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Antibiotic Uptake into Gram-Negative Bacteria

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Antibiotics taken up into gram-negative bacteria face two major diffusion barriers, the outer and cytoplasmic membranes. Of these, the former has been most studied and is discussed in detail here. Evidence from antibiotic MIC studies on porin-deficient mutants compared with their porin-sufficient parent strains has provided strong support for the proposal that some antibiotics, particularly β-lactams, pass across the outer membrane through the water-filled channels of a class of proteins called porins. Nevertheless substantial evidence has accumulated for the importance of non-porin pathways of antibiotic uptake across the outer membranes of gram-negative bacteria. Examples discussed include the uptake of polycationic antibiotics via the self-promoted pathway, the uptake of hydrophobic antibiotics in some bacterial species and in mutants of others via the hydrophobic pathway, and the possible importance of poorly understood non-porin pathways of uptake of a variety of antibiotics. Other potential barriers to diffusion, including the cytoplasmic membrane, are briefly discussed.

Since the initiation of the modern era of antibiotic usage in the 1940s, considerable research effort has been expended in determining the mechanism of uptake and mode of action of all groups of antibiotics. In many cases the mode of action of individual antibiotics is quite well understood, however antibiotic uptake mechanisms have remained more elusive. This is perhaps best illustrated by the aminoglycosides which have been studied in enormous detail. Despite this, the mechanism of aminoglycoside uptake across the cytoplasmic membrane of bacteria remains controversial. It is of little assistance that all known aminoglycoside resistant mutants influence antibiotic uptake (1), since it is difficult to differentiate mutants that exclusively affect uptake from those that additionally influence mode of action. As a result of this and similar problems, this brief review will concentrate on an area of antibiotic uptake which has become increasingly well understood, that is uptake across the outer membranes of gram-negative bacteria. Brief mentions will be made of other cell layers which might be considered to be influential in antibiotic uptake into cells.

The outer membranes of gram-negative bacteria vary somewhat in composition, but may generally considered to be lipopolysaccharide (LPS): phospholipid bilayers (in the outer and inner monolayers respectively) studded with proteins (Figure 1). The outer membrane, where studied, is in direct physical contact with the underlying peptidoglycan by means of strong non-covalent or sometimes covalent interactions. The structure of the outer membrane has been described in some detail recently (2, 3) and only two concepts of direct relevance to antibiotic uptake will be discussed here. Firstly, the outer membrane is usually described as a semi-permeable barrier (or molecular sieve), in which those hydrophilic molecules of sizes below a given exclusion limit can pass through the channels of proteins called porins (2, 3). In contrast the remainder of the outer membrane has been considered to exclude uptake of hydrophilic and, in all except a few cases, hydrophobic molecules. As described below, this generalization is probably an oversimplification. Secondly, the pioneering work of Leive (4) on the mode of interaction of EDTA with *Escherichia coli* demonstrated that the interactions of divalent cations with LPS molecules were important determinants of outer membrane structural stability and barrier function. Because of space limitations, review articles have been extensively utilized as references rather than original manuscripts.

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Porin-Mediated Antibiotic Uptake Across the Outer Membrane

General Porin Properties

The structure, genetics and in vitro model membrane properties of porins have been reviewed in some detail elsewhere (2, 3, 5). In general, porins comprise

