

Potassium Permeability of *Rickettsia prowazekii*

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The potassium permeability of *Rickettsia prowazekii* was characterized by chemical measurement of the intracellular sodium and potassium pools and isotopic flux measurements with $^{86}\text{Rb}^+$ as a tracer. *R. prowazekii*, in contrast to *Escherichia coli*, did not maintain a high potassium-to-sodium ratio in their cytoplasm except when the potassium-to-sodium ratio in the extracellular medium was high or when the extracellular concentrations of both cations were low (ca. 1 mM). Both influx and efflux assays with $^{86}\text{Rb}^+$ demonstrated that the rickettsial membrane had limited permeability to potassium and that incorporation of valinomycin into these cells increased these fluxes at least 10-fold. The transport of potassium showed specificity and dependence on rickettsial metabolism. The increased flux of potassium which results from the incorporation of valinomycin into the rickettsial membrane was detrimental to both lysine transport and lysis of erythrocytes by the rickettsiae.

Rickettsia prowazekii, an obligate intracellular parasitic bacterium, grows only in a high-potassium environment, the cytoplasm of a eucaryotic cell. It would be interesting to know what effect this environment has had on the evolution of potassium transport systems and the cation permeability of the rickettsial cell membrane. Unfortunately, we do not know how to evaluate these processes when the rickettsiae are within the cytoplasm of the host: the rickettsiae must be removed from this environment and separated from cell organelles to obtain a useful preparation with which to experiment. Although these purified rickettsiae cannot be made to grow in axenic medium, they are metabolically active and can do work. For example, isolated rickettsiae can accumulate the amino acid lysine and lyse erythrocytes and fibroblasts—processes which necessitate the rickettsiae being energetically competent with an intact proton motive force (PMF) (4, 6, 8, 11).

To maintain a PMF, the rickettsial membrane must have a cation and proton conductance that is less than the rate of extrusion of protons from the rickettsiae by their electron transport system or their ATP translocase and adenosine triphosphatase (7, 10, 11). Under these conditions the membrane potential will be maintained rather than short-circuited. Myers et al. suggested, based principally on plasmolysis data, that *Rickettsia typhi*, a closely related species, becomes highly permeable to sodium and potassium ions as a result of the purification process (3). Since such a high ion conductance would be seemingly incompatible with the known capabilities of purified rickettsiae to maintain and use their PMF, their permeability to potassium ions and the effect of this permeability on energy-requiring processes were investigated.

MATERIALS AND METHODS

Rickettsia preparation and growth. *R. prowazekii* Madrid E was propagated in embryonated, antibiotic-free chicken eggs by inoculation with 0.2 ml of a seed pool. Rickettsial suspensions were prepared from heavily infected yolk sacs by a modification of the methods of Bovarnick and Snyder (2) and Wisseman et al. (9) as previously described (11). Only fresh, unfrozen rickettsiae were used. The medium for the rickettsial suspension in the purification procedure was a

sucrose-potassium-phosphate-glutamate (SPG) solution originally devised by Bovarnick et al. (1).

The purified rickettsiae were suspended in a sucrose-Tris-phosphate-glutamate medium (STPG), identical to SPG except that Tris replaced potassium. The metabolic activity of the rickettsiae did not appear to be adversely affected by the presence of Tris, and this medium allowed the potassium concentration to be adjusted to the desired range. Rickettsiae were treated with valinomycin, unless otherwise indicated, by incubation with 15 μM valinomycin for 1 h at 0°C at a protein concentration of 0.5 mg/ml.

