ANTIBIOTIC MIXTURES*

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The merits of treatment with more than one antibiotic have been debated for years and are still a subject of controversy. Of the 5 usually recognized indications for such treatment[1] 3 have little to commend them, and 2 deserve more serious consideration.

INDICATIONS FOR COMBINED TREATMENT

Diagnosis unknown. A patient may clearly be suffering from a serious infection, but its nature and even its site may not be evident. It is then tempting for the time being to administer 2 antibiotics to cover the main possibilities. This should never be done until all material necessary for a bacteriological diagnosis has been obtained, and is even so of doubtful utility. It may well be preferable to make a specific provisional diagnosis and treat accordingly, changing the treatment if it fails or if the true diagnosis proves different.

Diminished risk of toxicity. This assumes that a diminished dose of each component of a mixture will suffice, but most authorities maintain that full doses of each are necessary. (On the other hand this claim is valid for mixtures of sulphonamides, since their individual doses are smaller and there is hence a reduced risk of tubular or ureteric blockage.)

Mixed infections. These may be best controlled by a broad-spectrum antibiotic: tetracyclines, for instance, are the drugs of choice for peritonitis due to perforation of the lower bowel. On the other hand, if two bacteria with totally different antibiotic sensitivities are demonstrably involved, such as *Strep. pyogenes* and *Ps. pyocyanea,* it may be necessary to aim an antibiotic at each: in this example these might be penicillin and polymyxin.

These are the lesser indications: the two following are of more importance.

Prevention of acquired bacterial resistance. There are sound theoretical grounds for supposing that it is far more difficult for bacteria to acquire resistance to an antibacterial agent in the presence of another one, provided that they are sensitive to both. Practical confirmation of this has been abundantly forthcoming in the field of tuberculosis, where, owing to the long duration of treatment, it is consequently imperative to use mixtures. There is no similar evidence about any other infection, but nor is there any reason for supposing that the same mechanism will not operate, whatever the bacterial species. On the other hand, there are not many situations in which combined treatment is advisable for this reason in the interests of the patient himself; it has been used with apparent success for the benefit of a whole hospital population, to discourage the acquisition of multiple resistance in staphylococci.[2]

To achieve synergy. This term refers properly to an effect unobtainable with either component alone, or exceeding the sum of those produced by each component acting alone. The clearest example of it is the combined action of penicillin and streptomycin on *Strep. faecalis:* this is totally bactericidal, whereas that of penicillin is only to reduce the number of survivors, and that of streptomycin—in the concentration used in a test or achieved in the body—may actually be *nil* (Fig. 1). The efficacy of

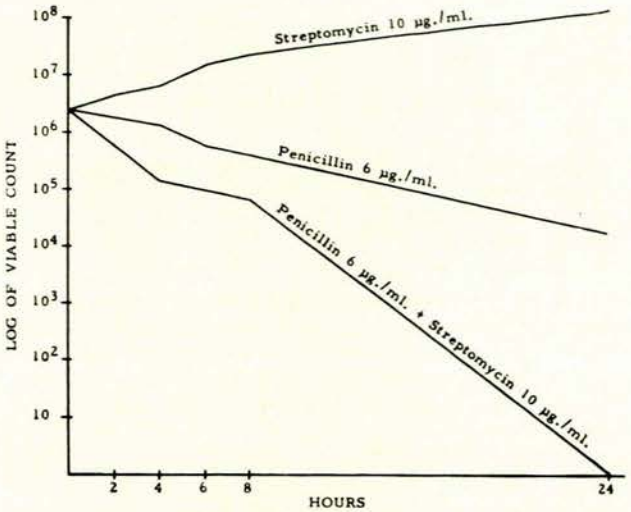

Fig. 1. Viable counts in broth containing an optimal concentration of penicillin (6 µg./ml.), streptomycin (10 µg./ml.) or both, and inoculated with a strain of *Strep. faecalis* (G.F.) from the blood of a patient with bacterial endocarditis. Penicillin causes only a slow fall in the viable count. Streptomycin fails to prevent growth (MIC was 16 µg./ml.): the combination sterilizes the preparation. This combination was used successfully in treating not only the original attack of endocarditis but a recurrence eight months later. [From Barber, M. and Garrod, L. P. (1963): *Antibiotic and Chemotherapy.* Edinburgh: E. & S. Livingstone—reproduced with the publishers' kind permission.]