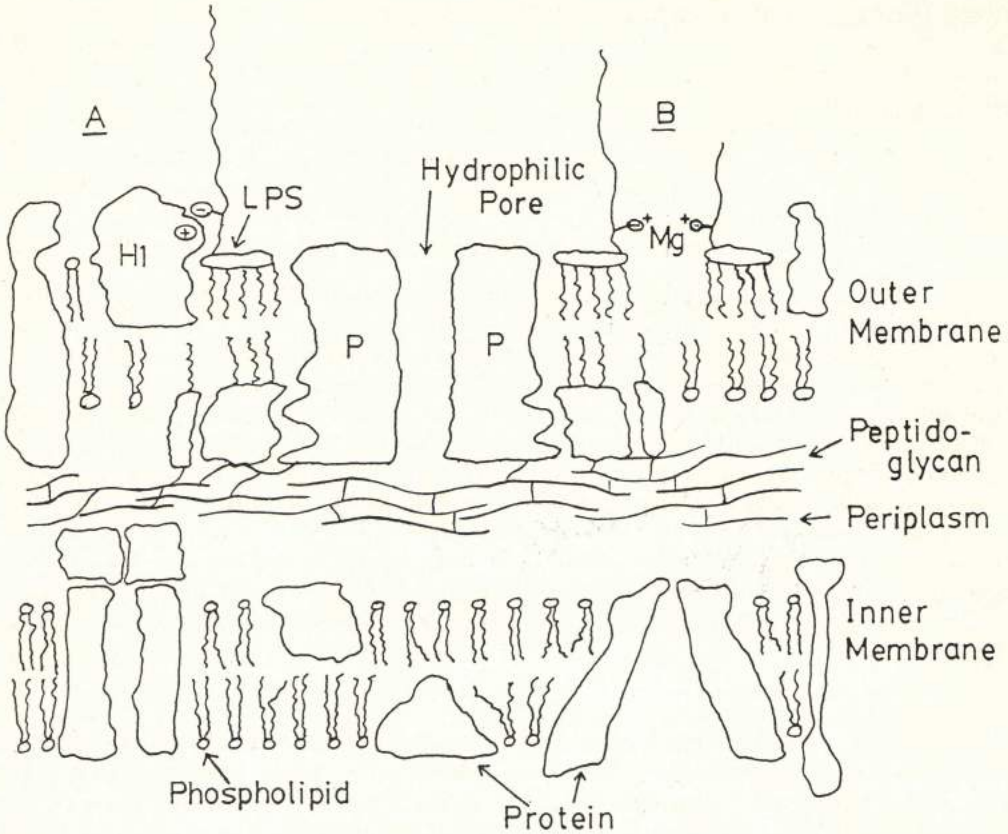

Figure 1: Schematic representation of a cross section of the cell envelope of gram-negative bacteria. P = porin protein involved in uptake of hydrophilic antibiotics, LPS = lipopolysaccharide. Hl = other membrane protein Hl. A represents sites at which self-promoted uptake is blocked by protein Hl in *Pseudomonas aeruginosa* (see text). B represents sites at which polycations and chelators can displace divalent cations from LPS, resulting in self-promoted uptake. Alteration in the nature of the B sites (e.g. reduction in the affinity of LPS for divalent cations) might result in a non-porin pathway of uptake for antibiotics that are not polycations (including hydrophobic antibiotics).

oligomeric aggregates (usually trimers) of monomer molecular weights in the range of 28,000 to 48,000. Where studied they have a high content of β-sheet structure which confers extraordinary structural stability on many porins such that they often resist denaturation upon heating in sodium dodecyl sulphate. Porins are tightly but non-covalently associated with the underlying peptidoglycan and with LPS. However, porins often vary from this general scheme in one or more properties (5).

Nakae first demonstrated by liposome reconstitution experiments that porins contained water-filled channels capable of allowing size-dependent uptake of saccharides (6). Subsequently, a variety of model membrane studies (5) have demonstrated that porin channels have the following intrinsic properties. They are large (diameter 0.6–2.3 nm), water-filled channels, the dimensions of which apparently determine their exclusion limits for hydrophilic molecules. Most porins demonstrate little chemical selectivity, although exceptions exist. The interior of a porin channel contains charged amino acids. The number and positioning of these charged amino acids relative to the most constricted portion of the channel apparently determines the ion selectivity of porin channels (which are usually weakly selective for small ions). Generally, model membrane studies have suggested that porin molecules contain passive diffusion channels which can influence uptake of β-lactams by virtue of their channel size relative to the permeating β-lactam, and by their intrinsic ion selectivity relative to the charge on the β-lactam. Thus these studies predict that β-lactam passage through porins should be definable in terms of general channel theory as described by specific equations (5). In many cases this has been proven to be correct.

