THE USE OF COMBINATIONS OF ANTIMICROBIAL DRUGS[1]

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Twenty years have passed since synergism between antibiotics was first described (1, 2). Several reviews have covered the earlier studies (3–7). As recounted by Dowling (6), the subject of combined antibiotic action has aroused a good deal of emotion and controversy from time to time. In recent years, interest in the investigation of combinations of antibiotics has only "simmered along," but the subject continued to be of practical concern to the drug industry and to practicing physicians. The high-pressure sale of fixed drug combinations has violently antagonized clinical teachers and investigators (8–10), but it has provided a handsome return to pharmaceutical houses. Many advertisements, the "siren song of the purveyor of pills" (11), still advise physicians that administration of drug combinations helps to "cover all possibilities." Physicians believe that, in general, antimicrobial drugs are widely effective and fairly harmless; and in their search for "security" in treatment, they prescribe antibiotic combinations. This desire for security, shared by people of all ages and all walks of life, has led to the impression that if one antimicrobial drug is good, two should be better, and three should cure virtually everybody of virtually everything. Emotions and thoughts such as these may have contributed to the continuing extensive use of combinations of antimicrobial drugs. Perhaps the best explanation of this phenomenon was provided by Mr. Charles Schulz, that leading observer of the American scene.

Yet, the practice of prescribing more than one antibiotic is not limited to the much abused LMD or general practitioner. At the reputable Johns Hopkins Hospital, 11 per cent of 7094 patients, admitted during a three-month period in 1963, received from two to five antibiotics (12). Only an additional 7 per cent received a single antibiotic. The widespread use of antimicrobial drug combinations is not limited to the United States. Some years ago, a correspondent surveying the German drug market found hundreds of preparations containing several drugs and concluded: "It has become unfashionable to use one drug by itself" (13).

[1] I have not attempted to conduct a formal survey of the literature or to list all papers that bear some relationship to the topic of discussion. I have mentioned a few papers published as late as May 1967. Work carried out in my laboratory has been supported for many years by grants from the Burroughs Wellcome Fund. I have freely drawn on other reviews prepared by myself and by others, because I could not think of a truly novel way of presenting material which I considered important. I apologize for these unconventional features.

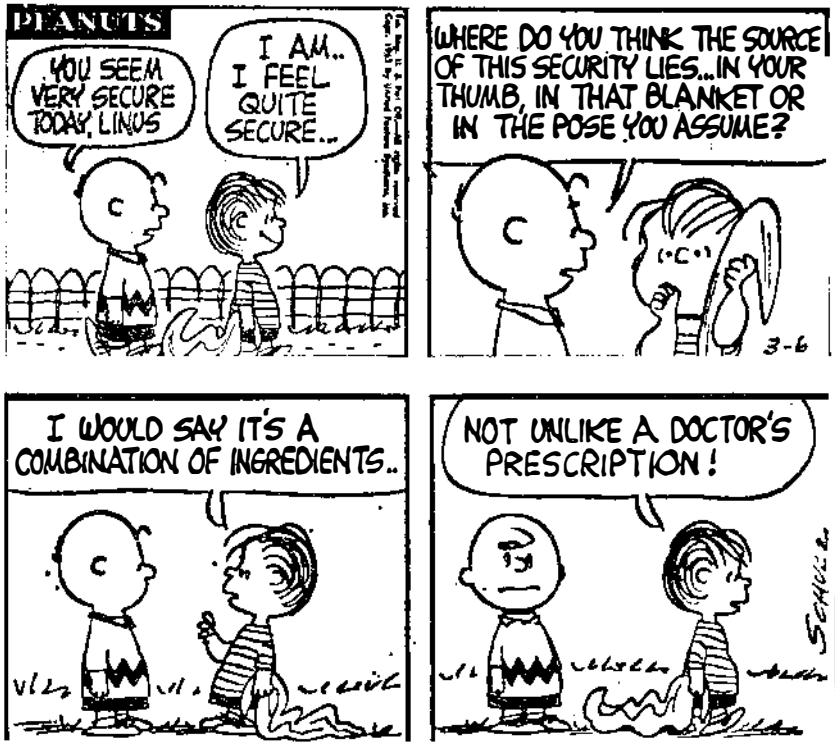

FIG. 1. (Reproduced by permission of Charles M. Schulz.)

While the indiscriminate use of antibiotic mixtures and combinations should be condemned, surely there must be some clinical situations in which the initial administration of more than one antimicrobial drug is medically accepted. Some of these possible indications for combined antimicrobial drug therapy are discussed below.

SOME CLINICAL INDICATIONS FOR COMBINED ANTIMICROBIAL DRUG THERAPY

Overwhelming infections.—In certain seriously ill patients suspected of having bacterial infections, it might be desirable to administer more than one antimicrobial drug in the hope of attacking the unknown etiologic agent. Prior to such treatment, all steps must be taken to initiate laboratory tests designed to make a prompt etiologic diagnosis. The drugs are chosen by "aiming" at the types of organisms most likely to cause the clinical picture in a given patient, and are administered only until laboratory identification of the etiologic agent permits specific treatment. In the past, bacterial meningitis of childhood was a leading example in this category. Simultaneous administration of penicillin, sulfonamide, and chloramphenicol was believed de-

sirable to cover the most likely etiologic organisms: pneumococcus, meningococcus, hemophilus (14). However, recently ampicillin used alone was shown to give more satisfactory results in childhood meningitis than the drug combinations employed earlier (15, 16). Furthermore, in at least one study (17), the addition of other drugs to ampicillin apparently reduced its therapeutic efficacy. Therefore, for the time being, it must be concluded that ampicillin may be the drug of choice in bacterial meningitis of unknown etiology, and that drug combinations are not indicated.