this combination in treating endocarditis caused by this organism is well known. It may also be effective in endocarditis caused by highly penicillin-resistant strains of *Strep. viridans*[3] and by penicillin-resistant staphylococci (Fig. 2).

Few combinations exert so striking an effect: others may be merely additive or indifferent, and some are actually antagonistic, one antibiotic interfering with the action of the other. The type of effect to be expected can be deduced on the following principles:

THEORY OF COMBINED ACTION

The law of combined action propounded by Jawetz and Gunnison[4] classifies antibiotics according to whether they are bactericidal (e.g. penicillin, streptomycin, neomycin, kanamycin, polymyxin) or merely bacteristatic or growth-inhibitory (of which the clearest examples are chloramphenicol and tetracyclines). The type of action depends

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on how these classes are combined, as follows:

Bactericidal + bactericidal — synergic
Bacteristatic + bacteristatic — additive
Bactericidal + bacteristatic — antagonistic

It should be understood that none of these effects is invariable: they are conditioned by antibiotic concentrations and by not only bacterial species but also the properties of individual strains. An *ad hoc* test is strongly advisable to confirm, for instance, that a given combination is synergic.

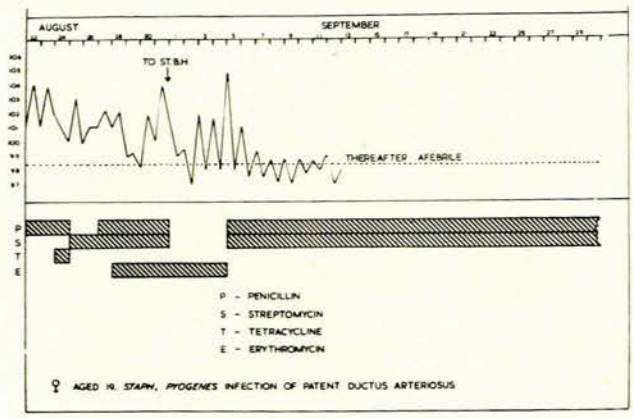

Fig. 2. Successful treatment of *Staphylococcus pyogenes* infection of a patent ductus arteriosus with penicillin and streptomycin.

Female patient aged 19 was admitted to St. Bartholomew's Hospital (St.B.H.) after treatment elsewhere. The staphylococcus was resistant to penicillin, but sensitive to other antibiotics. Tests of combined bactericidal action showed that only penicillin plus streptomycin was completely bactericidal. Penicillin (10 mega units) and streptomycin and dihydrostreptomycin (1 G each) were given daily in divided doses for 3 weeks, followed by 3 weeks of the same dose of penicillin and half the previous dose of streptomycins. Recovery was complete and the patent ductus was later ligated. [From Dormer, A. E. (1960): Brit. Med. Bull., **16**, 61—reproduced by kind permission of author and editor.]

Antagonism has been the subject of much dispute, and some question its reality. Its explanation is clear: most bactericidal antibiotics can only kill multiplying bacteria, and if an inhibitor is also present, multiplication is prevented and killing cannot occur. This is easily verified both *in vitro* and in the experimental animal: a dose of penicillin achieving a high cure-rate in streptococcal infection in mice loses much of its effect if chloramphenicol or a tetracycline is given in addition.[5] The clearest example of antagonism in the clinical field is the comparison made by Lepper and Dowling[6] of the effect of penicillin alone (given in large, frequent doses intramuscularly) and that of the same dose of penicillin together with chlortetracycline, in pneumococcal meningitis. It is enough to say that the mortalities in the two series of patients were respectively 30 and 79%. In this special example the relatively low concentrations attained in the cerebrospinal fluid must have favoured the antagonistic effect. Another illustration is Strom's[7] studies of the treatment of scarlet fever with each of these antibiotics or both together. After combined treatment haemolytic streptococci reappeared in the throat more often than after treatment with the same dose of penicillin alone.

