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ALTERATIONS IN OUTER MEMBRANE PERMEABILITY

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INTRODUCTION

The outer membrane serves as a physical and functional barrier for gram-negative bacteria. In recent years, considerable efforts have been made to understand the functions, synthesis, chemical composition, and structure of outer membrane components (34, 84, 101, 102, 119, 129). Parallel with these studies, a number of experiments have been addressed to integrating these data in an attempt to understand the functioning of the outer membrane as a whole. This review will examine those studies that concentrate on alterations of the outer membrane barrier properties and will attempt to explain these permeability alterations on the basis of a single class of critical outer membrane sites. To provide a framework for discussion, the structure and permeability properties of wild-type outer membranes will first be briefly discussed.

Structure of the Cell Envelope

The gram-negative cell envelope consists of two membranes separated by a layer of peptidoglycan and a cellular compartment called the periplasm. The innermost, cytoplasmic membrane is generally a phospholipid bilayer liberally studded with a wide variety of polypeptides. The major functions of cytoplasmic membrane proteins are in energy generation, in active and facilitated transport of nutrients and export of toxic byproducts, and in enzymatic synthesis and translocation of cell envelope components (148). The cytoplasmic membrane serves as a major barrier for hydrophilic or charged molecules (in the absence of a utilizable transport system), but it is generally accepted that even moderately hydrophobic molecules can enter into or even cross the lipid bilayer at growth temperatures (117).

The periplasm (previously called the periplasmic space) is probably a matrix of polypeptides and saccharides with net negative charges (89, 171). It contains a variety of enzymes, some of which function as scavenger or processing enzymes for conversion of nontransportable metabolites to transport substrates. Little is known about the barrier function of the periplasm, nor, for that matter, of the peptidoglycan that serves as a primary shape and osmotic stability–maintaining determinant of the cell.

The outer membrane is an unusual biological membrane in that its outer monolayer contains lipopolysaccharide (LPS) as its major lipidic molecule, while the inner leaflet contains phospholipids rather than LPS (102, 119). LPS is an amphiphilic molecule containing a hydrophobic region (Lipid A, also known as endotoxin) that has 5 or 6 fatty acids linked to diglucosamine phosphate. Covalently attached to this is the rough oligosaccharide core containing in its proximal portion an unusual sugar, 2-keto-3-deoxyoctanate (KDO), as well as a variety of heptose and hexose residues (48, 102). The rough oligosaccharide core may be substituted with a variable number of repeated tri- to penta-saccharide units called the O-antigen (132).

The LPS carries a net negative charge resulting in the strong negative surface charge of gram-negative cells (16, 162). One of the most important features of LPS is that it appears to be anchored in the outer membrane by binding to outer membrane proteins (159, 212), possibly through hydrophobic interactions with Lipid A (212), and by noncovalent cross-bridging of adjacent LPS molecules with divalent cations (61, 98, 102, 119). Thus treatment of gram-negative cells with ethylenediaminetetraacetate (EDTA) generally results in removal, by chelation, of divalent cations and consequent disruption of the outer membrane (see below).

In the absence of such chelators, however, the combination of negative charge and divalent cation cross-bridging of LPS provides gram-negative cells with many of their more important properties, including resistance to hydrophobic antibiotics, bile salts, detergents, proteases, lipases, and lysozyme (74, 98, 102, 117, 119). The outer membrane also contains a small number of so-called "major" proteins present in high copy number (10^5 copies per cell) (102). These proteins may have structural roles in that they anchor, either covalently [in the case of Braun's lipoprotein (14)] or more usually noncovalently (102), the outer membrane to the underlying peptidoglycan. Little is known about the function of outer membrane proteins with the exception of porins that form relatively nonselective, water-filled channels of defined exclusion limits for hydrophilic compounds (9, 119).

Recent studies have defined structural discontinuities in outer membranes that appear in freeze fracture electron microscopic studies as particles and pits on opposing fracture faces of the outer membrane (102). These particles are 4–8 nm in diameter and probably contain both proteins and lipopolysaccharide. As many as 60,000 particles may be present in the outer membrane of a single cell. Of interest to this review is that some of the particles probably contain the chelator-sensitive sites of the outer membrane, in that EDTA and Ca^{2+} can markedly influence the observed number of such sites (102, 197). In addition, evidence has been presented that suggests that some of these sites contain porin proteins (102).

Uptake Across the Outer Membrane

THE HYDROPHILIC PATHWAY It is now well established that hydrophilic compounds can pass across the outer membrane via the water-filled channels of proteins, called "porins" (9, 119). The channel area of individual porin pores and therefore the effective exclusion limit of porins and of outer membranes varies from organism to organism. For example, *Escherichia coli* allows the passage of trisaccharides (119) or tetrapeptides (133), whereas *Pseudomonas aeruginosa* (72) and *Neisseria gonorrhoeae* porins (37) exclude only those saccharides of molecular weights greater than about 6000. The rate of uptake, by different porins, of different hydrophilic compounds is determined by the molecular properties of the porin channel, with channel size and ionic selectiv-

ity relative to the size and charge of the compound (9, 119, 120) as well as the total number of available channels per cell (2, 114) playing major roles.

In addition, because porin channels are filled with water and have charged amino acid residues at the mouth of or within the channel (9), solute passage is a function of the intrinsic viscosity (i.e. the hydrophobicity) of the permeating molecule (120). The movement of small, very hydrophobic compounds through porin channels will be severely restricted, as demonstrated experimentally by Nikaido (117).

THE HYDROPHOBIC PATHWAY Many lipid bilayer membranes allow the passive uptake of hydrophobic compounds into the membrane interior and the passage of amphiphilic (moderately hydrophobic) molecules across the membrane (e.g. 160, 169). In contrast, Nikaido (117) has demonstrated that the outer membranes of wild-type *Salmonella typhimurium* and *E. coli* cells do not allow the passage of hydrophobic or amphiphilic molecules. Symptomatic of the lack of a hydrophobic uptake pathway is cellular resistance to hydrophobic antibacterial agents like actinomycin D, phenol, and crystal violet, as well as resistance to detergents and bile salts (119).

Because this is a common property of many wild-type gram-negative bacteria, with the possible exception of some *Neisseria* strains (see below), it would seem that the hydrophobic uptake pathway is very inefficient in gram-negative bacteria in contrast to some but not all gram-positive bacteria (46). Even in mutants of *Salmonella* with heptose-deficient LPS, which were shown by Nikaido to have a hydrophobic uptake pathway (117), the pathway can apparently be blocked by the addition of divalent cations (85, 168). This and other data suggest that the combined effects of divalent cation bridging of LPS molecules and high-surface negative charge may be responsible for the absence of a hydrophobic uptake pathway in most gram-negative bacteria.

THE SELF-PROMOTED PATHWAY The self-promoted pathway has been postulated for the uptake of polycationic antibiotics, like polymyxins and aminoglycosides, across the outer membrane of *P. aeruginosa* (70, 74, 75). It involves the displacement of divalent cations from LPS by these polycations, thus destroying the LPS cross-bridging and destabilizing the outer membrane (74, 75). Because this can result in enhancement of uptake of lysozyme, β-lactams (75), and hydrophobic fluorescent dyes (100) across the outer membrane, we have proposed that such interactions promote the uptake of the interacting polycationic antibiotic itself.

As further evidence in favor of self-promoted uptake, EDTA, a divalent cation chelator that removes Mg^{2+} from outer membrane sites, causes similar enhancement of uptake of lysozyme and β-lactams (74) as well as enhanced killing by the polycationic antibiotics (177). Furthermore, a single point

mutation in *P. aeruginosa* renders the cell resistant to not only the polycationic antibiotics, but also EDTA (113), while external Mg^{2+} inhibits the action of both classes of agents (112, 216).

Although the self-promoted pathway has not been demonstrated in other bacteria, the similarity of polymyxin and EDTA effects on many bacteria, including *P. aeruginosa* (see below), suggests it may well be a common pathway of uptake. Interestingly, a number of the membrane-active agents effective against gram-negative bacteria are cationic, including the quaternary ammonium disinfectants, chlorhexidine and other diguanidine compounds, gramicidin S and tyrocidin, as well as the polymyxins.

Intrinsic Defects in Outer Membrane Permeability in Some Bacteria

The opportunitistic pathogen *Pseudomonas aeruginosa* demonstrates intrinsic resistance to a wide range of antibiotics (17). It has now been clearly demonstrated that this is due to the poor permeability of its outer membrane (2, 114, 214). Despite the fact that the major porin of *P. aeruginosa* forms substantially larger channels than enteric organisms (8, 72), its activity is substantially lower, and it has been estimated that only 0.2–1% of the available porin molecules form open functional channels (2, 114).

Because the rate of diffusion of hydrophilic compounds is proportional to the total available area of water-filled porin channels across the outer membrane, the rate of uptake of all hydropilic compounds is reduced (114). Presumably this property of *P. aeruginosa* is of advantage in its major ecological niche, the soil, in which many antibiotic-secreting organisms compete. Although similar molecular studies have not been performed on other soil-derived, pathogenic pseudomonads, their general property of high intrinsic antibiotic resistance (42) is suggestive of a common defect in outer membrane permeability.

In contrast to other gram-negative bacteria, some organisms, notably *N. gonorrhoeae*, demonstrate higher uptake of crystal violet (210) and high susceptibility to hydrophobic agents such as erythromycin, rifampicin, acridine orange, ethidium bromide, and free fatty acids (151). This has lead to the proposal that these bacteria have a functioning hydrophobic uptake pathway (119).

