Some Principles in the Chemotherapy of Bacterial Infections—II*

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British Medical Journal, 1969, 2, 209–212

Drug Resistance

Infectious Drug Resistance

"Transferred," or "infectious," drug resistance consists in the transfer of genetic material from a strain or species resistant to a particular drug to another strain previously sensitive. The discovery that such a transfer of drug resistance from a non-pathogenic species to a pathogenic species of Gram-negative bacilli can occur within the intestine of animals or man (Meynell and Datta, 1967; Watanabe, 1967) has caused justifiable apprehension. Such a possibility exists not only in the intestine but in the upper respiratory tract and perhaps in hospital environments. The phenomenon is being very actively explored at the present time, and is of great theoretical interest for molecular biology. It is possible here to give only a brief and simplified account of it.

Genetic material in bacteria is mostly concentrated in the single chromosome, but as much as 20% of the D.N.A. in most species of staphylococci and Enterobacteriaceae exists in the cytoplasm, associated with cytoplasmic bodies known as plasmids or episomes. Genes for drug resistance may be associated with D.N.A. in chromosome or cytoplasm, and under appropriate conditions may be transferred to sensitive strains. One of the alarming things about the phenomenon is that resistance to several different drugs may be transferred at the same time (Watanabe, 1963; Medeiros and O'Brien, 1966; Anderson, 1967). Organisms apt to acquire transferred resistance include those causing dysentery, typhoid, urinary infections, cholera, and plague (Watanabe, 1967). Transferred resistance has also been demonstrated in *Klebsiella* species (Medeiros and O'Brien, 1966) and staphylococci (McDonald, 1966), both of which have obvious practical implications in the hospital environment. Genes transferred from the cytoplasmic bodies may confer resistance by altering the functions of the cell membrane. This alteration may consist in either decreasing the permeability of the membrane to the drug in question or in inducing the manufacture of drug-destroying enzymes which are released between the membrane and the cell wall (Richmond, 1966).

Mechanisms of Transfer of Resistance

There are three main mechanisms by which genetic material conferring resistance may be transferred from a resistant to a sensitive strain (Watanabe, 1967).

(1) *Transformation.*—This consists in transfer of parts of the D.N.A. of the chromosome, which become recombined within the host bacterial cell. The genes are actually released into the medium. Transformation occurs only under optimal laboratory conditions and is probably of little importance in clinical practice.

(2) *Conjugation.*—This consists in the transfer of genetic material by physical contact between individual bacterial cells by means of a cytoplasmic bridge or by a "pilus."

(3) *Transduction.*—This consists in the carrying of genetic material from a resistant to a sensitive strain by means of bacteriophage.

Conjugation.—This depends on a "male" type of bacterial cell with the capacity for producing a pilus which allows conjugation with a "female" or receptor cell. It is at present uncertain whether the genetic material actually passes through the pilus or whether the pilus merely acts as a "grappling iron," allowing the formation of a cytoplasmic bridge elsewhere in the cell (W. Hayes, personal communication). There are two main types of conjugation at present described, the Hfr (high frequency) type and the R (resistance) factor type. In the Hfr type (Fig. 8) the male type cells may transfer portions of the chromosome, perhaps including a gene for resistance, to a female type cell which may incorporate the gene for resistance in its own chromosome, a phenomenon known as "recombination." In the R factor type (Fig. 9) a cytoplasmic episome, or R factor, which is responsible for multiple drug resistance, replicates itself within the cytoplasm and also induces the formation

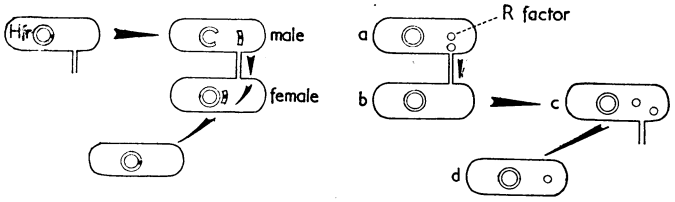

FIG. 8 FIG. 9

FIG. 8.—Transfer of drug resistance by conjugation (Hfr type) (after Watanabe, 1967). FIG. 9.—Transfer of drug resistance by conjugation (cytoplasmic R factor) (after Watanabe, 1967).

of a pilus which allows transfer of an R factor to a previously sensitive female type of cell. This makes the recipient cell resistant and also induces the formation of a pilus in this cell, rendering it a male in its turn and able for a time to affect others, until in due course an inhibitory process comes into action which prevents the formation of further pili (Meynell and Datta, 1967; Watanabe, 1967).