There are several exceptions to the 3 simple rules on combined action defined above. It has rightly been pointed out by Manten and Wisse[8] that antibiotics such as polymyxin which kill not only multiplying but resting cells, are not antagonized by bacteristatic antibiotics, although these authors' inclusion of streptomycin, neomycin and kanamycin in this special category must be contested. They also point out that sulphonamides, although purely bacteristatic, do not antagonize the action of bactericidal antibiotics such as penicillin: this has long been recognized. A further qualification suggested by Garrod and Waterworth[9] concerns erythromycin and novobiocin, which are bacteristatic in low concentrations and bactericidal in higher: their concentration can therefore determine whether the combination with a bactericidal antibiotic is antagonistic or synergic. This dual effect is readily demonstrable by the cellophane transfer technique.[9]

LABORATORY TESTS OF COMBINED ACTION

Although the action of a mixture on a given organism can be predicted on this theoretical basis, the behaviour even of different strains within a species is inconstant. All authors who have studied multiple strains have found this: the rules are never invariably obeyed, either because of minor differences in the degree of sensitivity to the individual antibiotics or for reasons unknown. It is therefore strongly advisable that the expected efficacy of a mixture should be verified by an appropriate test.

Bacterial endocarditis caused by an organism not fully sensitive to penicillin most imperatively demands this. This is a disease in which treatment must exterminate the entire bacterial population of the lesion: if only a few cocci survive, relapse is almost certain. It is therefore necessary to find an antibiotic or mixture which will actually sterilize a preparation containing large numbers of the organism. There are two methods for this, each perfected by Chabbert, and both also described by Garrod and Waterworth:[9] only their outlines are given here.

Test in liquid medium with subculture. To tubes of a suitable liquid medium antibiotics are added singly and in every possible combination, all in a fixed concentration of 10 μg./ml., this being taken as that obtainable in the blood by full dosage. Each tube receives a measured large (at least 10^5 per ml.) inoculum of the organism. After overnight incubation the tubes are plated: if any subculture yields no growth, bactericidal action has been total and that antibiotic or mixture can be recommended for therapy.

Cellophane transfer method. The inoculum is carried on a sheet of sterilized cellophane stretched over a shallow glass cylinder ('tambour') slightly narrower than a Petri dish. This is first placed on a plate of culture medium into which antibiotics have diffused from 2 strips of blotting paper placed at right-angles. Both nutrients and antibiotics pass through the cellophane and the bacteria will grow where the antibiotic concentration is not inhibitory. The tambour is then transferred to a plate of normal medium, and bacteria which have been inhibited but not killed will then also grow. The area of most interest after the second stage of incubation is that where both antibiotics are present (i.e., corresponding to the junction of the 2 original strips). This may be free of colonies although some grow along the more distal part of each strip (synergy) or show profuse growth, whereas in the distal areas growth is sparse (antagonism). The method is greatly superior to velvet pad transfer, since the whole population is transferred, without which total bactericidal action cannot be confirmed. Differing

effects according to the absolute and relative concentrations of the two antibiotics can also be observed.