MUTATIONAL ALTERATIONS IN OUTER MEMBRANE PERMEABILITY

A large number of potential mutants altered in outer membrane permeability have been isolated. One of the primary problems in assigning the mutational defects in these mutants is the lack of specific experiments demonstrating that the mutants have outer membrane permeability alterations. Relatively unam-

biguous techniques for assessing outer membrane permeability changes include hydrolysis of β-lactams by periplasmic β-lactamase in whole cells [using the methodology of Zimmerman & Rosselet (217)] as a measure of hydrophilic permeability and uptake of gentian violet (210) as a measure of hydrophobic permeability.

Often, however, more ambiguous means are employed. Generally speaking, I have considered as mutants in outer-membrane permeability those strains with clearly defined biochemical alterations in the outer membrane together with enhanced susceptibility to antibiotics, dyes, chelators, or detergents. In addition, given the importance of the outer membrane as a barrier to moderately hydrophobic antibiotics (see above) and detergents, any mutants altered in susceptibility to these compounds must be considered potential outer membrane permeability mutants. A more detailed summary of the properties of the individual outer membrane permeabilty mutants is contained in Table 1.

Porin-Deficient Mutants

Porin-deficient mutants have been isolated in a variety of *Enterobacteriaceae* and in *P. aeruginosa*. *E. Coli* porin-deficient mutants are specifically altered in the uptake across the outer membrane of hydrophilic molecules, including a variety of β-lactam antibiotics (78, 87, 91, 152), tetracyclines (45), chloramphenicol (45), nucleotides (4, 195), methionine (103), saccharides (3), Ca^{2+}, and silver (103). For the smaller substrates mentioned above, these effects manifest themselves at low substrate concentrations when outer-membrane permeation is rate-limiting. Porin-deficient mutants grow relatively normally at higher external concentrations, where the diffusion rate across the outer membrane creates a periplasmic concentration of substrate substantially higher than the affinity constant (Km, Kd) for transport or binding of the substrate.

Measurement of the rate of permeation of β-lactams into porin-deficient mutants has demonstrated a 10- to 100-fold lower rate of permeation into these strains (3, 114, 118, 121, 122, 130). This suggests that the total area of porin channels in the outer membrane is reduced to 1–10%: presumably the residual uptake of hydrophilic substrates is due to alternative (7) or residual (3) porin molecules. A single bacterium can have up to five different porins. For example, *E. coli* has the OmpC, OmpF (121), LamB (11), PhoE (121), and protein K (205) porins. The molecular properties of these porins and their activities (i.e. proportion of open functional channels) can vary considerably (9, 11).

Thus, deletion of one of these porins by mutation is not necessarily equivalent to deletion of another. For example, *ompF* mutants have greater alterations in the uptake of nucleotides than *ompC* mutants (195), leading to the erroneous conclusion that OmpF pores are "specific" for nucleotides. It is now known that the real reason for this observation is the lower activity and/or smaller size of

the OmpC protein pore (121). However, mutations in alternative porins can result in a specific alteration in the permeability of the outer membrane; for example, maltose and maltodextran transport are preferentially reduced in mutants deficient in the Lam B porin protein (11).

While substantial alterations in the outer membrane permeability and, consequently, the resistance of cells to hydrophilic antibiotics are observed in porin-deficient mutants, the sensitivity of cells to polycationic (91, 110) and hydrophobic antibiotics and detergents (83) is unaltered.

Pseudomonas aeruginosa wild-type cells have a 12-fold lowered permeability to β-lactam antibiotics compared to *E. coli* (114, 214) and a consequent higher resistance to hydrophilic antibiotics (17). Therefore, wild-type *P. aeruginosa* cells behave like porin-deficient mutants despite the high copy number of their major porin protein F (2, 114). This is primarily due to the low activity of this porin [only 0.4% of the protein F in the outer membrane forms pores (114)]. Loss of protein F by mutation decreases the outer membrane permeability to β-lactams by six-fold (114) but has only a small effect on sensitivity to many antibiotics (112a).

Adaptation and Resistance to Antibiotics

An effective method of isolating porin-deficient mutants in the *Enterobacteriaceae* is by selection for resistance to mono- or di-anionic β-lactams (78, 87, 91, 152). Alternatively, such mutants can be selected as resistant to low levels of chloramphenicol (45). The *pen*B2 mutant of *N. gonorrhoeae* results in low-level resistance to hydrophilic antibiotics, possibly due to a porin alteration (67).

Outer membrane–altered mutants, resistant to polymyxin B, have been isolated in both *P. aeruginosa* (113) and *S. typhimurium* (105, 191). In both cases, cells become refractory to the effects of EDTA (113, 187, 192). In addition, the mutants are cross resistant to polycationic compounds like aminoglycosides (for *P. aeruginosa*), protamine, and polylysine (for *E. coli*). The permeability of the outer membrane towards β-lactam antibiotics is, however, unaltered (114, 185). In each case, the characterized mutational defect is consistent with these cells having a defective self-promoted uptake pathway. The *P. aeruginosa* polymyxin B resistant mutants have an increased level of a major outer membrane protein H1 and a corresponding decrease in cell envelope Mg^{2+} (113). This led us to propose that protein H1 may replace Mg^{2+} (presumably via protonated amino groups) at LPS Mg^{2+}-crossbridging sites that were proposed to be the sites of interaction of polymyxin B, aminoglycosides, and EDTA with the cell surface (70, 113, 114).

In support of this, a phenotype identical to the one appearing in these polymyxin resistant mutants is acquired by wild-type cells growing in Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}-deficient medium and reversed by addition of any of these

Table 1 Mutants affecting outer membrane permeability[a]

Mutant	Primary alteration	Outer membrane hydrophilic permeability	Gentian violet uptake	Alterations in susceptibility[b]					References
				DET	EDTA	HPI	HPO	PCAT	
Porin^{-} [c]	porin-deficient	6-100X ↓	—	U	—	R	U	U	3, 4, 45, 75, 78, 83, 87, 91, 103, 110, 114, 118, 121, 130, 152, 195
PX^{r} [d]	OM protein H1 over-produced	U	—	U	R	U	U	R	113–115
*pmr*A[e]	LPS Lipid A	U	—	SS	R	U	U	R	105, 185, 187, 191, 192
mtr	—	—	↓	R	—	R	R	U	66, 104, 166
Z61[d]	LPS Lipid A	6X ↑	—	—	—	SS	SS	SS	2, 93
1–4[d]	—	↑	—	SS	—	SS	U	SS	128, 141
*acr*A[g]	Lipid A phosphate	—	—	SS	—	SS	SS	SS/U	26, 111
*env*A[g]	LPS (?)	4.5X ↑	↑	U	—	SS	SS	SS	64, 126, 127
*env*B,M–T[g]	—	—	—	SS	—	SS	SS	SS	38, 125
env[f]	—	—	↑	SS	—	SS	SS	—	66, 151
DC2[g]	—	↑	—	—	—	SS	SS	—	140
H101[g]	—	—	—	SS	—	U	SS	—	40
lky[e,g]	periplasmic-leaky mutants	—	—	SS/U	SS/—	SS	SS	SS/—	1, 96

*tol*A,B[g]	colicin tolerant	—	—	SS	SS	SS	SS	—	10, 30
*tol*C[g]	TolC OM protein	—	—	SS	SS	SS	SS	—	30, 109
*tol*D,E[g]	LPS	—	↑	SS	—	—	—	—	41
*rfa*E[c,g]	heptose-deficient LPS	—	↑	SS	SS	U	SS	SS/U	6, 76, 85, 88, 117, 142, 149, 156–158, 163, 168, 178, 193, 194, 210
*rfa*D[e]	LPS	—	—	SS	—	U	SS	—	12
*lps*A[g]	LPS	—	↑	SS	—	—	—	—	65
*rfa*H[e,g]	leaky LPS rough mutation	—		—	—	—	SS	SS	150
*rfa*Rd[e]	glucose-deficient LPS	—	—	SS	—	U	SS	SS	76, 134
AK43[d]	rough LPS	—	—	—	—	SS	SS	SS	92
pss[g]	PSS-deficient		—	—	—	SS	SS	SS	135a
lpo[g]	Braun lipoprotein (BLP)	U	—	SS	SS	U	SS	—	7, 176, 213
*lky*D[e]	PG-bound BLP	—		SS	SS	—	SS		47, 201

[a]Abbreviations used: LPS = lipopolysaccharide; OM = outer membrane; PSS = phosphatidyl serine synthetase; BLP = Braun's lipoprotein; PG = peptidoglycan.

[b]Abbreviations for classes of compounds: DET = detergents; EDTA = ethylenediaminetetraacetate; HPI = hydrophilic antibiotics; HPO = hydrophobic antibiotics; PCAT = polycationic compounds. R signifies resistant; SS = supersensitive; U = unaltered susceptibility.

[c,d,e,f,g]Denotes the bacterial species in which the mutant was isolated, as follows: c = many different species; d = *Pseudomonas aeruginosa;* e = *Salmonella typhimurium;* f = *Neisseria gonorrhoeae;* g = *Escherischia coli.*

four cations to the growth medium (115). In the case of the *S. typhimurium pmr*A mutant, an increase in the amount of 4-amino-4-deoxy-L-arabinose bound to Lipid A phosphate in the LPS (191) would convert the negative charge on phosphate to a positive charge on the arabinosamine. This might reduce Mg^{2+}-crossbridging of the LPS due to the formation of ion pairs between the arabinosamine and an adjacent phosphate (rather than Mg^{2+}-crossbridging between phosphates on adjacent LPS molecules). Such an ion pair should not be attacked by polymyxin or EDTA.

