30 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. None</td>
<td>6.6</td>
</tr>
<tr>
<td>2. Ascorbate + phenazine methosulfate</td>
<td>225</td>
</tr>
<tr>
<td>3. Ascorbate</td>
<td>49.9</td>
</tr>
<tr>
<td>4. Phenazine methosulfate</td>
<td>9.8</td>
</tr>
<tr>
<td>5. d-Lactate</td>
<td>103</td>
</tr>
<tr>
<td>6. Succinate</td>
<td>22.9</td>
</tr>
<tr>
<td>7. L-Lactate</td>
<td>43.8</td>
</tr>
<tr>
<td>8. dl-α-Hydroxybutyrate</td>
<td>39.3</td>
</tr>
<tr>
<td>9. NADH</td>
<td>21.7</td>
</tr>
</tbody>
</table>

* The following compounds, when tested at a final concentration of 20 mM, produced no significant stimulation of Rb⁺ uptake: glucose, 6-P-glucose, glucose-6-P, glucose-1-P, fructose-6-P, fructose-1-P, fructose-1,6-P₂, dl-α-glycerol-P, dihydroyacetone-P, 3-P-glycerate, 1,3-diphosphoglycerate, 2-P-glycerate, P-enolpyruvate, pyruvate, acetate, acetyl-CoA, citrate, isocitrate, aconitate, α-ketoglutarate, fumarate, DL-malate, oxaloacetate, formate, DL-α-hydroxybutyrurate, α-ketobutyrate, ATP, 3',5'-AMP, NAD, NADP, NADPH, FMN, FAD, acetyl-P, and carbamyl-P.

* Employed at a final concentration of 0.1 mM.

Table II

Respiration and valinomycin-induced Rb⁺ uptake by ML 308-225 membrane vesicles at 25°C

A suspension of ML 308-225 membranes in 0.1 M sodium phosphate (pH 6.6) containing 1.0 mg of membrane protein per ml was divided into two equal portions; to one portion was added 0.75 µl of dimethylsulfoxide per ml of suspension, and to the other portion was added 0.75 µl of 1.0 mM valinomycin in dimethylsulfoxide per ml. For the oxygen uptake experiments (Column I), assay mixtures were prepared by diluting 0.4 ml aliquots of control or valinomycin-treated membranes to a final volume of 4.0 ml containing, in final concentrations, 50 mM sodium phosphate (pH 6.6), 10 mM MgSO₄, and (where indicated) 20 mM electron donor (except for NADH and phenazine methosulfate, which were employed at concentrations of 5 mM and 0.1 mM, respectively). Rates of oxygen uptake were measured at 25°C as described under "Methods." For the Rb⁺ uptake studies (Column II), reaction mixtures were prepared by diluting 50-µl aliquots of valinomycin-treated membranes to a final volume of 100 µl containing 50 mM sodium phosphate (pH 6.6), 10 mM MgSO₄, and the indicated electron donor at the same final concentration as employed in the oxygen uptake studies. The reactions were initiated by the addition of 10µRbCl (11.5 mCi per mmole, 8.0 mM final concentration), the incubations were continued at 25°C for 30 s, the reactions were terminated, and the samples were assayed as described (16, 17). Samples containing ascorbate were gassed with oxygen as reported previously (31). With the exception of glucose and phenazine methosulfate, all energy sources were supplied as sodium or lithium salts.

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Oxygen uptake rate</th>
<th>Initial rate of Rb⁺ uptake</th>
<th>Ratio of II:I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng atom/mg membrane protein/min</td>
<td>μmol/mg membrane protein/min</td>
<td></td>
</tr>
<tr>
<td>1. None</td>
<td>1</td>
<td>16</td>
<td>8.0</td>
</tr>
<tr>
<td>2. p-Lactate</td>
<td>300</td>
<td>533</td>
<td>1.81</td>
</tr>
<tr>
<td>3. Succinate</td>
<td>489</td>
<td>68.7</td>
<td>0.14</td>
</tr>
<tr>
<td>4. L-Lactate</td>
<td>104</td>
<td>139.1</td>
<td>1.34</td>
</tr>
<tr>
<td>5. dl-α-Hydroxybutyrate</td>
<td>65</td>
<td>114.7</td>
<td>1.75</td>
</tr>
<tr>
<td>6. NADH</td>
<td>592</td>
<td>53.4</td>
<td>0.03</td>
</tr>
<tr>
<td>7. Ascorbate-phenazine methosulfate</td>
<td>1400</td>
<td>1400</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* Rates of oxygen uptake by control and valinomycin-treated membranes are indistinguishable and are unaffected by the addition of RbCl (8.0 mM final concentration).

Rb⁺ uptake to oxygen utilization (expressed as nanomoles of Rb⁺ transported per ng atom of oxygen consumed) by vesicles in the presence of d-lactate yields a value of nearly 2:1. This result is in marked contrast to values obtained for the sugar and amino acid transport systems, where ratios of only 0.002:1 to 0.1:1 are observed (18, 21). Ratios in excess of 1:1 are also obtained with L-lactate and dl-α-hydroxybutyrate (Table II), whereas succinate and NADH yield much lower values. Ascorbate-phenazine methosulfate yields a ratio of approximately 1:1.¹

Kinetics of Valinomycin-induced Rb⁺ Transport—The initial rate of valinomycin-induced Rb⁺ uptake in the presence of d-lactate is a saturable function of Rb⁺ concentration (Fig. 2A).

¹ The ratio of one Rb⁺ ion taken up per electron pair which is observed with ascorbate-phenazine methosulfate (Table II) may be fortuitous, since this electron donor may reduce other acceptors (e.g. the succinate dehydrogenase respiratory branch) in reactions that are unproductive for Rb⁺ uptake.
Fig. 2. Kinetics of D-lactate-stimulated Rb⁺ uptake by ML 308-225 membrane vesicles. A, effect of external Rb⁺ concentration on initial rates of valinomycin-induced Rb⁺ uptake. Reactions were assayed in valinomycin-treated ML 308-225 membranes prepared as described in the legend to Fig. 1 and contained, in a final volume of 100 μl, 0.050 mg of membrane protein, 0.0375 nmole of valinomycin, 50 mM sodium phosphate (pH 6.6), and 10 mM MgSO₄. After a 2-min incubation at 25°C, lithium D-lactate and 86RbCl (8.42 mCi per mmole) were employed at a final concentration of 4.0 mM throughout. Inset, data plotted by the method of Hofstee (33). B, effect of valinomycin concentration on initial rates of Rb⁺ uptake. Reaction mixtures were prepared as in A except that valinomycin was omitted. The antibiotic was then added (in 1 μl of dimethylsulfoxide) to the reaction mixtures to yield the final concentrations indicated in the figure. The incubations were carried out for 5 s at 25°C, the reactions were terminated, and the samples were assayed as described (16, 17). Inset, data plotted by the method of Hofstee (33).

The data yield an apparent Kₘ for Rb⁺ of 0.89 mM. Moreover, initial rates of Rb⁺ uptake are saturable with respect to valinomycin (Fig. 2B), exhibiting an apparent Kₘ of 2.0 × 10⁻⁴ M. Virtually all of the valinomycin added to the reaction mixtures under these experimental conditions, however, is apparently bound to the membrane vesicles.2 Repeated washing and dilution of valinomycin-treated membranes with aqueous buffers causes only minimal losses of Rb⁺ transport activity. These findings suggest that the apparent Kₘ for valinomycin-induced Rb⁺ uptake might be more accurately expressed as 0.40 nmole of valinomycin per mg of membrane protein (cf. legend to Fig. 2).