Transduction.—This consists in the transfer of genetic material conferring resistance from a resistant to a sensitive strain by means of bacteriophage (Fig. 10). This has been shown particularly in staphylococci. The phage may infect a coccus containing a cytoplasmic plasmid capable of inducing, for instance, penicillinase formation. During the replication of the phage in the coccus it may happen to incorporate the genetic material of the plasmid. In due course the replicated

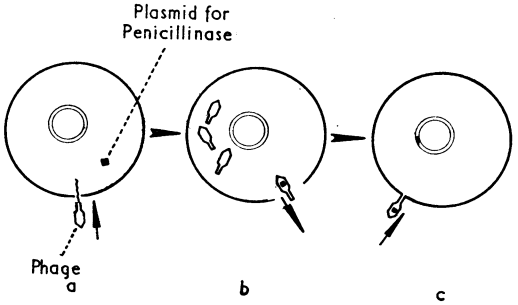

FIG. 10.—Transduction of penicillin resistance in *Staphylococcus aureus* (after Watanabe, 1967).

* The conclusion of the first Sir Robert Philip lecture given at the Royal College of Physicians of Edinburgh, November 1968. The first part appeared in last week's issue.

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phages are released, and a plasmid-bearing individual phage may infect a previously penicillin-sensitive coccus. The D.N.A. of the plasmid will then induce the newly infected coccus to manufacture penicillinase (McDonald, 1966 ; Novick and Morse, 1967 ; Watanabe, 1967).

Cross-resistance between Drugs

" Cross-resistance " is a term used to indicate that if a strain is resistant to one drug it is also resistant to another. There are three main reasons for the clinician to be aware of this possibility. (1) If a particular drug has been unsuccessful in treating the patient's infection then there is usually little point in changing to another to which there may be cross-resistance ; this is particularly important if the first drug belongs to a group to which resistance is readily acquired. (2) If there is cross-resistance between several drugs which are regarded as drugs reserved to treat particularly dangerous organisms, then all members of the group should be reserved in case the free use of one of them results in an ecological situation in which many strains are resistant to the others. (3) In preventing acquired resistance by drug combinations there is little point in combining two drugs liable to cross-resistance, as each may be ineffective against the mutants in the population resistant to the other.

An example of the first group is the use of tetracyclines. There is little point in changing from one tetracycline to another to obtain a greater effect, as there is virtually complete cross-resistance between them, though it is true that in certain patients one tetracycline produces fewer side-effects than another, and this may be a reason for change. Similarly with the group of drugs comprising erythromycin, oleandomycin, carbomycin, spiramycin, and lincomycin cross-resistance is relatively common in vitro though somewhat less common clinically. There is therefore little point in changing from one drug of this group to another unless it has been definitely shown that the organisms are sensitive to the second drug proposed.

In the group of reserve drugs the most important clinical consideration is the risk of cross-resistance between methicillin, cloxacillin, and cephaloridine. There is virtually complete cross-resistance between methicillin and cloxacillin. This does not seem to be related to destruction by bacterial enzyme but possibly to some difference in the cell wall (Richmond and Stewart, 1966). Strains resistant to methicillin are relatively rarely resistant to cephalosporin, but induction of resistance to cephalosporin is said usually to result in resistance to methicillin (and presumably cloxacillin) (Ott and Godzeski, 1966). There does not yet seem to be sufficient clinical information on this point, but meantime it seems wisest to give cephalosporin only under isolation conditions, as with methicillin and cloxacillin. Lists of known cross-resistance between drugs may be found elsewhere (Garrod and O'Grady, 1968 ; Crofton and Douglas, 1969).