Sepsis, caused by gram negative bacteria, has been a problem of increasing magnitude in medicine, particularly in hospitalized patients (18–20). Such gram negative rod bacteremia occurs most frequently in persons subjected to urinary tract manipulation, or as a complication in the management of neoplastic disease with immunosuppressive drugs. If gram negative rod bacteremia is permitted to proceed to the development of endotoxin shock, the process may become irreversible. Consequently, there is urgent need to arrest bacteremia as early as possible. Until the laboratory can identify a specific etiologic organism, treatment with drug combinations aimed at the most likely culprits are often recommended. The most common organisms in gram negative rod bacteremia are coliforms, Proteus and Pseudomonas. Among the drug combinations to be considered are streptomycin plus chloramphenicol (21), kanamycin plus chloramphenicol (20, 22), kanamycin plus ampicillin (22), kanamycin plus polymyxin (20), streptomycin plus chloramphenicol plus penicillin (21), and many other permutations. Preferences vary from hospital to hospital and from year to year, depending on the antibiotic susceptibility of the organisms most commonly enountered in a given setting. Similar considerations are often applied to the management of sepsis during the neonatal period, which is most commonly caused by coliform bacteria.

Mixed infections.—In mixed infections, it is possible that two or more drugs, each acting on a separate portion of a complex microbial flora, may be more effective than one. This indication seems theoretically sound and might appear to justify the fairly frequent use of antimicrobial drug combinations. Actually, however, the occasions are rare when a mixed flora requires two drugs to be given together. In mixed infections of skin or wounds, mixtures of poorly absorbed, or systemically toxic, peptide antibiotics, (e.g. bacitracin, polymyxin) have been used topically with success. Secondarily infected joint or body cavities may occasionally require the local administration of drug combinations in order to eradicate a mixed infection. On the other hand, mixed infections of the peritoneal cavity, following perforation of a viscus, usually respond well to a single drug (tetracycline, ampicillin); the same applies to mixed infections of the respiratory tract (23). Mixed infections of the urinary tract are a common feature of chronic infections associated with anatomical and physiological abnormalities which make permanent eradication of the infection unlikely.

Rapid development of mutant strains.—In some clinical situations, the

rapid emergence of bacterial mutants which are resistant to one drug may impair the chances for cure. The addition of a second drug sometimes delays emergence of resistant strains. This effect has been demonstrated unequivocally in chronic tuberculosis and is the basis for the combined use of isoniazid, aminosalicylic acid, and streptomycin (and occasionally other, non-cross-reaction drugs) in that disease (24–27). The same principle may apply to other chronic infections and is the basis for the recommendation that erythromycin, novobiocin, streptomycin, or similar agents should not be given singly for prolonged periods because resistance to each drug is likely to develop (28–30). The recent success of pyrimethamine in combination with a sulfonamide in the treatment of chloroquine-resistant falciparum malaria is probably based on the delay in the emergence of resistant forms of the parasite (31–33).

Reduction of adverse reactions.—Sometimes the use of drugs in combination may reduce the incidence or intensity of adverse reactions. A given microorganism may be susceptible to each of two drugs but only in concentrations that are likely to give rise to severe adverse reactions. However, if the drugs are given concomitantly, each may be administered in half the concentration, below the threshold of adverse reactions. For example, a strain of Pseudomonas might be inhibited *in vitro* by polymyxin 5 μg/ml or by chloramphenicol 30 μg/ml, used singly; or by a mixture of polymyxin 2 μg/ml plus chloramphenicol 15 μg/ml. The latter levels can be achieved by the use of reasonably well-tolerated doses of each drug, whereas a dose of polymyxin B sufficient to sustain a serum level of 5 μg/ml may give rise to troublesome adverse reactions (34). However, such events are rare and require detailed laboratory evidence for support. Combinations have also been used in orthopedic surgery. A high concentration of a drug considered too toxic for systemic use is applied topically, and a second drug which enhances the activity of the first is given systemically. It is hoped that the two drugs meet in tissue to provide antibacterial activity without toxicity (35).

Drug synergism.—At times, the simultaneous use of two drugs achieves an effect not obtainable by either drug alone. One drug may specifically enhance the antibacterial activity of the second drug against a specific microorganism. Such an effect may be called, synergism–a term that has been much abused. Unfortunately, such desirable combined action is rare, unpredictable, and quite specific. A uniformly synergistic antimicrobial drug combination does not exist. A given combination of drugs must be specifically tailored by laboratory test to fit a specific microorganism, isolated from a specific patient. One of the best established examples of synergism is the cure of enterococcus endocarditis by penicillin in combination with other drugs, as compared to the usual failure of treatment with penicillin alone. Some features of the dynamics of combined antimicrobial drug action are discussed below.

In my opinion, a drug combination might be effective because of the simultaneous operation of several of the foregoing features, particularly those mentioned in the last three sections, when applied to particularly resistant

microorganisms against which, at a given time, no single highly effective drug is available. For example, a high proportion of cases of endocarditis caused by β-lactamase producing staphylococci was cured in pre-methicillin days by specially selected drug combinations which were effectively bactericidal *in vitro*, delayed the rapid emergence of resistance to individual drugs, and permitted the use of tolerable amounts of potentially toxic drugs (36). A similar approach to the selection of drug combinations may have to be applied to the treatment of methicillin-resistant staphylococcal infections which are now emerging (37). At present, there is a dearth of outstandingly effective drugs for the treatment of infections with certain gram negative bacteria. As discussed above, the management of gram negative rod bacteremia often involves a choice of drug combinations. The proper selection of such combinations–after an initial "informed guess"–may involve relatively complex laboratory tests, discussed below. These tests usually cannot distinguish unequivocally mechanisms involving synergism from those involving delay in the emergence of resistant mutants. Quite possibly, both may be involved. There are also claims for the efficacy of maximally tolerated doses of antimicrobial drugs given simultaneously to severely burned patients (38). Only comparative controlled studies will tell whether this effort to eliminate the microbial world by means of drugs is successful.

