ANTIBIOTIC SYNERGISM AND ANTAGONISM: AN ASSESSMENT OF THE PROBLEM*

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The last decade has witnessed an unprecedented development in the therapy of infectious diseases and a significant change in the practice of medicine. The variety of antibiotic substances discovered and made freely available during this short span of time permitted the ready control of many microbial infections and thus prevented many deaths. The developments were exceedingly rapid. As one drug speedily followed another, pressure from public, industry and physicians continuously urged the achievement of practical objectives. As a result, fundamental investigation of the properties and behavior of these antimicrobial substances lagged far behind their widespread clinical use.

It was fortuitous (and possibly unfortunate) that the first of the antibiotics to become widely used, penicillin, possessed characteristics closely approaching those of an ideal chemotherapeutic agent: virtual lack of toxicity for the host (60 grams daily have been injected without noticeable side action) (41); highly lethal action against susceptible parasites, which under most conditions has permitted only limited development of microbial resistance; satisfactory absorption, distribution and action in host tissues and fluids. Physicians soon developed the habit of administering penicillin quite indiscriminately because "it might help and could do no harm." As other antibiotics became available this same attitude was applied. Unfortunately, the "newer" drugs were not as close to the chemotherapeutic ideal as was penicillin and their widespread use and abuse were followed by a surge of reports on drug toxicity, bacterial resistance, and treatment failures.

Still influenced by the experience with penicillin, physicians began to administer two or more antimicrobial drugs concurrently on the theory that "if one drug is good, two should be better." Much combined antimicrobial therapy has been, and is being, carried out with no more cogent logic than that. The rationalization brought forward to defend the clinical use of drug mixtures against the criticism of "shotgun therapy" will be examined in this review.

Most experimental studies on combinations of antimicrobial agents prior to 1948, reviewed by Thatcher (89), dealt principally with the effects obtained by adding a sulfonamide to an antibiotic. It was the general impression that the addition of one good chemotherapeutic agent to another would usually result in an enhancement of activity (57). In 1949 Lankford and Lacy (58) pointed out that this was not necessarily true, and that a mixture of two antibiotics might well be less effective than a single agent. Price *et al.* (71) studied the effect of

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drug combinations on *Salmonella typhosa* infection in mice and were struck by the lack of correlation between *in-vitro* inhibitory tests and *in-vivo* results. These investigators also found marked differences among mixtures containing different proportions of the same drugs. They stressed the need "to employ proper concentrations of the drugs to demonstrate significant synergism." Spicer (85, 86) concluded on the basis of *in-vitro* examinations that "the effect of antibiotics used in pairs may be one of four types; namely, synergistic, additive, interfering or indifferent, depending on the particular combination of drugs for a given bacterial strain." That either synergism or antagonism might result from adding one antimicrobial substance to another was also pointed out by Bigger (11), Pratt and Dufrenoy (70), and others.

The study of combined antibiotic action received a new impetus from the problem of treatment-resistant bacterial endocarditis. Penicillin had revolutionized many aspects of this previously universally fatal disease. While sulfonadmides had virtually no effect, administration of penicillin resulted in the recovery of the majority of patients with this illness. In some instances, however, particularly in patients with infections due to *Streptococcus fecalis*, penicillin failed to effect a cure, although this drug apparently inhibited the organisms completely in the test tube. A possible explanation for this discrepancy was given by the discovery (15, 34, 39, 46, 74, 86) that penicillin did not kill the entire exposed microbial population in the test tube, but always permitted the survival of a certain number. These organisms, analogous to what Bigger had termed "persisters" (11), did not differ noticeably from the remainder of the population in apparent sensitivity to individual antibiotics, but could only be killed rapidly if two drugs were present simultaneously (5, 15, 21, 27, 34, 39, 42, 66). One of the outstanding characteristics of this positive summation of drug action was the marked increase in early *bactericidal* rate observed *in vitro* with combinations of penicillin and streptomycin acting on enterococci (*Streptococcus fecalis*). The marked potentiation of bactericidal action of this combination against several bacterial species had been noted by Nichols (69). When patients with enterococcal endocarditis were treated with penicillin and streptomycin, a large majority recovered (5, 15, 40, 73, 74). Here, then, was convincing clinical support for one form of positive summation of antibiotic action observed in the laboratory and it stimulated extensive further work along similar lines in the hope of discovering the principles underlying this extraordinary effect.