This second method cannot be commended for general use: it requires special apparatus, and minor technical difficulties are frequent except in experienced hands. The first should be within the capacity of any large hospital laboratory. It is time-consuming if many antibiotics are tested, but may be simplified by excluding (1) antibiotics which rarely participate in synergy (tetracyclines and chloramphenicol), (2) those which are more toxic or difficult to administer (bacitracin, neomycin, vancomycin and ristocetin). This leaves penicillin(s), streptomycin, kanamycin, erythromycin and novobiocin, a small group which has provided almost all the successful combinations for treating endocarditis in my own experience. If nothing more is done, even a simple 3-tube test with penicillin, streptomycin and both together will show whether this mixture is superior to penicillin alone for treating any *Strep. viridans* endocarditis.

PROPRIETARY MIXTURES

If manufacturers are to be believed, the indications for using antibiotic mixtures are very numerous. When no new antibiotic is available, the temptation to market a mixture which, whether new or not, can at least be given a new proprietary name, is evidently strong. Moreover, inclusion in a mixture may provide a commercial use for an inferior antibiotic. The objections to the widespread use of mixtures, and to their over-enthusiastic advocacy in advertising material, were forcibly voiced by Dowling[10] in a report to the Council of the American Medical Association.

There are three main types of mixtures now on the market: those of bactericidal antibiotics, which may have a synergic action; those of bacteristatic drugs, the action of which according to the Jawetz law is likely to be only additive; and those of an antibacterial antibiotic with an antifungal.

BACTERICIDAL MIXTURES

The best known of these is penicillin + streptomycin, and its merits for treating certain forms of endocarditis have already been commended. Even for this purpose, however, a proprietary mixture will not often be employed, because the fixed ratio between the two components may not be suitable: a higher proportion of penicillin will often be indicated. This type of action may well be exerted on other bacteria, although there is little proof of this. The commonest use of these products is not aimed at any specific infection, but at mixed or undiagnosed infections, or at simple prevention of infection, either of the operation site or of the lungs, in surgical patients.

Not only is there much evidence that antibiotic 'cover' for most kinds of operation fails in its object,[11] and even that septic complications are commoner when it is given, but this practice involves two grave dangers. Treatment with penicillin and streptomycin strongly predisposes to acute staphylococcal enterocolitis,[12,13] particularly after operations on the stomach or colon. Secondly, in patients with unrecognized impairment of renal function, only a few doses of streptomycin can cause irreparable vestibular damage. In Cawthorne and Ranger's[14] series of 21 patients with this condition, 12 had received a total dose of 10 G or less of streptomycin, and in several it had been given as cover for a clean operation. The deafness which may be caused by including dihydrostreptomycin in such mixtures is an even graver handicap: in the series of 32 such patients reported by Shambaugh *et al.*,[15] 18 had been given 10 G or less and 9 not more than 3 G, and in 6 patients the object was cover for surgery (appendicectomy, 3 cases, and hysterectomy, 3 cases). The infliction of such an injury for a purpose of very doubtful utility is intolerable.

The possibility of causing eighth nerve damage is reason enough, if there were no other, for restricting the use of streptomycin to specific and serious indications: 'shot-gun' treatment and preventive use can rarely be advisable.

BACTERISTATIC MIXTURES

Several of these have been devised, and two at least appear to be extensively used. According to the Jawetz law, they should have no more than an additive effect, but there are claims that bacteristatic synergy has been demonstrated by some form of *in vitro* test, and in some instances in therapeutic tests in mice. Much of the literature about them is concerned with clinical studies, few of which were properly controlled trials.

A method of studying the action of such mixtures, which commands attention, is the *in vivo—in vitro* method used by Finland and his colleagues. The antibiotics are given separately and together to normal subjects, and blood is withdrawn at intervals: the capacity of the serum to inhibit the growth of appropriate pathogenic bacteria is then determined quantitatively. The results of this form of test, which takes account of the extent and rate of absorption as well as other factors, must reflect the forces operating within the body, and should be accorded more significance than anything else less than a comparative therapeutic trial. In a series of studies by this method, involving 7 different mixtures referred to later, no more than an additive effect was ever observed, and the combined action of the mixture was sometimes inferior to that of the same dose of one of its components.