A critical reading of the preceding list of possible indications for antimicrobial drug combinations indicates that the choice of a combination on rational grounds is an infrequent event. In a large majority of clinical situations a single antimicrobial drug, properly chosen, will give optimal results. The basis for the initial selection of an antimicrobial drug must be the formulation of a specific etiologic diagnosis on clinical grounds, to be supported later by laboratory evidence. This process requires much thought; and hard thinking is hard work. Often, it is far easier to prescribe two or three antimicrobial drugs than to worry about defining the most probable etiologic agent. In my opinion, this aspect contributes heavily to the frequency with which physicians prescribe combinations. To balance this trend, it may be worthwhile to list a few of the disadvantages which may be incurred in the indiscriminate use of antimicrobial drug combinations.

Some Disadvantages of Combined Antimicrobial Drug Therapy

Due to the implied promise of "broader spectrum" and "greater efficiency," antimicrobial drug combinations, particularly fixed combinations, engender a false sense of security. This discourages efforts toward specific etiologic diagnosis, encourages inadequate dosage of drugs, and harms the patient.

The simultaneous administration of two or more antimicrobial drugs enhances the likelihood of adverse effects, and increases their variety. These reactions include direct toxic effects, the induction of hypersensitivity, and allergic reactions to each component drug of a combination. All these harm the patient and confuse the physician.

Unnecessary multiple drug therapy, especially in a hospital environment,

favors the emergence of microorganisms resistant to many drugs in that environment and thus contributes to the danger of hospital-borne infections (39).

It is incorrect and misleading to speak of a synergistic drug combination, without specifying a specific microbial strain. Thus, no fixed drug combination regularly results in such a desirable effect. Furthermore, the ratio of drugs in a fixed combination is unlikely to be suitable for a given patient, even if the individual constituents may be appropriate for the infecting microorganism. It is my opinion that none of the currently advertised fixed antimicrobial drug combinations has been proven by laboratory or clinical study to be more effective than one of the components of the mixture. Several editorials have vigorously condemned the sale and use of fixed antimicrobial drug mixtures (8–10).

Problems in Defining and Measuring the Effects of Combined Antimicrobial Drug Action

There is strong temptation to believe that the combined action of antimicrobial drugs must be governed by some general laws which can be formulated in mathematical models. Several such models have been published (40–44). I do not fully understand these mathematical models and, therefore, should not criticize them. However, I shall list some of my objections to the claims that the study of one model system is likely to reveal some generally applicable formula for combined antibiotic effects. Some studies (44) have employed a single test organism, and most of the studies have considered only a single drug combination. In my experience, different microorganisms and even different strains of the same type of organism behave differently toward the same mixture of drugs (3, 4), and the behavior toward one drug combination does not permit prediction of the behavior toward another drug combination. In terms of one criterion (bacteriostatic efficiency), the mixture of tetracycline plus erythromycin, used in two studies, might exhibit positive summation; in terms of another criterion (bactericidal efficiency), that same mixture generally fails to exhibit any advantage over the single drug. I have been unable to convince myself that the mathematical formulations permit any generalization. On the contrary, I feel that they point out the divergent results obtained with different methods of assessing antimicrobial activity of combinations and the difficulties of relating unequivocally any event *in vitro* to a desired therapeutic outcome in human diseases.

Even when technically feasible, chemical estimates of the concentration of antimicrobial drugs usually do not mirror accurately their antimicrobial activity. Direct evaluation of bacteriostatic or bactericidal activity is the only meaningful way of measuring their effects. Several methods can be employed and each raises questions as to its applicability to the measurement of combined drug action, and its pertinence to clinical therapy. It is obvious that the type of measurement performed will determine the type of definition which can be applied to different results of antibiotic interactions. The most

common methods are the following: (*a*) Bacteriostatic effect: Endpoints are expressed as the minimum amount of drug necessary to suppress visible growth of a microorganism under a given set of laboratory conditions for a given time. (*b*) Bactericidal effect as shown by the rate of killing: Results are expressed as the bactericidal rate, i.e. the slope of the plot of viable survivors at various time intervals, under a given set of laboratory conditions. (*c*) Bactericidal effect as shown by the completeness of killing: Results are expressed as the smallest concentration of drug resulting in a fixed number or proportion of viable survivors at a fixed time, under a given set of conditions. (*d*) Therapeutic effect in experimental infections is expressed as either the prevention of death, or of lesions, or the prolongation of life, or the eradication of infecting microorganisms. (*e*) Therapeutic effect in natural infection is expressed as either clinical cure of a disease with suitable follow-up, or suppression or eradication of infection with significant alteration of the natural course of the disease.

The results of any one method of evaluation need not coincide with those of another method because each may measure different events in the test system. *In vivo* evaluation need not coincide with *in vitro* results. The following example should indicate that even different *in vitro* evaluations may provide different answers. In a certain concentration, a drug may be markedly inhibitory but only slightly bactericidal against a given microorganism. The importance that the physician attaches to either of these measurements will determine his choice of method. If he believes that the principal action of an antimicrobial drug in a certain infectious process is microbial inhibition (e.g. bacterial pneumonia), then he will be satisfied with methods that primarily measure bacteriostatic effects. If he believes that the essential chemotherapeutic action in a given disease (e.g. bacterial endocarditis) requires rapid killing of microorganisms, he will insist on a method which estimates bactericidal effects. Neither method can be designated as better; it must be chosen according to the problem at hand.

