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TOXICITY OF ANTIBACTERIAL AGENTS: MECHANISM OF ACTION ON MAMMALIAN CELLS

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INTRODUCTION

Antibacterial agents may adversely affect the host either directly or indirectly. Direct injury, or toxicity, is the focal point of this review. Indirect injury may result from (*a*) induction of an allergic or hypersensitivity reaction in which components of the immune system (antibody, activated cells, complement) mediate damage to host tissues or (*b*) alteration of the ecological balance of the normal microbial flora which facilitates superinfection or impairs epithelial physiology or nutrition. These indirect effects have been reviewed elsewhere (1, 2) and will not be considered further.

An extraordinary number and a wide variety of toxic reactions have been reported following administration of antibacterial agents. Limitations of space have dictated that only a few of these be considered in this review. Priority was given those reactions for which the mechanism of drug action is either relatively well understood or currently the subject of intense interest or controversy. In certain instances, the mechanism of toxicity for mammalian (eucaryotic) cells is very similar to or identical with that for sensitive bacterial (procaryotic) cells. In order to simplify discussion, we refer to these very similar mechanisms as *pharmacological effects* and include a list of the mechanisms of antibacterial action as they are presently perceived for each group of drugs considered (Table 1). We attempt to differentiate those mechanisms that are unique to mammalian cells from those that appear to be pharmacological.

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Table 1 Mechanism of action of antimicrobial agents on procaryotic cells (3–7)

I. Agents affecting cell wall synthesis
   A. *Cycloserine:* Analogue of D-alanine. Competitively inhibits alanine racemase and dipeptidyl synthetase; thus prevents incorporation of D-alanyl-D-alanine into pentapeptide side chains.
   B. *Vancomycin:* Attaches to terminal D-alanyl-D-alanine on pentapeptides. Blocks peptidoglycan polymerase from catalyzing release of murein precursor unit from phospholipid unit and its subsequent attachment to growing cell wall.
   C. *Penicillins, cephalosporins:* Inhibit peptidoglycan transpeptidase and D-alanine carboxypeptidase (possibly bind covalently to enzymes); thus prevent cross-linking of peptidoglycan strands. Cell lysis may actually result from continued activity of autolysins in presence of inhibited cell wall synthesis.
   D. *Isoniazid:* Inhibits synthesis of mycolic acids by preventing elongation of 26-carbon fatty acids.

II. Agents affecting cell membrane
   A. *Polymyxin B, colistin:* Cationic surface active agents. Competitively displace Mg^{2+} or Ca^{2+} from phosphate groups on membrane lipids (especially phosphatidylethanolamine); fatty acid tail penetrates hydrophobic area of membrane, disturbing the normal packing of membrane phospholipid fatty acids.

III. Agents affecting protein synthesis
   A. Agents that bind to 30S ribosomal subunit
      1. *Tetracyclines:* Inhibit binding of aminoacyl-tRNA in the acceptor site of the ribosome.
      2. *Spectinomycin:* Interferes with interactions between mRNA and 30S subunit that occur during translocation.
      3. *Aminoglycosides:* Induce misreading of mRNA. Inhibit elongation by interfering with aminoacyl-tRNA binding to acceptor site. Produce frozen 70S-mRNA monosomes. Precise mechanism responsible for lethal effect unknown.
   B. Agents that bind to 50S ribosomal subunit
      1. *Chloramphenicol:* Prevents binding in acceptor site of amino acid–containing end of aminoacyl-tRNA; thus inhibits peptide bond formation without affecting initiation, translocation, or termination.
      2. *Erythromycin:* Inhibits late but not early peptide bond formation. Precise mechanism unknown.
      3. *Lincomycin, clindamycin:* Precise mechanism unknown. May inhibit peptidyl synthetase by interfering with substrate binding at acceptor or peptidyl site.

IV. Agents affecting nucleic acid synthesis
   A. *Rifampin:* Binds to DNA-dependent RNA polymerase, thus inhibits RNA synthesis.
   B. *Nalidixic acid:* Inhibits semiconservative DNA synthesis. Precise mechanism unknown. May bind to an as yet unidentified protein involved in DNA synthesis, as it does not bind to known polymerases or DNA template.

V. Antimetabolites
   A. *p-Aminosalicylic acid, sulfonamides:* Analogues of *p*-aminobenzoate (PABA). Inhibit incorporation of PABA into dihydropteric acid; may become incorporated into folate precursors.
   B. *Trimethoprim:* Analogue of pteridine portion of dihydrofolate. Inhibits reduction of dihydrofolate to tetrahydrofolate.

OTOTOXICITY

Administration of a variety of antimicrobial agents has been associated with ototoxicity in humans and experimental animals (8). Antiseptics, polymyxin B, chloramphenicol, erythromycin, tetracycline, neomycin, and streptomycin have each produced cochlear damage following either intratympanic instillation or application to the outer ear in the presence of a perforation in the tympanic membrane (8–10). Each of the available aminoglycosides when administered parenterally has been implicated as a cause of ototoxicity. Streptomycin, gentamicin, and tobramycin tend to impair vestibular function more frequently than hearing (8). Neomycin, kanamycin, and amikacin primarily affect hearing. However, most of these drugs on occasion may impair both auditory and vestibular functions (8). The vestibular toxicity of the aminoglycosides has been reviewed recently (11, 12). Also, the pharmacology of hearing and ototoxicity was reviewed in the preceding volume of this publication (8). Thus, we consider here only the possible mechanisms of aminoglycoside-induced cochlear damage that have been the subject of extensive investigation in the past few years: (*a*) interference with membrane polyphosphoinositide metabolism and (*b*) impairment of carbohydrate metabolism and energy utilization.

Impairment of Polyphosphoinositide Metabolism by Aminoglycosides

Polyphosphoinositides are constituents primarily of nervous and secretory tissues (13, 14). They are highly active metabolically and are presumed to play a role in regulation of membrane permeability and ion transport (13–16). Perfusion of the guinea pig cochlea with artificial perilymph containing neomycin promptly reduces the ability of this organ to generate an acoustical current in response to a sound stimulus (17). When radiolabeled phosphate is added to the artificial perilymph, it is incorporated into membrane phospholipids. Addition of neomycin inhibits incorporation of the labeled phosphate into phosphatidylinositol diphosphate, which is one of the components of the membranes (18, 19). Furthermore, parenteral treatment of guinea pigs with neomycin for three weeks has been shown to reduce incorporation of labeled phosphate into phosphatidylinositol diphosphate in both the organ of Corti and the stria vascularis (19, 20). The localization of the effect of neomycin on polyphosphoinositide metabolism correlates with the site of drug-induced histopathological changes (21, 22). In addition, neomycin, tobramycin, gentamicin, and kanamycin A have been shown to inhibit binding of calcium to polyphosphoinositide-rich homogenates of cerebral tissue from guinea pigs (19, 20). Neomycin has also been

shown to inhibit binding of calcium to polyphosphoinositides in artificial lipid monolayers (23).