It should be noted that initial rates of valinomycin-induced Rb⁺ uptake by ML 308-225 membranes in the presence of D-lactate (or ascorbate-phenazine methosulfate) at 25°C are linear during only the first 5 to 10 s of the reaction.3 Although not shown in Fig. 2B, addition of increasing amounts of valinomycin above a level of 1 μM (i.e. 2 nmole of valinomycin per mg of membrane protein) causes a progressive decrease in the initial rate of Rb⁺ uptake.4 This inhibition is also observed in the steady state level of Rb⁺ accumulation, as indicated in Line 2 of Table III.

Effect of Valinomycin Analogs and Ionophoric Antibiotics on Rb⁺ Uptake—As shown in Table III, open-chain valinomycin (Line 3), retrovalinomycin (in which the alternating peptide and ester linkages of valinomycin are interchanged5) (Line 4), and prolinomycin (a peptide analogue of valinomycin (34)) (Line 5) produce no significant enhancement of Rb⁺ uptake in the presence of D-lactate (Column I). Furthermore, these compounds cause little or no inhibition of valinomycin-induced Rb⁺ uptake (Column II). Open-chain valinomycin (35) and retrovalinomycin (36) have been shown to possess much lower binding affinities for K⁺ than valinomycin itself. On the other hand, prolinomycin binds K⁺ ions with approximately the same affinity as the natural antibiotic (34). These results suggest that the ability of valinomycin or its analogues to bind K⁺ or Rb⁺ is necessary but not sufficient for the induction of active Rb⁺ transport in the membrane vesicles.

Although not as effective as valinomycin, all four members of the nactin group of antibiotics induce concentrative Rb⁺ uptake

### Table III

<table>
<thead>
<tr>
<th>Ionophoric antibiotic or analogue Concentration</th>
<th>Rb⁺ uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I - Valinomycin</td>
</tr>
<tr>
<td></td>
<td>μM</td>
</tr>
<tr>
<td>1. None</td>
<td>5 x 10⁻⁶</td>
</tr>
<tr>
<td>2. Valinomycin*</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>3. Open-chain valinomycin*</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>4. Retrovalinomycin*</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>5. Prolinomycin*</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>6. Nonaactin</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>7. Monactin</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>8. Dinactin</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>9. Triactin</td>
<td>5 x 10⁻⁷</td>
</tr>
<tr>
<td>10. Monensin</td>
<td>1 x 10⁻⁷</td>
</tr>
<tr>
<td>11. Nigericin</td>
<td>1 x 10⁻⁷</td>
</tr>
<tr>
<td>12. Gramicidin</td>
<td>1 x 10⁻⁷</td>
</tr>
</tbody>
</table>


3. Open-chain valinomycin (35) and retrovalinomycin (36) have been shown to possess much lower binding affinities for K⁺ than valinomycin itself. On the other hand, prolinomycin binds K⁺ ions with approximately the same affinity as the natural antibiotic (34). These results suggest that the ability of valinomycin or its analogues to bind K⁺ or Rb⁺ is necessary but not sufficient for the induction of active Rb⁺ transport in the membrane vesicles.


5. B. F. Gisin and R. B. Merrifield, personal communication.
value at 55°C. In contrast, initial rates of Rb+ transport exhibit the peak and extent of valinomycin-induced Rb+ uptake (Fig. 3). Additional studies have shown that K+ inhibition is competitive with regard to valinomycin-induced optimum at 18°C, which falls steadily from 18°C to a negligible constant K+ or Rb+ follows the identical time course (Fig. 3). Furthermore, the exchange of intravesicular 86Rb+ with either KCl (V) and Control + KCl (O), respectively. The reactions were incubated at 45°C, and samples were assayed at the times shown. At 5 min (indicated by vertical arrow), KCl or RbCl was added at a final concentration of 12 mM to samples labeled Control → 12 mM KCl (V) and Control → 12 mM RbCl (O), respectively. The incubations were continued at 25°C, and samples were assayed at the times shown.

It is also noteworthy that the activity of nontoxic (Line 6) is manifested only at much higher concentrations than that of its higher homologues. The cation-binding activity of these antibiotics, like that of valinomycin, exhibits a marked preference for K+ or Rb+ over Na+ and Li+ ions (9).

Valinomycin-induced Rb+ uptake by ML 308-225 membranes in the presence of valinomycin and n-lactate. Reaction mixtures containing valinomycin-treated ML 308-225 membranes were prepared as described in the legend to Fig. 1 and contained, in a final volume of 100 μl, 0.050 mg of membrane protein, 0.0375 mM of valinomycin, 50 mM sodium phosphate (pH 6.6), and 10 mM MgSO4. 86RbCl (5.83 mCi per mmole, 2.0 mM final concentration) was then added to all samples, followed by the addition of KCl to samples labeled 5 mM KCl (△), 6 mM KCl (V), and 12 mM KCl (●) at the final concentrations indicated. Control samples (○) and samples labeled Control → 12 mM KCl (V) and Control → 12 mM RbCl (●) contained no KCl. After a 2-min incubation at 25°C, the reactions were initiated by the addition of lithium n-lactate (20 mM final concentration). Incubation was continued at 25°C, and samples were assayed at the times shown. At 5 min (indicated by vertical arrow), KCl or RbCl was added at a final concentration of 12 mM to samples labeled Control → 12 mM KCl (V) and Control → 12 mM RbCl (●), respectively. The incubations were continued at 25°C, and samples were assayed at the times shown.

As demonstrated for the β-galactoside and amino acid transport systems (19, 20), steady state levels of Rb+ accumulation at 20°C and 45°C represent a balance between rates of influx and efflux which can be shifted rapidly by changing the temperature (Fig. 5). After a 3-min incubation at 45°C in the presence of n-lactate and valinomycin, membrane vesicles accumulate Rb+ to a steady state level of approximately 120 nmoles per mg of membrane protein. When the samples are shifted to 20°C, there is further accumulation of Rb+ for 5 min until a new steady state level of approximately 190 nmoles per mg of membrane protein is established. If the reaction mixtures are then shifted back to 45°C, there is a rapid efflux of Rb+ such that the intravesicular Rb+ concentration returns to the same steady state level observed during the initial 45°C incubation.

Effect of Temperature on Valinomycin-induced Rb+ Transport.—As shown in Fig. 4, steady state levels of n-lactate-stimulated Rb+ uptake increase from 0°C to the steady state temperature optimum at 18°C, then fall steadily from 18°C-45°C to a negligible value at 55°C. In contrast, initial rates of Rb+ transport exhibit a temperature optimum at 50°C, increasing steadily from 0°C to about 25°C, and rising more rapidly from 25-50°C; above 50°C the initial rate drops abruptly. These results are very similar to those reported previously for β-galactoside transport by ML 308-225 membranes (19).