The *E. coli* polymyxin-resistant mutants *(pmx)* of Dame & Shapiro are not well characterized biochemically. However, they are quite sensitive to detergents and hydrophobic antibiotics. Since *pmr*A and *pmx* mutants share susceptibility to sodium deoxycholate and resistance to polymyxin B (29, 105), it may well be that these mutants are analagous or identical.

Seratia marcescens and *P. aeruginosa* strains grown in increasing concentrations of polymyxin B become adapted to high levels of polymyxin, in that resistance is dependent on the presence of polymyxin B and reverts rapidly upon growth of the strains in the absence of polymyxin B (52). These strains apparently develop multiple outer membrane changes (51), and many different explanations have been forwarded for this adaptation to polymyxin B. One possibility is the cells are able to establish a stable state in which polymyxin B is bound to or incorporated into the outer membrane (182). In any case, *S. marcescens* and *P. aeruginosa* adapted to polymyxin B become cross-resistant to polycationic compounds, but hypersensitive to detergents and rifampicin (52, 182).

The reason for this latter observation is probably the action of polymyxin B (which must be present during growth to stabilize the adaptation) on the outer membranes to alter the detergent susceptibility, as shown for a naturally polymyxin-resistant isolate of *Proteus mirabilis* (173). In addition, polymyxin B apparently stimulates the action of a phospholipase (23) [perhaps phospholipase A1 (94) of the outer membrane] in polymyxin-adapted strains. How these cells manage to maintain a stable state in which they interact with polymyxin B but are able to grow in its presence is an unresolved paradox worthy of further investigation.

Antibiotic-Supersensitive Mutants

A number of mutants have been described to be supersensitive to a wide variety of antibiotics (Table 1). Mutant Z61 of *P. aeruginosa* is 4- to 10,000-fold more susceptible to 30 different antimicrobial agents (2). This strain has a 6-fold enhancement in outer membrane permeability to the β-lactam, nitrocefin (2), although there is no apparent alteration in the major porin protein. Instead, mutant Z61 has an alteration in lipid A, and we have proposed that the altered LPS of mutant Z61 interacts with porin and favors a higher percentage of open,

functional pores (93). Similar, but less well-characterized mutants of *P. aeruginosa* have been described (128, 141).

E. coli strains selected as supersensitive to acridine orange, *acr*A, are also more sensitive than wild-type cells to six hydrophobic antibiotics and two detergents (111). The *acr*A mutation apparently results in a reduction in lipid A phosphate (26). This mutation could be explained if the reduction in critical anionic sites involved in stabilization of the outer membrane by divalent cations resulted in a less stable outer membrane that could allow increased uptake of hydrophobic compounds.

Other biochemically undefined mutants of *E. coli* resulting in enhanced sensitivity to hydrophobic antibiotics have been described. These have been isolated as having enhanced susceptibility to various agents (38, 40, 125) or as periplasmic-leaky mutants that secrete various periplasmic components (see below). One of the best characterized of the former class, the *env*A mutant, has enhanced permeability to β-lactams and to gentian violet and enhanced susceptibility to a wide variety of antibiotics and detergents. It is thought to have a subtle LPS alteration (64), although it is difficult to reconcile this with its tendency to form long filiaments (126). The *env* mutation of *N. gonorrhoeae* results in hypersensitivity to a variety of hydrophobic and hydrophilic antibiotics and enhanced uptake of gentian violet (66, 151). In contrast, the *mtr* locus (also called *ery*) encodes the inverse phenotype (i.e. resistance to the same hydrophobic and hydrophilic antibiotics and reduced uptake of gentian violet) (66, 104, 166).

LPS Alterations

Perhaps the best-studied example of mutational alterations in outer membrane permeability is afforded by deep-rough (heptose-deficient) mutants of *E. coli* and *S. typhimurium* (Table 1). These mutants demonstrate enhanced sensitivity to a variety of hydrophobic antibiotics and detergents (Table 1). In addition, they demonstrate enhanced uptake of the hydrophobic dye gentian violet (112, 168), enhanced susceptibility to EDTA (163), Tris-lysozyme (8, 163), and phospholipases (57, 88), and leakage of periplasmic enzymes (163, 193). Despite this leakiness to water-soluble periplasmic enzymes, deep-rough mutants do not have enhanced susceptibility to hydrophilic compounds (78, 157, 158) (although no direct measurements of hydrophilic permeability have been performed).

The biochemical nature of the surface of deep-rough organisms is the source of considerable controversy. The molar ratios of phospholipids to LPS that are present in the outer membrane have been described as varying between 2.5:1 and 8.9:1 (mean = 5.0:1) for wild-type strains and between 1.2:1 and 4.4:1 (mean = 3.3:1) for deep-rough (*rfa*E) mutants (57, 58, 102, 161, 164). It

should be noted that each of the above studies employs many assumptions, the major of which is that extracted outer membranes have a composition identical to native outer membranes, an assumption of doubtful validity. From their data, Smit & Nikaido have suggested that deep-rough mutants have patches of exposed phospholipids on their surfaces, resulting in areas of phospholipid bilayer in the outer membrane (164).

In contrast, the data of Gmeiner & Schlecht (57, 58) suggests that the asymetric nature of the outer membrane (LPS in the outer monolayer, phospholipids in the inner) may be maintained in deep-rough mutants. The susceptibility of phospholipids in deep-rough strains to phospholipase digestion (57, 88) has been cited in favor of the former model, but given the permeability of deep-rough mutants to proteins, including lysozyme and periplasmic proteins (85, 163, 193), it seems possible that these outer membranes may also be permeable to phospholipases. One piece of evidence in favor of the latter model is the reversal of the phenotype of deep-rough mutants by Mg^{2+}. It might be that the higher amount of LPS in deep-rough strains [as reported by two groups (57, 58, 161) but not by Smit & Nikaido (164)] brings about a higher requirement of these cells for Mg^{2+} in order to stabilize their outer membranes.

Furthermore, the effects of polymyxin, which extracts Mg^{2+} (but little LPS) (115, 189) from the outer membrane mimic the effects of the deep-rough mutation (see Tables 1 and 2). Thus, while I personally favor the concept of maintenance of outer membrane asymmetry in deep-rough mutants, considerable work must still be performed to distinguish between these models.

Other less drastic alterations in LPS composition, including the *lps*A, *gal*U, *rfa*Rd1, *rfa*D, *rfa*H, and *tol*D,E mutants of *E. coli* or *S. typhimurium,* and mutant AK43 of *P. aeruginosa,* cause supersusceptibility to hydrophobic antibiotics, detergents, and EDTA and, where studied, enhanced uptake of gentian violet (Table 1). Generally, the magnitude of these effects is related to the extent of the LPS alterations.

Other Mutational Alterations

Mutants deficient in Braun's liprotein *(lpo)* or in the covalently peptidoglycan-bound form of lipoprotein (*lky* D) in *E. coli* and *S. typhimurium* respectively, have conditional alterations in the permeability of the outer membrane to hydrophobic compounds (47, 176, 201, 213) and in the ability of the outer membrane to prevent leakage of periplasmic components (47, 213). The effects on permeability are reversible by Mg^{2+} (165, 213). The alteration in permeability may occur specifically at the site of the division septum formation, because the *lky*D mutation has been shown to cause blebbing of the outer membrane at this position (201).

A variety of colicin-tolerant mutants (cells able to bind specific colicins but not susceptible to their lethal action) in *E. coli* K-12 have been shown to be

more susceptible to hydrophobic antibiotics and detergents and, in some cases, are periplasmic-leaky (1, 10, 30, 41, 96, 109). One of these mutants, *tol* C, is thought to be altered in a minor outer-membrane protein [from gene-cloning experiments (P. Reeves, personal communication)]. Another mutant, *tol*A,B may be identical to the periplasmic leaky mutant *lky*::MuCts (1, 10, 30), which has been proposed to be altered in its LPS (1). Mapping and the phenotypes of another pair of periplasmic-leaky mutants *lky*-207 and *lky*-236 suggest that these too may be the same as the *tol* A,B mutations, although small differences exist (96).

In addition to the above specific examples, a variety of other mutants in outer membrane permeability to hydrophobic agents have been isolated and characterized (see Table 1). The types of permeability alterations in these are frequently similar, although minor differences exist in susceptibility to given agents. Some of these differences can be explained by different assay conditions; for example, Mg^{2+} is important (see Table 3), as is the concentration of the agent being tested. Taken overall these data argue for a multicomponent hydrophobic uptake system that can be "activated" by a variety of different mutational events. In addition to hypersensitivity to hydrophobic antibiotics, these mutants are usually somewhat more susceptible to EDTA and polycations.

ENHANCEMENT OF OUTER MEMBRANE PERMEABILITY BY CHEMICALS AND ANTIBIOTICS

EDTA

EDTA causes increased outer membrane permeability to many compounds in a wide range of gram-negative bacteria (Table 2). Central to the mode of action of EDTA is its strong divalent cation-chelating function (98), although it should be stressed that, with the exception of *P. aeruginosa* (59), an organic cation (usually Tris is used) and an appropriate mild alkaline pH are also required. The actual mechanism of outer membrane permeabilization is unknown. EDTA apparently causes the loss of substantial amounts of LPS [between 30% and 67% of the total in *E. coli* (60)], at either 37° C or 4° C (99), although the outer membrane apparently remains continuous after such treatment (54, 197), in that there do not appear to be easily observeable holes in the outer membrane.

