which are interlinked by short peptide bonds. In the resting cell, this molecule is apparently united in every direction over the cell's surface and there are no free ends available for further growth (Tomasz, 1981). For cell growth to proceed, this mucopeptide lattice must be broken to enable new cell wall material to be inserted. In the dividing cell, there is the additional complex process of the formation of a septum or cross-wall (consisting of cytoplasmic membrane and wall), which splits in a special way to produce progeny identical to the parent cell. Lytic enzymes (autolysins) are involved in both of these processes. Obviously in the normal growing cell, synthesis and lysis must be balanced to allow cell division, without cell destruction. In Gram-positive bacteria the interstices within the mucopeptide net, communicating as they do with the cytoplasmic membrane, constitute a periplasmic space. A number of degradative enzymes may be located in this space, which are capable of destroying a variety of antibiotics; these include beta-lactamases (penicillinases and cephalosporinases), which hydrolyze the beta-lactam ring of susceptible penicillins and cephalosporins. The type and amount of the enzymes present in the periplasmic space depend on the bacterial species. In pathogenic Gram-positive bacteria, the bacterial envelope is usually completed by a third component, a protein coat or a carbohydrate capsule, exterior to the mucopeptide layer.

In Gram-negative bacteria, the envelope is more complex and consists of four layers. A mucopeptide net again is exterior to and supports the cytoplasmic membrane, but the periplasmic space formed by the niches in the mucopeptide is extended out beyond the mucopeptide layer, by protruding lipoprotein bundles that meet an extra outer membrane (Nikaido and Vaara, 1985; Nikaido, 1988; Nikaido, 1989). Exterior to this outer membrane there is also usually a protein or a carbohydrate capsule. The periplasmic space of Gram-negative bacteria therefore consists of an area spreading from the cytoplasmic membrane through the mucopeptide net, to the outer membrane. This outer membrane plays a specific role in permeability because it contains several porin proteins with pores that allow small molecules to diffuse into the periplasmic space (Jaffe *et al*., 1982; Piddock and Wise, 1985; Nikaido, 1988; Nikaido, 1989). *Escherichia coli* mutants lacking one or more of these proteins have increased resistance to some antibiotics, although these drugs may utilize other pathways to enter the bacterial cell (Mortimer and Piddock, 1993). The outer membrane normally has selective permeability and thereby preserves the microenvironment of the periplasmic space. For instance, it prevents the outward passage of periplasmic enzymes and prevents the inward passage of some antibiotics. The penetrability of the outer membrane to various antibiotics is often specific for particular bacterial species, but may be altered by a number of factors, including the acquisition of plasmids. Inability to penetrate the various layers of the envelope is one explanation for the intrinsic resistance of Gram-negative bacteria to antibiotics. The effect of beta-lactamase activity and the outer membrane barrier on the elevation of MICs is also synergistic; the contribution of beta-lactamase is more effectively expressed in the bacterial cells with a higher outer membrane barrier (Sawai *et al*., 1988). However, the bacteria cannot make their outer membrane completely impenetrable; this then would exclude all essential nutrients as well. It has now been shown that in an organism like *P. aeruginosa*, in addition to the permeability barrier there is also the membrane-associated energy-driven efflux. This actively pumps antibiotics out of the periplasmic space and so prevents their access to their target proteins (Nikaido, 1994).

Degradative enzymes in the periplasmic space that are confined within the cell by the outer membrane of Gram-negative bacteria are also important in determining antibiotic resistance. Of special importance are the beta-lactamases, which confer resistance to beta-lactam antibiotics (Sykes, 1982; Bauernfeind, 1986; Bush and Sykes, 1986; Bush, 1988; Bush 1989a; Bush, 1989b; Bush, 1989c; Sanders, 1992). A number of factors influence the efficacy of beta-lactam antibiotics. These include the amount of the beta-lactam antibiotic that has penetrated through into the space, the amount of enzyme present, the affinity or specificity of the enzyme for the particular beta-lactam antibiotic involved, and its "efficiency" in hydrolyzing the antibiotic. In addition, the amount of beta-lactamase present in the periplasmic space can be altered in many Gram-negative bacteria by chromosomal mutation, induction, or by the acquisition of plasmids. Beta-lactamase inhibitors (see Chapter 13, Beta-Lactamase Inhibitors) have been developed to overcome the destruction of beta-lactam antibiotics by some of these enzymes.

Most, if not all, of the penicillin molecules that have diffused through the outer boundaries of the bacterial cell and have not been destroyed by beta-lactamases in the periplasmic space become strongly bound by the plasma membrane. The components of the membrane responsible for this binding are called penicillin binding proteins. There is a wide variation in both the number and the amount of PBPs in different bacteria, but related bacteria tend to have similar patterns of PBPs (Tomasz, 1982; Tomasz, 1986). The PBPs are proteins that normally play essential roles in a variety of physiologic functions in the bacterial cell, such as maintenance of structural integrity, shape, and cell division (Tomasz, 1979; Tuomanen *et al*., 1986; Georgopapadakou, 1993). For instance, in *E. coli* PBPs 1a, 1b, 2, 3, 4, 5, and 6 have been identified. PBPs 1a and 1b are jointly concerned in cell elongation, PBP2 in shape determination, and PBP3 in cell division (Curtis, 1981). Pen G and other beta-lactam antibiotics mainly bind to PBP 1a, 1b, 2 and 3 of *E. coli*. Rapid lysis of the cell is caused by beta-lactams that bind to PBP1 (e.g. cephalosporins). Inhibition of PBP2 in *E. coli* results in the generation of stable round forms and not spheroplasts, as associated with exposure to some beta-lactams. These continue to grow for several generations before further aberrations occur and lysis ensures. Mecillinam (see Chapter 11, Mecillinam (Amdinocillin) and Pivmecillinam), for instance, binds exclusively to PBP2 and causes these changes. Most beta-lactam antibiotics inhibit PBP3, the protein involved in cell division of *E. coli*. By inhibiting PBP3, cell division, and in particular cross-wall synthesis, is prevented, resulting