As demonstrated for the β-galactoside and amino acid transport systems (19, 20), steady state levels of Rb+ accumulation at 20°C and 45°C represent a balance between rates of influx and efflux which can be shifted rapidly by changing the temperature (Fig. 5). After a 3-min incubation at 45°C in the presence of n-lactate and valinomycin, membrane vesicles accumulate Rb+ to a steady state level of approximately 120 nmoles per mg of membrane protein. When the samples are shifted to 20°C, there is further accumulation of Rb+ for 5 min until a new steady state level of approximately 190 nmoles per mg of membrane protein is established. If the reaction mixtures are then shifted back to 45°C, there is a rapid efflux of Rb+ such that the intravesicular Rb+ concentration returns to the same steady state level observed during the initial 45°C incubation.
source by the addition of 40-fold higher than that of the medium and deprived of an energy membrane vesicles under the acid transport systems (19, 21). The failure of oxamate to to those obtained previously with the figalactoside and amino Rb+ uptake (Fig. 6) and n-lactate oxidation6 causes no efflux of contrast, oxamate, at a concentration which markedly inhibits cyanide produce a rapid efflux of Rb+ from membrane vesicles in the presence of n-lactate. Reaction mixtures containing valinomycin-treated ML 308-225 membranes were prepared as described in the legend to Fig. 1 and contained in a final volume of 100 µl, 0.050 mg of membrane protein, 0.0375 nmole of valinomycin, 50 mM sodium phosphate (pH 6.6), and 10 mM MgSO4. One of the inhibitors shown above was then added to the indicated samples to give the following final concentrations of inhibitors: NaCN (A), 10 mM; 2-heptyl-4-hydroxyquinoline-N-oxide (HOQNO) (Δ), 80 µM; sodium amytal (V), 10 mM; sodium oxamate (●), 10 mM. Control samples (○) and samples labeled (—)O2 (□) contained no inhibitor. Lithium n-lactate was then added to all samples at a final concentration of 20 mM and the tubes were preincubated for 5 min at 25°, during which time the samples labeled (—)O2 were gassed with argon by methods described previously (31). The reactions were initiated at this time by the addition of 4RbCl (11.5 nCi per nmole, 2.0 mM final concentration). The incubations were carried out at 25° for the times indicated, the reactions were terminated, and the samples were assayed as described (16, 17). For the samples labeled (—)O2, the incubations were carried out under argon.

Fig. 7 (right). Effect of anaerobiosis and electron transfer inhibitors on Rb+ efflux. Reaction mixtures containing valinomycin-treated ML 308-225 membranes were prepared as described in the legend to Fig. 1 and contained in a final volume of 100 µl, 0.050 mg of membrane protein, 0.0375 nmole of valinomycin, 50 mM sodium phosphate (pH 6.6), and 10 mM MgSO4. Lithium n-lactate and 4RbCl (0.18 nCi per nmole) were added at final concentrations of 20 mM and 1.5 mM, respectively, and the samples were incubated at 25° for 10 min. At this time (i.e. zero time in the figure), one of the inhibitors shown above was added to the indicated samples to give the following final concentrations of inhibitors: NaCN (A), 10 mM; 2-heptyl-4-hydroxyquinoline-N-oxide (HOQNO) (Δ), 80 µM; sodium amytal (V), 10 mM; sodium oxamate (●), 10 mM. Control samples (○) contained no inhibitor. Samples labeled (—)O2 (□) contained no inhibitor but were gassed with argon at zero time by methods described previously (31). All samples were then incubated at 25° under aerobic conditions (room air) except the samples labeled (—)O2, which were maintained under argon. The incubations were terminated at the times indicated and the samples were assayed as described (16, 17).

As judged by oxygen uptake measurements, oxamate produces 69% inhibition of the n-lactate dehydrogenase activity of membrane vesicles under the conditions employed in these experiments. presence of valinomycin. This finding suggests that, at the concentrations used in these studies, valinomycin does not catalyze the passive transfer of Rb+ across the vesicle membrane.

Addition of the uncoupling agent carbonyl cyanide m-chlorophenylhydrazo to vesicles previously loaded with Rb+ in the presence of n-lactate and valinomycin causes rapid efflux of accumulated Rb+ ions. Moreover, initial rates of CCCP-induced Rb+ efflux exhibit a Km for intravesicular Rb+ in excess of 100 mM (data not shown). The Kma for Rb+ efflux is thus at least 100-fold greater than the Kma determined for valinomycin-induced Rb+ uptake (Fig. 2), a finding which is analogous to results reported previously for the respiration-linked lactose (19) and proline (21) uptake systems.

Effect of Sulfhydryl Reagents on Rb+ Uptake, Efflux, and Exchange—Initial rates of n-lactate-stimulated Rb+ uptake by valinomycin-treated ML 308-225 membranes are substantially inhibited by N-ethylmaleimide at concentrations below 1 mM (Fig. 8A); approximately 60% inhibition is observed at a concentration of 0.5 mM NEM. As the concentration of NEM is increased, however, no further inhibition of Rb+ uptake is observed. In contrast to these results, NEM causes only minimal inhibition of Rb+ uptake when ascorbate-phenazine methosulfate is employed as electron donor. Prolonged incubation of membrane vesicles in the presence of NEM produces no change in the effects of this compound.

The effects of p-chloromercuribenzenesulfonate on initial rates of Rb+ uptake are substantially different from those produced by NEM. As shown in Fig. 8B, p-CMB causes pronounced inhibition of both n-lactate- and ascorbate-phenazine methosulfate-stimulated Rb+ transport; in both cases, inhibition is reversed almost completely by dithiothreitol. Furthermore, high concentrations of p-CMB produce nearly complete inhibition of n-lactate-stimulated Rb+ uptake. It should be noted, moreover, that p-CMB inhibition observed with n-lactate (and to a lesser extent with ascorbate-phenazine methosulfate) as electron donor exhibits a concentration dependence which is markedly biphasic. Thus, as the concentration of p-CMB increases from 0 to 0.025 mM, n-lactate-stimulated Rb+ uptake falls steeply to approximately 50% of the control value and then decreases more slowly as the p-CMB concentration increases above 0.025 mM. These results, together with those obtained with NEM, suggest that both lactate-dependent Rb+ uptake involves at least two specific populations of sulfhydryl groups with differing susceptibilities to attack by sulfhydryl reagents.

This conclusion implies a functional heterogeneity of the n-lactate dehydrogenase activity of ML 308-225 membranes, and is in accord with present6 and earlier results (18) showing that n-lactate oxidation by these membrane preparations is inhibited only partially (i.e. approximately 70%) by NEM.7

As shown in Fig. 9, p-CMBS, at a concentration which causes pronounced inhibition of Rb+ uptake and n-lactate oxidation,6 produces little or no Rb+ efflux from previously loaded membranes in the presence of valinomycin and n-lactate. In this respect, the effects of p-CMBS on Rb+ uptake and efflux are similar to those of oxamate (Figs. 6 and 7), and are identical with the effects of p-chloromercuribenzoate and NEM on the uptake and efflux of sugars and amino acids reported previously (19, 21).