Clinical Implications of Drug Resistance

It will be clear from the above discussion that drug resistance is a very complex matter. Acquired drug resistance is of great importance to the clinician, for he must always seek to prevent it, when he is giving the relevant drugs, by using at least two drugs to which the organism is sensitive and which do not have cross-resistance between them. The problems associated with natural drug resistance imply that in any hospital environment, and preferably also in a community, there should be an antibiotic policy by which certain crucial drugs are reserved only for the treatment of dangerous pathogenic organisms, such as the staphylococcus, which are often resistant to routine therapy. It is desirable that patients who are treated with these reserve drugs should be isolated. The discovery of infectious drug resistance has also very important ecological implications, for if antibiotics are used widely non-pathogenic strains may acquire resistance which they subsequently transfer to pathogenic strains. Non-pathogenic drug-resistant bacteria might be transferred from animals to man. This possibility suggests the need for an antibiotic policy in the treatment of domestic animals as well as in the treatment of man. Drugs crucially necessary for treating dangerous infections should be kept in reserve.

Another disturbing thought has been the suggestion that the R factor might be transferred from a resistant to a sensitive strain during resistance testing by the disc method, resulting in a misleading bacteriological report (Smith and Stewart, 1966).

Bacterial Persistence

Bacterial persistence is a term used to describe the survival of fully sensitive bacteria in the presence of a concentration of antibiotic which kills the great majority of the bacterial population. The phenomenon was described for streptococci and staphylococci by Hobby *et al.* (1942) and for staphylococci by Bigger (1944), who coined the term. The subject has been extensively reviewed by McDermott (1958), and recently and more briefly by Yow *et al.* (1961) and Yow (1966). Persisters usually form only a very small proportion of a bacterial population exposed to a drug. Their clinical significance is that they may be responsible for relapse. They occur particularly in old bacterial populations or in the presence of pus, poor drainage, or a foreign body. They are less likely to occur if an infection is treated early and with adequate doses of a bactericidal drug. Appropriate timing of doses may possibly diminish the chances of persistence (Yow, 1966). It is probable that persisters are bacterial cells which happen to be relatively dormant, and so only very slowly metabolizing, at the time of exposure to the drug. They are therefore less readily eliminated (Eagle, 1952 ; Yow, 1966 ; Rogers, 1967).

In some cases persistence may be due to the induction of L-forms which have lost their cell walls and thereby ceased to be sensitive to drugs acting on cell walls (Kubota *et al.*, 1966). Persisters of this kind might be eliminated by other drugs, as has been shown in vitro for kanamycin in the case of L-forms of enterococci derived from bacterial endocarditis (Hewitt and Deigh, 1965), and possibly also in the case of experimental rat pyelonephritis (Montgomerie *et al.*, 1965). In most cases persisters are eventually eliminated by the host defences (McCune *et al.*, 1960 ; Kalmanson *et al.*, 1966). The duration of chemotherapy may also have an effect, particularly if the host defences are poor. A good example of this is pulmonary tuberculosis. Tubercle bacilli can be killed in the test-tube within a few days, but, if there is a large bacterial population, may take 18 months to eliminate in a clinical lung infection. If proper chemotherapy is given the long-term survivors still remain drug-sensitive (Stewart *et al.*, 1956). Presumably the dormant persisters either begin metabolizing and are then killed by the chemotherapy or, if they remain dormant, are eventually dealt with by the host defences.

Another well-known instance of persistence is bacterial endocarditis. Chronic bronchitis and chronic genitourinary infections are other possible examples.

Poor Host Defences

The role of host defences in eliminating persisters has already been mentioned. The patient with poor defences is more likely to suffer from diseases due to pathogenic bacteria, less likely to recover under chemotherapy, and more likely to be superinfected with other organisms resistant to the antibiotics being used. There is no space here to elaborate all the factors lowering host resistance. There may be a congenital lack of immunoglobulins or of cellular factors. Congenitally abnormal mucus, as in cystic fibrosis, may lead to poor drainage. Other factors include old age, other diseases such as diabetes or the reticuloses,

alcohol, and the use of drugs such as corticosteroids, immunosuppressants, or cytotoxic agents. Poor drainage of a body space notoriously encourages its infection and causes difficulty in eliminating the infecting agent. The absence of a flow of tissue fluids through the space may diminish the access of defending cells and antibodies and may decrease the penetration of antibiotics (Sabath *et al.*, 1962 ; Verwey *et al.*, 1965).