The observations on the interactions of aminoglycosides with polyphosphoinositide metabolism have led Schacht (20) to propose a detailed model of the possible interaction of these drugs with cell membranes, such as those in the organ of Corti. Briefly stated, it is postulated that normally a phosphomonoesterase acts upon phosphoinositides to cleave phosphate groups, liberate bound calcium, and thereby alter permeability of the membrane to cations. Restoration of permeability would involve phosphorylation of the same groups. Aminoglycosides, which are strongly cationic, would compete with calcium for the exposed negatively charged phosphate groups. Since aminoglycosides are known to cross membranes ineffectively (24), their presence in low concentrations might competitively and reversibly impair membrane permeability. At higher concentrations, it is possible that binding would become noncompetitive and irreversible thereby imposing conformational changes in the membrane that might be lethal for the cell. Similar second-level effects at higher concentrations have been observed in interactions of neomycin with membranes at nerve endings and with synthetic lipid monolayers (23). This model would be consistent with the observations in humans that aminoglycoside ototoxicity may be reversible or permanent (24, 25). Although speculative, this model may be tested further with currently available microtechniques, cell cultures, organ slices, and synthetic membranes. In addition, it has been suggested that the model may be applicable to aminoglycoside-induced neuromuscular blockade and nephrotoxicity, since the involved tissues are also rich in polyphosphoinositides (13, 14). Finally, further work in this area may provide clues to facilitate elucidation of the mechanism of the bactericidal activity of aminoglycosides, which at present is unknown.

Impairment of Carbohydrate Metabolism and Energy Utilization by Aminoglycosides

The second proposed mechanism of ototoxicity of aminoglycosides involves effects on carbohydrate metabolism and energy utilization by the outer hair cells of the organ of Corti and possibly the stria vascularis. Kanamycin has been implicated in (*a*) suppression of respiratory enzyme activity in outer hair cells (26, 27); (*b*) inhibition of the Embden-Myerhof pathway, but not the hexose monophosphate pathway, in tissues prepared from the organ of Corti (28); and (*c*) impairment of ATP-ase activity in the organ of Corti (26). These effects would logically be expected to result in inefficient utilization of glucose and depletion of glycogen (8). In fact, an inverse relationship has been observed between the glycogen content of outer hair cells and their susceptibility to damage by the aminoglycosides (29–32). Depletion of

glycogen storage granules has been noted as a component of the drug-induced morphological damage to the cells (31, 32). Also, the outer hair cells of alloxan-treated diabetic animals become relatively more resistant to the effects of kanamycin with increments in the concentration of glucose obtained in the blood (33). This apparent protective effect of the hyperglycemia may have resulted from competition with the aminoglycoside for transport into the cochlea or an osmotic diuresis that facilitated excretion of the drug. Nevertheless, the hypothesis that the aminoglycosides act through impairment of carbohydrate metabolism and energy utilization is plausible.

The two proposed models for ototoxicity provide a useful framework for further study of the effects of aminoglycosides in the cochlea, nervous system, and kidney. Unfortunately, much of the work that led to the two hypotheses was done in large part with two different aminoglycosides, kanamycin and neomycin. Although these two closely related drugs have been presumed to act by the same mechanism and therefore to be interchangeable, they have not been compared simultaneously in many critical experiments. In addition, study of the effects of the other aminoglycosides including those that are less prone to induce ototoxicity might prove useful. The two models are not necessarily mutually exclusive and this possibility should be considered in the design of future experiments. Since the evidence in support of each model is largely indirect or circumstantial, both should be considered highly tentative at present.

NEUROMUSCULAR BLOCKADE

Since the first report by Pridgeon in 1956 of prolonged apnea following use of neomycin-ether combinations (34), numerous accounts of antibiotic-induced neuromuscular blockade have appeared in the literature. It appears that four major groups of antibiotics are capable of inducing neuromuscular blockade in humans. These include (*a*) the aminoglycosides—streptomycin, dihydrostreptomycin, neomycin, kanamycin (35), gentamicin (36), and tobramycin (37); (*b*) the polymyxins—colistin, colistimethate, and polymyxin B (35); (*c*) the tetracyclines—rolitetracycline and oxytetracycline (35); and (*d*) the lincosamides—lincomycin (38, 39) and clindamycin (40, 41). These episodes of antibiotic-induced blockade almost always have been associated with concomitant use of other neuromuscular depressants or local instillation of the antibiotics or both. They have also tended to occur more frequently in patients with preexisting neuromuscular disease or serum electrolyte imbalance. Pittinger & Adamson extensively reviewed antibiotic blockade of neuromuscular function in the 1972 issue of this publication (42). Thus this section deals primarily with developments since that time.

Table 2 summarizes the general features of the neuromuscular blockade induced by the four groups of antibiotics. All four groups appear to potentiate the effects of competitive neuromuscular blocking agents while two also interact with depolarizing agents. Reversibility of the antibiotic-induced blockade by a variety of agents varies greatly between the four groups. These features suggest that each of the four acts differently and that none are classic competitive or depolarizing agents.

Aminoglycosides

The aminoglycosides are polycations containing amino sugars glycosidically linked to streptidine or deoxystreptamine. The streptidine or deoxystreptamine portions are involved in the neuromuscular blocking activity (42). However, other portions of the molecules must also contribute since the blocking potency of the aminoglycosides containing deoxystreptamine varies widely (42). Theories on the mechanism of aminoglycoside-induced blockade were reviewed by Pittinger & Adamson (42). The "competitive hypothesis" of Vital Brazil & Prado-Franceschi (43) still appears to be most consistent with available data. This hypothesis states that aminoglycosides, like magnesium, compete with calcium ions and thus inhibit the prejunctional release of acetylcholine (ACh). They also decrease the sensitivity of the motor end plate to the depolarizing action of ACh, and have a depressant effect on denervated muscle which, unlike the presynaptic effect, is enhanced by calcium (42). More recent studies have focused on determining which component (presynaptic or postsynaptic) is more important in the neuromuscular blocking activity and the molecular basis for the blockade.

The relative contributions of the pre- and postsynaptic effects in aminoglycoside-induced blockade have been the subject of controversy for many years. It is possible that the disparities in the published literature result solely from differences in experimental design and methodology. The rela-

Table 2 General features of antibiotic-induced blockade

	Aminoglycosides	Polymyxins	Tetracyclines	Lincosamides
Effect on neuromuscular blocking activity of				
Competitive agents	potentiate	potentiate	potentiate	potentiate
Depolarizing agents	potentiate	potentiate	none	none
Reversibility of antibiotic-induced blockade by				
Calcium	complete	inconsistent	inconsistent	none
Anticholinesterases	inconsistent	inconsistent (may augment)	inconsistent	partial (may augment)
3,4-diaminopyridine	complete	partial	none	inconsistent
Presynaptic effect	yes	possible	no	yes
Postsynaptic effect	yes	yes	yes	yes

tive pre- and postjunctional effects of the aminoglycosides vary with the drug employed (44, 45), the concentrations of the drug (46) and ionized calcium (44, 45), and the type of muscle preparation studied (47). Prejunctional effects predominate in more studies with gentamicin or neomycin (42, 44) than kanamycin or streptomycin (42, 46). A low concentration of ionized calcium has been shown to potentiate the presynaptic block without affecting the postsynaptic block (42, 44). In contrast, high concentrations of ionized calcium decrease the presynaptic component, but enhance or do not affect the postsynaptic component (42, 44, 45). A recent study by Adams et al (47) showed predominantly prejunctional effects in fast-twitch muscles and postjunctional effects in slow-twitch muscles. Thus a predominant prejunctional effect would occur in studies with neomycin or gentamicin, low ionized calcium, or fast-twitch muscles while postjunctional effects would predominate in studies with streptomycin, high ionized calcium, or slow-twitch muscles. However, the prejunctional effects are probably the more important component in aminoglycoside-induced blockade in humans since the blockade is predictably reversible by calcium but not anticholinesterases. Also, the blocking potency of the aminoglycosides varies directly with their prejunctional effects (42).