Determination of sodium and potassium ion pools. A 400- μl portion of the suspension of bacteria at a protein concentration of 2 to 3 mg/ml in STPG containing [^3H]sucrose (1 $\mu\text{Ci/ml}$) and the indicated additions were added to three 450- μl plastic microfuge tubes and centrifuged in a Beckman Microfuge for 6 min. The supernatant fluid was immediately and thoroughly removed from the pellet and placed in another tube. Nitric acid (50 μl) was added to each pellet, and the pellets were placed in a refrigerator overnight. Water (350 μl) was added to the nitric acid extract, mixed well, and centrifuged above. The clarified extract was removed and placed in a clean tube. The sodium and potassium concentrations were determined on 25- μl and 200- μl portions of the supernatant fluid and pellet extract, respectively, with lithium as an internal standard by flame photometry. The radioactivity was also determined so that the amount of cation in the pellet extract could be accurately corrected for extracellular fluid to determine the true intracellular pool.

Transport of potassium and lysine. The uptake of potassium was measured by membrane filtration of a portion (0.1 ml) of a suspension of rickettsiae at 0.5 mg of protein per ml in STPG plus $^{86}\text{Rb}^+$ (5 $\mu\text{Ci/ml}$; 1 $\mu\text{g/ml}$) as a potassium tracer. The potassium concentration was 1 mM unless otherwise indicated. Efflux of potassium was measured by filtering 25 ml of a rickettsial suspension formed by dilution of the uptake suspension 250-fold. Lysine transport and hemolysis were assayed as previously described (4, 6).

RESULTS

Comparison of the sodium and potassium ion levels in *R. prowazekii* and *Escherichia coli*. The inability of *R. prowaze-*

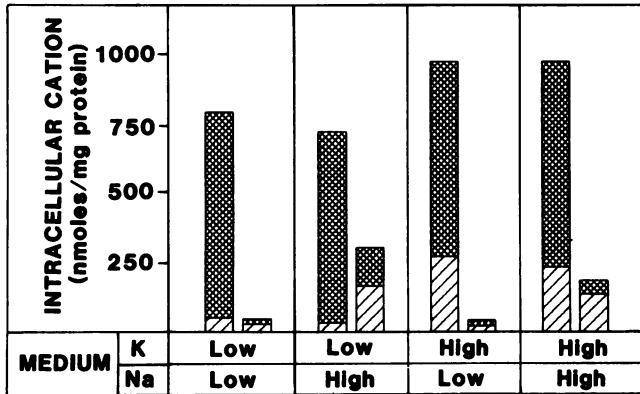


FIG. 1. Intracellular Na⁺ and K⁺ composition of *E. coli*. Strain K-12 was grown to exponential phase, harvested, washed, and suspended to about 2 mg of protein per ml in STPG (0.218 M sucrose, 10 mM Tris-phosphate [pH 7.0], 5 mM glutamic acid) to which KCl or NaCl was added at 1 or 20 mM. These media are referred to as low and high Na and K on the figure. After incubation, the supernatant was removed, the radioactivity was determined by liquid scintillation techniques, and the Na⁺ and K⁺ concentrations were assayed by flame photometry. The pellet was digested overnight in HNO₃, and the radioactivity and K⁺ and Na⁺ concentrations were determined. The total K⁺ or Na⁺ in the pellet was corrected for the K⁺ or Na⁺ in the extracellular (sucrose) space of the pellet to give the intracellular concentration. The left bar in each column depicts K⁺, and the right bar depicts Na⁺. The Na⁺ and K⁺ composition of heat-killed organisms is indicated by the lower bars.

kii to regulate its intracellular cation pools was in marked contrast to *E. coli* (see reference 5 for review). The intracellular potassium pool in *E. coli* was maintained at ca. 800 nmols per mg of protein (about 150 mM) at all extracellular potassium and sodium concentrations from 1 to 20 mM (Fig. 1). The intracellular sodium pool in *E. coli* increased from 50 to 300 nmols per mg of protein as the extracellular sodium concentration was increased. The contribution of nonspecific binding of cations was evaluated by determining the cations associated with the pellet of heat-killed (56°C; 30 min) bacteria after correction for extracellular space in the pellet. This contribution in *E. coli* remained relatively small in all media.