Role of Porins in Beta-Lactam Uptake

Zimmermann and Rosselet (7) first described outer membrane permeability to β-lactams in terms of

Fick's first law of diffusion. Thus Vd = C (So–Si) in which Vd is the rate of diffusion across the outer membrane, So is the external and Si the periplasmic concentration of β-lactam, and C is a permeability parameter dependent on the total area of porin channels per outer membrane, the inverse of the length of porin channels and the diffusivity coefficient (5). This theoretical treatment of outer membranes, which assumes a semi-permeable membrane perforated by porins, makes three testable predictions. Firstly, the rate of diffusion Vd should increase proportionally as the concentration gradient (So–Si) across the outer membrane increases, a concept that has been tested in *Escherichia coli* (7) and *Pseudomonas aeruginosa* (8) (note that since So ⩾ Si, Vd is approximately proportional to So). Secondly, the total area of channels per outer membrane should influence antibiotic uptake, a concept that has been used to explain the greater intrinsic resistance to antibiotics of *Pseudomonas aeruginosa* compared to *Escherichia coli* (8). Thirdly, the diffusivity coefficient should be strongly influenced by the physicochemical nature of the β-lactam antibiotic and the channel properties. In agreement with this, Nikaido and colleagues have shown that small differences in channel size (e.g. comparing the OmpF and OmpC channels of *Escherichia coli*) and differences in the nature of individual β-lactams can influence the penetration rates of β-lactams in model membrane studies (2).

Nevertheless, the strongest data favouring an in vivo role for porins in uptake of some β-lactam antibiotics across the outer membrane comes from comparisons of porin-deficient mutants with their isogenic wild type strains. Such mutants have significant increases in MIC for some but not all β-lactams (Table 1) (8–13), as well as measurable decreases in the uptake rates of given β-lactams (14). However, the increased MICs occur for only a subset of β-lactams (12). Indeed in constructed mutants, e.g. oprF::Ω of *Pseudomonas aeruginosa* lacking the proposed porin protein F, Woodruff and Hancock (12) were unable to measure large increases in MIC compared to wild-type for any β-lactam (Table 1). There are several potential explanations for these data. For example, it has been suggested that the amount and kinetics of periplasmic β-lactamase and the kinetics of its action on substrates are influential in determining MICs and in overriding differences in outer membrane penetration rates due to altered porin content (2). Alternatively, Woodruff and Hancock (12) demonstrated increased hydrophobic permeability of *Pseudomonas aeruginosa* protein F-deficient mutants and suggested that this might reflect counteractive uptake of β-lactams via non-porin pathways (see below) caused by the loss of a major outer membrane structural component. Consistent with this latter proposal, Godfrey and Bryan (13) isolated a putative protein F-altered mutant with substantial increases in antibiotic MICs [cf. a protein F-deficient mutant (12)]. In addition, Siden and Boman (15) observed increased uptake of hydrophobic substances in several *Escherichia coli* OmpC (porin deficient) mutants but not in others. Similarly, Then and Angehrn (11) observed two types of mutants with drastic reductions in the amounts of two outer membrane proteins probably corresponding to *Enterobacter cloacae* porins. One, AZT-R, was supersusceptible to hydrophobic agents including acridine orange, trimethoprim and SDS, and demonstrated no decrease in antibiotic susceptibility to two β-lactams (ampicillin and piperacillin) but large changes in susceptibility to many others. The

Table 1: Influence of porin deficiencies on antibiotic resistance in various bacteria. Data from references No. 9, 10, 11, 12, and 13.