Both the pre- and postsynaptic components of aminoglycoside-induced blockade appear to depend upon a drug-membrane interaction which affects the passage of positive ions through the membrane (42, 48, 49). Only the effects on calcium uptake have been studied to any extent (44, 49–51). These investigations have shown that the aminoglycosides, acting at a superficial site, decrease total calcium uptake and increase calcium efflux. These effects appear to result from decreased binding of free calcium coupled with displacement of bound calcium. The interaction of aminoglycosides with molecular components of the membrane has not been studied directly. It has been hypothesized that the drugs bind to polyphosphoinositides (see section on ototoxicity).

Polymyxins

The polymyxins are cationic surface active agents composed of a polypeptide head and a fatty acid tail. They are the most potent neuromuscular blockers among the antibiotics, and this activity depends upon the charged, intact polypeptide head (42, 52). Their effects on myoneural function have been recently summarized by Wright & Collier (52). The primary effect of the polymyxins is to decrease the motor end plate sensitivity to ACh. They also produce a local anesthetic effect equivalent to lidocaine on desheathed nerves, and depress the response of muscle to direct stimulation. They do not appear to decrease evoked presynaptic release of ACh and may actually increase its spontaneous release (52). Although the precise mechanism

involved in polymyxin-induced blockade is not known, it is very likely related to their pharmacologic effects (Table 1). Were the polymyxins to interact with phospholipids on nerve and muscle membranes, this could affect ion-dependent transmitter release, postsynaptic depolarization, and muscle contractility. This mechanism would explain effects on transmission, local anesthetic action, interactions with other neuromuscular blocking agents, and the nonpredictable response of the blockade to certain reversing agents (52–55; Table 2).

Tetracyclines

The tetracyclines are congeneric derivatives of the polycyclic naphthacenecarboxamide. Studies on the neuromuscular blocking properties of these antibiotics have shown that they decrease the response of the motor end plate to ACh without affecting either presynaptic release of ACh (52) or muscle response to direct stimulation (56). Chelation of calcium had been proposed as a possible mechanism for tetracycline-induced blockade (42). However, the findings of only a postsynaptic effect and the inability of calcium or aminopyridines (55, 57) to effectively reverse the blockade negate this hypothesis. The tetracyclines are like classic competitive blocking agents. They are bulky, rigid molecules and appear to produce similar effects on the neuromuscular junction. However, their blocking activity is not consistently reversed by anticholinesterases (42, 56). Thus further studies are needed to clarify the mechanism of the blockade.

Lincosamides

The lincosamides are derivatives of the amino acid *trans*-L-4-*n*-propylhygrinic acid. The un-ionized form has been reported to possess greater activity against myoneural functions than the ionized form (58). Studies on lincosamide-induced blockade have yielded highly conflicting results. Several studies have shown that the lincosamides potentiate the effect of competitive blocking agents (40, 59, 60) and have no effect on the activity of depolarizing agents (59). However, conflicting reports have appeared which have shown (*a*) no interaction between lincomycin and *d*-tubocurarine if lincomycin is administered first (39), (*b*) no interaction between clindamycin and *d*-tubocurarine (58), and (*c*) a possible interaction between succinylcholine and clindamycin in man (41). Similar conflicting reports concerning the reversibility of lincosamide-induced blockade have appeared. Although most studies have shown little effect of calcium or anticholinesterases on lincosamide-induced blockade (58, 61), others have reported partial antagonism (42, 60), while some have suggested enhancement of the blockade by anticholinesterases (40, 42).

Studies on the mechanism of lincosamide-induced neuromuscular blockade have also yielded conflicting results. This is probably due to two factors. First, the predominant effect of lincosamides on neuromuscular functions appears to vary with the concentration employed. Second, the two lincosamides, lincomycin and clindamycin, vary in their abilities to produce certain effects. Lincomycin, at low concentrations had a nerve terminal stimulatory effect reflected by an increase in miniature end plate potential frequency (62). At moderate concentrations, lincomycin had depressant pre- and postsynaptic effects reflected by decreased ACh release, depressed miniature end plate potential amplitude, and decreased end plate sensitivity to directly applied ACh (62). At high concentrations, lincomycin depressed muscle response to direct stimulation (58). Similar concentration-dependent effects were seen with clindamycin. At low concentrations, clindamycin had a stimulatory effect reflected by increased miniature end plate potential frequency (62) and increased twitch tension in guinea pig lumbrical muscle-nerve preparations (60). At moderate concentrations, clindamycin had stimulatory presynaptic and inhibitory postsynaptic effects reflected by (*a*) increased release of ACh, (*b*) decreased miniature end plate potential amplitude, (*c*) decreased motor end plate sensitivity to directly applied ACh, and (*d*) initially increased twitch tension followed by a significant decrease (60, 62). At high concentrations, clindamycin decreased muscle response to direct stimulation, but this effect occurred with lower concentrations of clindamycin than lincomycin (55, 58) and clindamycin also produced a local anesthetic effect on desheathed nerves that was not observed with lincomycin (58). These concentration-dependent effects of the lincosamides would explain many of the apparently conflicting reports on the reversibility of lincosamide-induced blockade by a variety of agents as well as their ability to potentiate other neuromuscular blocking agents. However, further studies will be required before the precise mechanism responsible for the blockade is known.

NEUROLOGICAL TOXICITY

Isoniazid and Cycloserine

Isoniazid may produce a variety of dose-related effects on the peripheral and central nervous systems (63). Peripheral neuritis is most commonly encountered. Occasional patients develop symptoms of excitability, which may range from mild euphoria to irritability to generalized seizures. The isoniazid-induced peripheral neuritis may be prevented or reversed by administration of pyridoxine (63, 64). Early studies indicated that isoniazid may compete with pyridoxal phosphate for opotryptophanase in the involved

tissues (63, 64). More recently, it has been suggested that isoniazid induces a general pyridoxine deficiency that may be the sole explanation for the apparent neurological toxicity (65). Although some controversy persists, it appears that the mechanism of toxicity of isoniazid for peripheral nerves differs from that for susceptible mycobacteria (Table 1).