The major alteration in outer-membrane substructure is in the reduction of the number of particles and pits upon freeze fracture (197). The LPS that is released can be separated into two fractions by ultracentrifugation (99). One, fraction F1, contains associated protein and lipid. The other, F2, which may have different sugar composition than bulk LPS, is apparently pure LPS. Data from mutant (198) and reconstitution (60) studies have suggested that it is the release of F2 that results in outer membrane permeabilization. For example,

Table 2 Outer membrane permeabilizing agents[a]

Agent	Bacteria[b]	Outer membrane permeabilization to[c]				Enhanced susceptibility to[d]		Other effects	References
		HPO	HPI	Lysozyme	PER1	HPO	HPI		
EDTA	many	1, 2, 3, 4	5, 6, 7	+	−	9, 10, 11, 12, 13, 14	18, 19, 20, 21, 22	A, B	5, 18, 25, 31, 59, 65, 69, 75, 81, 98, 108, 139, 155, 167, 174, 175, 185, 202
Ascorbate	P	1	5	+		9, 15	18, 19, 20, 21, 22		77, 136
Tris	E, P		5, 8	+	+			C	75, 77, 86, 185
Polymyxin B	many	2, 3, 4	5	+	+	10, 12, 13, 15	19, 20	C, D	22, 62, 77, 95, 112, 155, 172, 173, 183, 187
Deacylated polymyxin	E				−	9, 10, 11, 12	18	C	186, 188, 190
Octapeptin	E				+	13		A	185

Aminoglycosides	P	1, 2	5	+	−		18	A	75, 77, 100, 177
Polylysine	E, S, P	1	5	+	−	9, 11, 13	18	A	77
Benzalkonium chloride	E				+	13			185
Protamine	E				−	9, 11, 13		A	185, 188
BPI	E, S					10, 14		B	39, 203
Serum and/or complement	E			+	+		18, 19, 21, 22	D	31, 32, 35, 56, 211
Benzyl penicillin	P				+	13			114, 124
Ca^{2+} pretreatment	E	4	6	+	+	14, 17			13, 147, 155

[a] Abbreviations: BPI = bactericidal/permeability increasing protein; HPO = hydrophobic compounds; HPI = hydrophilic compounds; PERI = periplasmic enzymes—often β-lactamase or alkaline phosphatase.

[b] Bacterial species studied: E = *E. coli;* P = *P. aeruginosa;* S = *S. typhimurum*.

[c] The numbers signify the following compounds: 1 = N-phenyl napthylamine; 2 = 1-anilino-8-napthosulphonate; 3 = Gentian violet; 4 = dansyl chloride; 5 = nitrocefin; 6 = AMP or DNA; 7 = benzyl penicillin; 8 = paranitrophenyl phosphate; blank = not studied; + equals positive result; − equals no permeabilization to periplasmic enzymes observed.

[d] The numbers signify the following compounds: 9 = erythromycin; 10 = rifampicin; 11 = novobiocin; 12 = serum and/or complement; 13 = deoxycholate and/or cholate; 14 = actinomycin D; 15 = trimethoprim; 16 = bacitracin; 17 = nigericin, valinomycin, CCCP; 18 = β-lactam antibiotics; 19 = tetracyclines; 20 = chloramphenicol; 21 = aminoglycoside antibiotics; 22 = polymyxin B.

[e] The block capital letters mean: A = causes LPS release; B = enhances phospholipase sensitivity of cells; C = binds to LPS; D = causes outer membrane disorganization.

Table 3 Mg^{2+} reversal of the enhancement, by agents or mutants, of outer membrane permeability

Permeabilizing agent or mutant	Properties reversed by Mg^{2+}	References
Porin$^-$ mutant	periplasmic β-lactamase leakage	11
Env mutant	Lysozyme sensitivity and autolysis	66
*tol*A,B mutant	Vancomycin sensitivity	10
*tol*D mutant	colicin tolerance; cell envelope stability; resistance to ampicillin; sensitivity to cholate	20, 123
Deep-rough (*rfa*) mutants	Hypersensitivity to Erythromycin, Bacitracin, Vancomycin, and Novobiocin; uptake of Gentian violet; leakage of periplasmic enzymes; outer membrane freeze-fracture morphology	85, 168
lpo mutant	Leakage of periplasmic β-lactamase; growth defect; outer membrane "blebbing"; EDTA hypersensitivity	7, 176, 213
Tris in growth medium	Release of alkaline phosphatase	24
Polymxin B	Killing; lytic activity, uptake of hydrophobic fluorescent compounds; formation of outer membrane blebs; release of periplasmic enzymes; enhanced nitrocefin uptake	22, 77, 112, 113, 153, 155
Octapeptin	Killing; phospholipid release	143
Aminoglycosides	Killing; uptake; permeability of outer membrane to nitrocefin, lysozyme, hydrophobic fluorescent compounds	19, 75, 100
Bactericidal/permeability increasing protein	Actinomycin D, rifampicin sensitivity	39, 203

EDTA-resistant mutants can be induced to release up to 30% of their LPS, primarily F1, without dramatic alterations in permeability (198).

In *P. aeruginosa*, a more substantial amount of protein is released during EDTA treatment. However, the outer membrane ultrastructural alterations are apparently similar to those of *E. coli* (54, 197).

Polymyxins

Polymyxin B (and octapeptin) causes rodlike projections (blebs) of the outer membranes of gram-negative bacteria (53, 154, 189). For this effect, and for a wide range of outer membrane permeabilization events mediated by polymyxin (Table 3), the fatty acyl tail is not required, because deacylated polymyxin [polymyxin B nonapeptide (189)] causes similar blebbling. Interestingly,

polymyxin B is different from EDTA in that it apparently releases little LPS into the medium (189). Because the polycationic part of polymyxin B is capable of displacing divalent cations by competition while EDTA removes divalent cations by chelation, it is tempting to speculate that these blebs represent an intermediate step in the release of LPS caused by EDTA treatment. In agreement with this proposal, not only do polymyxin B and EDTA cause similar permeabilization events, but outer membrane mutants exist in both *S. typhimurium (pmx)* and *P. aeruginosa* (PX^r) that are cross-resistant to both agents (see Table 1).

In any case, the blebbed outer membrane appears to be quite unstable, because polymyxin B–treated cells release substantial levels of periplasmic proteins (22) whereas EDTA-treated cells release only very small amounts of these proteins. Other polycations, like protamine and polylysine, are more like EDTA in their outer membrane effects because they cause substantial LPS release but no periplasmic protein release and little or no outer membrane blebbing (189).

The release of periplasmic proteins by polymyxin B, but not other outer membrane permeability effects, may be a function of possession of a hydrophobic tail (189). Model studies have suggested a possible mechanism for this polymyxin B effect, because it interacts with both the polar and nonpolar regions of lipids (131) to destabilize bilayers (106). This explanation, however, is probably an oversimplification, because it does not explain the similarities between the effects of polymyxins and EDTA. Polymyxin B is known to interact with the divalent-cation binding sites on LPS (74, 153), and such binding is almost certainly critical to its permeabilizing function, because polymyxin effects are blocked by Mg^{2+} (113).

Aminoglycosides

Polycationic aminoglycoside antibiotics (e.g., streptomycin, gentamicin) interact with divalent-cation binding sites on *P. aeruginosa* LPS (E. McGroarty, unpublished) and permeabilize the outer membranes of this organism (75, 100). A number of lines of evidence suggest that aminoglycosides act at the same site as do polymyxins and EDTA (70, 74, 113). We have proposed that the interaction of aminoglycosides with *P. aeruginosa* outer membranes at this site is part of an uptake mechanism (i.e. self-promoted uptake across the outer membrane of this organism) (see above).

Tris

The large organic cation, Tris, is not only capable of assisting the action of EDTA (59) but is also able to permeabilize outer membranes itself, when used in moderately high concentrations (75, 86). This effect is probably related to its ability to interact with LPS, albeit with lower affinity than e.g. polymyxin

(154). The results of Voss (199) are suggestive that this ability to permeabilize outer membranes may also be shared by other organic cations.

Ca^{2+} Pretreatment and DNA Uptake

The development of competence [i.e. the ability of a cell to be genetically transformed by exogenous DNA] usually involves two stages: (*a*) incubation of a concentrated cell suspension at O°C, in the presence of exogenous DNA, in buffer containing 25–100 mM $CaCl_2$; and (*b*) a brief (6–15 min) temperature shock caused by raising the temprature of the buffer (147, 180, 196). The temperature shock seems to be dispensible in some species of bacteria, and individual researchers use somewhat different procedures.

However, the net result of this procedure is to permeabilize the outer membrane to a variety of hydrophobic and hydrophilic compounds (Table 2). This treatment also allows vectorial transport (i.e. uptake) of macromolecules, including large DNA molecules (63), antibodies, amylopectin, and periplasmic maltose and galactose-binding proteins (13) across the outer membrane. However, development of competence does not cause large holes in the outer membrane (63, 87) and does not permit significant leakage of periplasmic enzymes into the environment (13).

The uptake of DNA across the outer membrane probably involves an LPS site, because rough mutants of *E. coli* are more easily transformed than smooth (O-antigen-containing) strains (179, 180). In addition, there is substantial variation in the competence of different isogenic rough LPS mutants (179). The involvement of divalent cations [usually Ca^{2+}, although Mg^{2+} and Ba^{2+} also work (33, 179, 204)] in the induction of competence may indicate that the same LPS divalent cation–crossbridging sites discussed above are involved. In agreement with this, Tris-EDTA-lysozyme–induced spheroplasts will also take up DNA, providing an appropriate polycationic compound is present (82).

The mechanism of DNA uptake is not well understood, but it has been postulated to involve the formation of transient channels through the membrane. These channels are proposed to be open only during the interaction of the nucleic acid with the membrane, and after nucleic acid uptake is finished the channel is destroyed or closed (63). [*Note:* Although this proposal was made for DNA uptake across the inner membrane, it seems equally valid for outer-membrane uptake that apparently occurs with higher efficiency (204).] Van Die et al (196) have suggested that a phase transition (caused by the temperature shock) and/or the formation of nonbilayer structures (caused by Ca^{2+}) may be important in formation of the temporary channels.

