ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1981, p. 803-808
0066-4804/81/120808-06$02.00/0

Vol. 20, No. 6

Role of the Membrane Potential in Bacterial Resistance to Aminoglycoside Antibiotics

P. DIANNE DAMPER† AND WOLFGANG EPSTEIN*

Department of Biochemistry, The University of Chicago, Chicago, Illinois 60637

Received 30 July 1981/Accepted 15 September 1981

The electrical potential difference ($\Delta\psi$) across the membrane of *Escherichia coli* was measured by the distribution of lipid-soluble cations and correlated with resistance to dihydrostreptomycin, where resistance is presumed due to reduced uptake of the drug. A good correlation between the two measured parameters was found under all conditions tested, which included effects of several mutations, inhibitors, changes in pH, and osmolarity. The most dramatic changes were seen when pH was varied; in wild-type strains resistance increased more than 100-fold, and $\Delta\psi$ fell by 70 mV when pH was reduced from 8.5 to 5.5. These results were interpreted as support for a model in which the uptake of the polycationic aminoglycosides is electrogenic and therefore driven by $\Delta\psi$. The factor common to mutations and conditions which increase resistance was a reduction in $\Delta\psi$. A simple model was developed which relates the minimal inhibitory concentration to the rate of aminoglycoside uptake and the rate of growth.

Resistance to aminoglycoside antibiotics is associated with a variety of metabolic lesions in bacteria. Mutations producing resistance include ones affecting the synthesis of heme (27, 29), menaquinone (26, 28), cytochromes (6, 28), and the proton-translocating adenosine triphosphatase (ATPase) (19). Because these mutations do not alter the ribosomal site of aminoglycoside action nor appear to result in inactivation of the antibiotics, it has been assumed that resistance in such mutants is due to reduced uptake of the drugs into the cells. Reduced uptake has been demonstrated in some cases (6, 8, 10, 28), making this mechanism a reasonable explanation for this type of resistance. This mechanism is of wider interest, for it has been reported in some clinically resistant strains (4). The coupling between metabolic defects and resistance to aminoglycosides was the subject of this study.

Mutations which produce resistance reduce or abolish the conversion of energy from oxidation-reduction reactions in the membrane to the synthesis of ATP or other uses. The connection of resistance with oxidative metabolism is supported by the observation that anaerobic growth is associated with resistance to aminoglycosides (5, 7). This type of resistance affects all aminoglycosides regardless of specific structure. By contrast, resistance in which R-factor-coded enzymes inactivate the drug or in which the ribosomal site of action is altered by mutation are specific for those aminoglycosides with the requisite structural features (3). Therefore, some relatively nonspecific property of the aminoglycosides must be involved. One common property of all aminoglycosides is a large net positive charge at physiological pH due to multiple amino groups with a high pK. Any positively charged molecule experiences a strong driving force for entry into bacteria which maintain a large electrical potential ($\Delta\psi$), interior negative, across their membrane (22). A reduction in $\Delta\psi$ reduces this driving force and could decrease the rate of aminoglycoside uptake to make the cells resistant.

In this study, we examined a number of conditions that alter $\Delta\psi$ in *Escherichia coli* and found a correlation between $\Delta\psi$ and susceptibility to aminoglycosides. The results indicate that the magnitude of $\Delta\psi$ is an important determinant of resistance to aminoglycosides. A preliminary account of some of this work has been presented previously (11).

MATERIALS AND METHODS

Bacterial strains. The strains of *Escherichia coli* K-12 and their genotypes are listed in Table 1. The *hem-28* and *unc-40* were obtained in selections for low-level neomycin resistance after ultraviolet mutagenesis. The *hem-28* mutant has a phenotype typical of *hem* mutants described by others (27). The mutation in this strain is close to and clockwise from *cya* near min 85 on the current map of *E. coli* (2), indicating it is either a *hemC* or *hemD* mutation. Strain TK1207 is unable to grow on succinate, has less than 2% of the

† Present address: Department of Microbiology and Immunology, University of Illinois Medical Center, Chicago, IL 60612.