Antibiotic	Ratio of MICs of porin-deficient mutant/porin-sufficient parent									
	Escherichia coli	*Proteus mirabilis*	*Proteus vulgaris*	*Morganella morganii*	*Providencia rettgeri*	*Providencia alcalifaciens*	*Enterobacter cloacae*		*Pseudomonas aeruginosa*	
							AMA-R	AZT-R	oprF::Ω	PCC-23
Ampicillin	4–16	2	2	2	2	2	16	0.5	–	–
Piperacillin	2	1	1	1	1	1	16	1	1.4	83
Cefotaxime	2	–	–	–	–	–	–	–	3.2	333
Ceftazidime	2	–	–	–	–	–	16	16	1.6	21
Cefoxitin	16–32	16	16	32	32	16	–	–	–	–
Cefazolin	8–16	32	4	16	8	32	–	–	–	–
Aztreonam	4	–	–	–	–	–	128	64	1.7	–
Imipenem	1	–	–	–	–	–	4	4	0.8	4
Tetracycline	3	4	4	4	4	4	–	–	–	0.5
Chlor-amphenicol	1.5	1	1	1	1	1	4	0.25	–	–
Norfloxacin	4	–	–	–	–	–	32	1	1	0.25
Minocycline	1.5	1	1	1	1	1	–	–	–	–

other, AMA-R, showed normal resistance to hydrophobic agents and resistance to all β-lactams including piperacillin and ampicillin. One explanation for these phenomena is that porin-deficient mutants are normally more susceptible to hydrophobic agents (due to the loss of a major outer membrane component and consequent increase in lipidic components) (12), but can undergo adaptive changes causing structural alterations and loss of supersusceptibility to such hydrophobic agents.

Role of Porins in Uptake of Other Antibiotics

The discovery of the role of porins in uptake of other antibiotics stems largely from examination of the antibiotic MICs of porin-deficient mutant compared with wild type strains. On this basis chloramphenicol, tetracycline, and some quinolones may be taken up by porin pathways (2, 16). Nevertheless, porin-deficient mutants demonstrate substantial residual uptake of these antibiotics, suggesting the possibility of additional significant uptake systems. In contrast, porin-deficient mutants are usually not more resistant to aminoglycosides and we consider the role of porins in uptake of aminoglycosides in any bacterium to be unresolved.

Other Porin-Like Pathways

There is as yet little definitive evidence for the involvement, in uptake of specific antibiotics, of porins other than the major diffusion channels of gram-negative outer membranes. However, certain possibilities have recently come to light. Iron transport in gram-negative bacteria often involves chelation of ferric iron by siderophores. Such chelates bind to specific outer membrane receptor proteins and are subsequently translocated across the cell envelope of *Escherichia coli* in a *tonB*-protein dependent step (2). It is as yet unknown how the ferric-siderophore complex is translocated across the outer membrane but it may involve the outer membrane receptor functioning as a specific porin. Recently, a potent anti-pseudomonal cephalosporin, E-0702, was developed and it apparently utilizes one of the *tonB*-dependent iron transport systems of *Escherichia coli* (17). Similarly, the semi-synthetic rifamycin derivative CGP4832 is taken up across the outer membrane via the *tonB*-dependent ferrichrome uptake system of *Escherichia coli* (18). *Pseudomonas aeruginosa* mutants resistant to the broad-spectrum carbapenem β-lactam antibiotic, imipenem, lack a specific 45 kDa outer membrane protein (19). It has been suggested that this protein serves as an imipenem-specific porin since mutants lacking this protein retain susceptibility to other antibiotics.

Non-Porin Pathways of Antibiotic Uptake Across the Outer Membrane

Self-Promoted Uptake

Certain antibiotics appear to cross the outer membrane by pathways other than diffusion through porins. One such pathway is known as self-promoted uptake. It was named on the basis of studies of the mechanism of uptake of polycationic antibiotics in *Pseudomonas aeruginosa* (3, 20). However, the earlier studies on EDTA interaction with the *Escherichia coli* outer membrane by Leive and colleagues, and on polymyxin uptake in a variety of bacteria were suggestive that such a mechanism of uptake is more widely distributed (2, 3, 20).