On the other hand, Grinius (63) suggests that Ca^{2+} binds and confers a net positive charge on DNA, converting it to a polycation. In agreement with this, divalent cations promote the binding of DNA to *E. coli* and *H. influenza* outer membranes (33, 204). Interestingly, this hypothesis would imply that DNA might interact with cells in ways similar to other polycations. Kahn et al (87)

have shown that competent *Haemophilus* cells produce outer membrane blebs reminiscent of those that appear due to polymyxin B and deacylated polymyxin B treatment of *E. coli* (189).

Serum and/or Complement

The bactericidal killing of gram-negative cells by serum usually involves one of two complement cascade pathways (107). Each of these pathways is activated in a unique fashion, although the final steps and proteins involved are common. The effect of at least one complement component is to disrupt the outer membrane, as judged by not only electron microscopic observation (207) but also enhanced uptake of lysozyme (56, 207) and release of periplasmic enzymes (211) by complement-treated cells. The permeabilization event has been proposed (211) to be important for allowing passage through the outer membrane and subsequent incorporation into the inner membrane of the channel-forming component C9.

The mechanism of complement permeabilization of outer membranes may well be similar to the self-promoted uptake mechanism discussed above. Evidence for this includes the synergy of aminoglycosides, polymyxins, and other polycationic compounds with serum and/or complement (31, 32, 43) as well as the binding of complement to the lipid A portion of LPS (50, 208) and the inhibition of this binding by polmyxin B or Mg^{2+} or Ca^{2+} (suggesting competition for an LPS site) (49, 208). In addition, rough LPS-altered organisms are more sensitive to serum bactericidal (complement) killing than smooth cells (145), a result usually taken to reflect differences in the carboyhydrate portion of LPS, but which may instead reflect the differential affinities of smooth and rough LPS for complement and Mg^{2+} (49).

Bactericidal/Permeability–Increasing Protein from Polymorphonuclear Leukocytes

A protein from polymorphonuclear leukocytes with an isoelectric point of 9.5–10.0 kills several species of gram-negative bacteria (203). Rough strains are more susceptible than smooth strains and, like polymyxin, this highly cationic protein (called bactericidal/permeability–increasing protein) is quite inactive against gram-positive cells (39). In addition, Mg^{2+}-limited cells of *Pseudomonas aeruginosa,* which have been shown to be resistant to polymyxin B, aminoglycosides, and EDTA (113; see PX^r in Table 1), are resistant to the action of this protein (44). The protein results in permeabilization of outer membranes to actinomycin D and rifampicin, and the permeabilization event is antagonized by either Mg^{2+} or Ca^{2+} (39, 203). Permeability induction requires the continuous presence of bound bactericidal/permeability–increasing protein, in addition to at least two postbinding steps. The proposed mechanism of interaction of this cationic protein bears many similarities to the self-promoted uptake mechanism (203).

ENERGIZATION STATE AND HYDROPHOBIC PERMEABILITY

A number of authors have demonstrated that de-energization of cells in a variety of ways results in increased fluorescence of hydrophobic fluorescent probes added to the cell suspension (80). This is true of negatively charged (81, 116, 181), positively charged (15), or neutral (81, 166) probes added to cells de-energized in a variety of ways. Membrane de-energization will increase the fluorescence of exogenously added cis-parinaric acid but not biosynthetically incorporated, esterified parinaric acid (181). This data, together with direct measurement of N-phenyl napthylamine (NPN) disappearance from the environment, led Helgerson & Cramer (80, 81) to propose that de-energization resulted in an alteration in outer membrane permeability to the probes; that is, the enhancement was due to partitioning of the probes into the more hydrophobic membrane interiors.

In agreement with this it could be shown that EDTA (81), polymyxin B (112), and aminoglycosides (100) cause similar uptake of hydrophobic fluorescent probes. Also, added Mg^{2+} decreased the rate of fluroescence increase due to polymyxin B (112), aminoglycosides (100), and two colicins that are presumed to cause alterations in membrane energization (15, 184). In addition, deep-rough LPS-altered mutants of *Salmonella,* which exhibit many other outer membrane permeability alterations (see Table 1), showed no enhanced uptake of the hydrophobic, photolabile probe azidopyrene upon de-energization, in marked contrast to wild-type *Salmonella* strains, which showed a 2–5-fold enhancement of uptake (209; Wolf & Konisky, submitted). This data implies that either de-energization of cells results in a marked structural change in the outer membrane, or there is an energized secretion mechanism for hydrophobic compounds in untreated wild-type cells.

In favor of the latter view, re-energization of cells causes rapid loss of NPN from the cells (80, 81). In addition, energy metabolism is required to reverse EDTA-mediated outer membrane permeability alterations (45). In support of the former idea, de-energization of cells by CCCP results in enhanced rates of DNA uptake into Ca^{2+}-treated cells in the absence of a temperature shock (146), prevents irreversible binding of bacteriophages T1 and 80 to their outer membrane receptors (71), and blocks a variety of *ton* B–protein–mediated transport systems (73), possibly at the level of the outer membrane.

PHYSICAL DAMAGE OF OUTER MEMBRANES

Outer membranes can also be damaged by physical treatments. For example, *E. coli* cells exposed to their aquatic or reagent grade water become susceptible to deoxycholate and lysozyme (215). Electron microscopic examination of

such cells showed outer membrane blebbing. Similarly, aerosolization of *E. coli* makes the outer membrane permeable to lysozyme (68). The sensitivity of aerosolized *E. coli* to lysozyme can be partly reversed by Mg^{2+}, although as with EDTA treatment of cells, full repair of outer membranes required energy metabolism.

Freeze drying of *Salmonella anatum* results in enhanced susceptibility of cells to EDTA, actinomycin D, penicillin, tetracycline, and chloramphenicol, suggesting outer-membrane damage (138). Once again energy metabolism is required to repair outer membrane damage. Similarly, freeze-thawing *E. coli* strains results in release of cyclic phosphodiesterase (21) as well as enhanced susceptibility to dyes, enzymes, and detergents (6), and loss of LPS. The effects of freeze-thawing on outer membrane permeability are more extreme in deep-rough mutants than in smooth strains (6).

GROWTH AND MEDIA EFFECTS

A number of studies have examined the influence of growth in various media on susceptibility to antibiotics, but only rarely have outer membrane permeability alterations been definitively demonstrated. Perhaps the best example is *P. aeruginosa* cells grown in Mg^{2+}-limited medium, which are phenotypically indistinguishable from the genetically polymyxin-, aminoglycoside-, EDTA-resistant mutants (PX^r) described in Table 1. Another example is *P. fluorescens* cells grown on succinate, which demonstrate higher sensitivity to actinomycin D and to the permeabilizing effects of EDTA than the same cells grown on glucose (200). In addition, phosphate limitation of *P. fluorescens* renders these cells polymyxin B–resistant and decreases the capacity of outer membranes to bind polymyxin B (36).

EXCRETION OF PROTEINS ACROSS THE OUTER MEMBRANE

Whereas some gram-negative bacteria (e.g. *E. coli*) have few, if any, proteins that are excreted out of the cell, others like *P. aeruginosa* are known to excrete a range of polypeptides (55, 170). The secretion of proteins across the outer membrane may well relate to the specific nature of the organism and its outer membrane. The excretion by *P. aeruginosa* of phospholipase C (and another enzyme, alkaline phosphatase, which is often used in other bacteria as a periplasmic marker enzyme) is specific, because it has been shown that its excretion is not accompanied by the excretion of periplasmic β-lactamase or phosphate-binding protein (135).

In addition, during phospholipase secretion, outer membrane permeability to the β-lactam nitrocefin is unaltered (135). Despite this, the levels of alkaline

phosphatase and phospholipase C increase in both the periplasm and the external medium after phosphate limitation. Thus it seems that either a specific alteration in outer membrane permeability has occurred, or these enzymes are secreted to the outer membrane and from there released either inwards (to the periplasm) or outwards (to the external medium).

DIVALENT CATION REVERSAL OF OUTER MEMBRANE PERMEABILITY ALTERATIONS

One of the most interesting aspects of the alteration of outer membrane permeability by mutants and by various agents is that in those instances where it has been examined, exogenously added Mg^{2+} reverses the permeability changes (Table 3). This is true of even the classical outer-membrane permeabilizing agents and mutants (e.g. deep-rough mutants, polymyxin B, EDTA). This provides strong evidence that a single class of outer membrane site is involved in all of these permeability alterations, presumably a Mg^{2+}-binding site. As clearly demonstrated by Schindler & Osborn (153) and by McGroarty and coworkers (27), LPS has a strong divalent cation–binding site.

A HYPOTHESIS TO EXPLAIN OUTER MEMBRANE PERMEABILITY ALTERATIONS

It is my belief that the majority of the outer membrane permeability alterations discussed in this review can be simply explained by postulating that they involve sites at which divalent cations noncovalently cross-bridge adjacent LPS molecules. This proposal is not unique and has been made for a number of the pemeabilization events discussed; however, the universality of the proposal has not been widely recognized. The evidence suggesting a single class of outer membrane sites involved in permeability alterations includes (*a*) the chemical similarities of permeabilizing agents (usually polycations, organic cations, or divalent cation chelators—Table 2), (*b*) the antagonism of agents and mutants by Mg^{2+} (Table 3), (*c*) the similar types of lesions caused by most mutants and agents (Tables 1 and 2) (with destruction of the permeability barrier to hydrophobic compounds featuring prominently), and (*d*) the occurence of outer membrane mutants altered in their susceptibility to many outer-membrane permeabilizing agents (especially PX^r and *pmr*A in Table 1).