Earlier studies also showed that p-CMB and NEM block

7 Detailed studies on the localization of sulfhydryl-containing components of the respiratory chain and their relationship to transport will be the subject of a subsequent paper (F. J. Lombardi and H. R. Kaback, manuscript in preparation).
and 9), it seems likely that the exchange reaction observed in the presence of functional sulfhydryl groups. Since p-CMBS-treated membranes labeled with 86RbCl (2.09 mCi per mmole, 2.0 mM final concentration) was then added, and the samples were incubated for 5 min at 25°C. 86RbCl (2.09 mCi per mmole, 2.0 mM final concentration) was then added, and the reactions were initiated immediately thereafter by the addition of lithium D-lactate (D-LAC) (0) or sodium ascorbate-phenazine methosulfate. Incubations were terminated, and the samples were assayed at the times shown. At 2 min (indicated by vertical arrow), KCl (20 mM final concentration) was added to samples labeled Control → KCl (●) and +PCMB → KCl (▲). The incubations were continued at 25°C and samples were assayed at the times shown.

Fig. 8. Effect of sulfhydryl reagents on valinomycin-induced Rb⁺ uptake by ML 308-225 membranes in the presence of D-lactate or ascorbate-phenazine methosulfate. A, effect of NEM on initial rates of valinomycin-induced Rb⁺ transport. Incubation mixtures containing valinomycin-treated ML 308-225 membranes were prepared as described in the legend to Fig. 1 and contained, in a final volume of 100 μl, 0.000 mg of ascorbate protein, 0.000 mg of valinomycin, 50 mM sodium phosphate (pH 6.0), and 10 mM MgSO₄. NEM was added at the final concentrations indicated, and the samples were incubated for 5 min at 25°C. 86RbCl (2.09 mCi per mmole, 2.0 mM final concentration) was then added, and the reactions were initiated immediately thereafter by the addition of lithium D-lactate (D-LAC) (0) or sodium ascorbate-phenazine methosulfate (ASC-PMS) (▲) at final concentrations of 20 mM, 20 mM, and 0.1 mM, respectively, as indicated. The incubations were carried out for 15 s at 25°C, the reactions were terminated, and the samples were assayed as described (16, 17). Samples containing ascorbate-phenazine methosulfate were gassed with oxygen for 2 min prior to initiation and throughout the incubation period as described previously (31). B, inhibition of valinomycin-induced Rb⁺ uptake by p-CMBS and reversal by dithiothreitol. Incubations were the same as in A except that NEM was replaced by p-CMBS at the concentrations indicated, and dithiothreitol (20 mM final concentration) was added to samples labeled D-LAC + DTT (●) and ASC-PMS + DTT (▲) just prior to initiation. Initial rates of Rb⁺ uptake in control samples were 177 and 605 nmoles per mg of membrane protein per min for D-lactate and ascorbate-phenazine methosulfate, respectively.

Fig. 9. Effect of p-CMBS on efflux and exchange of Rb⁺ by ML 308-225 membranes in the presence of valinomycin and D-lactate. Reaction mixtures containing valinomycin-treated ML 308-225 membranes were prepared as described in the legend to Fig. 1 and contained, in a final volume of 100 μl, 0.060 mg of membrane protein, 0.060 mmole of valinomycin, 50 mM sodium phosphate (pH 6.0), and 10 mM MgSO₄. 86RbCl (5.83 mCi per mmole) and lithium D-lactate were added at final concentrations of 2.0 and 20 mM, respectively, and the samples were incubated at 25°C for 10 min. At this time (i.e. at zero time in the figure), a control sample was assayed, and p-CMBS (0.5 mM final concentration) was added to samples labeled +PCMBS; NO KCl (▲) and +PCMBS → KCl (▲); samples labeled Control; NO KCl (●) and Control → KCl (●) received no p-CMBS. Incubation was continued at 25°C and samples were assayed as indicated. At 2 min (indicated by vertical arrow), KCl (20 mM final concentration) was added to samples labeled Control → KCl (●) and +PCMBS → KCl (▲). The incubations were continued at 25°C and samples were assayed at the times shown.

The lipophilic cations tetraphenylarsonium and triphenylmethylphosphonium cause pronounced inhibition of D-lactate-dependent Rb⁺ uptake (Experiment 3, Lines 2 and 3) at 5 mM.

FIG. 8

Fig. 8. Effect of sulfhydryl reagents on valinomycin-induced Rb⁺ uptake by ML 308-225 membranes in the presence of D-lactate or ascorbate-phenazine methosulfate. A, effect of NEM on initial rates of valinomycin-induced Rb⁺ transport. Incubation mixtures containing valinomycin-treated ML 308-225 membranes were prepared as described in the legend to Fig. 1 and contained, in a final volume of 100 μl, 0.000 mg of ascorbate protein, 0.000 mg of valinomycin, 50 mM sodium phosphate (pH 6.0), and 10 mM MgSO₄. NEM was added at the final concentrations indicated, and the samples were incubated for 5 min at 25°C. 86RbCl (2.09 mCi per mmole, 2.0 mM final concentration) was then added, and the reactions were initiated immediately thereafter by the addition of lithium D-lactate (D-LAC) (0) or sodium ascorbate-phenazine methosulfate (ASC-PMS) (▲) at final concentrations of 20 mM, 20 mM, and 0.1 mM, respectively, as indicated. The incubations were carried out for 15 s at 25°C, the reactions were terminated, and the samples were assayed as described (16, 17). Samples containing ascorbate-phenazine methosulfate were gassed with oxygen for 2 min prior to initiation and throughout the incubation period as described previously (31). B, inhibition of valinomycin-induced Rb⁺ uptake by p-CMBS and reversal by dithiothreitol. Incubations were the same as in A except that NEM was replaced by p-CMBS at the concentrations indicated, and dithiothreitol (20 mM final concentration) was added to samples labeled D-LAC + DTT (●) and ASC-PMS + DTT (▲) just prior to initiation. Initial rates of Rb⁺ uptake in control samples were 177 and 605 nmoles per mg of membrane protein per min for D-lactate and ascorbate-phenazine methosulfate, respectively.

the exchange of intramembranous lactose (19), proline (38), and tyrosine (91) with external solute. In contrast, p-CMBS has no effect on the exchange of internal 86Rb⁺ with extravascular K⁺ (Fig. 9). Similar results were obtained with p-CMB and NEM (data not shown). These findings demonstrate that, unlike the sugar and amino acid uptake systems (19, 21), certain aspects of energy-independent Rb⁺ transport do not exhibit a requirement for functional sulfhydryl groups. Since p-CMBS-treated membranes do not catalyze net uptake or efflux of Rb⁺ (Figs. 8 and 9), it seems likely that the exchange reaction observed in the presence of p-CMBS is limited to a one-for-one interchange between internal and external cations.

Effect of Various Buffers, Metabolic Inhibitors, and Lipophilic Cations on Rb⁺ Uptake—Data presented in Table IV demonstrate that D-lactate-stimulated Rb⁺ uptake by valinomycin-treated membranes is unaffected when sodium phosphate buffer and lithium D-lactate are replaced by choline phosphate and choline D-lactate, respectively (Experiment 1, Line 3). Furthermore, membrane vesicles suspended in choline cacodylate (Experiment 1, Line 4) catalyze active Rb⁺ uptake approximately half as effectively as control samples. These findings indicate that Na⁺, Li⁺, and phosphate ions are not specifically required for active Rb⁺ accumulation by the vesicles.