TABLE 1. *Bacterial strains*

Strain	Genotype	Origin or reference
FRAG-5	F⁻ *thi rha lacZ gal kdpABC5*	(13)
TK1207	F⁻ *thi rha lacZ gal kdpABC5 unc-40*	Ultraviolet mutant; derived from FRAG-5
TK1208	F⁻ *thi rha lacZ gal kdpABC5 unc-42*	Spontaneous; from TK1207
TK1235	F⁻ *thi rha lacI gal kdpABC5 hem-28*	Ultraviolet mutant of *lacI* derivative of FRAG-5

wild-type level of membrane ATPase (9), is defective in proline transport, and has a mutation in the *unc* cluster near min 83 of the map. These properties indicate that it is a proton-leaky *unc* mutation. (12). This mutant grows somewhat slowly on all media; spontaneous mutations to more rapid growth arise frequently. Strain TK1208, which carries one of these mutations to more rapid growth, is neither resistant to aminoglycosides nor defective in proline transport, but remains unable to grow on succinate. The genetic lesion in strain TK1208, here called *unc-42*, is a double mutation combining the original *unc-40* mutations with a second, closely linked mutation which abolishes the high proton permeability produced by *unc-40*.

Media. Minimal medium contained 10 mM $(NH_4)_2SO_4$; 0.4 mM $MgSO_4$; 0.5 mM K_2HPO_4; 1 mM Na citrate; 6 μM $FeSO_4$; and a buffer component, which was usually 100 mM K *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.5. To vary pH, 100 mM K-morpholineethanesulfonic acid (MES) was used for pH 5.5 to 6.5, 100 mM K-HEPES was used for pH 7 to 8, and 100 mM tris(hydroxymethyl)aminomethane (Tris)-chloride was used for pH above 8. Glucose (50 mM) and 8 g of Casamino Acids per liter were used as carbon and energy sources, and vitamins at 1 mg/liter were added as needed. Solid medium contained 15 g of agar per liter.

Antibiotic sensitivity. The minimal inhibitory concentration (MIC) of dihydrostreptomycin was determined by growth on solid medium. Mid-log phase cells were diluted in minimal medium to a concentration of 10^4/ml, and 0.02 ml of this suspension was spotted on a series of plates, each differing from the next by at most a twofold difference in drug concentration. The MIC is the lowest concentration of drug that prevented growth of any colonies visible with a low-powered (×20) microscope after 24 to 36 h of incubation at 37°C.

Electrical potential measurement. The magnitude of the membrane potential was determined from the distribution ratio of lipid-soluble phosphonium ions (25). Both [*methyl*-^{3}H]triphenyl phosphonium (TPMP) (New England Nuclear Corp.) and [^{3}H]tetraphenyl phosphonium (TPP, kindly provided by H. R. Kaback) were used with comparable results. Cells growing in minimal medium were collected by filtration on a membrane filter, washed with and suspended in 0.1 M Tris-chloride (pH 8.0) at 10^9 cells per ml and incubated at 37°C for 10 min. Then Tris-ethylenediaminetetraacetic acid (EDTA) (pH 8.0) was added to 2 mM, and 5 min later $MgCl_2$ was added to 4 mM. The cells were collected by filtration, washed, and suspended in 20 mM morpholinepropanesulfonic acid (MOPS)–135 mM choline-chloride (pH 7.5) at 2×10^9 cells per ml and kept on ice for up to 1.5 h until used. For uptake assays, the cell suspension was diluted with an equal volume of 135 mM NaCl–50 mM glucose–20 mM K-MOPS (pH 7.5)–2 μM phenylcarbaundecarborane and incubated at 23°C for 10 min with aeration. Uptake was initiated by the addition of labeled $TPMP^+$ or TPP^+, usually to 10 μM. Samples of 0.5 ml were removed at intervals, washed with 5 ml of 10 mM MOPS–200 mM choline-chloride (pH 7.5), dried, and counted in a Packard liquid scintillation counter. Inhibitors were added to cell suspensions 10 min before initiation of uptake of the lipid-soluble cation. For measurements of $\Delta\psi$ at a different pH, either K-MES (pH 5.5 to 6.5) or Tris-chloride (pH 8.0 to 8.5) was substituted for K-MOPS (pH 7.0 to 7.5) in the post-EDTA suspension and washing buffers.