Isoniazid-induced seizures have been studied in chicks (66). Subconvulsant doses of isoniazid produced only a slow decline in brain levels of γ-aminobutyric acid (GABA). Convulsant doses induced first a fall and then a rise to above normal in levels of GABA. These events were paralleled by changes in the relative activities of glutamic acid decarboxylase (GAD) and the enzyme that degrades GABA, α-oxoglutarate aminotransferase (GABA-T). Administration of pyridoxine with convulsant doses of isoniazid prevented seizures and returned GABA levels toward normal in the chicks. Large doses of pyridoxine have also been demonstrated to terminate seizure activity in patients with overdosage of isoniazid (67). Although no cause-and-effect relationship has been proven, further studies of GABA metabolism may offer insights into the pathogenesis of seizures and provide useful clues to prevention.

Cycloserine may also produce toxicity for the CNS similar to that of isoniazid. Cycloserine-induced seizures also may be prevented or ameliorated by administration of large doses of pyridoxine (68). The mechanism of cycloserine toxicity and its relationship to pyridoxine metabolism has not been elucidated; most certainly it is not a pharmacological effect of the drug (Table 1).

Chloramphenicol

Chloramphenicol may induce optic neuritis, especially in children with mucoviscidosis who have been treated for prolonged periods (2). Retinal ganglion cells are lost symmetrically and optic nerve fibers may degenerate. Administration of large doses of B-complex vitamins may reverse the neuritis despite continuation of chloramphenicol. It is unlikely that optic neuritis represents a pharmacological effect of the drug (Table 1).

Ethambutol

The major toxicity of ethambutol is optic neuritis, first manifested by loss of ability to perceive the color green followed by diminution of visual acuity (2). Optic lesions have been produced by large doses of the drug in experimental animals (69, 70). Concentrations of copper and zinc in the tissues of these animals were reduced (70). Most notably, the content of zinc in the eyes was significantly depressed. It has been hypothesized that trace element deficiency plays an important role in pathogenesis of the optic neuritis (2,

70). However, the molecular mechanism of toxicity and of antimycobacterial activity of ethambutol has not been elucidated.

Penicillins

Neurotoxicity has been observed following use of almost any one of the many available penicillins (2, 71). Most often the toxicity has been manifested as a seizure disorder. Toxicity almost invariably has been associated with the very high serum levels that have followed "massive" doses of the drug or continued administration of usual doses to patients with renal failure (2, 71). A close correlation has been observed between the neurotoxic potencies of the various penicillins and their partition coefficients and chromatographic characteristics (72, 73). The more hydrophobic molecules were more neurotoxic when administered intravenously to rabbits (73) or when applied directly to the cortex of rats (72). The neurophysiological changes induced by the penicillins have been described recently by Mesher & Wyler (74) and by Davenport, Schwindt & Crill (75). The molecular basis of the neurotoxicity is unknown; most certainly it is not a pharmacological action of the drug (Table 1).

NEPHROTOXICITY

Many antibacterial agents may impair kidney function or damage renal tissues, or both (76). Examples include the penicillins, cephalosporins, sulfonamides, aminoglycosides, polymyxins, and tetracyclines. The molecular or cellular mechanisms of these adverse effects are poorly understood. In part, research on the mechanisms of nephrotoxicity has been impeded by the lack of an understanding of the action of many of these drugs on membranes and transport systems in procaryotic cells and by the variable responses obtained in different species of experimental animals. Toxicity of cephaloridine, the polymyxins, and the aminoglycosides has received the greatest attention recently.

Cephaloridine

Most of the adverse renal effects of the penicillins and cephalosporins result from immunologically mediated injury or from the cations of the drug salts (76). Cephaloridine appears to be an exception. Nephrotoxicity has been encountered more frequently with use of cephaloridine than other available cephalosporins (77). Declining renal function and varying degrees of tubular necrosis have been observed. The chemical nature and pharmacokinetics of cephaloridine differ in many respects from those of other cephalosporins (77). Cephaloridine is a zwitterion. It is excreted unchanged primarily by

glomerular filtration, while other cephalosporins are secreted to a large extent by the tubules. Studies in a variety of experimental animals have demonstrated that the proximal tubular cells are the major target for the toxicity of cephaloridine (76, 77). Damage to the proximal tubular cells has been observed by electron microscopy within hours of administration of the drug (78). A clear relationship between dose administered and the frequency and severity of toxicity has been noted in most of these studies. By autoradiography, cephaloridine has been shown to concentrate at the periphery of the proximal tubular cells (79), and the extent of injury correlated with the degree of intracellular accumulation of the drug (80). However, most physiological studies have failed to detect tubular secretion or reabsorption of the drug (76, 77). Cephaloridine appears to be transported into the tubular cell by the same secretory carrier as *p*-aminohippuric acid (81). The relative lack of nephrotoxicity in newborn animals and possibly in neonatal humans may relate to the known immaturity of this secretory carrier system in the very young (77, 81). Administration of probenecid to animals appears to protect against the nephrotoxicity of cephaloridine (80, 81). High doses of chlorothiazide have been shown to confirm some protection, while furosemide and ethacrynic acid have potentiated the nephrotoxicity of the drug in animals (77). Collectively, these studies indicate that cephaloridine is actively transported into proximal tubular cells where it is sequestered in high concentrations. It has been suggested that the drug may precipitate or polymerize to damage the cell (82); but the molecular basis for the injury remains unknown.

The Polymyxins

The polymyxins B and E (colistin) have been associated with a dose-related impairment of renal function, proximal tubular damage, and acute tubular necrosis (76). The polymyxins are excreted slowly by the kidneys (76), and high concentrations are bound to renal tissue (83). Polymyxin B binds avidly to renal cell membranes (84) and active drug can be extracted from renal phospholipids (83). It is therefore reasonable to postulate that the polymyxins pharmacologically alter renal cell structure and function as a result of binding to phospholipids in the membrane in a manner analogous, if not identical, with their binding to the membranes of sensitive bacteria and lipid monolayers (Table 1; 85, 86). However, this possibility has not been tested directly.

The Aminoglycosides

The aminoglycosides—streptomycin, kanamycin, neomycin, gentamicin, tobramycin, and amikacin—are each potentially nephrotoxic. The drug-induced morphological and physiological changes have been reviewed

recently by Appel & Neu (76). In comparisons between the drugs, quantitative differences in nephrotoxicity have been observed, but the qualitative effects on the kidney usually have been similar in both man and animals. Although toxicity is generally dose-related and reversible, exceptions have been noted. The proximal tubular cell appears to be the primary target of the nephrotoxicity of the aminoglycosides. Cloudy swelling, leakage of enzymes and ions, structural alterations of lysosomes, and necrosis have been observed in proximal tubular cells of animals treated with one or more of the aminoglycosides (76, 87, 88). Gentamicin has been shown to accumulate in the cortex and in proximal tubular cells (89–91); presumably the other aminoglycosides act similarly (76). More recently, gentamicin has been found to stimulate uptake and diminish efflux of *p*-aminohippurate in rat renal cortical slices, which was interpreted by the investigators to indicate an effect of the drug at the antiluminal membrane (92). However, a similar effect of gentamicin was not found when accumulation of iodohippurate was studied in rabbit renal cortical slices (93); only uptake was inhibited and only by very high concentrations of the drug.