Tetracycline and oleandomycin. A 2:1 mixture of these 2 antibiotics (sigmamycin) was found by English *et al.*[16] to have a synergic effect in simple tests of bacteristatic action in broth on 9 out of 22 strains of staphylococci. I found no such effect in tests with 56 strains of my own, or when I repeated the tests of English *et al.* with one of their own strains, which in their hands showed a high degree of synergy.[17] The *in vivo—in vitro* method[18] was applied in an extensive study not only of this mixture, but of that of tetracycline with other macrolides. So far from showing any virtues in the tetracycline-oleandomycin mixture, this study concludes that if any macrolide is to be added to tetracycline it should be erythromycin, which is much the most active of this group. When 250 mg. was given with 500 mg. of tetracycline, the expected additive effect was obtained, but the addition of the same amount of either oleandomycin or spiramycin had no perceptible effect.

Tetracycline and novobiocin. Hirsch and Finland,[19] in reporting their study of this mixture, refer to earlier papers in which it was found to be synergic, both *in vitro* and in therapeutic tests in staphylococcal infection in mice. It exhibited no synergy against the 4 organisms (1 strain of streptococcus and 3 of staphylococci) used in their

in vivo—in vitro tests, and the latter showed rather less than an additive effect: indeed the single antibiotics seem consistently to have produced rather more activity in the serum than the same total dose of the mixture.

Other combinations tested by this group of workers, with results consistently unfavourable to the idea that they have any therapeutic advantage, are those of erythromycin with chloramphenicol, and of penicillin with oleandomycin, chloramphenicol, tetracycline or novobiocin.

Tetracycline and antifungal antibiotics. These mixtures are in a different category, the purpose of the second component being simply to prevent the overgrowth of *Candida* (monilia) in the alimentary tract. Nystatin is certainly indicated when this occurs as a result of tetracycline therapy (when tetracycline should also preferably be stopped), and in special circumstances the initial administration of both together may be advisable. It is another question whether this type of mixture should be used regularly in place of tetracycline alone, as has been suggested.

Three aspects of this question deserve consideration, one of which is the efficacy of such treatment. Larkin[20] reports good results, but Rein, Lewis and Dick,[21] in a larger series of patients, found little difference in the frequency of gastro-intestinal side-effects between groups given tetracycline alone and tetracycline + nystatin. This is not to deny that nystatin will suppress monilial overgrowth, but to question how often this is responsible for symptoms. Diarrhoea may more often result from simple chemical irritation or from the overgrowth of resistant coliforms. The second question concerns *safety*. Nystatin is harmless, but the form of Mysteclin-V intended for use in children contains amphotericin. Although only a small proportion of an oral dose of this antibiotic is absorbed, its formidable toxicity demands extreme caution, and even an apparently remote risk deserves to be weighed carefully against the relatively trivial benefits obtained.

The third consideration is the possibility that widespread use of the polyene antifungal antibiotics may lead eventually to the appearance of resistant strains of *Candida*. Such resistance is difficult to induce *in vitro*, and has not been observed clinically, but with the example of penicillin resistance in gonococci, which took 15 years to develop, who can say what may happen in the future? There is cross-resistance between nystatin and amphotericin, and acquired resistance to either would therefore deprive us of the only means of treating systemic candidiasis. As pointed out in *Medical Letter*,[22] in an assessment concluding that 'the routine prophylactic use of antifungal agents along with tetracyclines cannot be justified' the margin between the inhibitory concentration of amphotericin for fungi and the 'maximum achievable blood levels' is ominously narrow, and a small increase in resistance could lead to loss of all therapeutic effect.

DISCUSSION

There are a few restricted purposes for which a combination of antibiotics is essential, even to save life. To determine which of these will serve, is one of the more difficult but most rewarding of laboratory exercises. A combination may also be directed against 2 different organisms in a double infection: its use in a mixed infection of a nature not fully ascertained is less commendable or likely to succeed. There are also circumstances in which combinations may be indicated in order to discourage acquired bacterial resistance.