In contrast, *R. prowazekii* (Fig. 2) had an intracellular potassium concentration that was greater than the intracellular

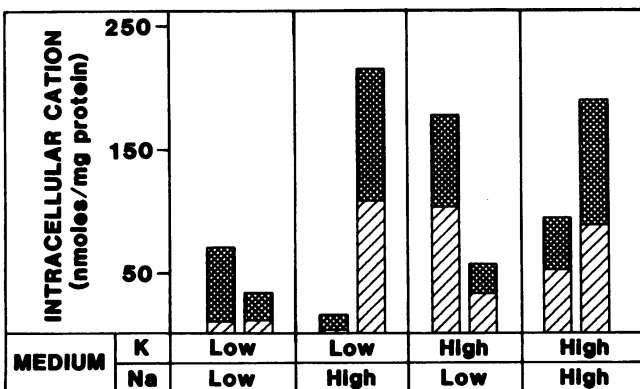


FIG. 2. Intracellular Na⁺ and K⁺ composition of *R. prowazekii*. The same procedure used for *E. coli* in Fig. 1 was adopted to *R. prowazekii* in this study.

lar sodium concentration only when the extracellular potassium concentration was greater than the extracellular sodium concentration or when the extracellular concentration of both cations was low (ca. 1 mM). (Based on cell water, 70 nmols/mg of protein is equal to about 44 mM.) When the extracellular concentration of both cations was 20 mM, the rickettsial cell became sodium rich relative to its potassium level. It was noteworthy that the potassium level, or the sum of the sodium plus potassium levels, was in all cases much less than that in *E. coli*. Also in contrast with *E. coli*, at higher extracellular cation concentrations (20 mM) the contribution of binding of both potassium and sodium to heat-killed rickettsiae was a significant fraction of the total pool in native rickettsiae.

Effect of valinomycin on potassium ion uptake. The potassium ionophore, valinomycin, was incubated with rickettsiae at 0°C for 60 min, and its effect on the rate and extent of potassium uptake was evaluated using ⁸⁶Rb⁺ as a tracer of potassium uptake. This isotope has been often used as a potassium tracer because of the expense and difficulty caused by the very short half-life of radioactive potassium. Figure 3 depicts the striking increase in the rate of potassium uptake (measured by ⁸⁶Rb⁺ influx) that resulted from the incorporation of increasing amounts of valinomycin into the rickettsial cell membrane. It was of interest that no special treatment of the rickettsiae was necessary for valinomycin to be effective.

The effects of valinomycin and heat treatment on the time course of uptake at an extracellular potassium concentration of 1 mM in *R. prowazekii* is shown in Fig. 4. The background binding, that observed in heat-killed rickettsiae, was the same in both valinomycin-treated and untreated rickettsiae. This background was a significant fraction of the total uptake observed in native rickettsiae, but not in rickettsiae in which the uptake was mediated by valinomycin. At a higher extracellular potassium concentration, the contribution to the total potassium uptake in native rickettsiae by the heat-killed cells was so large that the fraction of the uptake mediated by viable rickettsiae could not be accurately determined. From day to day there was variation in the level of uptake calculated on a nanomole per milligram of protein basis, but the addition of valinomycin always increased the

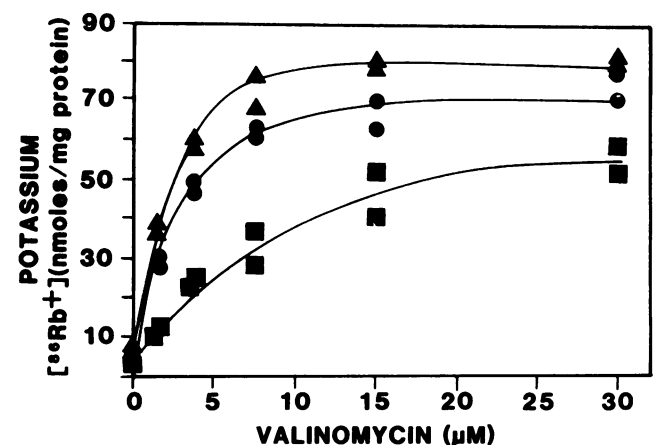


FIG. 3. The effect of valinomycin on K⁺ uptake in *R. prowazekii*. Valinomycin dissolved in ethanol was added to rickettsiae in STPG at 0.5 mg of protein per ml at 0°C for 60 min. The ethanol concentration was 1% under all conditions, and the KCl concentration was 1 mM. Samples were filtered at 0.5 (■), 1.2 (●), and 2.0 min (▲).