The intracellular ion concentration at equilibrium was the plateau level reached within 25 min minus nonspecific uptake as determined by measuring the amount of cation remaining after the addition of an uncoupling concentration of either 1 mM dinitrophenol or 20 μM carbonylcyanide *m*-chlorophenyl hydrazone. Intracellular concentrations were calculated by the Nernst equation and a value of 2.5 ml internal volume per g (dry weight) of cells (14).

RESULTS

The membrane potential in bacteria such as *E. coli* is generated by the extrusion of protons coupled to oxidative reactions in the cell membrane. This process generates a potential for protons, or protonmotive force (PMF), the term introduced by Mitchell (21). The PMF consists of an electrical component, represented by $\Delta\psi$, and a proton concentration component given by the difference in pH. Because the interior of bacteria is maintained near pH 7.5, there is little difference in pH across the membranes of cells in medium at pH 7.5, so that almost all of the PMF is expressed $\Delta\psi$ (22).

Drugs commonly called uncouplers act to increase the permeability of protons through the membrane, reducing the PMF by discharging it. At pH 7.5, uncouplers reduce $\Delta\psi$. Table 2 shows the effect of 1 mM dinitrophenol on two sensitive strains of *E. coli*. This concentration of uncoupler reduced the growth rate by 20% and modestly increased resistance to dihydrostreptomy-

TABLE 2. *Effect of 2,4-dinitrophenol on $\Delta\psi$ and MIC for dihydrostreptomycin*

Strain	Dinitrophenol (mM)	$\Delta\psi$ (mV)	MIC (μg/ml)
FRAG-5	0	−142	0.5
	1	−129	1
TK1208	0	−162	0.3
	1	−133	0.5

cin. Under the same conditions $\Delta\psi$ showed a moderate decrease in both strains, suggesting a correlation between $\Delta\psi$ and drug resistance.

A reduced PMF is associated with some *unc* mutations (1). Strain TK1207 with the *unc-40* mutation is presumed to have a reduced PMF because it has a markedly reduced rate of proline uptake, a process known to be driven by the PMF (17). The reduced PMF in such *unc* strains is due to abnormal leakage of protons through the membrane portion of the proton-translocating ATPase. This leak can be blocked by dicyclohexylcarbodiimide (DCCD) (1, 24). As seen in Table 3, strain TK1207 had a low $\Delta\psi$ that increased in the presence of 0.5 mM DCCD by 30 mV to values typical of wild-type strains. At the same time the MIC was reduced by a factor of 2. As control, we examined strain TK1208 with an *unc* mutation not associated with a proton leak. In this strain, treatment with DCCD caused no change in MIC and only a slight change in $\Delta\psi$, suggesting that the effect of DCCD on strain TK1207 is due to a reduction in the proton leak through the membrane component of the ATPase.

The addition of salt is known to increase resistance to aminoglycosides (20, 30) and to inhibit gentamicin uptake (10). We found that such resistance was associated with a reduction in $\Delta\psi$ in both wild-type and mutant strains (Table 4). In two of the strains 0.5 M NaCl allowed reasonable rates of growth and produced over a 10-fold increase in MIC and reduction in $\Delta\psi$ of 22 to 48 mV. In the two mutants in which the high salt concentration virtually abolished growth, the lower concentration of 0.2 M produced two- to fivefold increases in MIC and sizable reductions in $\Delta\psi$. We examined the effect of a non-ionic solute, sucrose, and found similar effects (Table 4). The concentration of sucrose used here, 0.3 M, is the osmotic equivalent of 0.17 M NaCl, indicating that osmotically equivalent concentrations of sucrose are at least as effective as NaCl in producing resistance. Thus the important parameter here appears to be osmolarity, not ionic strength. The mechanism whereby $\Delta\psi$ is reduced when osmolarity is increased is not known, but the correlation further supports a connection between resistance and $\Delta\psi$.