The differences in permeability alterations between individual mutations could be explained on the basis of the specific nature of the mutants, which are largely unknown. Similarly the differences in alterations of outer-membrane permeability caused by different agents could be explained by competition for the permeabilized site between the permeabilizing agent and the compounds being studied. With this in mind it should be noted that two distinct per-

meabilization events to hydrophobic agents and detergents like sodium dodecyl sulphate have been proposed (189).

Whereas the site of outer membrane permeabilization can be predicted, little is known or understood about the specific nature of the underlying lesions. Despite the secretion of copious amounts of LPS from EDTA-treated cells, the outer membrane remains continuous across the cell surface (54, 197), as indeed it does with other outer membrane permeabilizing agents (53, 63, 86, 90, 97, 153, 189) or for various hyperpermeable mutants (45, 85, 201). Therefore, it does not seem likely that the presence of holes through the outer membrane can explain the permeabilization (the specificity of the lesions also favors this argument). Nor does lipid flip-flop giving rise to areas of phospholipid bilayer in the outer membrane [as suggested for deep-rough mutants (164)] provide an easy explanation for these lesions, because it is difficult to understand how a lipid bilayer allows the enhanced passage of hydrophobic compounds and lysozyme (or DNA) as seen for many of the permeability alterations.

Although any explanation of the mechanism whereby compounds pass across permeabilized outer membranes is premature, a number of possibilities exist. For example, the removal or displacement of divalent cations from their LPS binding sites may cause a structural discontinuity, such as nonbilayer structures (28), membrane blebs (53, 153), elastic distortion of the bilayer (79), or regions of enhanced fluidity (144) in the outer membrane. The interaction of molecules with the surface charges surrounding such structures and partitioning into the hydrophobic regions of the structure may explain the permeability to both specific hydrophilic compounds and hydrophobic substances respectively. This proposal is deliberately vague and poses more questions than it answers. For example, (*a*) What is the nature of the discontinuity? (*b*) What is the role of particles and pits (102, 197) in the outer membrane interior? (*c*) Why do only some permeabilizing agents cause blebs? (*d*) What is the role of energization in preventing such structural discontinuities from forming?

The physiological importance of LPS divalent cation crossbridges (for outer membrane stability and as a component of the hydrophobic barrier) is clear. In contrast, the physiological significance of self-promoted uptake, aside from a possible role in transformation, is arguable. However, the existence of such an LPS site may have strong medical relevance, because it constitutes an "Achille's heel" for the cell. (This site is apparently attacked by complement, polymyxins, aminoglycosides (in *P. aeruginosa),* and cationic proteins from polymorphonuclear leukocytes). Given the strong influence of the outer membrane permeability barrier on rate of uptake and the susceptibility of gram-negative bacteria to antibiotics (2, 117, 119), agents that enhance outer membrane permeability should prove synergistic with antibiotics. This has been frequently demonstrated in vitro (see Table 2), while clinical trials with EDTA (206) and ascorbate (137) have been quite promising.

Thus, future studies on agents capable of enhancing outer-membrane permeability should be directed towards understanding the mechanism of permeabilization as well as searching for medically useful outer-membrane permeability–altering compounds.

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Literature Cited

1. Anderson, J. J., Wilson, J. M., Oxender, D. L. 1979. *J. Bacteriol.* 140:351–58
2. Angus, B. L., Carey, A. M., Caron, D. A., Kropinski, A. M. B., Hancock, R. E. W. 1982. *Antimicrob. Agents Chemother.* 21:299–309
3. Bavoil, P., Nikaido, H., von Meyenburg, K. 1977. *Mol. Gen. Genet.* 158: 23–33
4. Beacham, I. R., Haas, P., Yagil, E. 1977. *J. Bacteriol.* 129:1034–44
5. Beacham, I. R., Kahana, R., Levy, L., Yagil, E. 1973. *J. Bacteriol.* 116:957–64
6. Bennet, G. M., Seaver, A., Calcott, P. H. 1981. *Appl. Environ. Microbiol.* 42:843–49
7. Benz, R., Boehler-Kohler, B. A., Dieterle, R., Boos, W. 1978. *J. Bacteriol.* 135:1080–90
8. Benz, R., Hancock, R. E. W. 1981. *Biochim. Biophys. Acta* 646:298–308
9. Benz, R., Hancock, R. E. W., Nakae, T. 1982. *Transport in Biomembranes: Model Systems and Reconstitution.* pp. 123–34. New York: Raven 272 pp.
10. Bernstein, A., Rolfe, B., Onodera, K. 1972. *J. Bacteriol.* 112:74–83
11. Boehler-Kohler, B. A., Boos, W., Dieterle, R., Benz, R. 1979. *J. Bacteriol.* 138:33–39
12. Branes, L. V., Kay, W. W. 1983. *J. Bacteriol.* 154:1462–66
13. Brass, J. M., Boos, W., Hengge, R. 1981. *J. Bacteriol.* 146:10–17
14. Braun, V., Sieglun, U. 1970. *Eur. J. Biochem.* 13:336–46
15. Brewer, G. J. 1976. *Biochemistry* 15: 1387–92
16. Brinton, C. C., Buzzell, Z., Lauffer, M. A. 1964. *Biochim. Biophys. Acta* 15: 533–40
17. Brown, M. R. W. 1975. *Resistance of Pseudomonas Aeruginosa.* pp. 71–107. London: Wiley
18. Brown, M. R. W., Richards, R. M. E., 1965. *Nature* 207:1391–93
19. Bryan, L. E., van den Elzen, H. M. 1977. *Antimicrob. Agents Chemother.* 12:163–77
20. Burman, L. G., Nordstrom, K. 1971. *J. Bacteriol.* 106:1–13
21. Calcott, P. H., McLeod, R. A. 1975. *Can. J. Microbiol.* 21:1724–32
22. Gerny, G., Teuber, M. 1971. *Arch. Microbiol.* 78:166–79
23. Champlin, F. R., Gilleland, M. E., Conrad, R. S. 1983. *Antimicrob. Agents Chemother.* 24:5–9
24. Cheng, K.-J., Ingram, J. M., Costerton, J. W. 1970. *J. Bacteriol.* 104:748–43
25. Cleeland, R., Beskid, G., Grunberg, E. 1970. *Infect. Immun.* 2:371–75
26. Coleman, W. G., Leive, L. 1979. *J. Bacteriol.* 139:899–910
27. Coughlin, R. T., Caldwell, C. R., Haug, A., McGroarty, E. J. 1981. *Biochem. Biophys. Res. Commun.* 100:1137–42
28. Cullis, P. R., de Kruijff, B. 1979. *Biochim. Biophys. Acta* 559:399–420
29. Dame, J. B., Shapiro, B. M. 1976. *J. Bacteriol.* 127:961–72
30. Davies, J. K., Reeves, P. 1975. *J. Bacteriol.* 123:102–17
31. Davis, S. D., Iannett, A. 1972. *Appl. Microbiol.* 23:775–79
32. Davis, S. D., Iannett, A. 1978. *Antimicrob. Agents Chemother.* 13:820–25
33. Deich, R. A., Hoyer, L. C. 1982. *J. Bacteriol.* 152:855–64
34. Dirienzo, J. M., Nakamura, K., Inouye, M. 1978. *Ann. Rev. Biochem.* 47:481–532
35. Donaldson, D. M., Roberts, R. R., Larsen, H. S., Tew, J. G. 1974. *Infect. Immun.* 10:657–66
36. Dorrer, E., Teuber, M. 1977. *Arch. Microbiol.* 114:87–89
37. Douglas, J., Lee, M. D., Nikaido, H. 1981. *FEMS Microbiol. Lett.* 12:305–9
38. Egan, A. F., Russell, R. R. B. 1973. *Genet. Res.* 21:139–52
39. Elsbach, P., Weiss, J., Franson, R. C.,