When penicillin of increasing purity became available in the late 1940s, it was noted that the impurities in the earlier preparations had enhanced the antimicrobial effects of the drug (38). Similar enhancing properties were ascribed to borrelidin (9), a new antibiotic discovered in 1948. While this substance was not suitable for clinical use, bacitracin, which showed therapeutic potentialities in spite of some toxic side actions, also markedly increased the bactericidal effect of penicillin *in vitro*, in experimental infections, and in clinical disease (4, 19, 24, 43, 50, 90, 92).

In the course of studies on the early bactericidal action of drug combinations,

it was noted that while streptomycin increased the rate of the bactericidal action of penicillin on certain microorganisms, chloramphenicol decreased it, thus resulting in "antagonism" (34, 39, 46). It was soon shown that this phenomenon was not limited to chloramphenicol but was also demonstrable with aureomycin, terramycin (12, 15, 33, 39) and, under certain circumstances, with sulfonamides (35) as observed earlier by Hobby and Dawson (37). In all these *in-vitro* experiments, the bacteriostatic agent diminished the early bactericidal rate, but later aided in sterilizing the culture. It was doubtful therefore whether this phenomenon would be applicable *in vivo* or might be limited to the test tube. However, it was readily demonstrated that under suitable circumstances mice with various experimental infections could be cured at a much higher rate with penicillin or streptomycin alone than with a combination of either of these drugs with aureomycin, terramycin, or chloramphenicol (2, 6, 12, 47, 49, 60, 83, 84). A number of clinicians believed that they had observed instances of antibiotic antagonism in patients (15, 61, 77), but only one convincing report has appeared to date. Lepper and Dowling (59) studied a series of patients with pneumococcal meningitis. Of those treated with massive doses of penicillin alone, 30 per cent died, whereas the death rate was 79 per cent among comparable patients who received full doses of aureomycin in addition to the same amounts of penicillin. These authors stressed the comparable severity of the disease in both groups and the statistical significance of the difference. Thus, antibiotic antagonism apparently *can* occur in clinical situations. Concern over the danger of antibiotic antagonism in medical therapy was voiced editorially (20–22). Possible reasons for the rarity of antagonism as the end result of combined antibiotic treatment in patients are discussed below.

Combined antimicrobial drug action is a complex subject and its experimental analysis is in its infancy. The results obtained and the conclusions drawn by different investigators depend largely on the methods employed and the interpretations applied to the findings. Several recent reviews (7, 8, 51, 89, 96) have done little more than list the claims of different workers and state the points of disagreement. The subject is in such a state of flux that it is doubtful whether another summary of its status would be contributory. It may be more profitable to examine the possible reasons for disagreement and to attempt to integrate compatible results into working hypotheses. The conflicting reports on combined antibiotic action have centered around the following points: (a) definitions of terms; (b) methods of study; (c) interpretation and significance of results, particularly with respect to correlation between laboratory findings and clinical observations; (d) dynamics of the processes; (e) speculations on possible modes of action. It is proposed to assess the problems of combined antibiotic action under these headings, in the hope of developing leads toward constructive work in the future.

DEFINITIONS

Webster (94) defined synergism: "Cooperative action of discrete agencies such that the total effect is greater than the sum of the two effects taken inde-

pendently. Opposite of antagonism." Sollmann (82) stated that synergism is "a quantitative change in the sense of increasing the efficiency" and reserved the term "potentiated summation" for combined drug action in excess of simple algebraic summation. According to Goodman and Gilman (30), "positive summation is known as synergism," and "instances in which the combined action of two drugs is greater than that which can be anticipated from the sum of their individual action . . . (are) known as potentiation."

Thatcher (89), who reviewed the extensive literature on synergism between antibacterial substances up to 1948, chose to define synergism as "the ability of two agents acting simultaneously to bring about bacteriostasis at individual threshold concentrations which are lower than could be accounted for by a mere summation of the individual effects of the discrete substances." Our group at the University of California Medical Center (41, 44, 45, 49) has developed 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 experimental or clinical infections more effectively than could be expected from simple algebraic summation of single drug effects."

In the special cases that we studied initially (46), a synergistic drug combination achieved a result that was not only quantitatively but *qualitatively* different from that obtainable with the participating agents acting singly. In the selected cases of microorganisms showing optimal zone effects (18, 34) the drug combination achieved more than *any* concentration of either drug alone. This synergism truly denoted a "potentiated summation" in excess of that attributable to simple additive effects of the component drugs. In many instances, however, this special situation does not prevail and great difficulties exist in establishing clearly that the combined effect of two antibacterial substances acting together is actually the excess of simple addition. The reasons for these difficulties are presented in a later section. Methods for experimental design have been proposed (93) to yield statistically unequivocal results, but as yet they have not been applied to the exhaustive investigation of combined antibiotic action. For the purpose of this discussion, the term "synergism" will be reserved for those instances where the *in-vitro* or *in-vivo* result of combined antimicrobial drug action is unequivocally in excess of simple algebraic summation of single drug effects. To all other instances of positive summation of drug action, the term "addition" will be applied.