A wider range of values of $\Delta\psi$ was achieved by varying external pH. As external pH was reduced, the pH difference increased, resulting in a compensatory fall in $\Delta\psi$ such that the sum of the two, the PMF, remained virtually constant. Our results (Fig. 1) are similar to those reported by others (31). It is known that reducing external pH markedly increases resistance to aminoglycosides (30), an effect illustrated by our data in Fig. 2. The effects of external pH are another example, and a very striking one, of the corre-

TABLE 3. *Effect of DCCD on $\Delta\psi$ and MIC for dihydrostreptomycin*

Strain	DCCD (mM)	$\Delta\psi$ (mV)	MIC (μg/ml)
TK1207	0	−120	12
	0.5	−150	5
TK1208	0	−162	0.5
	0.5	−155	0.5

TABLE 4. *Effect of NaCl and sucrose on $\Delta\psi$ and MIC for dihydrostreptomycin*

Strain	Solute	Concn (M)	$\Delta\psi$ (mV)	MIC (μg/ml)
FRAG-5	—[a]		−142	0.5
	NaCl	0.5	−120	7
	Sucrose	0.3	−114	10
TK1208	—		−162	0.3
	NaCl	0.5	−114	5
	Sucrose	0.3	−106	0.6
TK1207	—		−120	12
	NaCl	0.2	−96	28
	Sucrose	0.3	−102	22
TK1235	—		−106	5
	NaCl	0.2	−55	24
	Sucrose	0.3	ND[b]	15

[a] —, None.
[b] ND, Not determined.

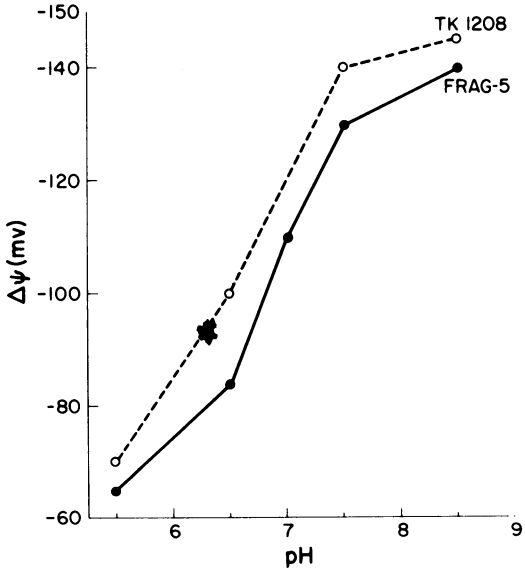

FIG. 1. *Variation of $\Delta\psi$ with external pH. The two strains examined were grown in medium at the indicated pH, and $\Delta\psi$ was measured at the same pH as described in the text. Each point is the average of several measurements performed in a single experiment.*

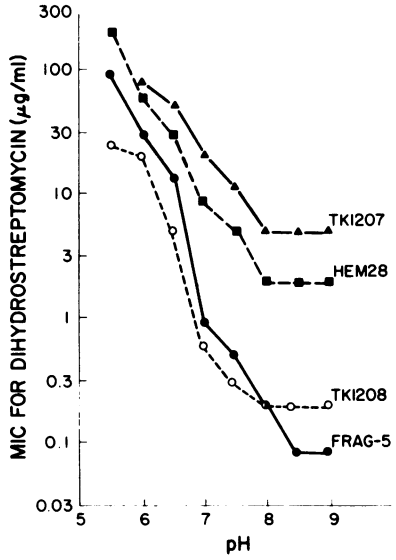

FIG. 2. *Effect of external pH on MIC for dihydrostreptomycin in the four strains used in this study. Data for strain TK1207 do not extend below pH 6 because this strain does not grow at more acidic pH.*

lation between $\Delta\psi$ and resistance to aminoglycosides.

To simplify the quantitative analysis of our results, we combined data in Fig. 1 and 2 and in Tables 2 through 4 for the single plot of Fig. 3. This figure shows the relationship of the logarithm of the MIC to $\Delta\psi$. In spite of some scatter, there is a reasonably good correlation between these two parameters. The solid line in Fig. 3, drawn by the method of least squares, is log MIC (μg/ml) = 3.3 − 0.025 $\Delta\psi$ (mV). The correlation coefficient, r, is −0.84.

Resistance to two other aminoglycoside antibiotics, kanamycin and neomycin, was also measured under many of the conditions studied here. In each case the relative changes in MIC were identical within experimental error to those observed for dihydrostreptomycin. We thus infer that the correlation between resistance and $\Delta\psi$ is true for the three aminoglycosides we studied and is probably true for all aminoglycosides.