uptake greatly. The cause of this variability is not known. However, it was not due to varying levels of exchange diffusion resulting from differing initial intracellular potassium concentrations in various batches of rickettsiae: rickettsiae loaded with potassium by preincubation in 20 mM extracellular salt had the same uptake as those depleted of potassium (data not shown).

Efflux of potassium ion. The efflux of potassium, again measured with $^{86}\text{Rb}^+$ as the tracer, from native and valinomycin-treated rickettsiae preincubated with an extracellular potassium concentration of 1 mM was determined (Fig. 5). Cells were diluted 250-fold into buffer containing no potassium, 20 mM potassium, no potassium plus 1 mM triphenylmethylphosphonium (TPMP), or buffer at 56°C. TPMP, a lipid-soluble cation, at this concentration eliminates the electrical component of the PMF and inhibits the active transport of lysine in rickettsiae (6, 11). The efflux from valinomycin-treated rickettsiae was rapid in all cases, and in the experiments where the efflux medium was 20 mM potassium or 1 mM TPMP it was complete, decreasing to the level of heat-killed rickettsiae. Native rickettsiae were, in contrast, much less permeable to potassium. Although efflux into a medium containing potassium (20 mM) was faster and more extensive than efflux into a medium without potassium, both efflux rates were clearly very slow relative to that mediated by valinomycin. The efflux from native rickettsiae into a medium containing TPMP was again relatively slow, but the intracellular potassium concentration ultimately decreased to the level observed in heat-killed cells. The concentration of TPMP in the efflux medium had to be high (ca. 1 mM) to obtain this extensive efflux: the efflux into 0.1 mM TPMP was the same as the control efflux, with only a slight increase in efflux at 0.2 mM TPMP. Presumably, the rickettsiae were able to maintain the PMF when the concentration, and thus the influx, of TPMP was low.

Effect of metabolic inhibitors on the influx of potassium. The uptake of potassium ions in both valinomycin-treated rickettsiae (data not shown) and native rickettsiae (Fig. 6)

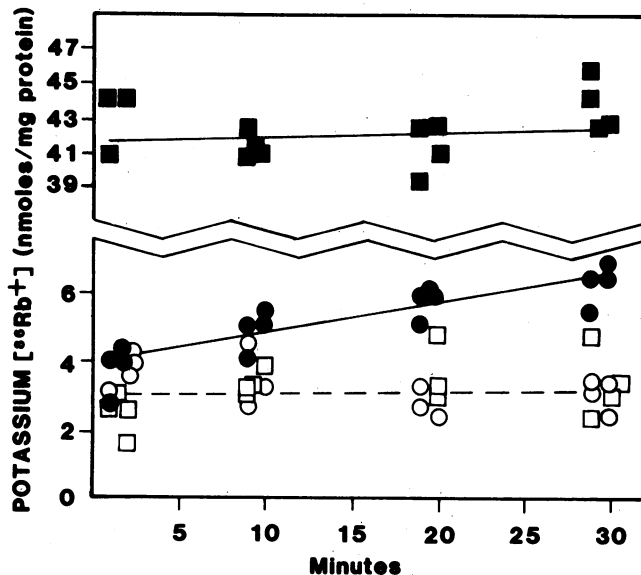


FIG. 4. Uptake of K^+ by *R. prowazekii*: effect of heat-killing and valinomycin. Rickettsiae treated with 15 μM valinomycin as in Fig. 3 are indicated by solid squares, native rickettsiae are indicated by solid circles, and the corresponding heat-killed rickettsiae are indicated by open squares and circles. Note break in scale.