Even within a given test system, the results may depend on drug concentration, time of interaction, inoculum size, and other laboratory variables and on the meaning attached to a given event. The example in Figure 2 is redrawn from our earliest publication on measurement of combined antibiotic effects (**45**). It is evident that at time *I*, fewer bacteria survive exposure to drug A than to the combination of drugs A plus B. Thus, in terms of early bactericidal effect, the combination of A plus B is less effective than A alone—an example of antibiotic antagonism. On the other hand, at time *II*, there are fewer surviving bacteria with A plus B than with A alone, which could be interpreted as an additive effect. The interpretation of such test results obviously depends on the importance attached to early bactericidal action and to completeness of bactericidal effect, respectively.

The fallacy of comparing single drugs with combinations on the basis of weight must also be considered. It is possible to determine experimentally one minimal effective dose (MED) (bacteriostatic, bactericidal, or curative)

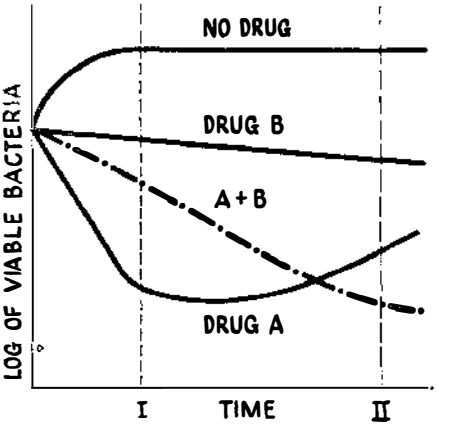

FIG. 2. Schematic representation of count of viable bacteria against time, indicating the effect of Drug A, Drug B, or the two drugs A+B.

and express it in terms of the weight of a drug. The temptation may then be great to claim synergism for a combination which contains less than one-half MED of each of two drugs and which possesses greater activity than would be expected on the basis of arithmetic summation. The fallacy of such a claim (inherent in some mathematical models, in my opinion) lies in the assumption of a linear relationship between drug weight and effectiveness. The relationship between weight and effect of a drug can be estimated experimentally above one MED only, by definition of the MED. However, below one MED, the relationship might be as shown in X or Y in Figure 3. Thus, the effect of

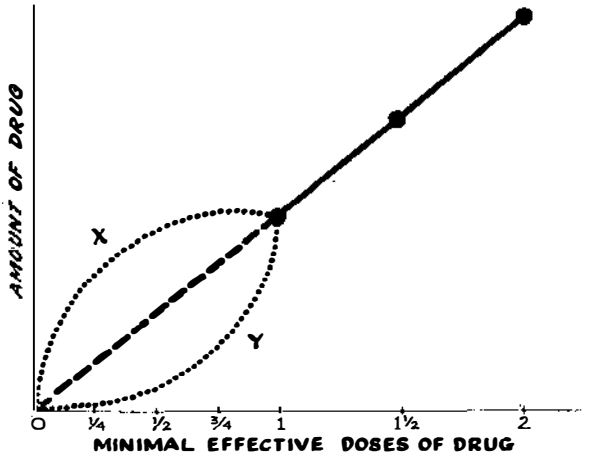

FIG. 3. Hypothetical relationship between the amount (weight) of a drug and its antimicrobial effect.

one-half MED of drug A plus one-half MED of drug B might be greater than, equal to, or less than the effect of one MED by weight of either drug alone. In the past, this point has often been used as the basis for claims of synergism (46), although it does not provide such evidence in my opinion (3, 4).

Dynamics of Combined Antimicrobial Drug Action

The literature prior to 1958 has been reviewed (4–6). Only a small number of publications have dealt with the topic since, and only a few selected ones will be covered here. A brief synopsis of my views, based largely on our own studies, will be given here.

When two antimicrobial drugs act simultaneously on a homogeneous microbial population, the resulting effect usually falls into one of three types—indifference, antagonism, synergism. By far the most common result is indifference (48). The effect of combining the two drugs is equal to that of the single more active component of the mixture or is equal to the arithmetic sum of the effects of the two individual drugs. The same total effect can be obtained with a single drug used in a dose equivalent to that of the mixture. I believe that the large majority of drug combinations employed by physicians give end results in the category of indifference. Nevertheless, the simultaneous use of two drugs in the indifferent category might be justified for one of the reasons given above under "Clinical Indications."

Antagonism

It has been pointed out by Lankford & Lacy (49) and by Spicer (48) that a mixture of two antimicrobial drugs might well be less effective than a single agent. Antagonism may be defined as a combined drug effect which is smaller than the algebraic sum of the effects of the single drugs present in the mixture. Since this is difficult to measure, it is preferable to restrict the term, antagonism, to those instances where a combination of antimicrobial agents results in a total effect smaller than that produced by the more effective single member of the combination when acting alone.

Antagonism can be demonstrated *in vitro* by a decrease either in the inhibitory activity (46) or in the bactericidal rate of a drug mixture below that of its components (50–52). The following general characteristics apply to the latter type of antagonism (3, 4, 47): For optimal demonstration of antagonism *in vitro*, a minimal bacteriostatic amount of the interfering agent must be added to a bactericidal quantity of the effective drug, as shown schematically in Figure 4. Antagonism can be demonstrated only when the drugs act on organisms that are capable of multiplication.