Replacement of sodium phosphate with sodium arsenate buffer has no effect on Rb⁺ uptake activity of membrane vesicles (Line 2 of Experiment 1). Moreover, dicyclohexylcarbodiimide (Experiment 2, Line 5), at concentrations which markedly inhibit the membrane-bound Mg²⁺,Ca²⁺-stimulated ATPase (39), produces only minimal inhibition of Rb⁺ uptake. As shown previously (39), valinomycin-induced Rb⁺ uptake is also normal in membrane vesicles prepared from recA mutants of E. coli (40) which are defective in Mg²⁺,Ca²⁺-activated ATPase. These results demonstrate that valinomycin-induced Rb⁺ transport, like the other D-lactate-dependent transport systems (18, 21), does not require the generation or utilization of high energy phosphate intermediates. Earlier studies also showed that respiration-linked sugar and amino acid transport are inhibited by the uncoupling agents 2,4-dinitrophenol and CCCP and by azide (16, 17). As shown in Lines 2 to 4 of Experiment 2, these same compounds produce marked inhibition of active Rb⁺ uptake.
2,4-Dinitrophenol, CCCP, and dicyclohexylcarbodiimide were incubated for 30 s at 25°C, the reactions were added at a final concentration of 20 mM. (In Lines 3 and 4 of Experiment 1, lithium n-lactate was added (where indicated) at the final concentrations given. After a 2-min incubation at 25°C, sodium u-lactate was replaced by 20 mM choline n-lactate.) The reactions were then initiated by the addition of [8RbCl (5.73 mCi per mmole, 2.0 mM final concentration). The incubation mixtures were prepared as described in the legend to Fig. 1 and contained, in a final volume of 100 μl, 0.050 mg of membrane protein, 0.0357 mmole of valinomycin, 50 mM sodium phosphate (pH 6.6) (where indicated), and 10 mM MgSO4. In Lines 2 to 4 of Experiment 1, sodium phosphate was replaced by the indicated buffer (50 mM final concentration) as described under "Methods." In Experiments 2 and 3, the appropriate inhibitor or lipophilic cation was added (where indicated) at the final concentrations 86RbCl shown in Table IV were carried out with ascorbate-phenazine buffer (50 mM final concentration) as described under "Methods." In Experiments 2 and 3, the appropriate inhibitor or lipophilic cation (where indicated) at the final concentrations given. After a 2-min incubation at 25°C, lithium n-lactate was added (where indicated) at the final concentrations given. After a 2-min incubation at 25°C, lithium n-lactate was replaced by 20 mM choline n-lactate. The reactions were then initiated by the addition of 86RbCl (5.73 mCi per mmole, 2.0 mM final concentration). The incubations were carried out for 30 s at 25°C. The reactions were terminated and the samples were assayed as described (16, 17). 2,4-Dinitrophenol, CCCP, and dicyclohexylcarbodiimide were added as aliquots of dimethylsulfoxide solutions. In Experiments 2 and 3, the appropriate inhibitor or lipophilic cation was added (where indicated) at the final concentrations given. After a 2-min incubation at 25°C, lithium n-lactate was added (where indicated) at the final concentrations given. After a 2-min incubation at 25°C, lithium n-lactate was replaced by 20 mM choline n-lactate. The reactions were then initiated by the addition of 86RbCl (5.73 mCi per mmole, 2.0 mM final concentration). The incubations were carried out for 30 s at 25°C. The reactions were terminated and the samples were assayed as described (16, 17).

**Table IV**

Effect of various buffers, metabolic inhibitors, and lipophilic cations on valinomycin-induced Rb+ uptake by ML 308-225 membrane vesicles

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Buffer (pH 6.6)</th>
<th>Additions</th>
<th>Rb+ uptake</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Sodium phosphate</td>
<td>137</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sodium arsenate</td>
<td>139</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Choline phosphate</td>
<td>133</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Choline cacodylate</td>
<td>00</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Sodium phosphate</td>
<td>147</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2,4-DNP+ (10⁻⁴ M)</td>
<td>70</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2,4-DNP+ (10⁻³ M)</td>
<td>24.6</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sodium phosphate</td>
<td>17.8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CCCP</td>
<td>9.0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sodium phosphate</td>
<td>56</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sodium arsenate</td>
<td>33</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Sodium phosphate</td>
<td>112</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DCCD</td>
<td>106</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sodium phosphate</td>
<td>143</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>φ4As-Cl (1.0 mM)</td>
<td>86</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>φ4As-Cl (5.0 mM)</td>
<td>13.6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>φMeP-Br (1.0 mM)</td>
<td>78</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>φMeP-Br (5.0 mM)</td>
<td>29.5</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sodium phosphate</td>
<td>119</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>DDA-Cl (1.0 mM)</td>
<td>107</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>DDA-Cl (5.0 mM)</td>
<td>107</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

Effect of Valinomycin, RbCl, and Dibenzyldimethylammonium Chloride on Efflux of Cations from ML 308-225 Membranes—In order to identify the compensating ion flux(es) required to satisfy electrical neutrality during Rb+ accumulation, the transport of the other ions present in the reaction mixtures was investigated. These studies showed that there is no accumulation of [32P]orthophosphate, [35S]sulfate, [33C]carbonate, or [3H]lactate, or [3H]pyruvate ions above equilibration levels during active Rb+ uptake. Furthermore, omission of MgSO4 from the incubation mixtures and replacement of lithium n-lactate with sodium n-lactate produces no significant effect on Rb+ transport activity, thus eliminating Mg2+ and Li+ ions from consideration. These studies account for all ionic species present in the standard reaction mixtures with the exception of Na+ and, potentially, H+ ions.

The results shown in Fig. 10 demonstrate that active Rb+ uptake by ML 308-225 membranes is accompanied by the efflux of intravesicular Na+ against its concentration gradient. Membrane vesicles were equilibrated with external εNa+ by passive diffusion at 0°C and were then tested for Na+ efflux under conditions similar to those used in Rb+ transport studies. As shown, there is a rapid efflux of Na+, in an amount comparable to that of active Rb+ uptake, from membrane vesicles incubated in the presence of n-lactate, valinomycin, and RbCl. In the absence of any one of these three components, i.e., in the absence of active Rb+ uptake, Na+ efflux is markedly reduced. Rapid efflux of Na+ is not observed when Rb+ is replaced by the lipophilic diberzylidimethylammonium ion. Moreover, dibenzyldimethylammonium ion does not inhibit Rb+-induced Na+ efflux. Additional experiments have shown that addition of n-lactate plus a mixture of lactose and amino acids to ML 308-225 membranes does not induce Na+ efflux nor does sodium acidification of the medium (data not shown). These results indicate that active Rb+ uptake is an electrogenic process resulting in passive efflux of Na+ ions from the intravesicular space.

It should be noted that Rb+-induced cation efflux is apparently not specific for Na+, since membrane vesicles suspended in choline phosphate catalyze Rb+ uptake normally (Table IV). As shown in Fig. 11, valinomycin in the presence of K+ has no significant effect on the rate or extent of transient H+ liberation observed upon addition of n-lactate to membrane vesicles (13). This finding is in marked contrast to results obtained with mitochondria (8), and suggests that valinomycin-induced uptake of K+ or Rb+ is unrelated to respiration-linked H+ release by bacterial membrane vesicles.