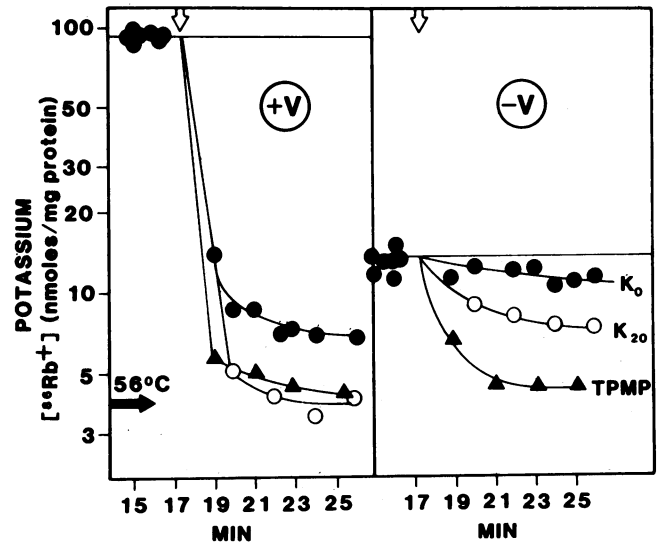


FIG. 5. Efflux of K^+ from rickettsiae. Cells (0.5 mg of protein per ml) were loaded in STPG with KCl (1 mM) and $^{86}\text{Rb}^+$ for 17 min. The cells were then diluted 250-fold into STPG (K_0) (●), STPG plus 20 mM KCl (K_{20}) (○), or STPG plus 1 mM TPMP (▲) and sampled (25 ml) at the indicated times. The solid arrow represents the K^+ in cells diluted into 56°C STPG. The left panel depicts valinomycin-treated rickettsiae; the right panel depicts native rickettsiae.

required metabolically active rickettsiae. Preincubation of rickettsiae for 10 min at 34°C with 1 mM concentrations of *N*-ethylmaleimide, 2,4-dinitrophenol, TPMP, or NaCN markedly decreased the uptake of potassium. These compounds have been shown to inhibit hemolysis and lysine accumulation in *R. prowazekii* (4, 6). Without preincubation, the inhibition was less pronounced with all inhibitors except TPMP.

Effects of cations on the uptake of potassium. The cation specificity of potassium uptake in valinomycin-treated and native rickettsiae was determined by measuring the uptake

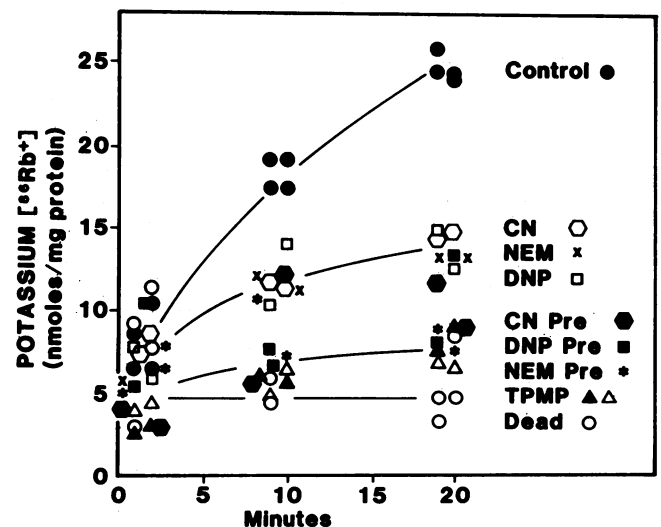


FIG. 6. Effect of metabolic inhibitors on K^+ uptake by rickettsiae. Rickettsiae (0.5 mg of protein per ml) were preincubated where indicated (Pre) with indicated poisons (1 mM) at 35°C for 10 min before the uptake of K^+ (1 mM KCl; $^{86}\text{Rb}^+$). Heat-killed rickettsiae (dead) were incubated at 56°C for 30 min.