Beckerdite-Quagliata, S., Schneider, A., Harris, L. 1979. *J. Biol. Chem.* 254:11000–9
40. Ennis, H. L. 1971. *J. Bacteriol.* 107: 486–90
41. Eriksson-Grennberg, K. G., Nordstrum, K. 1973. *J. Bacteriol.* 115:1219–22
42. Fass, R. J., Barnisham, J. 1980. *Rev. Infect. Dis.* 2:841–53
43. Fierer, J., Finley, F. 1979. *J. Infect. Dis.* 140:581–89
44. Finch, J. E., Brown, M. R. W. 1978. *Infect. Immun.* 20:340–46
45. Foulds, J. 1976. *J. Bacteriol.* 128:604–8
46. Franklin, T. J., Snow, G. A. 1981. *Biochemistry of Antimicrobial Action.* London: Chapman & Hall. 217 pp. 3rd Ed.
47. Fung, J., MacAlister, T. J., Rothfield, L. I. 1978. *J. Bacteriol.* 133:1467–71
48. Galanos, C., Luderitz, O., Rietschel, E. T., Westphal, O. 1977. *Int. Rev. Biochem.* 14:239–335
49. Galanos, C., Rietschel, E. T., Luderitz, O., Westphal, O. *Eur. J. Biochem.* 19: 143–52
50. Gewurz, H., Shin, H. S., Mergenhagen, S. E. 1968. *J. Exp. Med.* 128:1049–56
51. Gilleland, H. E., Conrad, R. S. 1982. *Antimicrob. Agents Chemother.* 22: 1012–16
52. Gilleland, H. E., Farley, L. B. 1982. *Can. J. Microbiol.* 28:830–40
53. Gilleland, H. E., Murray, R. G. E. 1976. *J. Bacteriol.* 125:267–81
54. Gilleland, H. E., Stinnett, J. D., Eagon, R. G. 1974. *J. Bacteriol.* 117:302–11
55. Glenn, A. R. 1976. *Ann. Rev. Microbiol.* 30:41–62
56. Glynn, A. A. 1969. *Immunology* 16: 463–68
57. Gmeiner, J., Schlecht, S. 1979. *Eur. J. Biochem.* 93:609–20
58. Gmeiner, J., Schlecht, S. 1980. *Arch. Microbiol.* 127:81–86
59. Goldschmidt, M. C., Wyss, O. 1967. *J. Gen. Microbiol.* 47:421–31
60. Graham, G. S., Treick, R. W., Brunner, D. P. 1979. *Curr. Microbiol.* 2:339–43
61. Gray, G. W., Wilkinson, S. G. 1965. *J. Gen. Microbiol.* 39:385–93
62. Greenfield, S., Feingold, D. S. 1970. *J. Infect. Dis.* 121:555–58
63. Grinius, L. 1980. *FEBS Lett.* 113:1–10
64. Grundstrom, T., Normark, S., Magnusson, K.-E. 1980. *J. Bacteriol.* 144:884–90
65. Gustafsson, P., Nordstrom, K., Normark, S. 1973. *J. Bacteriol.* 116:893–900
66. Guymon, L. F., Sparlin, P. F. 1975. *J. Bacteriol.* 124:757–63
67. Guymon, L. F., Walstad, D. L., Sparling, P. F. 1978. *J. Bacteriol.* 136:391–401
68. Hambleton, P. 1971. *J. Gen. Microbiol.* 69:81–88
69. Hamilton-Miller, J. M. T. 1965. *Biochem. Biophys. Res. Commun.* 20: 688–91
70. Hancock, R. E. W. 1981. *J. Antimicrob. Chemother.* 8:429–45
71. Hancock, R. E. W., Braun, V. 1976. *J. Bacteriol.* 125:409–15
72. Hancock, R. E. W., Decad, G. M., Nikaido, H. 1979. *Biochem. Biophys. Acta* 554:323–29
73. Hancock, R. E. W., Hantke, K., Braun, V. 1977. *Arch. Microbiol.* 114:231–39
74. Hancock, R. E. W., Nicas, T. I. 1984. *Antimicrobial Drug Resistance*, in press. New York: Academic 999 pp.
75. Hancock, R. E. W., Raffle, V. J., Nicas, T. I. 1981. *Antimicrob. Agents Chemother.* 19:777–85
76. Hancock, R. E. W., Reeves, P. 1976. *J. Bacteriol.* 127:98–108
77. Hancock, R. E. W., Wong, P. 1984. *Antimicrob. Agents Chemother.* In press
78. Harder, K. J., Nikaido, H., Mitsuhashi, M. 1981. *Antimicrob. Agents Chemother.* 20:549–52
79. Hartmann, W., Galla, H., Sackmann, E. 1978. *Biochim. Biophys. Acta* 510:124–39
80. Helgerson, S. L., Cramer, W. A. 1976. *J. Supramol. Struct.* 5:291–308
81. Helgerson, S. L., Cramer, W. A. 1977. *Biochemistry* 16:4109–17
82. Henner, W. D., Kleber, I., Benzinger, R. 1973. *J. Virol.* 12:741–47
83. Henning, Y., Haller, I. 1975. *FEBS Lett.* 55:161–64
84. Inouye, M., ed. 1979. *Bacterial Outer Membranes—Biogenesis and Functions.* New York: Wiley 534 pp.
85. Irvin, R. T., Chatterjee, A. K., Sanderson, K. E., Costerton, J. W. 1975. *J. Bacteriol.* 124:930–41
86. Irvin, R. T., MacAlister, T. J., Costerton, J. W. 1981. *J. Bacteriol.* 145:1397–403
87. Jaffe, A., Chabbert, Y. A., Semonin, O. 1982. *Antimicrob. Agents Chemother.* 22:942–48
87. Kahn, M. E., Maul, G., Goodgal, S. M. 1982. *Proc. Natl. Acad. Sci. USA* 79: 6370–74
88. Kamio, Y., Nikaido, H. 1976. *Biochemistry* 15:2561–70
89. Kennedy, E. P. 1982. *Proc. Natl. Acad. Sci. USA* 79:1092–95
90. Koike, M., Iida, K., Matsuo, M. 1969. *J. Bacteriol.* 97:448–52

91. Kropinski, Y., Murakami, K., Nishikawa, T. 1981. *Antimicrob. Agents Chemother.* 20:613–19
92. Kropinski, A. M. B., Chan, L., Milazzo, F. H. 1978. *Antimicrob. Agents Chemother.* 13:494–99
93. Kropinski, A. M., Kuzio, J., Angus, B. L., Hancock, R. E. W. 1982. *Antimicrob. Agents Chemother.* 21:310–19
94. Kusano, T., Izaki, K., Takahashi, H. 1975. *J. Antibiot.* 28:689–95
95. La Porte, D. C., Rosenthal, K. S., Storm, D. R. 1977. *Biochemistry* 16: 1642–48
96. Lazzaroni, J.-C., Portalier, R. C. 1981. *J. Bacteriol.* 145:1357–58
97. Leive, L. 1968. *J. Biol. Chem.* 243: 2373–80
98. Leive, L. 1974. *Ann. NY Acad. Sci.* 235:109–29
99. Leive, L., Shovlin, V. K., Mergenhagen, S. E. 1968. *J. Biol. Chem.* 243: 6384–91
100. Loh, B., Grant, C., Hancock, R. E. W. 1984. *Antimicrob. Agents Chemother.* In press
101. Lugtenberg, B. 1981. *Trends Biochem. Sci.* 6:262–66
102. Lugtenberg, B., van Alphen, L. 1983. *Biochim. Biophys. Acta* 737:51–115
103. Luktenhaus, J. F. 1977. *J. Bacteriol.* 131:631–37
104. Maier, T. W., Zubrzycki, L., Coyle, M. B., Chila, M., Warner, P. 1975. *J. Bacteriol.* 124:834–42
105. Makela, P. H., Sarvas, M., Calcagno, S., Lounatmaa, K. 1978. *FEMS Microbiol. Lett.* 3:323–26
106. Miller, I. R., Bach, D., Teuber, M., 1978. *J. Membr. Biol.* 39:49–56
107. Muller-Eberhard, H. J. 1975. *Ann. Rev. Biochem.* 44:697–724
108. Muschel, L. H., Gustafson, L. 1968. *J. Bacteriol.* 95:2010–13
109. Nagel de Zwaig, R., Luria, S. E. 1967. *J. Bacteriol.* 94:1112–23
110. Nakae, R., Nakae, T. 1982. *Antimicrob. Agents Chemother.* 22:554–59
111. Nakamura, H., Tojo, T., Greenberg, J. 1975. *J. Bacteriol.* 122:874–79
112. Newton, B. A. 1954. *J. Gen. Microbiol.* 10:491–99
112a. Nicas, T. I. 1983. *Ph.D. thesis.* Univ. British Columbia, Canada
113. Nicas, T. I., Hancock, R. E. W. 1980. *J. Bacteriol.* 143:872–78
114. Nicas, T. I., Hancock, R. E. W. 1983. *J. Bacteriol.* 153:281–85
115. Nicas, T. I., Hancock, R. E. W. 1983. *J. Gen. Microbiol.* 129:509–17
116. Nieva-Gomez, D., Gennis, R. B. 1977. *Proc. Natl. Acad. Sci. USA* 74:1811–15
117. Nikaido, H. 1976. *Biochim. Biophys. Acta* 433:118–32
118. Nikaido, H., Bavoil, P., Hirota, Y. 1977. *J. Bacteriol.* 132:1045–47
119. Nikaido, H., Nakae, T. 1979. *Adv. Microbiol. Physiol.* 21:163–250
120. Nikaido, H., Rosenberg, E. Y. 1983. *J. Bacteriol.* 153:241–52
121. Nikaido, H. Rosenberg, E. Y., Foulds, J. 1983. *J. Bacteriol.* 153:232–40
122. Nikaido, H., Song, S. A., Shaltiel, L., Nurminen, S. 1977. *Biochem. Biophys. Res. Commun.* 76:324–30
123. Nordstrom, K., Burman, L. G., Eriksson-Grennberg, K. G. 1970. *J. Bacteriol.* 101:659–68
124. Nordstrom, K., Sykes, R. B. 1974. *Antimicrob. Agents Chemother.* 6:741–46
125. Normark, S. 1969. *J. Bacteriol.* 98: 1274–77
126. Normark, S. 1970. *Genet. Res.* 16:63–78
127. Normark, S., Westling, B. 1971. *J. Bacteriol.* 108:45–50
128. Ohmori, H., Azuma, A., Suzuki, Y., Hashimoto, Y. 1977. *Antimicrob. Agents Chemother.* 12:537–39
129. Osborn, M. H., Wu, H. C. P. 1980. *Ann. Rev. Microbiol.* 34:369–422
130. Oberbeeke, N., Lugtenberg, B. 1982. *Eur. J. Biochem.* 126:113–18
131. Pache, W., Chapman, D., Hillaby, R. 1972. *Biochim. Biophys. Acta* 255:358–64
132. Palva, E. T., Makela, P. H. 1980. *Eur. J. Biochem.* 107:137–43
133. Payne, J. W., Gilvarg, C. 1968. *J. Biol. Chem.* 243:6291–301
134. Pazoles, C. J., Kulpa, C. F. 1977. *Biochim. Biophys. Acta* 466(1):160–75
135. Poole, K., Hancock, R. E. W. 1983. *FEMS Microbiol. Lett.* 16:25–29
135a. Ractz, C. R., Foulds, J. 1977. *J. Biol. Chem.* 252:5911–15
136. Rawal, B. D. 1978. *Chemotherapy* 24: 166–71
137. Rawal, B. D., McKay, G., Blackhall, M. I. 1974. *Med. J. Aust.* 1:169–74
138. Ray, B., Jezeski, J. J., Busta, F. F. 1971. *Appl. Microbiol.* 22:401–7
139. Reid, P., Speyer, J. 1970. *J. Bacteriol.* 104:376–86
140. Richmond, M. H., Clark, D. C., Wotton, S. 1979. *Antimicrob. Agents Chemother.* 10:215–18
141. Richmond, M. H., Sykes, R. B. 1973. *Adv. Microbiol. Physiol.* 9:31–86
142. Roantree, R. J., Kuo, T. T., MacPhee, D. G. 1977. *J. Gen. Microbiol.* 103:223–24
143. Rosenthal, K. S., Swanson, P. E., Storm, D. R. 1976. *Biochemistry* 15: 5783–92