The initial stages of self-promoted uptake seem to occur at negatively-charged sites (phosphate and/or carboxyl groups) on LPS, which bind divalent cations such as Mg^{2+} and Ca^{2+} strongly. The non-covalent association between LPS and divalent cations is an essential component of outer membrane integrity (4). Its disruption by the chelator ethylenediaminetetraacetate (EDTA) results in the release of large quantities of LPS from *Escherichia coli* and *Pseudomonas aeruginosa*, and increased susceptibility to various hydrophobic and hydrophilic compounds. EDTA is one of a class of compounds, known as permeabilizers, that can enhance outer membrane permeability to other agents in a number of gram-negative bacteria (20). The mode of action of permeabilizers has been investigated by using hydrophobic (e.g. N-phenylnaphthylamine) and hydrophilic (e.g. nitrocefin) probes of outer membrane permeability, measuring release of LPS and periplasmic enzymes, and examining outer membrane morphological changes. Compounds that can act as outer membrane permeabilizers include polymyxin B and related compounds, other polycations, monovalent organic cations, divalent cation chelators and host defense factors. The polycations are presumed to act by displacing divalent cations from binding sites on LPS, and the kinetics of this process have been investigated using a fluorescently-labeled polymyxin derivative and a cationic spin label probe (21). The actions of these permeabilizers are generally antagonized by added divalent cations including Mg^{2+} and Ca^{2+}.

Mutants of *Pseudomonas aeruginosa* cross-resistant to EDTA/Tris, polymyxin B and aminoglycosides were isolated (3, 20) and found to constitutively overproduce an outer membrane protein, Hl. Wild type cells grown in media deficient in certain divalent cations had similar resistance properties and were induced for protein Hl expression. Cells with mutational overproduction of protein Hl displayed altered kinetics of streptomycin uptake and had reduced Mg^{2+} levels in their cell envelopes. There was no change, however, in

susceptibility to other antibiotics such as β-lactams and tetracyclines (20), or in the outer membrane permeability to the β-lactam nitrocefin (8). It was concluded that protein Hl probably inhibited a common uptake pathway that was essential to the bactericidal action of polycations and EDTA/Tris (1, 3, 20). Since these compounds were known to disrupt LPS-divalent cation interactions and permeabilize the outer membrane, they could presumably enhance their own uptake. Protein Hl was hypothesized to inhibit self-promoted uptake by replacing divalent cations at negatively charged sites on LPS (1). The protein, being stably anchored in the membrane, could not be displaced by the permeabilizers, and thus could prevent membrane disruption and consequent uptake of the disrupting polycation. Figure 1 illustrates how the overproduction of protein Hl could lead to decreased self-promoted uptake. The exact nature of the protein Hl binding site on LPS is unknown, but several LPS mutations abolished protein Hl-mediated polymyxin B resistance in *Pseudomonas aeruginosa* (unpublished data).

Protein Hl-mediated resistance affected all aminoglycoside antibiotics tested, although these compounds had previously been assumed to cross the outer membrane through porins in *Escherichia coli* (1). Nevertheless, the above data strongly suggests that self-promoted pathway is a plausible mechanism of uptake of aminoglycosides, at least in *Pseudomonas aeruginosa*.

A somewhat similar type of mutation has been described in *Salmonella typhimurium* (2). The *pmr*A mutant was resistant to polymyxin B and EDTA/Tris killing and permeabilization, apparently owing to alterations which reduced the negative charges on LPS. In addition, polymyxin B and its deacylated derivative polymyxin B nonapeptide have been demonstrated to bind to *Escherichia coli* LPS, and to permeabilize the outer membranes of *Escherichia coli* and other bacterial species. Thus, we conclude that self-promoted uptake of polymyxin also occurs in a variety of bacteria including *Escherichia coli. Pseudomonas cepacia*, closely related to *Pesudomonas aeruginosa*, was apparently resistant to self-promoted uptake even without induction of any protein analogous to Hl (22). However, serious investigation into the possibility that self-promoted uptake operates widely in gram-negative bacteria has yet to be undertaken.