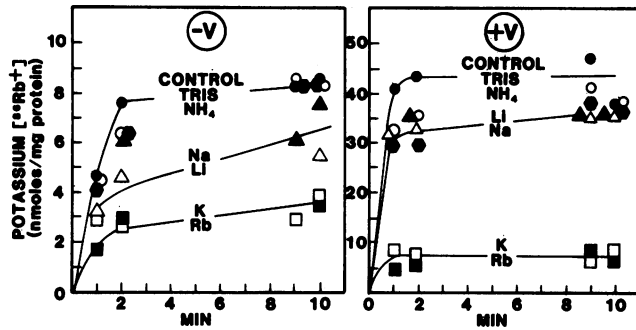


FIG. 7. Effect of various cations on K^+ uptake by rickettsiae. Rickettsiae were incubated in STPG plus 1 mM KCl, $^{86}\text{Rb}^+$, and 20 mM of the chloride salt of putative inhibitory cations and sampled at the times indicated. The left panel depicts native rickettsiae; the right panel depicts rickettsiae incubated with 15 μM valinomycin as in Fig. 3.

of 1 mM potassium, with $^{86}\text{Rb}^+$ as a tracer, in the presence of 20 mM concentrations of various monovalent cations (Fig. 7). Potassium or rubidium chloride markedly inhibited uptake in both native and valinomycin-treated cells. This result is consistent with these two cations having similar affinities for the same transport system. Sodium and lithium chloride at 20 mM caused a significant, but incomplete, inhibition of uptake in native rickettsiae. However, at a concentration of 4 mM, sodium ions had no significant effect on uptake in such rickettsiae, whereas at 4 mM, the potassium ions had a large effect (data not shown). Tris and ammonium cations at 20 mM had no effect in native rickettsiae on the uptake of potassium. Tris, ammonium, sodium, and lithium cations all caused a small inhibition of valinomycin-mediated uptake, whereas potassium and rubidium caused a complete inhibition.

Effect of potassium on metabolic processes. If the influx of potassium ions was so rapid that the electrical potential across the cell membrane could not be maintained, the influx of potassium would be detrimental to the metabolism of rickettsiae. A highly permeant potassium ion would affect cellular processes that depend on energy in the same manner that TPMP and 2,4-dinitrophenol do. The accumulation of lysine and the hemolysis of sheep erythrocytes in solutions with various concentrations of sodium or potassium, or both, in the presence and absence of valinomycin was evaluated (Table 1). Either cation at 20 mM caused about 20% inhibition of the accumulation of lysine in rickettsiae not treated with valinomycin. However, in valinomycin-treated rickettsiae, a cation specificity was observed. The effect of sodium in these rickettsiae was about the same as in native rickettsiae, but the inhibitory effect of potassium in valinomycin-treated rickettsiae was more than twice that in native rickettsiae. Similar inhibitory effects caused by the valinomycin-mediated increased potassium permeability could be seen at lower concentrations (5 mM) and in the hemolytic system (Table 1).

DISCUSSION

It was clear from the chemical analysis of rickettsiae that they were permeable to sodium and potassium ions and that their intracellular cation concentrations reflected the extracellular cation concentrations except at low extracellular concentrations where there was potassium selectivity. Rickettsiae in a high sodium medium had a higher sodium than potassium intracellular level, in marked contrast to *E. coli*,

which maintained a predominantly potassium intracellular milieu regardless of the nature of the extracellular cations (5).

The amount of potassium ion per milligram of protein was significantly lower in *R. prowazekii* than in *E. coli*. This lower level of potassium may be explained, in part, by the presence of nonviable rickettsiae in the rickettsial preparation (usually less than 50%) which could not accumulate potassium ions, whereas an *E. coli* culture would almost exclusively consist of viable organisms. The percentage of the native potassium level that was measured in the dead organisms was much higher in *R. prowazekii* than in *E. coli*, but the absolute amount of potassium ion per milligram of protein that was associated with the dead bacteria was about the same.