Antagonism is obscured by a large excess of either one of the participating agents. Antagonism is limited, not only by dose, but also by time relationships: The interfering agent must act on bacteria either simultaneously or before the bactericidal drug, not after. These doses and time limitations explain, in part, the ease with which antagonism can be demonstrated *in vitro* and in single-dose treatment of experimental animals (53, 54), and the diffi-

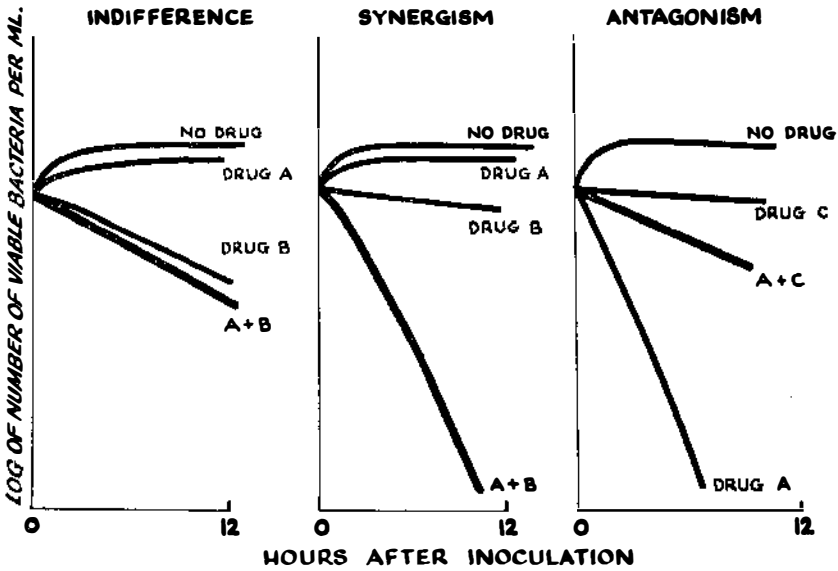

FIG. 4. Types of combined action of antimicrobial drugs. Schematic representation of bactericidal action *in vitro* showing the possible types of results seen when one drug, or two drugs, act on a homogeneous population of bacteria, under conditions permitting growth.

culty of observing antagonism in multiple-dose treatment of animals or man (55, 56). In the latter case, the ever-changing blood and tissue levels of two drugs reduce the opportunity for observing antagonism as the outcome of treatment.

Speculations on the mechanism of antagonism.—Physical or chemical interaction of drugs prior to their attachment to microorganisms may be excluded (3). Minor degrees of antagonism have been demonstrated in occasional laboratory experiments between almost any pair of drugs, but generally the most consistent interfering drugs are bacteriostatic agents like chloramphenicol, tetracyclines, erythromycins. All these agents appear to act predominantly as inhibitors of protein synthesis in microorganisms. They actively antagonize such agents as the penicillins which primarily block synthesis of cell wall mucopeptides. Drugs which block cell wall synthesis (bacitracin) or those which act on cell membrane (polymyxins) do not antagonize penicillin (57). It can be postulated that protein synthesis must proceed actively in order to permit active mucopeptide synthesis; therefore, inhibitors of protein synthesis can antagonize inhibitors of cell wall synthesis. The interference with the action of other bactericidal drugs, such as streptomycin, which do not influence cell wall synthesis by inhibitors of protein synthesis, suggests that antagonism may result when active protein synthesis is a required early event in any sequence leading up to the essential

bactericidal step (50). While these are only very general statements to explain observed phenomena,
nism cannot be proposed at present.

Clinical evidence of antimicrobial drug antagonism.—In clinical therapy, there is usually a large excess of antimicrobial drugs, given in multiple doses. It is, therefore, unlikely that the stringent time-dose requirements will be met. Consequently, antagonism cannot be expected as a frequent outcome of clinical antimicrobial therapy. The most dramatic examples of antagonism in clinical therapy come from the treatment of bacterial meningitis. Lepper & Dowling (58) noted a mortality rate of 30 per cent in patients with pneumococcal meningitis treated with penicillin alone, compared to a mortality rate of 79 per cent among comparable patients receiving chlortetracycline in addition to the same regimen of penicillin. Similar effects were recorded by Olsson (59) and by Appelbaum (60). Quite recently, Wehrle's group at the Los Angeles General Hospital noted a fatality rate of 3.9 per cent in 129 patients with bacterial meningitis treated with ampicillin alone, whereas among comparable 111 patients receiving chloramphenicol in addition to the same regimen of ampicillin, the fatality rate was 11.7 per cent (17). The antagonistic effect of chloramphenicol to penicillin in experimental pneumococcal meningitis in dogs was shown to obey the time relationships outlined above (61).

In bacterial infections other than meningitis, antagonism can occur, but its demonstration is more difficult and the effects are less dramatic. In streptococcal pharyngitis, the addition of chlortetracycline or erythromycin interfered somewhat with the eradication of streptococci by penicillin (62, 63). However, there was no discernible difference in the clinical course of patients receiving the combination and those given penicillin alone. In urinary tract infections, McCabe & Jackson (64) were able to cure seven of eight patients, who received drug combinations selected *in vitro* to exhibit synergism against the infecting microorganism. Only one of seven similar patients was cured when treated with a combination exhibiting antagonism *in vitro* against the infecting organism.

It is my opinion that antagonism between antimicrobial drugs undoubtedly occurs in man, but that with the exception of special situations, the effect is not of sufficient magnitude to interfere with a successful outcome of treatment. Antagonism may present the greatest risk in infections caused by relatively drug-resistant organisms when a drug is likely to reach only minimal effective levels in tissue, or in infections localized in areas where only marginally effective drug levels are likely to be reached.