Absorption, Protein-binding, Excretion, and Destruction of Antibiotics

The effect of an antibiotic depends on its concentration in the immediate neighbourhood of the bacteria in the lesion. This in turn is affected by a number of factors shown diagrammatically in Fig. 11. As is outlined below, the concentration of an antibiotic in the tissues and the lesion depends on the concentration of free antibiotic, unbound to protein, in the blood. Besides diffusing into the tissues some of the free antibiotic is,

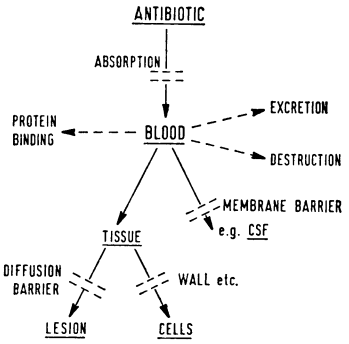

FIG. 11

in the case of certain drugs, destroyed by metabolic processes in the liver and elsewhere, and some is excreted by the kidneys (Verwey *et al.*, 1965). The concentration drops sharply with the distance from the blood vessel unless the serum levels can be maintained for long periods, giving time for equilibrium to occur. This may be important in relatively avascular lesions, such as thick-walled abscesses. The drainage of an abscess will maintain tissue flow and thus assist diffusion (Sabath *et al.*, 1962 ; Verwey *et al.*, 1965).

Membrane Barriers

The most well known of these is the blood-brain barrier. A relatively small amount of penicillin penetrates into the cerebrospinal fluid of normal people, though this may be larger in meningitis. Because of the low protein content of cerebrospinal fluid there is little protein-binding, so that most of the penicillin present is in the free state and therefore effective. Other drugs, such as isoniazid, penetrate the blood-brain barrier well.

Cell Barriers (Kessel, 1965)

In some infections many bacteria are in the cytoplasm of host cells. The ability of antibiotics to penetrate the cells is therefore relevant to the outcome of treatment. The bacteria lie within a phagocytic vacuole in the cell. If the antibiotic penetrates the cell wall and membrane it lies in a pinocytic vacuole within the cytoplasm (Fig. 12). After this the drug still requires to be transferred across the cytoplasm and to penetrate the wall of the vacuole containing the bacteria. It has been shown in the case of tetracycline, which can be identified by its fluorescent properties, that the drug in the pinocytic vacuole often fails to come in contact with the organisms in the phagocytic vacuole (du Buy *et al.*, 1964). Infections in which such barriers may be important include brucellosis, salmonella infections, leprosy, and tuberculosis.

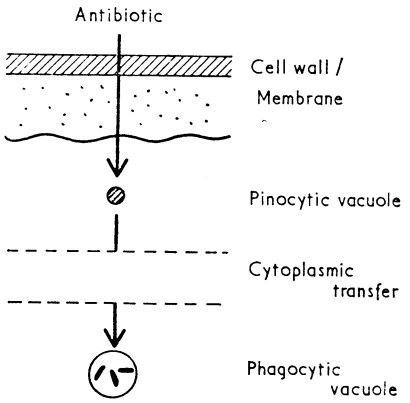

FIG. 12.—Cell barriers to drug action (after Kessel, 1965).

Protein-binding of Antibiotics

Though most clinicians know that there is binding of antibiotics to serum proteins, they usually have little idea of its significance. The mass of confusing data in the literature has recently been coherently summarized by Rolinson (1967) in a penetrating review. The proportion of an antibiotic which is bound varies greatly from one drug to another. A useful table is given by Kunin (1967). The proportion also varies from one animal species to another. Most drugs are bound to albumin, but erythromycin binds to α_1-globulin.