In contrast, dibenzyldimethylammonium, another lipophilic cation (32), produces only minimal inhibition (Experiment 3, Line 4).

Essentially identical results were obtained when the studies shown in Table IV were carried out with ascorbate-phenazine methosulfate as electron donor (data not shown).
The relatively low level of ascorbate-phenazine methosulfate-stimulated Rb\textsuperscript{+} uptake observed in Table V probably results from the tendency of the reaction mixtures to become anaerobic during transfer by syringe.
nearly complete inhibition of D-lactate-stimulated Rb\(^+\) release in membranes treated with 0.2% Tween 40 exhibit normal rates of D-lactate oxidation and steady state of steady state levels fall steeply to very low values at pH 5.0, and both activities exhibit a decline above pH 5.0. 

Effect of Tween 40 and CCCP on D-Lactate-stimulated H\(^+\) Release—Treatment of membrane vesicles with non-ionic detergents, phospholipases, or ascobic acid results in loss of the barrier function of the membrane with regard to retention of transported solutes but has little or no effect on membrane-associated catalytic activities (15, 39, 35, 42, 43). The data presented in Fig. 13 demonstrate that membranes treated with 0.2% Tween 40 exhibit the same initial rate and steady state level of D-lactate-stimulated Rb\(^+\) uptake as control membranes. Furthermore, the steady state level of acidification is greater than 50% of the controls. The uncoupling agent CCCP, which markedly inhibits D-lactate-dependent Rb\(^+\) uptake (Table IV), causes nearly complete inhibition of D-lactate-stimulated H\(^+\) release in both control and detergent-treated membranes (Fig. 13). Similar results were obtained with phospholipase A-B- and acetone-treated vesicles (data not shown). Membranes treated with 0.2% Tween 40 exhibit normal rates of D-lactate oxidation and P-enolpyruvate-dependent phosphorylation of \(\alpha\)-methylglucoside (15) but are unable to accumulate Rb\(^+\) in the presence of D-lactate and valinomycin and fail to retain D-methylglucoside (15) but are unable to accumulate Rb\(^+\) in the presence of D-lactate and valinomycin and fail to retain D-methylglucoside. The results suggest that D-lactate-dependent H\(^+\) liberation is unrelated to the generation of a transmembrane proton gradient. Moreover, since the detergent-treated preparations are devoid of a diffusion barrier, it is unlikely that inhibition by CCCP is the result of transmembrane proton conduction. 

**Valinomycin Inhibition of Sugar and Amino Acid Transport—** Valinomycin, in the presence of K\(^+\), causes pronounced inhibition of many of the D-lactate-dependent sugar and amino acid transport systems, as indicated by its failure to inhibit P-enolpyruvate-dependent accumulation of D-\(\alpha\)-methylglucoside-P. 

---

**Table V**

**Uptake of Rb\(^+\)** and Dibenzylidinemethylammonium Ions by ML 308-225 Membrane Vesicles

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Transport</th>
<th>I Rb(^+)</th>
<th>II DDA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.0</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>D-Lactate</td>
<td>261</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Ascorbate-phenazine methosulfate</td>
<td>105</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

| pH | Effect of pH on valinomycin-induced Rb\(^+\) transport and on H\(^+\) release by ML 308-225 membranes in the presence of D-lactate. A, effect of pH on steady state levels and initial rates of valinomycin-induced Rb\(^+\) uptake. Aliquots of membranes in 0.1 M sodium phosphate (pH 6.6) were centrifuged for 10 min at 20,000 X g. Each pellet was washed twice and resuspended in 0.1 M sodium phosphate at a pH indicated above, and 50-ml aliquots were assayed for Rb\(^+\) uptake at 25\(^\circ\)C. Assay mixtures contained, in a final volume of 100 ml, 0.050 mg of membrane protein, 50 mM sodium phosphate at a pH shown, 10 mM MgSO\(_4\), 20 mM lithium \(\alpha\)-lactate (where indicated), and 0.10 mM of valinomycin (added as 1-ml aliquots in dimethylsulfoxide). The reactions were initiated by the addition of 86RbCl (8.81 mCi per mmole) and 2.68 mCi per mmole, 2.0 mM final concentration) (DDA\(^+\), Column II). The samples were incubated at 25\(^\circ\)C for 1 min, at which time 25-ml aliquots were withdrawn by Hamilton syringe and transferred directly onto dry Millipore filters (13-mm diameter) under suction. Filtration was continued for 30 s, the filters were removed and dissolved in 10 ml of Fumarsilil-1X A (Beckman) containing 0.5 ml of water, and the samples were counted on a Beckman LS-100 liquid scintillation counter (combined H-I-C channel) with an efficiency of 91% for \(\text{Rb}^+\) and 88% for \(\text{I}^-

---

**Fig. 12. Effect of pH on valinomycin-induced Rb\(^+\) transport and on H\(^+\) release by ML 308-225 membranes in the presence of D-lactate. A, effect of pH on steady state levels and initial rates of valinomycin-induced Rb\(^+\) uptake. Aliquots of membranes in 0.1 M sodium phosphate (pH 6.6) were centrifuged for 10 min at 20,000 X g. Each pellet was washed twice and resuspended in 0.1 M sodium phosphate at a pH indicated above, and 50-ml aliquots were assayed for Rb\(^+\) uptake at 25\(^\circ\)C. Assay mixtures contained, in a final volume of 100 ml, 0.050 mg of membrane protein, 50 mM sodium phosphate at a pH shown, 10 mM MgSO\(_4\), 20 mM lithium \(\alpha\)-lactate (where indicated), and 0.10 mM of valinomycin (added as 1-ml aliquots in dimethylsulfoxide). The reactions were initiated by the addition of 86RbCl (8.81 mCi per mmole) and 2.68 mCi per mmole, 2.0 mM final concentration) (DDA\(^+\), Column II). The samples were incubated at 25\(^\circ\)C for 1 min, at which time 25-ml aliquots were withdrawn by Hamilton syringe and transferred directly onto dry Millipore filters (13-mm diameter) under suction. Filtration was continued for 30 s, the filters were removed and dissolved in 10 ml of Fumarsilil-1X A (Beckman) containing 0.5 ml of water, and the samples were counted on a Beckman LS-100 liquid scintillation counter (combined H-I-C channel) with an efficiency of 91% for \(\text{Rb}^+\) and 88% for \(\text{I}^-\) respectively. Each sample was corrected for binding of radioactivity to the filter by subtracting values obtained for controls containing no membranes. Samples containing ascorbate-phenazine methosulfate were gassed with oxygen as reported previously (31).
transport systems of *E. coli* membrane vesicles (16, 17, 14). As indicated in Table VI, however, the concentration of valinomycin required to cause 50% inhibition of steady state accumulation levels differs considerably depending on the transport system under study. Thus, \((I)_{1/2}\) values obtained for valinomycin inhibition of lactose, proline, lysine, serine, and glutamic acid uptake range from 0.006 to 0.10 mM of membrane protein per mg of membrane protein as described in Fig. 11.