144. Rotten, S., Leive, L. 1977. *J. Biol. Chem.* 252:2077–81
145. Rowley, D. 1968. *J. Bacteriol.* 95:1647–52
146. Sabelnikov, A. G., Domardsky, I. V. 1979. *Mol. Gen. Genet.* 172:313–17
147. Sabelnikov, A. G., Domaradsky, I. V. 1981. *J. Bacteriol.* 146:435–43
148. Saier, M. H. 1979. *The Bacteria*, VII:168–227. New York: Academic.
149. Sanderson, K. E., MacAlister, T., Costerton, J. W., Cheng, K. J. 1974. *Can. J. Microbiol.* 20:1135–45
150. Sanderson, K. E., Stocker, B. A. D. 1981. *J. Bacteriol.* 146:535–41
151. Sarubbi, F. A., Sparling, P. F., Blackman, E., Lewis, E. 1975. *J. Bacteriol.* 124:750–56
152. Sawai, T., Hiruma, R., Kawana, N., Kaneko, M., Taniyasu, F., et al. 1982. *Antimicrob. Agents Chemother.* 22:585–92
153. Schindler, M., Osborn, M. J., 1979. *Biochemistry* 18:4425–30
154. Schindler, P. G., Teuber, M. 1975. *Antimicrob. Agents Chemother.* 8:95–104
155. Schindler, P. R. G., Teuber, M. 1978. *J. Bacteriol.* 135:198–206
156. Schlecht, S., Schmidt, G. 1970. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig.* 212:505–511
157. Schlecht, S., Westphal, O. 1970. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig.* 213:356–81
158. Schmidt, G., Schlecth, S., Westphal, O. 1969. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig.* 212:88–96
159. Schweizer, M., Hindennach, I., Garten, W., Henning, U. 1978. *Eur. J. Biochem.* 82:211–17
160. Sha'afi, R. I., Gary-Bobo, C. M., Solomon, A. K. 1971. *J. Gen. Physiol.* 58:238–58
161. Shales, S., Chopra, I. 1982. *J. Antimicrob. Chemother.* 9:325–31
162. Sherbert, G. V., Lakshmi, M. S. 1973. *Biochim. Biophys. Acta* 298:50–58
163. Singh, A. P., Reithmeier, R. A. F. 1975. *J. Gen. Appl. Microb.* 21:109–18
164. Smit, J., Kanno, Y., Nikaido, H. 1975. *J. Bacteriol.* 124:942–58
165. Sonntag, I., Schwarz, H., Hirota, Y., Henning, U. 1978. *J. Bacteriol.* 136: 280–85
166. Sparling, P. F., Sarrubi, F. A., Blackman, E. 1975. *J. Bacteriol.* 124:740–49
167. Spicer, A. B., Spooner, D. F. 1974. *J. Gen. Microbiol.* 80:37–50
168. Stan Lotter, H., Gupta, M., Sanderson, K. E. 1979. *Can. J. Microbiol.* 25:475–85
169. Stein, W. D. 1967. *The Movement of Molecules Across Cell Membranes*. New York: Academic
170. Stinson, M. W., Hayden, C. 1979. *Infect. Immun.* 25:558–64
171. Stock, J. B., Rauch, B., Roseman, S. 1977. *J. Biol. Chem.* 252:7850–61
172. Storm, D. R., Rosenthal, K. S., Swanson, P. E. 1977. *Ann. Rev. Biochm.* 46:723–63
173. Sud, I. J., Feingold, D. S. 1975. *Antimicrob. Agents Chemother.* 8:34–37
174. Suginaka, H., Kotani, S., Takata, N., Ogawa, M. 1980. *FEMS Microbiol. Lett.* 8:79–82
175. Suginaka, H., Schimatani, M., Kotani, S., Ogawa, M., Hama, M., et al. 1979. *FEMS Microbiol. Lett.* 5:177–79
176. Suzuki, H., Nishimura, Y., Yasuda, S., Nishimura, A., Yamada, M., et al. 1978. *Molec. Gen. Genet.* 167:1–9
177. Sykes, R., Morris, A. 1975. *Prog. Med. Chem.* 12:333–93
178. Tamaki, S., Sato, T., Matsuhashi, M. 1971. *J. Bacteriol.* 105:968–75
179. Taketo, A. 1977. *Z. Naturforsch.* 32c:429–33
180. Taketo, A. 1978. *J. Gen. Appl. Microbiol.* 24:51–58
181. Tecoma, E. S., Wu, D. 1980. *J. Bacteriol.* 142:931–38
182. Traub, W. H. 1982. *Chemotherapy* 28:363–68
183. Traub, W. H., Kleber, I. 1975. *Antimicrob. Agents Chemother.* 7:874–76
184. Uratani, Y., Kageljama, M. 1977. *J. Biochem.* 81:333–41
185. Vaara, M. 1981. *J. Bacteriol.* 148:426–34
186. Vaara, M. 1983. *FEMS. Microbiol. Lett.* 18:117–21
187. Vaara, M., Vaara, T. 1981. *Antimicrob. Agents Chemother.* 19:578–83
188. Vaara, M., Vaara, T. 1983. *Antimicrob. Agents Chemother.* 24:107–13
189. Vaara, M., Vaara, T. 1983. *Antimicrob. Agents Chemother.* 24:114–22
190. Vaara, M., Vaara, T. 1983. *Nature* 303:526–28
191. Vaara, M., Vaara, T., Jensen, M., Helander, I., Nurminen, M., et al. 1981. *FEBS Lett.* 129:145–49
192. Vaara, M., Vaara, T., Sarvas, M. 1979. *J. Bacteriol.* 139:664–67
193. Van Alphen, W., Lugtenberg, B., Berendsen, W. 1976. *Mol. Gen. Genet.* 147:263–69
194. Van Alphen, L., Lugtenberg, B., van Boxtel, R., Verhoef, K. 1977. *Biochim. Biophys. Acta* 466:257–68

195. Van Alphen, W., van Selm, N., Lugtenberg, B. 1978. *Mol. Gen. Genet.* 159: 75–83
196. Van Die, I., van Oosterhout, A., Bergmans, H., Hoekstra, W. 1983. *FEMS Microbiol. Lett.* 18:127–30
197. Verkeleij, A., van Alphen, L., Bijvelt, J., Lugtenberg, B. 1977. *Biochim. Biophys. Acta* 466:269–82
198. Voll, M. J., Leive, L. 1970. *J. Biol. Chem.* 245:1108–14
199. Voss, J. G., 1967. *J. Gen. Microbiol.* 48:391–400
200. Walker, C. A., Durham, N. N. 1975. *Can. J. Microbiol.* 21:69–74
201. Weigand, R. A., Rothfield, L. I. 1976. *J. Bacteriol.* 125:340–45
202. Weisser, R., Asscher, A. W., Wimpenny, J. 1968. *Nature* 219:1365–66
203. Weiss, J., Victor, M., Elsbach, P. 1983. *J. Clin. Invest.* 71:540–49
204. Weston, A., Brown, M. G. M., Perkins, H. R., Saunders, J. R., Humphreys, G. O. 1981. *J. Bacteriol.* 145:780–87
205. Whitfield, C., Hancock, R. E. W., Costerton, J. W. 1983. *J. Bacteriol.* 156: 873–79
206. Wilson, L. A. 1970. *Brit. J. Opthamol.* 54:195–213
207. Wilson, L. A., Spitznagel, J. K. 1968. *J. Bacteriol.* 96:1339–48
208. Wilson, M. E., Morrison, D. C. 1982. *Eur. J. Biochem.* 128:137–41
209. Wolf, M. K., Konisky, J. 1981. *J. Bacteriol.* 145:341–47
210. Wolf-Watz, H., Elmros, T., Normark, S., Bloom, G. D. 1975. *Infect. Immun.* 11:1332–41
211. Wright, S. D., Levine R. P. 1981. *J. Immunol.* 127:1146–51
212. Yamada, H., Mizushima, S. 1980. *Eur. J. Biochem.* 103:209–18
213. Yem, D. W., Wu, H. C. 1978. *J. Bacteriol.* 133:1419–26
214. Yoshimura, F., Nikaido, H. 1982. *J. Bacteriol.* 152:636–42
215. Saske, S. K., Dockins, W. S., McFeters, G. A. 1980. *Appl. Environ. Microbiol.* 40:386–90
216. Zimelis, V. M., Jackson, G. G. 1973. *J. Infect. Dis.* 127:663–69
217. Zimmerman, W., Rosselet, A. 1977. *Antimicrob. Agents. Chemother.* 12: 368–76