

# PRINCIPLES AND PROBLEMS OF COMBINED ANTIBIOTIC THERAPY

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In 1922 Browning and Gulbransen made the observation that one antiprotozoal agent may interfere with the action of another. With the discovery of a large number of chemotherapeutic agents acting on bacteria, the problem of therapeutic interference gained fresh interest. In 1943 Ungar demonstrated that penicillin and sulphonamides reinforce each other's action both in the test tube and in experimental animals, and since that time many studies have been made in this field. In general, it may be assumed that when two or more antibiotics act simultaneously on a bacterial population they may reinforce one another, there may be no interaction between them or unwanted interference may occur. To understand the nature of antibiotic interactions, it is necessary to consider the mode of action of antibiotics on bacteria. Only a brief outline of the principles involved can be attempted here, however, and for a full discussion of combined chemotherapy reference should be made to the admirable review of Garrod (1953).

## The Effect of Antibiotics on Bacteria

Antibiotics interfere with the life-processes of micro-organisms, and those which are of therapeutic usefulness do so without being excessively toxic to the tissues of the host. The chemical nature of this metabolic interference is in most instances not understood, but from the practical point of view two distinct results may be observed. Some antibiotics block vital processes of bacteria, rendering them unable to divide and multiply. Such organisms are 'inhibited' but not dead, and on withdrawal of the drug will resume their previous activities. Eventually organisms temporarily paralysed in this way may die, but not sufficiently quickly for practical purposes. Other antibiotics, perhaps by blocking more important or urgent vital functions, bring about the rapid death of the microbes sensitive to their action. This is referred to as the 'lethal' or 'bactericidal' effect and is manifested only by some of the antibiotics. It must be emphasized that

routine tests as they are at present performed only reveal inhibition of growth. They give no information as regards the presence or absence of the bactericidal effect. This approach arose through the fact that penicillin happens to be both bacteriostatic and bactericidal in approximately the same concentration to those organisms that are sensitive to its action. The same, however, does not apply to most of the newer antibiotics, some of which exert only minimal bactericidal effect, or do so only in concentrations in gross excess of those obtainable in the body of a patient. Furthermore, considerable variations exist between different strains of organisms even of the same species. To some a given drug may be only inhibitory, while to another the same drug is lethal. By and large it is possible to generalize. Thus penicillin and streptomycin are generally bactericidal, while the tetracyclines are only bacteriostatic in the concentrations obtainable in the tissues of a patient. For a given pathogenic organism the effect of a drug cannot be safely forecast, however, from observing the 'sensitivity' to growth-inhibition, and this fact has considerable bearing on interference. The third result of antibiotic action is inherent in the actual number of bacteria present in disease. A bacterial population is not homogeneous in the sense that all individuals are identical. Some can by-pass the metabolic block which inhibits or kills his brethren, by utilizing some other biochemical function to achieve the same end. These individuals and their progeny, being resistant to the action of the antibiotic, can then proceed and replace the original sensitive population. The emergence of these resistant mutants is not strictly a result of the antibiotic, but is inherent in the biology of the microbe. However, experience has shown that with certain drugs resistant variants are frequently encountered, indicating that such mutants occur in relatively small bacterial populations, while with other drugs resistant mutants occur only in very large populations. With the latter, the resistant mutants often show only slightly increased resist-

ance, although their progeny may then show further steps in resistance.

These three modes of action of antibiotics: bacteriostatic, bactericidal and the ability to prevent resistant mutants, represent the print of view of practical therapeutics. In the attempt to effect a cure in a microbial disease, the aim is the 'Therapia sterilisans magna' of Ehrlich, the complete and utter annihilation of the pathogen. Fortunately, in this sense all antibiotic treatment is combined therapy, since the body defences of the patient are added to the action of the drug. Indeed, it is doubtful whether a cure could ever be achieved with any of the antibiotics we have at present if it was used singly and without the natural sterilizing effect of the body.

### The Effect of Combinations of Antibacterial Agents on Micro-organisms

Interference between two antibiotics can only be expected if their mode of action is different. Antibiotics may masquerade under different names, and may even be obtained from widely different sources, and yet be the same as regards their antibacterial action even though their pharmacological properties may be different. In such a case the acid test is whether the development of resistance to one antibiotic is accompanied by simultaneous development of resistance to the other. Combination of such related drugs have the same effect as increasing the effective concentration of either alone, and is therefore of little interest. The term 'additive' may be applied to such a combination. For true interaction the drugs must have a different point of attack in the bacterial metabolism. If two vital processes are blocked at the same time, the result may be either a greater or a lesser effect than with either of the two drugs alone. If two antibiotics, each with a bactericidal effect, interfere even in subminimal concentration with two distinct and essential vital processes, death of the bacterial cell may result although neither of the two drugs was present in an amount necessary to cause death by itself. The opposite effect may also occur however. A bactericidal drug requires rapid incorporation into the bacterial metabolism to exert its action. In other words, bactericidal drugs are generally most active on growing, rapidly metabolizing cell-populations. If a bacteriostatic drug slows down the rate of turnover of the microbe, a bactericidal drug may be unable to exert its full deadly blow. Unfortunately, the picture is further complicated by the relative concentrations of the drugs, and the further fact that *in vivo* such ratios are bound to vary with the intermittent administration of antibiotics, which is the only practical method. It also follows that if an antibiotic is totally inert

against a given pathogen, its combination with an active agent is not likely to be of any benefit. Resistance to a drug is, however, a relative term, and occasionally slight sensitivity may be made use of. This is illustrated by the case of penicillin-resistant penicillinase producing staphylococci. Although such strains show high resistance to penicillin alone, the addition of another drug by suppressing penicillinase production may increase the penicillin effect.