DISCUSSION

The mechanism of aminoglycoside uptake is not well understood. Höltje has suggested that uptake is by a transport system for polyamines (18). This model is not readily reconciled with some findings of Bryan and colleagues (6), nor with the discordant effects of polyamine starvation on polyamine and aminoglycoside transport (10). Nor is this model supported by the fact that mutants resistant from loss of a specific transport system have not been reported (8). Although it is possible that the transport system involved is essential, we prefer the view that aminoglycosides enter by many different transport systems, which have slight affinity for aminoglycosides. Candidates for such uptake include systems for sugars, aminosugars, polyamines, and amino acids. If uptake is by many different systems, any competitive effect by substrates of one system would probably be small, accounting for the failure to observe competition by a large number of substrates each tested singly (8). We assume that uptake of the polycationic aminoglycosides by all systems is electrogenic so that changes in the driving force have a profound effect on the rate of uptake. For the PMF-driven proline and lactose transport systems of *E. coli*, it is known that a reduction in the driving force markedly reduces the rate of uptake (23).

Bryan and colleagues have proposed that aminoglycosides enter cells by transport on quinones or other components of the electron transport chain (8). More recently, they have modified the model to include $\Delta\psi$ as the driving force for entry via electron transport components (6). In our view, the available data do not support any direct role of electron transport, only an indirect role in creating and maintaining $\Delta\psi$, which serves as a driving force. The role of $\Delta\psi$ is strongly supported by our data. Every condition we studied, whether a mutation, addition of an inhibitor, change in external pH, or change in osmolarity,

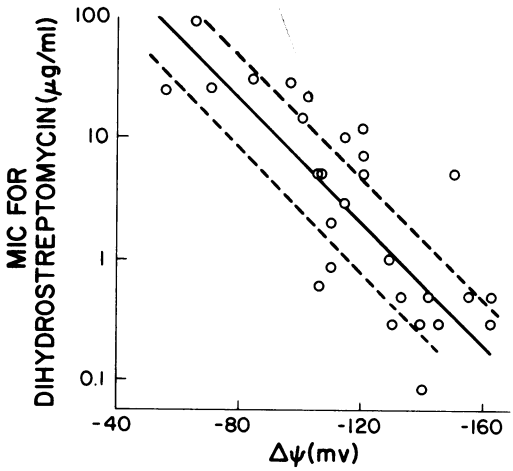

FIG. 3. *Composite plot of all data obtained in this study, showing the relationship between the logarithm of the MIC for dihydrostreptomycin and $\Delta\psi$. The solid line, drawn by the method of least squares, is log MIC (μg/ml) = 3.3 − 0.025 $\Delta\psi$ (mV). Dashed lines represent the range of variation expected from random errors as mentioned in the text.*

showed corresponding changes in resistance and $\Delta\psi$. If an electron transport component, for example, a cytochrome, were involved in transport, then the *hem* mutant we studied would have much higher resistance than would be predicted on the basis of $\Delta\psi$, because it would have both a lower driving force and lost the uptake system. Data for the *hem* mutant fit well the data for other conditions included in Fig. 3, indicating that such unusual resistance is not seen. The increased sensitivity to and uptake of aminoglycosides in some mutants that overproduce terminal respiratory enzymes are readily explained by the evidence of a greater driving force (6) and therefore do not require any specific role of these enzymes in transport.

The validity of using lipid-soluble cations to measure $\Delta\psi$ in bacteria has recently been demonstrated in giant *E. coli*, in which direct measurements of the potential can be made with microelectrodes (15). Our measurements of $\Delta\psi$ are subject to an error of about $\pm$ 10 mV based on variations we saw in values obtained under the same conditions on different days. The measurement of MIC by twofold dilution assay results in a standard deviation factor of 1.2 (16). The combination of these random errors, indicated by the dashed line in Fig. 3, accounts for a good deal of the scatter in our data. We thus conclude that the value of $\Delta\psi$ is the major determinant of aminoglycoside resistance in the types of mutants and under the conditions studied here where reduced uptake appears to be the mechanism of resistance. However, not all of our scatter is likely to be accounted for by random errors of measurement. Some of the more extreme variations, such as the 10-fold difference in MIC for very similar values of $\Delta\psi$ (Table 3, data with DCCD) suggest that other factors also play a role, albeit a minor one, in this type of resistance.