It is only the free antibiotic which has antibacterial effect, but there is ready dissociation of the bound drug from protein as the free drug diffuses out into the tissues and is excreted through the kidney (Fig. 13). In this way the protein-drug complex fulfils a storage function similar to that of haemoglobin for oxygen. Nevertheless the gradient of drug concentration between the blood, the tissues, and the lesion depends on the level of free drug in the blood, its duration, and the diffusion gradient. There is only slight diffusion of protein-bound drug from the blood stream, though this is somewhat increased in inflammation. The peak level in the lesion can virtually never exceed the peak level of *free* drug in the blood,

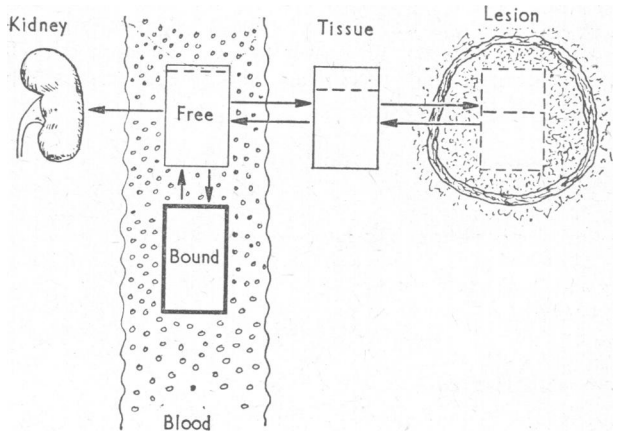

FIG. 13.—Protein-binding of drugs.

though, of course, the peak level in the lesion is reached later, and its height depends partly on the duration of the peak level in the blood. As the blood level falls, drug will tend to diffuse back into the blood stream from the tissues and from the lesion.

Protein-binding in tissues is relatively unimportant, partly because there is less free protein and partly because that in tissue binds less drug (Verwey *et al.*, 1965). Very low levels of total

drug, such as penicillin, in the cerebrospinal fluid may be effective because the drug is almost all free.

The estimation of drug levels in the blood by the cup-plate technique may be misleading. As free drug diffuses out into the agar, protein-bound drug dissociates so that the effective drug level may be overestimated. A tube method is more reliable, though even here the dilution factor will cause some dissociation.

All this sounds a little depressing for the physician, but Rolinson (1967) shows in a useful table that in the case of drugs effective against staphylococci and taken in usual doses the peak serum level of free drug is several times the minimal inhibitory concentration as measured in vitro. A drug like novobiocin, which is 94% bound in serum, may compensate for this by maintaining its maximal level of free drug over long periods, allowing effective diffusion into the lesion. Rolinson (1967) also points out that it is much more meaningful to give the percentage of *free* drug in the blood rather than that of *bound* drug. For two drugs a difference in binding of between 90 and 95% sounds very little, but, in fact, the first gives a proportion of free drug twice that of the second.

Inactivation of Antibiotic by Host Flora

Theoretically an antibiotic such as penicillin might in some sites be inactivated by penicillinase produced by bacteria other than those causing the illness. For instance, in the prophylaxis of rheumatic fever by penicillin it was found that the elimination of haemolytic streptococci by penicillin might be prevented by the presence of penicillinase-producing staphylococci in the throat (Massell *et al.*, 1966). This is probably not a frequent phenomenon. Hafez *et al.* (1965) found no evidence that it occurred in the sputum of patients with respiratory infections treated with penicillin.

Conclusions

In this brief survey I have tried to outline some of the principles which underlie the formerly empirical subject of chemotherapy. I have done so partly because it is intellectually more satisfying to be able to visualize the processes which are going on both in the patient and in his invaders during the treatment of a bacterial infection. But, even more important, a grasp of the nature of these processes should help the clinician to find answers to the minority of challenging problems of bacterial infection which, if inadequately met, may result in severe disability or death. A knowledge of principles enables the physician to ask the bacteriologist the right questions, and helps the bacteriologist to answer them. For both the whole subject becomes far more fascinating, and the ultimate beneficiary is the patient.

I am indebted to Professor William Hayes, of the Department of Molecular Biology, Edinburgh University, for constructive criticism. I am also grateful to Mr. C. Shepley and Mrs. A. MacNeill, of the department of medical illustration, and to Mr. W. Paterson, Mr. J. McInnes, Mr. J. Pizer, and Mr. I. Lennox, of the department of medical photography, University of Edinburgh, who drew the diagrams.

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