Synergism

Webster's Dictionary (65) defines synergism as "cooperative action of discrete agencies such that the total effect is greater than the sum of the two effects taken independently." In Goodman & Gilman's text, it is suggested that "synergism is often equated with supra-additive, but it has also been

used to refer to combined effects that exceed summation. . . . the term is best avoided for homergic drugs. Synergism should be reserved to describe combined effects of heterergic drugs" (i.e. a pair of drugs only one of which produces an effect) "that are greater than those of the active component alone" (66). In the discussion of antibiotic combinations in that text, the term, synergism, is not used at all. There is probably wisdom in this restraint.

While there are no universally accepted definitions to separate additive from synergistic action, the term synergism has been used widely in the past. There is little agreement between the various methods designed to determine positive summation of antimicrobial drug action, *in vitro*. In tests employing bacteriostatic activity (46), positive summation may indicate: (*a*) increased bacteriostasis; (*b*) selective suppression by each drug of a separate portion of the bacterial population; (*c*) delay by one drug of the emergence of mutants resistant to the other drug; (*d*) increased bactericidal action. One or more of these events may play a role in the clinical application of drug mixtures, as described in an earlier section, but there is no single definable set of characteristics to describe these various events.

By contrast, the dynamics of synergism, evidenced by increased, early bactericidal activity, have been fairly well defined and have been correlated with clinical evidence. Our group at the University of California Medical Center in San Francisco prefers the following definition: "Synergism implies the ability of two antimicrobial drugs acting together to increase markedly the rate of early bactericidal action, as compared to the rate with either drug alone, and to kill greater numbers of bacteria, or to cure infections more effectively than could be accomplished by simple algebraic summation of the single drug effects." (3). For *in vitro* studies, we have expanded this definition: "The addition of one drug to another results in a marked increase in bactericidal rate within the first 8 to 24 hours of exposure *in vitro*, and the bactericidal rate of the combination is more rapid than the rate with twice the concentration of each single drug participating in the mixture" (4). A few characteristics of this type of synergism may be listed. The effect extends over a fairly wide range of concentrations of each member of the drug pair and is not significantly influenced by the proportions in the mixture. Only one member of the pair need exhibit antibacterial activity alone, the other may be ineffective in the concentration entering into synergism, although active against the particular organism in a 10- to 100-fold larger amount (47). (From that aspect, this type of synergism fits into Goodman & Gilman's definition.) The bactericidal rate increases rapidly upon addition of the second member of a synergistic drug pair. However, the simultaneous presence of the two drugs is essential. If the first drug is removed before the second is added, no synergism occurs (67).

Speculations on the mechanism of synergism.—It is unlikely that chemical or physical interaction between members of the synergistic drug pair is responsible. It might be postulated that one drug alters surface characteristics of microorganisms so that penetration by the second drug might be easier (3). Plotz & Davis (68) showed that brief treatment of growing *E. coli* with

penicillin hastened subsequent killing of these cells by streptomycin, whereas the reverse sequence had no effect. The suggestion has been made that penicillin damaged cell membranes which, in turn, promoted uptake of streptomycin and damage at intracellular sites. Regrettably, *E. coli* is not often subject to synergistic action by penicillin and streptomycin. With enterococci, which are often subject to such synergism, the sequence of penicillin and streptomycin is not of importance.

While the type of bactericidal synergism described in our work deals with a microbial population which is relatively homogeneous in terms of drug resistance (a fairly crude determination), any population is undoubtedly heterogeneous in terms of the metabolic activity of individual cells at a given time. Metabolically inactive organisms are phenotypically resistant to penicillin, although genotypically susceptible. Such "persisters" can survive exposure to penicillin and be responsible for recrudescence of the infection later. It has been proposed that synergistic drug combinations can prevent the emergence of persisters through the rapid initial bactericidal effect (69, 70). Persisters might be L-phase variants. The absence of cell wall synthesis makes them resistant to penicillins. An aminoglycoside drug (streptomycin, kanamycin) might specifically act on these L-phase variants. Synergism might be due to the action of penicillin on one part of the population and of the aminoglycoside on the L-phase part (71, 72). Antibiotic synergism could also be explained by the simultaneous blocking of multiple interacting pathways essential for the growth of the microorganism (3, 73). If one pathway were blocked by one drug, the organism's growth might be temporarily inhibited until a second pathway could be utilized with greater efficiency. If, however both alternate pathways were blocked, the organism might die. I have no practical suggestion as to the nature of the pathway, or of the block, but this purely speculative hypothesis is perhaps no more unreasonable than the others mentioned.

One specialized type of synergistic drug effect needs to be mentioned. With organisms producing β-lactamase, benzylpenicillin and ampicillin are without effect because they are rapidly destroyed by the enzyme. If β-lactamase production could be inhibited, or the enzyme bound firmly by some other substance, the penicillin might have a chemotherapeutic effect. Chandler (74) proposed this mechanism for the occasional apparent synergistic effect observed when tetracycline and penicillin acted simultaneously on lactamase-producing staphylococci. We observed a similar combined effect between penicillin and a second drug acting on an occasional lactamase-producing staphylococcus (75) and attributed it, albeit without any specific evidence, to probable suppression of lactamase formation by the second drug. This might be a reasonable supposition in view of the earlier demonstration that tetracyclines and chloramphenicol could inhibit adaptive enzyme formation in microorganisms (76).