Any combined drug effect that is less than the algebraic sum of the effects of the single drugs can be called antagonism. This might include situations where the observed total result of combined drug action is greater than that of one or both single agents, but less than that which would be expected from algebraic summation of agents acting singly in the same direction ("deficient summation" (82)). While this is a valid theoretical possibility, it is somewhat difficult to establish experimentally and impossible to prove clinically in the treatment of human infections. Consequently, it may be 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 member of the combination when acting alone.

METHODS

In order to compare the relative antimicrobial activity of single drugs with that of drug combinations, the following techniques of evaluation have been applied by various investigators.

I. *In vitro*

(a) Inhibition of growth, *i.e.*, preventing organisms from multiplying normally in a given time in a suitable environment, without regard to the rate at which they may be killed. Endpoints are expressed as the minimal amounts of drug necessary to suppress visible growth for a fixed time in bacteriologic media or to delay growth as measured by turbidimetry or nephelometry.

(b) Rate and completeness of killing, *i.e.*, injuring the organisms so that they are unable to grow when removed from the influence of the drug after a certain time of exposure. The results are expressed as the early bactericidal rate or as the smallest concentration of drug capable of killing a given number of bacteria in a certain time.

(c) Alteration of some measurable metabolic activity (*e.g.*, respiration) or of morphologic characteristics.

II. *In vivo*

(a) Experimental infection in animals, in which one of the following criteria is used: (1) prevention of death or of lesions, (2) prolongation of life, (3) eradication of infecting microorganisms.

(b) Clinical infections in man, in which one or more of the following criteria are used: (1) clinical cure of the disease with suitable follow-up study, (2) bacteriological evidence of suppression or eradication of infection, (3) significant alteration of the natural course of the disease.

(c) Normal animals or man: Alteration in some measurable quantity, *e.g.*, body weight, fecal bacteria, fecal pigments, attributable to suppression of microorganisms by the drug.

The results of any one type of technique need not coincide with those of any other technique for reasons discussed below. However, any method that uses comparison of single drugs with combinations on the basis of their weights must take into account the following fallacy. It is possible to determine by experimental means one minimal effective dose (*e.g.*, minimal inhibitory dose or minimal lethal dose *in vitro* or 50 per cent curative dose *in vivo*) and express it in weight of drug. The temptation may then be great to claim synergism for a combination containing a fraction of the weight of one such minimal effective dose (MED) of each of two drugs and possessing greater activity than would be expected on the basis of algebraic summation. The fallacy of this reasoning resides in the assumption of a linear relationship between drug weight and effectiveness for which there can be no direct evidence below the level of 1 MED,

which by definition is the least effect that any method permits to be estimated. Above 1 MED there may or may not exist such a linear relationship in a given drug, but at least the points of such a curve can be established experimentally. Thus, it is possible to test whether 1½ MED of drug A plus 1 MED of drug B equals or is more effective or less effective than 2½ MED of either drug. Such simple arithmetic is not valid for the region below 1 MED where the relationship between weight and effect of drug (Fig. 1) cannot be estimated experimentally. Since this relationship might lie on curve X or curve Y (Fig. 1), as well as on a straight line, the effect of ½ MED of drug A plus ½ MED of drug B might be greater, equal or less than the effect of 1 MED by weight of either drug, even though each drug acts independently of the other. This point is discussed in detail because it is used frequently as a basis for claims of synergism found in the literature (3, 17, 68, 86), yet does not provide proof for synergistic effect. On the other hand, if 1 MED of drug A plus 1 MED of drug B achieve

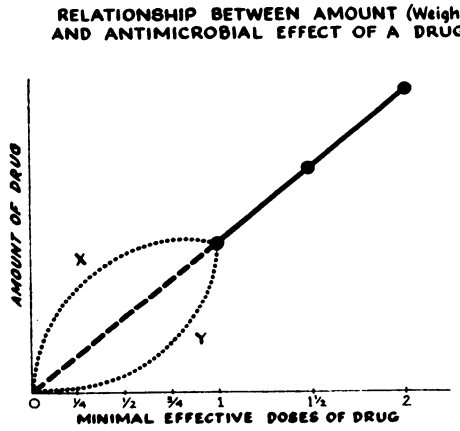


FIG. 1

much more than 2 MED of either drug, this finding suggests positive summation of drug action in excess of simple addition, because both 1 MED and 2 MED can be estimated quantitatively (Fig. 1).