The precise molecular nature of the uptake process following disruption of LPS-cation interactions remains obscure, and its elucidation probably depends on a better understanding of abnormal structures in LPS-containing membranes. There is electron microscopic evidence for the accumulation of transient holes in gentamicin-treated outer membranes (23). Analysis of the effects of polycations on LPS using a cationic spin probe has led to the proposal that displacement of cations causes rigidification of LPS-aggregates and allows antibiotics to rearrange LPS packing, causing "cracks" in the structure (21). In any event, a better understanding of the nature of self-promoted uptake would assist in the design of agents capable of crossing outer membranes.

Hydrophobic Uptake

Most wild type gram-negative bacteria exclude moderately hydrophobic substances (2). For example, the hydrophobic fluorescent probe 1-N-phenylnaphthylamine is excluded from wild type *Escherichia coli* and *Pseudomonas aeruginosa* cells (3, 20). The reason for this impermeability to hydrophobic substances seems to be that the external surface of the outer membrane prevents or resists the partitioning of moderately hydrophobic substances into the interior of the membrane. Permeabilization to hydrophobic substances can be achieved by addition of compounds which remove, be chelation (e.g. EDTA), or competively displace (e.g. polycations) divalent cations from their LPS binding sites at the cell surface (3, 20). Thus the stabilizing influence of divalent cations and LPS at the cell surface is a primary factor in exclusion of moderately hydrophobic substances. Some antibiotics can be considered moderately hydrophobic in that they will partition into organic solvents in two phase partitioning experiments. Nevertheless, most of these antibiotics are water-soluble at therapeuticallyrelevant concentrations. The high MICs of bacterial species for such antibiotics (e.g. Table 2) are probably indicative of the barrier effect of the outer membrane. In agreement with this, alteration of this barrier by treatment with permeabilizers or by specific outer membrane mutations affecting LPS (20, 25) will decrease MICs for these antibiotics (Table 2). In some bacterial species (e.g. *Neisseria* and *Haemophilus*), MICs for moderately hydrophobic antibiotics are substantially decreased (Table 2) and it can be assumed that these bacteria present outer surfaces to the environment that are less effectively stabilized.

Other Non-Porin Pathways

Hinma and colleagues (26) have demonstrated that certain β-lactam antibiotics have substantial permeation rates across reconstituted lipid bilayers. These include the moderately hydrophilic antibiotics, ampicillin and benzyl penicillin, although almost all β-lactams measured had some degree of permeability. The authors suggested that such permeation across lipid bilayers is regulated by lipopolysaccharide and can explain differential susceptibility to β-lactams of certain mutants. In vivo data with at least two classes of mutants suggest that this is true. The *Pseudomonas aeruginosa* antibiotic supersusceptible mutant Z61 contains at least two separate mutations which cause

Table 2: MICs for moderately hydrophobic antibiotics in various gram-negative bacteria. Data from references No. 20 and 24.

Antibiotic	MIC (μg/ml)						
	Pseudomonas aeruginosa		*Salmonella typhimurium*		*Neisseria gonorrhoeae*	*Neisseria meningitidis*	*Haemophilus influenzae*
	Wild type	Mutant (Z61)	Wild type	Mutant (Deep rought)	Wild type	Wild type	Wild type
Erythromycin	200	–	75	2	< 0.5	< 2	0.5– 8
Novobicin	> 128	0.05	500	5	4	< 4	0.2– 0.8
Fusidic acid	300	–	300	–	< 1	< 0.25	–
Clindamycin	> 64	–	> 64	–	< 4	–	0.5–16
Rifampicin	50	0.2	10	< 1	0.5	–	–
Minocycline	100	–	–	–	0.4	1.6	1.6