With the development of the lactamase-resistant penicillins and cephalosporins, it became apparent that these substances might have a much higher affinity for the enzyme than benzylpenicillin, protect the latter from

rapid destruction and, thus, permit an antibacterial effect to take place (77, 78). Sabath has pursued this line of investigation (79). He found that cloxacillin or methicillin served as potent inhibitors of the hydrolysis of benzylpenicillin and cephalosporins by a β-lactamase from Pseudomonas. The intact benzylpenicillin was then capable of strong antibacterial action against the Pseudomonas, albeit in a concentration of 0.5 to 8 μg/ml. Such concentrations and the amounts of inhibitor necessary for binding the lactamase could only be achieved in the urine, not in the systemic circulation. We have found that ampicillin plus isoxazolylpenicillins give similar results (80), and that this type of effect is highly inoculum-dependent. Synergistic action can only be demonstrated, if less than 10^6 Pseudomonas/ml are present, an infrequent situation in urinary tract infections. In spite of many drawbacks, this interesting system of combined antimicrobial drug action calls attention to the possibility of enhanced chemotherapeutic activity through the use of competitive inhibition of enzymatic drug destruction.

Clinical evidence of antimicrobial drug synergism.—Since the type of synergism considered here manifests itself principally as an increase in the rate of bactericidal action, the best clinical evidence for its occurrence should come from diseases in which cure depends on bactericidal drug action, capable of eradicating microorganisms without significant assistance from host mechanisms. Such diseases include bacterial endocarditis, and sepsis in the deficient host, among others. The best examples of antimicrobial drug synergism in clinical medicine come from the treatment of bacterial endocarditis. The most common infecting organism, *Streptococcus viridans*, is susceptible to the action of many antimicrobial drugs. However, the primarily bacteriostatic tetracyclines, chloramphenicol, or erythromycin usually fail to achieve cure, whereas the primarily bactericidal penicillin usually succeeds. *In vitro* the addition of streptomycin increases the bactericidal rate of penicillin acting on many strains of *viridans Streptococci* (81). The combined use of penicillin and streptomycin further improves the cure rate and permits a shorter course of treatment for this otherwise regularly fatal disease (82, 83).

Enterococci (*S. fecalis*) are the etiologic organisms in 5 to 15 per cent of cases of bacterial endocarditis. Most strains of enterococci are inhibited by penicillins *in vitro*, but there is little bactericidal effect (45, 81). Treatment with penicillin alone failed to cure a majority of patients with enterococcal endocarditis. Hunter (84) and Robbins & Tompsett (85) discovered empirically that such patients were cured often by a combination of penicillin and streptomycin. This drug combination was strikingly bactericidal for many strains of *S. fecalis* (45, 81, 84). Subsequent clinical experience (86–88) confirmed the efficacy of penicillin and streptomycin combinations in eradicating *S. fecalis* infections and curing enterococcal endocarditis. Synergistic action of this combination became widely accepted, and it was believed that strains of enterococci were uniformly susceptible to this synergistic effect. This belief was clearly incorrect. Occasionally, enterococcal strains were isolated which failed to be killed by penicillin-streptomycin combinations. Pa-

tients infected with such strains died of endocarditis unless a different bactericidal drug combination, e.g. penicillin plus neomycin (89) or penicillin plus kanamycin (72), was discovered by laboratory tests and applied promptly. Thus, even with enterococci, synergistic drug effects were quite specific for a given microbial strain. We examined ten recently isolated strains of *S. fecalis* for their susceptibility to penicillin and to the combined action of penicillin and streptomycin (88). Two of ten strains were killed *in vitro* as readily by penicillin alone as by the combination; four strains could only be killed by the combination; four others were susceptible to the combination but equal bactericidal rates could be achieved by much larger amounts of penicillin used alone. Eight of nine patients infected with some of these strains were clinically cured.

Many investigators believe that the rapid bactericidal effect often achieved *in vitro* by penicillin-streptomycin combinations is responsible for cure in patients with bacterial endocartitis caused by these enterococci (6, 28). However, Tompsett & Pizette (90) reported four patients cured of enterococcal endocarditis by penicillin-streptomycin treatment when the strains isolated from them were apparently not subject to the bactericidal action of these drugs *in vitro*. A critique of the technical features leading to this statement has been presented elswhere (88). Other disagreements between *in vitro* and *in vivo* results have also been recorded (91). The growth of many strains of enterococci is inhibited by smaller concentrations of ampicillin than of benzylpenicillin (91, 92). The suggestion has been made that ampicillin is sufficiently bactericidal for enterococci to permit its use as a single drug in enterococcal endocarditis (91, 92). A few patients have been treated and cured by ampicillin alone (92–94).

Recently, we compared the bactericidal action of ampicillin with that of benzylpenicillin, each drug alone or in combination with streptomycin, on 20 strains of enterococci (95). Again, there was considerable heterogeneity among strains. However, in no case was ampicillin as rapidly bactericidal alone as in the presence of streptomycin 20 μg/ml. Based on such tentative evidence, I believe that, for the time being, enterococcus endocarditis should be treated with a combination of drugs.

Bacterial endocarditis caused by other organisms has provided additional, though less conclusive, evidence for the occurrence of synergism in clinical therapy. Before the advent of methicillin, endocarditis and sepsis, resulting from β-lactamase producing staphylococci, presented serious problems in clinical management. Between 1956 and 1959, a group of patients were treated with drug combinations selected for their bactericidal capacity against the specific infecting staphylococcus *in vitro* (36). Treatment results were unexpectedly good. In a few cases, it was shown that earlier treatment with single drugs or nonsynergistic combinations had failed whereas the selected synergistic combination succeeded in eradicating the infection. Endocarditis caused by *Pseudomonas aeruginosa* usually fails to respond to drug therapy unless a concomitant removal of the infected site or tissue is

possible. However, one successfully treated case received a combination of cephalolothin and polymyxin, and recovery was attributed to this bactericidal combination (96).

In brucellosis, combined use of tetracycline with streptomycin has been advocated from time to time. Dowling (6) has recently reviewed the evidence and concluded that combination treatment had no advantage over streptomycin alone. However, Richardson & Holt (97) determined experimentally that neither streptomycin alone nor tetracycline alone could prevent the intracellular multiplication of brucellae, whereas the combination was rapidly bactericidal for such intracellular organisms.