Apart from these uses, deliberately aimed at a specific object, the proper place of combinations in therapeutics is doubtful, and in particular that of fixed commercial combinations, the properties of which have been very briefly reviewed here. There is one objection to their use which has not been mentioned: that it is detrimental to rational therapy. It would be possible to devise a mixture of antibiotics active against every known susceptible microorganism, and to treat every patient with it, not troubling to attempt a bacteriological diagnosis. This would be shotgun therapy at its worst, but the frequent use of combinations is a long step on the same road. It is surely more in accordance with the principles of scientific medicine to make a precise diagnosis and to prescribe the antibiotic known to be most active against the infection concerned. Not only is the maximum therapeutic effect obtained, but experience gained in this way is a better guide for the future.

SUMMARY

Of the 5 usually admitted reasons for prescribing a mixture of antibiotics, the 2 most acceptable are the prevention of acquired bacterial resistance and the achievement of a synergic effect.

The action of mixtures may be synergic, additive or antagonistic, depending on whether the antibiotics are bactericidal or bacteristatic.

A synergic mixture may be essential for the treatment of some forms of bacterial endocarditis. Two forms of test with the responsible organism enable its action to be verified before treatment is begun.

Proprietary mixtures of antibiotics fall into 3 main classes, 2 of which are used for less well-defined purposes. Evidence of their efficacy is conflicting, and their use is not without drawbacks.

REFERENCES

1. Garrod, L. P. (1953): Brit. Med. J., **1**, 953.
2. Barber, M., Dutton, A. A. C., Beard, M. A., Elmes, P. C. and Williams, R. (1960): *Ibid.*, **1**, 11.
3. Cates, J. E., Christie, R. V. and Garrod, L. P. (1951): *Ibid.*, **1**, 653.
4. Jawetz, E. and Gunnison, J. B. (1952): Antibiot. et Chemother. (Basel), **2**, 243.
5. Speck, R. S. and Jawetz, E. (1952): Amer. J. Med. Sci., **223**, 280.
6. Lepper, M. H. and Dowling, H. F. (1951): Arch. Intern. Med., **88**, 489.
7. Strom, J. (1955): Antibiot. Med., **1**, 6.
8. Manten, A. and Wisse, M. J. (1961): Nature (Lond.), **192**, 671.
9. Garrod, L. P. and Waterworth, P. M. (1962): J. Clin. Path., **15**, 328.
10. Dowling, H. F. (1957): J. Amer. Med. Assoc., **164**, 44.
11. Taylor, G. W. (1960): Brit. Med. Bull., **16**, 51.
12. Fairlie, C. W. and Kendall, R. E. (1953): J. Amer. Med. Assoc., **153**, 90.
13. Fowler, B. J. (1955): Brit. Med. J., **1**, 1313.
14. Cawthorne, T. and Ranger, D. (1957): *Ibid.*, **1**, 1444.
15. Shambaugh, G. E. jnr., Derlacki, E. L., Harrison, W. H., House, H., House, W., Hildyard, V., Schuknecht, H. and Shea, J. J. (1959): J. Amer. Med. Assoc., **170**, 1657.
16. English, A. R., McBride, T. J., Van Halsema, G. and Carlozzi, M. (1956): Antibiot. and Chemother, **6**, 511.
17. Garrod, L. P. (1957): Brit. Med. J., **2**, 57.
18. Jones, W. F. and Finland, M. (1957): New Engl. J. Med., **257**, 481.
19. Hirsch, H. A. and Finland, M. (1960): *Ibid.*, **262**, 209.
20. Larkin, R. (1959): Lancet, **1**, 1228.
21. Rein, C. R., Lewis, L. A. and Dick, L. A. (1957): Antibiot. Med., Brit. Ed., **4**, 771.
22. The Medical Letter on Drugs and Therapeutics (New York) 1961, **3**, 33.