As indicated in Table VI, however, the concentration of valinomycin required to cause 50% inhibition of steady state accumulation levels differs considerably depending on the transport system under study. Thus, \((I)_{1/2}\) values obtained for valinomycin inhibition of lactose, proline, lysine, serine, and glutamic acid uptake range from 0.006 to 0.10 mM of membrane protein as described in Fig. 11. These findings are inconsistent with the suggestion (14) that valinomycin inhibition in the vesicle system is due to its ability to dissipate a presumptive transmembrane electrical potential required for active uptake of sugars and amino acids, since a mechanism such as this would predict that steady state accumulation levels exhibited by all such transport systems be inhibited equally by the antibiotic. Furthermore, for lactose transport the \((I)_{1/2}\) for K+ in the presence of optimal inhibitory concentrations of valinomycin is 0.9 mM, a value equal to the \(K_m\) for uptake of K+ and Rb+. This observation is very difficult to explain in chemiosmotic terms since it cannot be maintained that K+ ions dissipate the potential responsible for their own accumulation at low concentrations.

**DISCUSSION**

The data presented in this paper demonstrate that valinomycin-induced Rb+ uptake by *E. coli* membrane vesicles is analogous in nearly all respects to the transport of \(\beta\)-galactosides and amino acids by these membrane preparations. Moreover, the results indicate that the action of valinomycin in the vesicle system is different from that established for black lipid films (7) and postulated for mitochondrial and erythrocyte membranes (2, 8). Thus, when *E. coli* vesicles are loaded with Rb+ to intravesicular concentrations 40- to 50-fold higher than that of the external medium, and then deprived of an energy source by addition of oxamate or p-CMBS, there is little or no efflux of Rb+ in the presence of valinomycin, even though Rb+ exchange occurs at a normal rate (Figs. 7 and 9). It is highly unlikely, therefore, that valinomycin, at the concentrations employed here, catalyzes the passive transfer of Rb+ across the *E. coli* vesicle membrane.

As demonstrated previously for the \(\beta\)-lactate-dependent sugar and amino acid transport systems (16, 17, 19, 21), valinomycin-induced active Rb+ uptake is dependent on respiration (Fig. 6) but independent of oxidative phosphorylation (39) or the presence of high energy phosphate intermediates (Tables I and IV), and the primary site of energy coupling for transport is located between \(\beta\)-lactic dehydrogenase and cytochrome b1 in the *E. coli* membrane.

---

**Table VI**

<table>
<thead>
<tr>
<th>Transport substrate</th>
<th>Concentration (mM)</th>
<th>Uptake (1 min control)</th>
<th>((I)_{1/2}) for valinomycin inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\mu) mmoles/mg protein</td>
<td>(X 10^{-9}) M</td>
</tr>
<tr>
<td>Lactose</td>
<td>400</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>Proline</td>
<td>9.6</td>
<td>8.5</td>
<td>10</td>
</tr>
<tr>
<td>Lysine</td>
<td>11.8</td>
<td>6.2</td>
<td>14</td>
</tr>
<tr>
<td>Serine</td>
<td>17.0</td>
<td>12.1</td>
<td>6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>23.4</td>
<td>9.0</td>
<td>100</td>
</tr>
</tbody>
</table>

---

\(1)\) As shown in Fig. 2, \(\beta\)-lactate-stimulated Rb+ uptake is a saturable function of valinomycin concentration up to a level of 1 \(\mu\)M (i.e. 2 nmoles of valinomycin per mg of membrane protein) which suggests that, at these concentrations, valinomycin-induced Rb+ uptake may require binding of valinomycin to specific component(s) of the membrane. The inhibition of active Rb+ uptake observed with concentrations of valinomycin above saturating levels (Table III) might result therefore from passive Rb+ efflux facilitated by excess valinomycin molecules dissolved in the phospholipids of the membrane.
respiratory chain (18; Table II). Anaerobiosis and electron transfer inhibitors which block electron flow after the site of energy coupling (i.e., amytal, HOQNO, and cyanide (18)) cause rapid efflux of Rb+ from the vesicles, whereas oxamate, which inhibits electron flow prior to the site of energy coupling (18), produces no efflux of accumulated Rb+ (Fig. 6). These results demonstrate that reduction of the respiratory chain at the site of energy coupling is responsible for Rb+ efflux. Since p-CMBS (47) and NEM do not cause Rb+ efflux when employed at concentrations which markedly inhibit Rb+ uptake (Fig. 8) and n-lactate oxidation (18), it is apparent that these sulfhydryl reagents, like oxamate, interrupt electron flow prior to (or at) the site of energy coupling.

Chemiosmotic coupling has been suggested by Mitchell (12, 14, 45, 46) as a mechanism for oxidative phosphorylation in mitochondria, and Harold (14, 36) and West (47) have applied this theory to active transport in bacteria. According to this hypothesis, active efflux of protons results in a proton gradient or electrical potential across the membrane, or both. It is postulated that this electrochemical gradient, in conjunction with membrane carriers catalyzing facilitated diffusion and coupled exchange reactions (14), is responsible for the accumulation of transport substrates. Moreover, the inhibitory effects of uncoupling agents on mitochondrial (48) and bacterial (49) transport systems are attributed to the ability of these compounds to conduct protons across the membrane and thus collapse the membrane potential (50). It is a characteristic feature of this hypothesis that energy coupling for active transport is visualized as an indirect process which is mediated by the electrochemical gradient.

According to chemiosmotic coupling theory, valinomycin-induced K+ or Rb+ uptake by bacterial membrane vesicles would take place by the mechanism shown on the left-hand side of Fig. 14 (electrogenic influx). In this scheme, energy derived from n-lactate oxidation is coupled to the active extrusion of protons, resulting in a membrane potential which is positive outside and negative inside. By this means, valinomycin would facilitate the passive movement of K+ or Rb+ ions through the membrane in response to the electrical gradient. Accordingly, the pH changes observed upon addition of n-lactate to membrane vesicles would reflect the generation of proton gradients across the membrane and would be expected to exhibit properties analogous to active Rb+ uptake. Moreover, it would be necessary to postulate the existence of a coupled H+ Na+ exchange system in the membrane vesicles in order to account for the extensive Na+ efflux observed during Rb+ uptake (Fig. 10). Finally, the electrogenic efflux model would predict that lipid-soluble cations such as the dibenzyldimethylammonium ion (51) should be accumulated under conditions in which active uptake of normal substrates is observed; accumulation of this ion has been reported for mitochondrial (52) and some bacterial systems (32).

In the alternative mechanism shown on the right-hand side of Fig. 14 (electrogenic efflux), valinomycin-induced uptake of K+ or Rb+ is represented as an active process coupled directly to respiration. This scheme is thus analogous to that proposed previously for the sugar and amino acid transport systems of E. coli membrane vesicles (19), and implies that energy coupling for active transport involves direct, local interactions between individual membrane carriers and discrete energy-coupling sites. According to this hypothesis, active influx of K+ or Rb+ is itself electrogenic, creating a transmembrane potential which is positive inside and negative outside, thereby causing the passive efflux of protons or Na+ ions down the electrical gradient and against their own concentration gradient. In this model, moreover, n-lactate-induced H+ liberation and n-lactate-stimulated Rb+ uptake are independent phenomena and would not be required to exhibit similar properties.

The following results indicate that the properties of valinomycin-induced Rb+ transport are consistent with the electrogenic influx model, and that proton or potential gradients are not the primary driving force for respiration-linked transport.