Proteus infections of the urinary tract are notoriously resistant to antimicrobial drugs, and on rare occasions, they may give rise to sepsis. Whereas *Proteus mirabilis* is susceptible to penicillins, kanamycin or neomycin are the most likely drugs to exhibit *in vitro* activity against other Proteus species. The addition of tetracycline, or chloramphenicol, strikingly enhances the bactericidal activity of kanamycin-neomycin against an occasional Proteus strain. The use of such a combination may have clinical success—a possible synergism (98, 99). Other types of drug combinations have also exhibited both enhanced bactericidal activity against Proteus and some clinical success (100, 101). In patients with chronic urinary tract infection associated with various gram negative bacteria, McCabe & Jackson (64) selected specific drug combinations which exhibited synergistic action against the offending microorganism. Treatment with these synergistic combinations was highly effective in eliminating the infecting organism, whereas treatment with combinations exhibiting antagonism *in vitro* was singularly ineffective. The significant difference between these two groups supports the clinical efficacy of specifically selected synergistic antimicrobial drug combinations.

The Selection of Drug Combinations in Clinical Practice

In the foregoing sections, evidence has been summarized for the existence of synergistic and antagonistic effects between antimicrobial drugs. I have stressed repeatedly that such effects are strictly specific for a given microorganism and that no universally synergistic or antagonistic drug pairs exist. In order to find a desirable synergism, the microorganism isolated from a given patient must be matched against a variety of antimicrobial drugs, singly and in combination, in laboratory experiments. A variety of laboratory methods have been proposed for this purpose. Ideally, such laboratory tests should include many different concentrations of each drug matched in many ratios with all other drugs, acting on bacterial populations of different size. The results should permit expression of the inhibitory as well as the bactericidal capacity of each combination, compared with that of its constituent members. Obviously, the complexity of such a procedure would preclude its use in the clinical laboratory. Therefore, simplified methods have been employed which sacrifice some aspects of completeness and accuracy for the sake of convenience.

Most methods include two steps. In the first, bacteria are exposed to

single or combined drugs and, after incubation for varying periods, inhibition of growth is noted. In the second step, bacteria are removed from the influence of drugs and placed in an environment which will permit growth if the organisms have survived the initial contact with drug. The first step may be carried out in broth tubes containing measured amounts of drug in which bacterial growth or its inhibition are observed (102, 103). Alternatively, it may involve growth of bacteria on solid media into which drugs are incorporated or diffused from paper discs or strips (104–106). The second step consists of subculture from the drug-containing medium into drug-free solid media by transferring a loopful of broth (103) or by "replica plating" from one solid surface onto another (104, 105). The tests involving diffusion have the advantage of a gradient of drug concentrations, but the resulting patterns may be more influenced by the physico-chemical features of the drugs than by their biological interaction with microorganisms. If two drugs are incorporated into one paper disc, the only meaningful combined effect that is observed concerns the suppression of mutants resistant to one drug but susceptible to the other (107).

After performing many thousands of tests on various microorganisms by a "checkerboard" method, and evaluating the results of many experiments in laboratory animals, we proposed a tentative scheme to serve as experimental basis for combined antimicrobial drug treatment (108). Following considerable amplification (109, 110), antimicrobial drugs were placed into two groups: *I.* penicillins, streptomycin, neomycin-kanamycin, bacitracin, polymyxins; *II.* tetracyclines, chloramphenicol, erythromycins, novobiocin, sulfonamides

Synergism, manifested by enchancement of bactericidal activity *in vitro*, occurred most frequently among members of group *I*, and hardly ever among members of group *II*. The effect of a combination of groups *I* and *II* depended largely on the behavior of the organism toward the group *I* drug. If the organism was killed rapidly by the group *I* drug, addition of a group *II* drug might slow the bactericidal rate and result in antagonism. If the organism was relatively resistant to the group *I* drug, addition of a group *II* drug might produce synergism under some circumstances.

This empirical, very loose scheme was intended to serve only as a framework for laboratory studies. Its essential validity was confirmed in several laboratories (6, 7, 51, 111). Manten & Wisse proposed an alternate scheme for which they claimed predictive value (52). We have never suggested that this scheme, proposed by us, can be used as a clinical guide, nor that the behavior of a microorganism toward drug combinations could be predicted a priori. Regrettably, our scheme became known as "Jawetz' formula," or even "Jawetz' law" (112). It must, therefore, be stressed once again that in the absence of specific laboratory information and of clinical experience, the scheme *cannot* serve as a guide for combined therapy.

Jones & Finland (113, 114) attempted to evaluate combined drug action in man in a standard system. Each of two antibiotics was administered singly and then together to the same volunteers at different times. Blood

from these volunteers was then assayed for antimicrobial action against standard strains of staphylococci and streptococci. Active single drugs were often more effective than combinations. While there was some agreement with the tentative scheme proposed by us, the results were often equivocal, and lent no support to the use of standardized or fixed combinations. We have employed the serum assay technique of Schlichter & McLean (115), in a modified form (116), as the most reliable guide for the adequacy of bactericidal drug levels after the administration of single or combined antimicrobial drugs. In such diseases as bacterial endocarditis, this serum assay is the best guide available to the physician to check on drug selection and dosage.

Conclusions

A famous physician is said to have written 800 years ago: "If one can manage well with one individual drug, one should not use a compound one. . . . one should use medications compounded of multiple ingredients only when compelled to do so" (117). I hope that this review will help physicians feel compelled less often to use combinations of antimicrobial drugs and provide them with a rational basis for decision in the infrequent situations where a combination may be indicated.

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