In recent studies with guinea pigs, neomycin has been shown to profoundly alter polyphosphoinositide metabolism by the kidney in vivo and by renal homogenates in vitro (15). In addition, neomycin appears to complex with the polyphosphoinositides in vitro and in vivo (15, 23, 94). The leakage of lysosomal and cytoplasmic enzymes, alterations in lysosomal structure, changes in transport of organic acids and ions, and interference with polyphosphoinositide metabolism each suggest that structural and functional integrity of proximal tubular cell membranes may be impaired. Since polyphosphoinositides are metabolically very active constituents of secretory tissues and possibly function in regulation of membrane permeability and ion transport (13–15), some have hypothesized that the acidic polyphosphoinositides complex with the polycationic aminoglycosides to induce these changes in the membrane (see section on ototoxicity above). However, it should be emphasized that the evidence to support this hypothesis is circumstantial and indirect at best. To date, not all of the nephrotoxic aminoglycoside antibiotics have been shown to concentrate in proximal tubular cells, and only a few have been demonstrated to produce each of the physiologic changes that are generally ascribed to the group as a whole (76, 87). Moreover, the precise role of polyphosphoinositides in maintenance of membrane structure and function has not been firmly established. Finally, alternative hypotheses that invoke other mechanisms may be equally plausible. For example, kanamycin has been shown to inhibit selectively the Embden-Meyerhof pathway without affecting the hexose monophosphate pathway in tissues from the kidney of guinea pigs (28), and very recently, several aminoglycosides have been observed to inhibit protein

synthesis and elicit misreading of natural messenger RNA in extracts of eucaryotic cells, including cultured human cells (95, 96). Thus, it is conceivable that any, or all, of these effects may occur within the tubular cell where aminoglycosides appear to accumulate in high concentrations. The recent finding that D-glucarates and related compounds prevent accumulation of aminoglycosides in renal tissue and reduce tubular damage in rats may provide useful tools for study of mechanism and possible prophylaxis of aminoglycoside nephrotoxicity (97, 98).

HEMATOLOGICAL TOXICITY

Antimicrobial agents have been implicated as causes of anemia, leukopenia, thrombocytopenia, coagulation defects, immunosuppression, and a variety of autoimmune phenomena. Hypersensitivity accounts for the majority of these adverse reactions. A description of the drug-induced, immunologically mediated effects may be found in several comprehensive reviews (1, 2, 99). Several toxic reactions may occur and the mechanisms of a few are becoming increasingly well understood.

Hemolysis in G-6-PD Deficiency

Hemolysis has resulted from administration of sulfonamides, sulfones, nitrofurans, chloramphenicol, quinine, primaquine, and *p*-aminosalicylic acid to patients with genetically determined deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) (100). The mechanism of the hemolysis clearly is not a pharmacologic action of these drugs (100; Table 1). The G-6-PD enzyme is involved at the beginning of the hexose monophosphate shunt pathway, which normally accounts for a small fraction of glucose metabolism of the red cell. When normal cells are exposed to oxidant drugs or related stresses, the proportion of glucose metabolized through the shunt pathway increases manyfold. The resultant increases in the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and reduced glutathione (GSH) protect the cell from these oxidant stresses. In enzyme-deficient cells, this protective mechanism is impaired. The oxidant drugs may then inactivate essential thiol moieties on membrane proteins, cause intracellular formation of methemoglobin, and degrade hemoglobin to form Heinz bodies. The more potent antimicrobial oxidant drugs, sulfanilamide and diaminodiphenylsulfone, may produce dose-related methemoglobinemia even in normal individuals (100).

Coagulation Defects

Antibody-mediated thrombocytopenia has followed administration of penicillins, cephalosporins, aminoglycosides, tetracyclines, rifamycins,

p-aminosalicylic acid, isoniazid, sulfonamides, and several antimalarial drugs. These immunological reactions have been reviewed in detail by Miescher (99). More recently, bleeding associated with use of high doses of the penicillins (101–103), especially carbenicillin, has been ascribed to a direct effect upon platelets and components of plasma that regulate blood coagulation. High concentrations of carbenicillin have been shown to inhibit platelet aggregation, diminish conversion of fibrinogen to fibrin, and increase antithrombin-III activity, possibly through release of heparin. Presumably, the other penicillins act similarly. Although the precise molecular mechanism is unknown, these effects appear not to be pharmacologically or immunologically mediated (Table 1).

Reversible Anemia Due to Chloramphenicol

Toxicity for bone marrow is a frequent complication of therapy with chloramphenicol. Two types of toxicity have been observed: (*a*) a dose-related, reversible suppression that involves primarily erythroid cells and (*b*) bone marrow aplasia.

The mechanism of the reversible erythroid suppression is relatively well understood. The extensive literature that contributed to this understanding has been reviewed by Yunis (104). Chloramphenicol appears to have little or no effect on ribosomal protein synthesis in mammalian cells. On the other hand, the drug has been shown to inhibit the independent protein synthesis that occurs in mammalian cell mitochondria. Other antibiotic inhibitors of bacterial protein synthesis that are not myelotoxic do not exert this effect (105). The mechanism of this inhibition of mitochondrial protein synthesis appears to be similar to, if not identical with, that observed in sensitive bacteria (Table 1). To date, this appears to be the only metabolic pathway in mammalian cells that is sensitive to therapeutically attainable concentrations of chloramphenicol (105).

Condensation of matrix without change in overall mitochondrial volume has been observed in nearly all bone marrow cells obtained from patients during therapy with chloramphenicol. This change disappears following discontinuation of the drug. The concurrence of the reversible mitochondrial changes and suppression of erythroid cells during chloramphenicol administration suggested a cause-and-effect relationship; however, the greater vulnerability of erythroid than myeloid cells observed clinically was unexplained (104).

The subsequent observation that chloramphenicol suppresses activity of ferrochelatase, which is intimately associated with the inner mitochondrial membrane, provides a possible explanation for (*a*) the apparently greater vulnerability of erythroid than myeloid cells and (*b*) the clinically apparent block in the synthesis of heme (104). Chloramphenicol has also been shown

to inhibit mitochondrial respiration in mammalian cells (106) which may be a direct result of suppression of synthesis of the membranous mitochondrial cytochromes $a + a_3$ and *b*. Respiration then may be impaired to a degree sufficient to inhibit cell proliferation (104). It has been speculated that the impairment of respiration may account for the reversible leukopenia and thrombocytopenia that occasionally accompanies the erythroid suppression induced by chloramphenicol (104). Although many questions remain unanswered, it is possible that most, if not all, of the manifestations of reversible toxicity of chloramphenicol may be ascribed ultimately to the pharmacological inhibition of protein synthesis by mitochondria.

Marrow Aplasia Due to Chloramphenicol

Bone marrow aplasia is a rare, but devastating, consequence of use of chloramphenicol. It has been estimated to occur in 1 in 19,000 to 1 in 40,000 courses of therapy (107). The aplasia is most often manifested as pancytopenia. It is usually irreversible and lethal. The rate of occurrence does not correlate with total dosage of drug administered. Onset may occur during therapy or months after the drug has been discontinued. The mechanism of this reaction is unknown, but it has been subjected to extensive investigation. Relevant literature published before 1973 has been reviewed by Yunis (104) and summarized in the proceedings of an international symposium (108).