different changes in LPS (25). One of these, the *absA* mutation, causes substantial increases in susceptibility to hydrophobic agents, in addition to a wide range of β-lactams and aminoglycosides. The other, the *absB* mutation, has an apparent alteration in LPS-divalent cation binding and an increase in susceptibility to many β-lactams and to aminoglycosides but not to hydrophobic agents. Studies from two laboratories (2, 25) have failed to identify any changes in porin function in the mutant Z61. Similarly, the *Escherichia coli* antibiotic supersusceptible mutant DC2 has an alteration in LPS that alters divalent cation binding (27), but no porin alterations (2). These mutants are 16-fold more susceptible to ampicillin, as well as several more hydrophobic antibiotics.

Based on these findings, we feel that the case for the existence of non-porin pathways of antibiotic uptake is strong, at least in mutants. One might predict that in organisms with a less effective porin-mediated uptake pathway, like *Pseudomonas aeruginosa* and *Pseudomonas cepacia*, these non-porin pathways might become more important.

Influence of Other Cell Layers on Antibiotic Uptake

Cytoplasmic Membrane

Beta-lactam antibiotics act upon a series of penicillin binding proteins located in the periplasm and thus do not need to cross the cytoplasmic membrane. Others, including a variety of hydrophobic antibiotics (see below), apparently cross the cytoplasmic membrane by passive diffusion.

In the case of aminoglycosides, transport across the cytoplasmic membrane is apparently tightly coupled to the bactericidal action of these antibiotics (1). Despite 40 years of research, there is little concensus concerning either the mode of action or transport mechanism of aminoglycosides (1, 28, 29). Nevertheless, some generalizations can be made. Most researchers agree that streptomycin uptake across the cytoplasmic membrane is energized by the protonmotive force (1). Transport is unidirectional (i.e. inwards) and substantially irreversible. Bryan and colleagues (28) have suggested that respiratory quinones are involved in transport, but this is controversial (29). Streptomycin uptake involves two energized phases, a slow phase EDPI, followed by a more rapid phase EDPII, which appears to be initiated at the same time as or subsequent to the lethal event (1, 28). Thus one way of rationalizing the two points of view expressed in the literature is that quinone-dependency is restricted to EDPI, whereas the bulk of energy dependent uptake (represented by EDPII) is not absolutely dependent on quinones.

Tetracycline can be taken up across the cytoplasmic membrane of *Escherichia coli* via two systems, an initial rapid passive diffusion system which is followed by a slower energized system (28). The driving force for energy-dependent transport of tetracycline appears to be the protonmotive force. Apparently, the tetracycline variant, minocycline, can be taken up by passive diffusion but does not serve as a substrate for energized transport.

It is generally accepted that hydrophobic antibiotics can cross the cytoplasmic membrane of bacteria (as well as phospholipid membranes of host cells) by passive diffusion. Whereas transport across the outer membrane of many bacteria is a problem for hydrophobic antibiotics, the inner membrane is assumed to offer no permeability barrier. However, not all authors agree on which drugs can be called hydrophobic and thus can be assumed to penetrate in this way. Trimethoprim, fusidic acid, rifampicin, novobicin and by some accounts sulphonamides, clindamycin, lincomycin and macrolides are assumed to

enter by passive diffusion (28). Chloramphenicol is lipophilic enough to diffuse across the cytoplasmic membrane, however some authors suggest it may enter by active transport. The 4-quinolones represent an unusual case, and are discussed separately below.