These data clearly show that the rickettsial membrane is not freely permeable to potassium ions. Incorporation of a known potassium carrier, the depsipeptide valinomycin, into the rickettsial membrane was able to increase the influx of potassium at least 10-fold. Similarly, the efflux of potassium was much more rapid when mediated by valinomycin than by the endogenous potassium transport systems of the rickettsiae.

The accumulation of potassium ions could be inhibited by a variety of metabolic poisons. Inhibition was most rapid with the lipophilic cation TPMP. The ability of rickettsiae to retain potassium was also abolished by TPMP. These results suggest that a membrane potential, interior negative, provided the driving force for the accumulation of potassium ions in a manner similar to that shown for lysine transport in rickettsiae (6, 11).

The system responsible for potassium uptake in *R. prowazekii* has apparent specificity. The uptake of $^{86}\text{Rb}^+$ was inhibited equally by potassium and rubidium ions, and the inhibition by these cations was significantly greater than that caused by sodium or lithium ions. Tris and ammonium ions had no effect. The known specific uptake mediated by valinomycin was inhibited almost totally by potassium and rubidium ions. A small inhibition of the extent, but not the rate, of uptake was characteristic of the addition of other monovalent cations. Inhibition by cations which do not

TABLE 1. Effect of potassium and valinomycin on lysine transport and hemolysis

Addition to STPG medium	Lysine uptake ^a		Hemolysis ^b	
	Without valinomycin	With valinomycin	+V/-V	+V/-V
None	100 (2.3 ± 0.4)	100 (1.2 ± 0.59)	1.0	1.0
5 mM KCl	90 ± 6	50 ± 3	0.6	0.6
5 mM NaCl	89 ± 4	107 ± 16	1.2	ND ^c
5 mM KCl + 5mM NaCl	83 ± 9	48 ± 1	0.6	ND
20 mM KCl	79 ± 12	25 ± 19	0.4	0.4
20 mM NaCl	84 ± 9	76 ± 18	0.9	0.9
20 mM KCl + 20 mM NaCl	65 ± 8	20 ± 6	0.3	0.5
1 mM DNP ^d	10 ± 1	15 ± 7	1.5	ND

^a Values are normalized to STPG without additions. Means and standard deviations are shown. Figures in parentheses are the lysine uptakes of these controls expressed as nanomoles per milligram of protein at 10 min.

^b Ratio of the value with valinomycin (+V) divided by that without valinomycin (-V).

^c ND, Not determined.

^d DNP, 2,4-dinitrophenol.

interact with valinomycin could be due to competition for nonspecific binding sites, shrinkage of the cell, the lowering of the membrane potential, or all three.

The effects of native and valinomycin-mediated transport of potassium ions on energy-requiring biological processes in *R. prowazekii* can serve as an indicator of that permeation and of the significance of that permeation to those processes. The uptake of lysine in untreated rickettsiae was inhibited slightly by the addition of either sodium or potassium ions. The inhibition by sodium ions was not changed when valinomycin-treated rickettsiae were tested, but the inhibition by potassium ions of both lysine uptake and hemolysis was greatly enhanced by valinomycin treatment of the rickettsiae.

Thus, the rate of electrogenic extrusion of protons from these purified rickettsiae, whether by the electron transport chain or by the rickettsial membrane adenosine triphosphatase, is adequate to allow this limited penetration of potassium ions without dissipation of the PMF. A 10-fold increase in potassium permeability, such as exists in valinomycin-treated rickettsiae, was shown to be very detrimental to energy-requiring processes in rickettsiae. At present, investigations of the metabolism of rickettsiae use rickettsiae that have been purified from contaminating host components. This is usually necessary because the specific activity of these host components may well be greater than that of the rickettsiae. Low-salt buffers, with that salt being potassium and with the osmolarity of the buffer contributed by sucrose, would seem appropriate to maintain rickettsiae. The SPG buffer devised many years ago by Bovarnick et al. (1) fulfills these criteria. The nature of potassium transport in a *R. prowazekii* organism growing within the cytoplasm of its host must remain for now a matter of speculation.

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