The chloramphenicol-induced aplasia may result from interaction of the drug with a genetically determined biochemical lesion that involves synthesis of nucleic acids. Support for this contention may be summarized as follows: 1. Despite its rarity, aplastic anemia has been observed in identical twins. 2. Therapeutically attainable concentrations of chloramphenicol have been shown to inhibit nucleic acid synthesis by marrow cells from patients who recovered from aplastic anemia. 3. In contrast, concentrations in excess of these attained clinically were required to induce an equivalent suppression of nucleic acid synthesis by marrow cells obtained from randomly selected normal subjects. 4. Susceptibility of marrow cells from parents of patients with chloramphenicol-induced aplasia more closely resembled that of their children than that of the randomly selected normal subjects (104).

The frequently encountered late onset of clinically apparent aplasia, the involvement of all bone marrow elements, and the lack of reversibility in most patients suggest that the stem cell is the primary, or sole, target of this toxic (or idiosyncratic) effect of chloramphenicol. However, there is no proof for the existence of a genetically determined predisposition or for the role of suppression of nucleic acid synthesis in stem cells specifically.

Many investigators have postulated that the *p*-nitrobenzene moiety of chloramphenicol may be responsible for the observed effects on nucleic acid synthesis by cultured marrow cells (104). Compounds that contain this moiety are known to undergo reduction to N-hydroxyl or nitroso derivatives in some mammalian cells (109). Chloramphenicol has been shown to be reduced by microsomal nitroreductases (109, 110), and similar reduction products have been shown to produce single-strand breaks in DNA (111). In general, inhibition of mammalian DNA synthesis requires very high concentrations of chloramphenicol and presence of intact cells (104, 112). These observations are consistent with the postulate that chloramphenicol must be altered by the cell prior to interference with nucleic acid synthesis; however, proof is lacking.

Thiamphenicol is a *p*-methylsulfonyl analogue of chloramphenicol (108). It has been widely used clinically in Europe (108). It produces erythroid suppression in patients and inhibits mitochondrial protein synthesis in mammalian cells with the same frequency and severity as chloramphenicol (108). To date, use of thiamphenicol in humans has not been followed by marrow aplasia (108). It is tempting to speculate that the lack of this effect is due to the absence of the *p*-nitrobenzene moiety. However, the failure to detect aplasia may reflect only the rarity of this complication. Clearly, close long-term follow-up of patients treated with thiamphenicol is necessary to confirm these early encouraging results.

The effects of chloramphenicol and thiamphenicol on DNA synthesis have been compared. Manyan, Arimura & Yunis (113) reported that thiamphenicol was less suppressive than chloramphenicol upon DNA synthesis by cultured lymphoblastoid cells. Subsequently, McLeod, Manyan & Yunis (114) observed that thiamphenicol penetrated a variety of mammalian cells less well than chloramphenicol. These investigators (114) and Krishna (115) also detected less covalent binding of thiamphenicol to macromolecules in rat bone marrow cells. Possibly the disparities in penetration and covalent binding may account for the differences in the effects of the two drugs on DNA synthesis (114, 115). Freeman, Patel & Haldar (112) have offered an alternative explanation. They found that both chloramphenicol and thiamphenicol in high concentrations inhibited DNA synthesis by Ehrlich ascites cells in the absence of glucose. However, in the presence of glucose, the inhibitory effect of thiamphenicol, but not chloramphenicol, was reversed. These investigators suggest that inhibition of DNA metabolism by the two drugs in the absence of glucose is secondary to suppression of NADPH oxidation. They hypothesize that in the presence of glucose with restoration of ATP levels chloramphenicol, but not thiamphenicol, is metabolized to derivatives that inhibit DNA synthesis by a

mechanism other than NADH oxidation. Once again, this raises the possibility that the reduced nitrobenzene moiety of chloramphenicol is involved in the observed impairment of DNA synthesis. Further research with thiamphenicol and other *p*-substituted congeners of chloramphenicol clinically and in marrow cell cultures may clarify (*a*) the relationship of impaired nucleic acid synthesis to aplastic anemia and (*b*) the role, if any, of the *p*-nitrobenzene moiety in this reaction.

HEPATOTOXICITY

Many antimicrobial agents have been implicated as causes of hepatic damage. In general, hepatotoxicity is most frequently encountered among those drugs that are metabolized or excreted by the liver. Examples include isoniazid, rifampin, and erythromycin estolate. The drugs that are excreted primarily by the kidney in an unmodified form tend not to induce hepatic injury. Examples include many of the penicillins and cephalosporins and the aminoglycosides. General biochemical mechanisms of drug-induced liver injury have been reviewed recently (116). Hepatotoxicity of isoniazid and erythromycin estolate has been studied extensively in the past few years.

Erythromycin Estolate

Hepatic damage is an infrequent complication of therapy with the erythromycins. However, cholestatic hepatitis may occur. Almost all cases have been ascribed to use of erythromycin estolate. Many of the clinical features of this reaction suggest that it may be immunologically mediated: (*a*) rare occurrence on first exposure to the drug, unless therapy has been continued for 10 or more days; (*b*) more frequent occurrence in individuals who have received the drug on several occasions; (*c*) lack of relationship to dose administered; (*d*) eosinophilia, and (*e*) rapid reappearance of symptoms after challenge with very small doses (117–119). However, studies in one patient with this reaction failed to detect presence of immunoglobulins in the liver, changes in serum complement, or lymphocyte transformation following challenge with the drug (120). This experience with but one patient does not exclude the possibility that the reaction is due to hypersensitivity. Interestingly, challenge of this patient with erythromycin estolate and erythromycin propionate resulted in reappearance of the reaction, while challenges with erythromycin base, stearate, gluceptate, and ethylsuccinate did not elicit the reaction. Others also have noted a lack of response to rechallenge of their patients with the base or stearate (121, 122). This lack of cross-reactivity to closely related drugs prompted a search for mechanisms other than hypersensitivity.

Erythromycin estolate is a lauryl sulfate salt of erythromycin propionate. The propionate form of the drug has been shown to rapidly reduce flow of bile in the isolated perfused rat liver, whereas the base had little effect (123). Furthermore, the propionate or the estolate or both have been shown to damage intact hepatocytes in concentrations at which other forms of erythromycin have little or no effect (124–126). Hepatocyte damage was indicated by loss of viability or leakage of hepatic enzymes. Several hypotheses have been proposed to explain the mechanism of injury to hepatocytes. Since the estolate is known to lower surface tension of bile, it has been suggested that this may facilitate disruption of hepatocyte membrane structure or function (123, 124). The hypothesis that is perhaps most attractive couples toxicity and hypersensitivity. It has been proposed that the propionate or the lauryl sulfate salt must first damage the hepatocyte and that a hypersensitivity reaction then follows (124). In this instance, the antigen (or allergen) might be a product of the damaged cell or a complex between the drug and a specific component of the injured cell. This hypothesis incorporates the clinical features that suggest hypersensitivity, the lack of effect of other erythromycins, and the known direct toxicity of the propionate and its salt. For several reasons, it appears unlikely that the hepatic damage is a direct result of a pharmacological action of the drug: 1. Not all congeners of erythromycin that act similarly on procaryotic cells (Table 1) are toxic for hepatocytes. 2. Recurrent episodes may be induced in the patient within a very short interval by very low doses of the estolate (118, 119). 3. Although the erythromycins may inhibit protein synthesis by isolated mammalian mitochondrial ribosomes, there is no effect on protein synthesis in the presence of intact mitochondrial membranes (127).