### Interactions at the Inhibitory Level

Although interference between drugs is easiest to test for at the level of growth inhibition, it is now generally agreed that this is of little clinical significance. The most that can happen is a slight increase or decrease of the growth-inhibition, which with the fluctuations in drug concentration *in vivo* also occurs with a single drug. If there is any increase of inhibition, the same can generally be achieved by increased dosage of one or the other drug. The argument that one drug maintains bacteriostasis, while the other is not present in sufficient concentration, is fallacious, since intermittent treatment should never be given in a way which allows actual multiplication between two administrations.

### Interference at the Bactericidal Level

As sterilization of the infection is the ultimate aim of all antibacterial treatment, the reinforcement or reduction of the bactericidal action of antibiotics upon each other is of great importance. A combination of drugs is synergistic when its lethal effect upon the bacteria is greater than that of the more bactericidal drug alone, and antagonistic when its effect is less (Elek, Hilton and Jewell, 1953). It must be stressed that the terms 'synergism' and 'antagonism,' unless otherwise specified, refer to the interactions at the bactericidal level (Jawetz and Gunnison, 1952). Not infrequently there is no demonstrable interreaction: the more potent drug exerts its characteristic lethal effect on the bacteria, without any apparent interaction by the other drug.

Such interference effects depend not only upon the drugs but also on the bacteria tested. On the assumption that fundamentally the action of a given drug upon a sensitive microbe is always qualitatively the same, certain generalizations have been suggested. Jawetz and his associates suggested that antibiotics may be divided into two broad groups: group I (penicillin, streptomycin, bacitracin, neomycin and polymixin) comprises drugs with marked bactericidal effect, while group II drugs (chloramphenicol, the tetracyclins and erythromycin) exert a bacteriostatic, but no significant, lethal effect. They found that pairs

of group I antibiotics are generally synergistic, while pairs consisting of group I and group II drugs are often antagonistic (Jawetz, Gunnison, Gruff and Coleman, 1952). Clearly these are only general indications, to which there are many exceptions, and the results on any given organism by a pair of antibiotics cannot be forecast.

### Laboratory Tests of Combined Action at the Bactericidal Level

There is no alternative method to the enumeration of surviving bacteria after exposure to the combined action of drugs. Comparison of the findings with those obtained under identical conditions and concentrations, using the drugs singly gives the required information. The complexities of such tests are apparent. Repeated viable counts with various mixtures are impossible to carry out in the routine laboratory and simplified methods have been suggested. It must be borne in mind that *in vivo* theoretically all kinds of concentration-ratios can occur between antibiotics and that the effect observed may also vary with the time chosen to measure the bactericidal effect. Thus the laboratory tests represent a compromise. Provided only a single concentration of each drug to be tested is used and a simplified viable count is employed, synergism or antagonism is relatively easy to determine (Martin, Sureau and Chabbert, 1952). It is customary to base the concentrations employed on the levels obtainable in the body by ordinary dosage. Varying concentrations in all combinations can be tested by diffusion methods followed by sampling for surviving organisms (Elek and Hilsen, 1954). Some of the work can be curtailed by omitting combinations unlikely to be of value, and by employing only one member of a group of antibiotics which have a related mode of action, i.e. the tetracyclines (Jawetz, Gunnison, Coleman, Kempe, Miedema, Merrill, Chandler and Stone, 1955).

### Clinical Requirements of Combined Therapy

Clinical experience indicates that adequate treatment by a single drug is generally successful. A bactericidal or even a bacteriostatic drug combined with the defences of the body generally turns the tide in favour of the patient, provided that bacteriostatic concentrations can be achieved in the tissues and maintained for a sufficient length of time, preferably without intermissions. There are only two sets of circumstances known in which treatment with single drugs has proved ineffective, although the organism appears sensitive *in vitro* to the usual bacteriostatic tests. One consists of bacterial infections where the normal body defences are inadequate. This is exemplified by subacute bacterial endocarditis, where the

anatomical arrangement of the valves does not allow an adequate inflammatory reaction to develop. Agammaglobulinaemia may possibly be another example. The second set of circumstances is more commonly encountered. Either because of the chronicity of the disease, as in tuberculosis, or because of the naturally high mutation rate of an organism to resistance to a given drug, antibiotic-resistant mutants emerge and viciate treatment. Combined therapy is essential in both circumstances, though for different reasons.