1. The lipophilic cation dibenzyldimethylammonium, at relatively high concentrations, produces only minimal inhibition of Rb+ uptake (Table IV) and is not accumulated in the presence of n-lactate under conditions in which Rb+ is actively taken up (Table V). Moreover, the vesicles are permeable to this cation, as evidenced by the observation that it equilibrates with the intravesicular pool. These results are incompatible with the presence of a membrane potential which is positive outside and negative inside (Fig. 14, electrogenic efflux).

2. Rapid efflux of Na+ against its own concentration gradient is observed only under conditions in which the membranes catalyze active Rb+ accumulation, i.e., in the presence of n-lactate, valinomycin, and Rb+ (Fig. 10). Rapid Na+ efflux does not occur when Rb+ is replaced by the dibenzyldimethylammonium ion, and this cation does not inhibit Rb+-induced Na+ efflux. These findings are compatible with a membrane potential which is positive inside and negative outside, generated as a consequence of active Rb+ uptake (Fig. 14, electrogenic influx).

3. Rapid Na+ efflux is not observed following sudden acidification of the medium, indicating the absence of a coupled H+-Na+ exchange mechanism in the membrane vesicles.

4. Addition of valinomycin to membrane vesicles suspended in KCl has no effect on the rate or extent of n-lactate-dependent H+ release (Fig. 11).

5. The lipid-soluble weak acid 5,5-dimethylloxazolidine-2,4-dione (36) is not taken up by the membranes in the presence of n-lactate or other electron donors under conditions in which active transport of Rb+ occurs.13 Since the distribution of this

---

13 J. I. Reeves, unpublished data.
compound is thought to reflect pH differences across the membrane (56, 53), this finding indicates that, under conditions of active Rb⁺ accumulation, the internal compartment of the membrane is not alkaline with respect to the medium.

6. The steady state level of β-lactate-stimulated H⁺ release exhibits a relatively sharp pH optimum at pH 5.5 to 6.0 where it reaches a maximum value of approximately 60 nmoles of H⁺ per mg of membrane protein (Fig. 12B). On the other hand, steady state levels of β-lactate-stimulated Rb⁺ uptake exhibit a broad pH optimum from pH 6.0 to approximately 8.5 and attain levels in excess of 150 nmoles of Rb⁺ per mg of membrane protein (Fig. 12A). Furthermore, at pH 8.0, where the level of H⁺ release is negligible, both the rate and extent of Rb⁺ uptake are more than 75% of maximum. Although these measurements were carried out under different experimental conditions making quantitative comparison difficult, the qualitative behavior of β-lactate-stimulated H⁺ release does not support the hypothesis that this process is involved in active Rb⁺ uptake.

7. Membranes treated with the non-ionic detergent Tween-40 such that they retain the catalytic activities associated with transport (i.e. P-enolpyruvate-dependent phosphorylation of α-methylglucoside and β-lactate oxidation) but are unable to retain transported solute (i.e. α-methylglucoside-P and Rb⁺) exhibit β-lactate-induced pH changes similar to those of untreated membranes (Fig. 13). Furthermore, the uncoupling agent CCCP has similar effects on normal and detergent-treated vesicles. Since the Tween-treated preparations are devoid of a diffusion barrier, it is unlikely that the observed pH changes are due to proton gradients. Recent studies demonstrated that β-lactate-induced changes in 1-anilino-8-naphthalensulphonate fluorescence are related to conformational changes in membrane components (54). The pH changes observed here may be a reflection of the same phenomenon.

8. The sugar and amino acid transport systems of E. coli membrane vesicles are stimulated with different relative efficiencies by various electron donors, i.e. β-lactate, succinate, l-lactate, NADH, and ascorbate-phenazine methosulfate (15, 21). Moreover, there is no competition among these transport systems for energy coupling via β-lactate dehydrogenase (19). As shown in Table II, the order of effectiveness of the various electron donors in stimulating Rb⁺ uptake (i.e. ascorbate-phenazine methosulfate > β-lactate > l-lactate > dl-α-hydroxybutyrate > succinate > NADH), though comparable to results obtained for the transport of β-galactosides and amino acids, is not identical with that observed with any of these uptake systems (19, 21). Thus, active Rb⁺ uptake exhibits the same uniqueness with regard to energy source specificity as noted previously for the other respiration-linked systems. These results are difficult to reconcile with an indirect coupling between energy generation and active transport mediated via proton or potential gradients. Were this the case, the relative ability of various electron donors to stimulate transport would be expected to be the same for all of the transport systems.

9. Addition of lactose plus a mixture of amino acids to E. coli membrane vesicles has no effect on the rate or extent of β-lactate-stimulated H⁺ release.13 Furthermore, addition of β-lactate plus a mixture of lactose and amino acids does not induce Na⁺ efflux, and dibenzylidimethylammonium chloride, at a concentration of 5 mM, produces only minimal inhibition of β-lactate-stimulated lactose uptake.8 These results support the conclusion that respiration-linked sugar and amino acid transport by E. coli membrane vesicles, like valinomycin-induced Rb⁺ uptake, is not dependent on proton or potential gradients.

Although the precise mode of action of valinomycin in concentrative Rb⁺ uptake is unknown, one possibility is that the antibiotic may make Rb⁺ accessible to part of a K⁺ transport system of E. coli (55, 56) which is not otherwise demonstrable in the vesicles. Alternatively, in view of the earlier findings regarding fluorescence of ANS in these membrane preparations (54), it might be speculated that the valinomycin-Rb⁺ complex binds to some component in or near the respiratory chain which undergoes a conformational change upon reduction. This conformational change might distort the geometry of the bound valinomycin-Rb⁺ complex such that the affinity for the complexed cation would be diminished, resulting in its release on the interior surface of the membrane. A mechanism such as this bears a resemblance to that proposed earlier for the respiration-linked sugar and amino acid transport systems (19). Current studies with fluorescent-labeled valinomycin analogues may clarify the mechanism of valinomycin action in the bacterial membrane vesicles.

Note Added in Proof.—While this paper was in press, the authors received a personal communication from Dr. Karlheinz Altenendorf, National Jewish Hospital, Denver, Colorado, to the effect that β-lactate-stimulated uptake of dibenzylidimethylammonium ion by ML 308-225 membranes is demonstrable with vesicles suspended in sodium phosphate buffer containing low concentrations of the lipophilic anion tetrathylphosphorionate. These observations have been confirmed by the authors. Since the vesicles do not accumulate the dibenzylidimethylammonium cation in potassium phosphate buffer, the significance of these findings with regard to uptake of physiological transport substrates is obscure.

Acknowledgments.—The authors are grateful to Dr. W. Benz, Hoffman-La Roche, Nutley, New Jersey, for carrying out the mass spectroscopy, to Mrs. Grace K. Kerwar for her excellent technical assistance during part of this work, and to Dr. W. W. Umbreit, in whose laboratory portions of this study were carried out.

REFERENCES


13 These studies are being carried out in collaboration with Dr. B. F. Gisin of Rockefeller University.
Mechanisms of Active Transport in Isolated Bacterial Membrane Vesicles: XIII. VALINOMYCIN-INDUCED RUBIDIUM TRANSPORT
Frank J. Lombardi, John P. Reeves and H. Ronald Kaback


Access the most updated version of this article at http://www.jbc.org/content/248/10/3551

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/248/10/3551.full.html#ref-list-1