Isoniazid

Isoniazid may produce hepatic injury that clinically and histopathologically resembles viral hepatitis. The hepatocyte appears to be the target of the drug-induced injury. The extent of the problem of hepatotoxicity was not recognized until 1970, which coincided with an increase in use of isoniazid alone in preventive therapy (128). A task force was organized to identify risk factors and other epidemiological characteristics of the problem. The data generated then stimulated a number of investigations into the pathophysiology of the hepatic injury. The results of studies performed between 1970 and 1975 are reviewed in the proceedings of a conference held at the National Institutes of Health (129).

The epidemiological and clinical investigations revealed the following characteristics of the drug-induced hepatitis: 1. Approximately 10–23% of patients given isoniazid develop at least subclinical evidence of hepatic injury (129, 130). 2. The incidence of clinically apparent hepatitis increases

substantially with advancing age (129, 131). 3. The reaction may appear any time up to 12 months after institution of therapy (131). 4. There is no correlation between plasma levels of isoniazid and susceptibility to hepatic injury (130, 131). 5. The risk of the reaction appears to be greater in rapid than slow acetylators of the drug (129, 132). These data tended to implicate a metabolite of isoniazid, rather than the drug itself, in the etiology of this reaction.

Isoniazid is metabolized at rates that vary in comparisons between individuals, and the rate of any given individual is genetically determined (132, 133). There are two phenotypes: rapid and slow acetylators of the drug. Studies of the metabolism of isoniazid have shown that rapid acetylators hydrolyze much more of the drug to isonicotinic acid and the free hydrazine moiety than slow acetylators (132). The hydrazine moiety is liberated as acetylhydrazine (132, 134). Evidence is mounting to suggest that acetylhydrazine or its metabolites, or both, may be responsible for damage to hepatocytes. Many hydrazines are potent hepatotoxins, mutagens, and carcinogens in experimental animals (135–137), and acetylhydrazine has recently been shown to induce hepatic necrosis in rats (138, 139). Induction of the P450 hepatic drug-metabolizing enzymes increases the susceptibility of rats to acetylisoniazid- and acetylhydrazine-induced hepatic necrosis (129). Furthermore, if rats are given acetylisoniazid that is radiolabeled in the acetyl side chain or labeled acetylhydrazine, significant covalent binding to hepatic macromolecules can be demonstrated (129). Although acetylhydrazine and its metabolites have been convincingly linked to hepatic injury, the precise molecular basis of lethality for the hepatocyte has not yet been elucidated.

Very few patients with isoniazid-induced hepatitis meet standard criteria for the clinical diagnosis of an immunologically mediated allergic or hypersensitivity reaction (129, 131, 140). Lymphocytes obtained from one patient with hepatitis transformed after exposure to isoniazid (141); however, this phenomenon was not demonstrated with lymphocytes obtained subsequently from a group of patients with isoniazid-induced hepatitis (142). Although unproven, it appears likely that occasional patients given isoniazid may develop hepatitis that is immunologically mediated. Of course, the possibility remains that the final pathway of acetylisoniazid-induced hepatic cell injury is mediated by components of the immune system.

Because of the known mutagenic properties of hydrazines (136–138), concern has been expressed that isoniazid indirectly may be carcinogenic (129). Preliminary evidence is conflicting. Prolonged administration of relatively high doses of isoniazid to rodents has been associated with appearance of hyperplastic and neoplastic lung lesions (143). However, a retrospective study in humans detected no evidence for an increased risk of malignancy (144), while another indicated that the risk of death from

cancer was increased following the introduction of isoniazid as a chemotherapeutic agent for tuberculosis (145). Careful long-term studies are clearly indicated. There is no evidence to suggest that any of these adverse reactions to isoniazid result from the pharmacological mechanism of action of the drug as it is presently perceived (Table 1).

GASTROINTESTINAL TOXICITY

Almost all antimicrobial agents have been implicated as causes of annoying, but seldom life-threatening, reactions such as nausea, vomiting, diarrhea, and intestinal cramping (146). These reactions are usually dose-related and occur more frequently when a given drug is administered orally. Although commonly attributed to "irritative" or toxic effects of the drugs, the mechanism(s) of these reactions remains obscure (146). Overgrowth of portions of the gastrointestinal tract by resistant organisms, such as staphylococci, candida, and pseudomonads, appears to result from antibiotic-induced alterations in the ecology of the normal microbial flora rather than any direct effect of the drugs upon the gastrointestinal epithelium (147, 148). Two additional reactions have been postulated to result from toxicity for the intestinal epithelium: malabsorption and pseudomembranous enterocolitis.

Malabsorption

Malabsorption has been reported following oral administration of a variety of broad-spectrum antimicrobial agents, usually in high doses or for prolonged periods, or both (146, 149). The aminoglycosides, especially neomycin, have been implicated most frequently. The oral administration of neomycin has resulted in malabsorption of fat, protein, carbohydrate, cholesterol, carotene, glucose, lactose, sodium, calcium, iron, and cyanocobalamin. Since malabsorption is a characteristic of germ-free animals, it was postulated that antibiotic-induced sprue-like syndromes resulted from suppression of the enteric microflora. However, it is unlikely that this is the sole explanation for neomycin-induced malabsorption (149). Oral, but not parenteral, neomycin has been shown to induce a marked decrease in serum cholesterol that is out of proportion to the degree of malabsorption. In addition, the drug has been found (*a*) to produce necrosis of intestinal crypt cells which are known to synthesize cholesterol and (*b*) to inhibit the activity of enzymes such as intestinal lactase. The molecular mechanism of lethality for crypt cells or of impairment of intestinal enzyme activity is unknown. However, a direct effect upon the intestinal epithelium, rather than an indirect effect resultant from suppression of the indigenous flora, appears to have been reasonably well substantiated.

Pseudomembranous Enterocolitis

Pseudomembranous enterocolitis may be a severe and potentially life-threatening complication of antimicrobial therapy (150). Clindamycin, lincomycin, ampicillin, the tetracyclines, and chloramphenicol have been implicated. Until very recently, the mechanism of this adverse effect was poorly understood. It had been hypothesized to result from direct toxicity to the epithelial cells, alterations in the intestinal microflora, interaction with the secretory immune system, or a combination of the foregoing (150, 151). However, scant evidence was available to support any of these or other hypotheses. Very recent studies with clindamycin in humans and experimental animals portend success in identifying the molecular basis for this reaction. Although the mechanism may not involve a direct effect of the drug on the intestinal epithelial cell, a brief review of these recent studies is indicated because of their future implications for prevention and treatment of pseudomembranous colitis and for a basic understanding of drug-induced epithelial cell damage.