To obtain a quantitative relationship between the rate of uptake of a drug and the sensitivity to the drug, we developed a simple model. In growing cells, the concentration of drug inside represents a balance between uptake and the dilution produced by cell growth. For our analysis, we assume the accumulated drug does not exit and is biochemically unchanged, that cells are in balanced exponential growth so that the ratio of area to volume is constant, and that uptake under given conditions is proportional to the surface area of the bacteria. Let A be the area in square centimeters, V be volume in cubic centimeters, J the influx rate per unit area in micromoles per minute per square centimeter, k be the growth rate constant per minute, and subscripts 0 and t refer to time of drug addition and a later time, respectively. The total amount accumulated at time t is:

$$\int_0^t JAdt = \int_0^t JA_0e^{kt}dt = JA_0(e^{kt} - 1)/k$$

Cell volume at time t is V_0e^{kt}, so the concentration C of drug at time t is $C = JA_0(e^{kt} - 1)/kV_0e^{kt}$. When t is large, e^{kt} is large relative to 1; thus this expression becomes $C = JD/k$, where D is the ratio of area to volume, A/V.

This result states that the concentration ultimately reached in the cell is directly proportional to the influx rate J and inversely proportional to the growth rate k. The MIC is the minimal external concentration at which internal concentration ultimately reaches the lethal concentration. The dependence of C on growth rate explains the well-known fact that rapidly growing bacteria are more resistant to a number of antibiotics, including the aminoglycosides (30). The effect of growth rate contributes only a part of the variance in the data of Fig. 3, because growth rates varied by only slightly more than a factor of 2. The major effect seems to be the dependence of J on $\Delta\psi$. This dependence is very steep; the MIC doubles for each 12 mV reduction in $\Delta\psi$ (Fig. 3). A steep dependence of influx on $\Delta\psi$ is consistent with uptake of a polycationic compound by electrogenic transport.

ACKNOWLEDGMENT

This work was supported by grant GM22323 from the Institute of General Medical Sciences of the National Institutes of Health and by grant PCM7904641 from the National Science Foundation.

LITERATURE CITED

1. **Altendorf, K., F. M. Harold, and R. D. Simoni.** 1974. Impairment and restoration of the energized state in membrane vesicles of a mutant of *Escherichia coli* lacking adenosine triphosphatase. J. Biol. Chem. **249**: 4587–4593.
2. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
3. **Benveniste, R., and J. Davies.** 1973. Mechanisms of antibiotic resistance in bacteria. Annu. Rev. Biochem. **42**:471–506.
4. **Bryan, L. E., R. Haraphongse, and H. M. Van Den Elzen.** 1976. Gentamicin resistance in clinical-isolates of *Pseudomonas aeruginosa* associated with diminished gentamicin accumulation and no detectable enzymatic modification. J. Antibiot. (Tokyo) **29**:743–753.
5. **Bryan, L. E., S. K. Kowand, and H. M. Van Den Elzen.** 1979. Mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. Antimicrob. Agents Chemother. **15**:7–13.
6. **Bryan, L. E., T. Nicas, B. W. Holloway, and C. Crowther.** 1980. Aminoglycoside-resistant mutation of *Pseudomonas aeruginosa* defective in cytochrome c_{552}

and nitrate reductase. Antimicrob. Agents Chemother. **17**:71–79.