In one respect bacterial endocarditis has a unique pathology. Although many forms of untreated bacterial diseases may prove fatal, there are few other examples which are *invariably* fatal. In this disease the body defences are powerless and chemotherapy must wipe out the last survivor if relapses are to be avoided. This is illustrated by the fact that bactericidal drugs like penicillin were found to effect cures, while the newer antibiotics, which are mostly bacteriostatic, were disappointing. Most cases of subacute bacterial endocarditis are caused by penicillin-sensitive streptococci, and their treatment with this drug alone is generally successful. At least a tenth of all cases is caused, however, by enterococci, which are relatively resistant to penicillin. If streptomycin is added even in concentrations ineffective alone, the bactericidal effect of penicillin on enterococci *in vitro* is accelerated and complete sterilization follows (Jawetz and Gunnison, 1950). This combination has been successfully employed in many cases (Hunter, 1950; Robbins and Tompsett, 1951; Cates, Christie and Garrod, 1951). The ordinary 'sensitivity tests' in subacute endocarditis may be most misleading: what matters is the bactericidal effect and whether this can be achieved by the concentration that is obtainable *in vivo*. In this disease the laboratory testing of combination for synergism is not only warranted but definitely required (Pilkington, Elek and Jewell, 1956).

### Combined Therapy for the Suppression of Drug-resistant Variants

One of the most important problems arising from antibiotic therapy is the emergence of resistant variants. 'Acquired resistance' is threatening the usefulness of all the antibiotics at present in use. Although the term is accepted, in fact it is now generally believed that the antibiotics are not the direct cause of the change. A bacterial population is made up of individuals of varying resistance, including some which through spontaneous mutation exhibit abnormally high resistance to various antibiotics. If their more sensitive brethren are inhibited by the antibiotic, these resistant individuals continue to grow, producing

a drug-resistant progeny which, if sufficiently virulent, will continue the disease. To some extent these resistant variants can be kept in check by a continuous high level *in vivo* of the drug, since very resistant organisms are rare in a bacterial population. The incidence of resistant variants is, however, a character of the bacterial strain, and also of the drug. Thus resistant variants to streptomycin occur in relatively small bacterial populations with most bacteria, and this valuable drug more than any other, requires a partner for this reason.

There is nothing we can do about the incidence of spontaneous drug-resistant mutants: the mutation rate is part of the creative force. It is, however, possible to restrict a bacterial population, that is the total number of live bacteria in the body of a patient. Since the incidence of mutants bears a relationship to the total number of bacteria, bactericidal drugs by reducing the viable population lower the chances of resistant mutants to a second drug. In chronic infections and in particular in tuberculosis the total population over a certain length of time may be very large indeed. Even though the disease is kept in check by a destruction of the same number of bacilli as the newly-formed ones, each individual contributes to the pool of potentially resistant mutants. Resistance frequently develops to streptomycin, isonicotinic acid and p-aminosalicylic acid when used singly. When the drugs are given in combination, before a mutant can actually grow it must be simultaneously resistant to two or more drugs. The mathematical chances of this are remote: if the incidence of mutants is 1 in a million to one drug, and 1 in a million to the other, the odds of a single mutant resistant to both drugs is 1 in a million times a million. In infection there may well be a few million organisms in the body, but a million times a million bacilli represent about 10 litres of a good laboratory culture of a fast-growing bacillus. Such enormous populations are unlikely to occur, and this is the explanation of the success of combined antibiotic treatment in preventing the emergence of resistant mutants.

Once a resistant strain to one drug has emerged, these considerations no longer hold. The use of this drug is then contraindicated in combined treatment. It is entirely without action and the other drug acts as if given singly. In this way, by giving *one drug after the other*, it is possible to breed out strains which are simultaneously resistant to many antibiotics. This is what happened in staphylococcal surgical infections acquired in hospitals. In preventing resistant

mutants by combined therapy both drugs must be at least bacteriostatic to the organism.

Mitchison (1954) advises in the treatment of tuberculosis that no chemotherapeutic drug should ever be given alone, since development of resistance limits its action and prevents its successful use in later combined therapy. This advice also holds for other chronic infections and also for the treatment of acute infections in circumstances when resistant mutants readily emerge. Thus treatment of urinary infections by streptomycin alone is generally disappointing, but combined therapy may utilize the bactericidal effect of streptomycin in full (Stern and Elek, 1955). Had combined treatment been used early in combating staphylococcal infections in hospital, it may be that the number of multiple antibiotic resistant strains would be less today.

### Conclusions

The indiscriminate use of 'shot-gun therapy' is undesirable. Many of the newer antibiotics have pharmacological properties which restrict their usefulness. These have to be weighed against the possible advantages which may accrue from their use in combinations. The fuller understanding of the way in which antibiotics help to bring about a cure indicates that combinations should not be given blindly in the hope that it will deal with any microbic infection. Combinations should be reserved for particular problems, in which they are capable of achieving results unobtainable by single drugs. This, however, requires not only the identification of the infecting organism, but also careful laboratory tests which can forecast the usefulness or otherwise of various combinations.

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