In 1977, two groups of investigators (152–154) reported identification of a toxin in preparations of stools from patients with clindamycin-associated colitis. The toxin was heat-labile and cytopathic in tissue cultures; its activity could be neutralized with polyvalent gas gangrene (clostridial) antitoxin. More recently, the syndrome has been reproduced in hamsters by intracecal injection of stools from patients with clindamycin-induced colitis (155). Injection of strains of *Clostridium difficile* from the stools and cell-free filtrates of cultures of these organisms also elicited colitis (156). Toxic activity of stools and culture filtrates was neutralized by clostridial antitoxin. Stools from normal subjects were devoid of cytotoxic activity in cell cultures and did not reproduce colitis when injected into hamsters (155). Appropriate experimental controls have excluded the possibility of carryover of clindamycin in transfers of stools to cultures or laboratory animals.

Strains of *C. difficile* that produce the toxin have been isolated from the stools of hamsters (156) and patients (157) with clindamycin-induced pseudomembranous colitis. In one patient, the numbers of *C. difficile* per gram of feces were 100-fold higher during colitis than following recovery (157). Oral administration of vancomycin, a nonabsorbable antibiotic active against clostridia, has been shown to prevent or ameliorate clindamycin-induced colitis in hamsters (158). Collectively, these observations strongly support the hypothesis that a clostridial toxin mediates the epithelial damage in clindamycin-associated pseudomembranous enterocolitis. Presumably, the drug suppresses much of the enteric microflora which in turn permits accelerated growth or metabolism of clindamycin-tolerant clostridia (*C. difficile* and possibly other species) and elaboration of the toxin.

If this hypothesis is proven to be correct, it may be possible to prevent or treat the colitis with antibiotics active against the toxin-producing bacteria. It may also be possible to administer antitoxin or to prepare a toxoid from the purified toxin. Future research should attempt to determine the mechanism of action of the toxin and its role, if any, in pseudomembranous enterocolitis induced by other antimicrobial agents.

CUTANEOUS TOXICITY

A variety of cutaneous reactions have been associated with use of each of the available antimicrobial agents. Most of these have been linked convincingly to host-mediated hypersensitivity to the drug administered. Three groups of reactions may be mediated by other mechanisms: phototoxicity, flushing, and many of the rashes resultant from use of ampicillin.

Phototoxicity

Phototoxic reactions have been associated with use of nalidixic acid (159), the sulfonamides (160), and the tetracyclines, especially demethylchlortetracycline and doxycycline (161, 162). The clinical spectrum of the reactions range from mild paresthesias and abnormal sunburn to severe bullous eruptions with or without onycholysis and pigmentation of the nails. The reaction is precipitated by light in the ultraviolet spectrum of 260–320 nm (2). It has been hypothesized that the ultraviolet irradiation stimulates the formation of photodecomposition products that mediate the response (163). However, there is little evidence to support this or other equally plausible hypotheses (160). Evidence that these reactions are not immunologically mediated is well summarized in a review by Harber & Baer (160). It is most unlikely that the phototoxicity results directly from a pharmacological action of these drugs (Table 1).

Flushing Due to the Polymyxins

Occasional patients given the polymyxins (polymyxin B or colistin) develop annoying, but seldom severe, flushing and other subjective side effects suggestive of histamine release (164). Many of these effects appear to have been ameliorated by administration of antihistamines (164). The polymyxins have been shown to induce release of histamine in a variety of experimental animals (165) and from mast cells in vitro (166). The in vitro degranulation of mast cells following addition of polymyxin B occurs in the absence of fresh serum and components of the immune system (immune globulin and complement), which suggests that the drug interacts directly with the mast cell (166, 167). Tizard & Holmes (167) have shown that in polymyxin-treated mast cells the histamine-containing granules migrate to the periph-

ery of the cell either freely or bound by a membrane and are released through the cell membrane into the extracellular fluid. It is possible that membranous pores in the mast cell are created by the polymyxins in a manner analogous to their action upon the membranes of sensitive bacterial cells (Table 1); however, this possibility has not been studied directly.

Rashes Due to Ampicillin

The rate of development of skin rashes is generally far greater for patients given ampicillin (168) than for patients given other penicillins. The disparity in rates is even more pronounced for patients with underlying infectious mononucleosis (169–171). The incidence of rashes in patients with infectious mononucleosis who are also given ampicillin approaches 90%, while the rate in patients with mononucleosis alone is approximately 10–15%. Concurrent therapy with allopurinol also is associated with an increased risk of development of rash during treatment with ampicillin (172). It is not clear whether this increased risk is resultant from allopurinol itself or the underlying hyperuricemia for which it was prescribed (172).

Maculopapular rashes appear to account for most of the excess incidence observed with ampicillin (168–172). These maculopapular rashes may not be due to allergy to the drug. The evidence against an allergic reaction may be summarized as follows: 1. The serum from patients with ampicillin-associated rash seldom promoted degranulation of mast cells (histamine release) in vitro, while serum from patients with classical penicillin allergy promptly induced degranulation (173). 2. Significantly fewer patients with ampicillin-related maculopapular rashes reacted to a variety of penicillin skin test reagents than did patients whose rash was manifested as urticaria (174). 3. Readministration of the drug to patients who recovered from the ampicillin-associated maculopapular rash did not produce a recurrence of the reaction (174). Thus many of the hallmarks of classical drug allergy—histamine release, skin test reactivity, and recall upon subsequent challenge—were absent in the majority of patients with the ampicillin-induced maculopapular rashes.

More recently, McKenzie, Parratt & White (175) have reported detection of antibody-like activity directed against an ampicillin-derived (ampicilloyl) antigen in serum of patients with infectious mononucleosis. Most important, this activity was detected in some patients without evidence for prior exposure to ampicillin. These investigators hypothesize the presence of polyclonally stimulated antiampicilloyl antibodies (analogous, but not identical, with heterophil antibodies) in infectious mononucleosis. Furthermore, they suggest that these antibodies may complex with ampicillin, localize in small vessels, and fix complement, which then mediates the injury to the host's cells. Knudsen et al (176) suggest that macromolecular complexes or

other impurities in some preparations of ampicillin may incite the cutaneous reactions. Thus it remains unclear whether these maculopapular rashes result from direct toxicity or immunologically mediated injury. Regardless, the reaction most certainly is not a pharmacological action of the drug (Table 1).

CONCLUSION

Despite extensive research, the molecular mechanisms of very few toxic reactions have been clearly elucidated. The importance of understanding these basic mechanisms cannot be overemphasized. Were this information at hand, prevention and treatment of reactions and engineering of more potent, less toxic antibacterials might be greatly facilitated. The well conceived and executed studies of the mechanism of isoniazid-induced hepatotoxicity underscore the importance of collective and often collaborative efforts of individuals with diverse "specialty" interests. A similar approach will undoubtedly be required to resolve the problems of aminoglycoside-induced ototoxicity, nephrotoxicity, and neuromuscular blockade—which possibly share a common molecular mechanism—and a variety of other toxic reactions.

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