Experimentally, the rates of passive diffusion of lipophilic molecules below about 250 Da correlate reasonably well with their lipid/water partition coefficients (2, 28). Partition coefficients in octanol/phosphate buffer are generally used as a measure of hydrophobicity of antibiotics, but high solubility in octanol is not necessarily a reliable index of ability to passively penetrate phospholipid bilayers. Detailed study of hydrophobic uptake was been largely avoided, probably because the experiments are difficult and often inconclusive. For example, Chopra (30) was unable to demonstrate decreased uptake of fusidic acid across the cytoplasmic membrane of *Staphylococcus aureus* strains with plasmid-mediated resistance to the drug, even though changes in phospholipid composition were found and other mechanisms of resistance had been ruled out.

Some hydrophobic antibiotics exert their bactericidal action by inserting into the cytoplasmic membrane. They may cause major disorganization of the membrane (e.g. polymyxin B), break down membrane integrity by pore formation (e.g. gramicidins) or act as ionophores (28). Antibiotics with targets inside the cytoplasmic membrane may also disrupt the membrane during penetration and/or indirectly.

Nalixidic acid is fairly hydrophobic and has been assumed to penetrate the cytoplasmic membrane by passive diffusion (28). The newer 4-quinolones such as norfloxacin and enoxacin have a similar mode of action (28) and are more active on the whole, but appear to be considerably less hydrophobic (16).

The mechanism of enoxacin uptake across the cytoplasmic membranes of *Escherichia coli* and *Bacillus subtilis* was studied by Bryan and colleagues (16). All of the kinetic data favoured the passive diffusion mechanism, and inhibitors of energized uptake had no effect. The pharmacokinetic properties of the 4-quinolones suggest that they readily traverse eukaryotic membranes. On the limited information available, it seems that low hydrophobicity as measured by oil/water partition need not necessarily be a barrier to passive diffusion across the membrane. More detailed experimental evidence is needed before assumptions about the passive diffusion of drugs of different degrees of hydrophobicity can be confirmed.

Peptidoglycan

Most bacteria contain peptidoglycan as an essential component of the cell wall. The peptidoglycan network is assumed to have no sieving effect on molecules in the size range of antibiotics (31).

Periplasm

The aqueous space between the outer and inner membranes of gram-negative bacteria, the periplasm, is not known to act as a barrier to antibiotics. Compounds which are sufficiently hydrophilic to diffuse through outer membrane porins would presumably continue until reaching the surface of the inner membrane. However, alteration of antibiotics during passage through the periplasm by protonation, binding to macromolecules, or alteration by enzymes (30) may affect the compound's subsequent penetration of the cytoplasmic membrane.

Extracellular Polymers

Many bacteria possess extracellular polymers, usually polysaccharides. If the polymer is present in a discrete layer around the cell it is usually called a capsule, whereas material casually associated with the cell is referred to as slime. In addition, the term "surface arrays" has been used for extracellular polymers with repeating subunit structures.

Theoretically, extracellular polymeric layers could act as a barrier to diffusion of antibiotics from the extracellular medium to the cell surface. This possibility has been studied seriously only in *Pseudomonas aeruginosa*. This organism is notoriously resistant to antibiotics and can be isolated in mucoid form, especially from the lungs of patients with cystic fibrosis (32). The mucoid exopolysaccharide (MEP) of these strains is chemically heterogeneous and distinct from the slime associated with non-mucoid isolates. Several studies, summarized by Slack and Nichols (32), have compared antibiotic susceptibilities of mucoid and non-mucoid isolates. In some cases the mucoid strains have been more resistant to certain antibiotics but in others they have been equally or more susceptible. This lack of concensus may be the result of inconsistent test conditions and the diversity of MEP chemotypes.

Purified MEP has been shown to retard diffusion of aminoglycosides, but not β-lactams, in vitro. The anionic uronic acid groups of the MEP were thought to act as cation exchangers for the positively charged aminoglycoside molecules. However, Slack and Nichols concluded that inhibition of aminoglycoside diffusion by MEP was unlikely to be the rate-limiting step of uptake (32). It is difficult to say at present whether extracellular polymers can present a significant barrier to antibiotic uptake.

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