7. **Bryan, L. E., and H. M. Van Den Elzen.** 1976. Streptomycin accumulation in susceptible and resistant strains of *Escherichia coli* and *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **9**:928–938.
8. **Bryan, L. E., and H. M. Van Den Elzen.** 1977. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. Antimicrob. Agents Chemother. **12**:162–177.
9. **Butlin, J. D., G. B. Cox, and F. Gibson.** 1971. Oxidative phosphorylation in *Escherichia coli* K-12. Mutations affecting magnesium ion- or calcium ion-stimulated adenosine triphosphatase. Biochem. J. **124**:75–81.
10. **Campbell, B. D., and R. J. Kadner.** 1980. Relation of anaerobiosis and ionic strength to the uptake of dihydrostreptomycin in *Escherichia coli*. Biochim. Biophys. Acta **593**:1–10.
11. **Damper, P. D., and W. Epstein.** 1980. Mechanism of low-level aminoglycoside resistance in *Escherichia coli*, p. 706–708. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease, vol. 1. American Society for Microbiology, Washington, D.C.
12. **Downie, J. A., F. Gibson, and G. B. Cox.** 1979. Membrane adenosine triphosphatases of prokaryotic cells. Annu. Rev. Biochem. **48**:103–131.
13. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108**:639–644.
14. **Epstein, W., and S. G. Schultz.** 1965. Cation transport in *Escherichia coli*. V. Regulation of cation content. J. Gen. Physiol. **49**:221–234.
15. **Felle, H., J. S. Porter, C. L. Slayman, and H. R. Kaback.** 1980. Quantitative measurements of membrane potential in *Escherichia coli*. Biochemistry **19**: 3585–3589.
16. **Finney, D. J.** 1952. Statistical methods in biological assay. C. Griffin, London.
17. **Hirata, H., K. Altendorf, and F. M. Harold.** 1973. Role of an electrical potential in the coupling of metabolic energy to active transport by membrane vesicles of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:1904–1908.
18. **Höltje, J.-V.** 1978. Streptomycin uptake via an inducible polyamine transport system in *Escherichia coli*. Eur. J. Biochem. **86**:345–351.
19. **Kanner, B. I., and D. L. Gutnick.** 1972. Use of neomycin in the isolation of mutants blocked in energy conservation in *Escherichia coli*. J. Bacteriol. **111**:287–289.
20. **Medeiros, A. A., T. F. O'Brien, W. E. C. Wacker, and N. F. Yulug.** 1971. Effect of salt concentration on the apparent in vitro susceptibility of *Pseudomonas* and other gram-negative bacilli to gentamicin. J. Infect. Dis. **124**(Suppl.):S59–S64.
21. **Mitchell, P.** 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. **41**:445–502.
22. **Padan, E., D. Zilberstein, and H. Rottenberg.** 1976. The proton electrochemical gradient in *Escherichia coli* cells. Eur. J. Biochem. **63**:533–541.
23. **Robertson, D. E., G. J. Kaczorowski, M.-L. Garcia, and H. R. Kaback.** 1980. Active transport in membrane vesicles of *Escherichia coli*: the electrochemical proton gradient alters the distribution of the *lac* carrier between two different kinetic states. Biochemistry **19**: 5692–5702.
24. **Rosen, B. P.** 1973. β-galactoside transport and proton movements in an adenosine triphosphatase-deficient mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. **53**:1289–1296.
25. **Rottenberg, H.** 1979. The measurement of membrane potential and ΔpH in cells, organelles, and vesicles. Methods Enzymol. **55**:547–569.
26. **Săsărman, A., M. Surdeanu, V. Portelance, R. Dobardzic, and S. Sonea.** 1969. Vitamin K-deficient mutants of bacteria. Nature (London) **224**:272.
27. **Săsărman, A., M. Surdeanu, G. Szegli, T. Horodniceanu, V. Greceanu, and A. Dumitrescu.** 1968. Hemindeficient mutants of *Escherichia coli* K-12. J. Bacteriol. **96**:570–572.
28. **Taber, H., and G. M. Halfenger.** 1976. Multiple-aminoglycoside-resistant mutants of *Bacillus subtilis* deficient in accumulation of kanamycin. Antimicrob. Agents Chemother. **9**:251–259.
29. **Tien, W., and D. C. White.** 1968. Linear sequential arrangement of genes for the biosynthetic pathway of protoheme in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U.S.A. **61**:1392–1398.
30. **Youmans, G. P., and M. W. Fisher.** 1949. Action of streptomycin on microorganisms *in vitro*, p. 91–111. *In* S. A. Waksman (ed.), Streptomycin, nature and practical applications. Williams & Wilkins Co., Baltimore.
31. **Zilberstein, D., S. Schuldiner, and E. Padan.** 1979. Proton electrochemical gradient in *Escherichia coli* cells and its relation to active transport of lactose. Biochemistry **18**:669–673.