While it may seem redundant to mention it, the results arrived at by any method must have quantitative validity in terms of the criteria chosen by the investigator. It is regrettable that some reports do not live up to that postulate. It is even more unfortunate that certain articles in scientific publications read like advertisements for specific commercial preparations of antimicrobial drug combinations based on most tenuous evidence (81).

INTERPRETATION OF RESULTS OF *IN-VITRO* TESTS AND THEIR CORRELATION WITH *IN-VIVO* OBSERVATIONS

Most antimicrobial agents possess a spectrum of activity similar to that of disinfectants (64). The lowest concentrations have no demonstrable effect,

whereas slightly higher ones often possess some growth-stimulating activity (27, 70). Further increase in the amount of drug results in growth inhibition, and finally the lethal concentrations are reached, sometimes with a zone of optimal action (18). The relative width of the range of concentrations exhibiting each of these effects determines the predominant character of the chemotherapeutic agent. Thus, a drug like penicillin is predominantly lethal for microbes, while one like aureomycin is principally bacteriostatic. Nevertheless, the entire spectrum can often be demonstrated for a given antibiotic agent (27, 70). Drug combinations undoubtedly reflect the behavior of individual components, as well as their interactions.

The different methods outlined above measure different events in the test systems, and consequently apparent disagreement in the results must be expected (12). The importance that an investigator attaches to any one of the measurable events will determine his choice of method. If he believes that the principal action of antibiotics in infectious diseases is microbial inhibition, then he will be satisfied with test methods that primarily measure inhibitory action (Method Ia). If he believes that antibiotics are most effective *in vivo* if capable of killing microorganisms, he will insist on measuring lethal action (Method Ib). Since the advent of the sulfonamides, it has been evident that many infectious processes, *e.g.*, respiratory infections, are curable with drugs whose predominant action is bacteriostatic (17), relegating to the host the eradication of the pathogen. There are other diseases, *e.g.*, subacute bacterial endocarditis, where a predominantly bacteriostatic drug rarely results in cure (28, 40, 42, 52), but where only agents capable of rapidly killing the causative microorganisms effect recovery of the patient (5, 28, 39, 40). Thus, it does not appear possible at this time to designate either Method Ia or Ib as objectively "better"; the method must be selected according to the problem at hand. This difficulty has been discussed recently by Waisbren (91).

The complexity in interpreting the results of these methods is further increased by the need to consider the element of time in antibiotic-microbe interaction *in vitro*. Thus, an inhibitory concentration of an agent might become lethal to microorganisms exposed for a long period (depending on factors of microbial activity and environment), and similarly a mixture of drugs which at first results in antagonism might some hours later progress to killing the exposed microbial population (33, 91). It thus becomes necessary to define, somewhat arbitrarily, the time limits for each experimental method.

Method Ic can be dismissed briefly, for it has been employed but rarely (10, 65, 89). As long as the metabolic basis of chemotherapeutic action remains unknown for any single antibiotic, it seems doubtful that such a method could provide more suggestive evidence than direct observation of microbial inhibition and death.

If it is conceded that the proof of antimicrobial effectiveness of single drugs or mixtures rests on the cure of experimental or clinical infections, then *in-vivo* methods would give the decisive answers, and those *in-vitro* methods which correlate best with the results obtained in living hosts should carry most weight.

The limited number of experimental infections suitable for chemotherapeutic trials (Method IIa), therefore, has been drawn upon heavily, while realizing their somewhat artificial nature. At least it is readily possible to formulate criteria and to observe quantitative end points in such experiments which can be treated statistically to assess their significance. Ideally, of course, clinical investigation in human disease (Method IIb) should yield the ultimate answers. This is only true in very special cases, however. Most human infections have a tendency toward spontaneous recovery, an element difficult to assess in the individual case. Because of the difficulty in using minimal effective amounts of antibiotics, data on combined antibiotic treatment of human disease have been most convincing where the drug combination achieved a *qualitatively* different effect from the single drugs, *e.g.*, clinical failure with large amounts of single drugs, success with smaller amounts in a combination.

In spite of the vast amount of antimicrobial drugs in combination administered to humans, only very limited evidence on combined drug action has evolved. For the most part the use of antibiotic combinations in humans has rested on conjecture and speculation. The following possible reasons for the simultaneous use of several antimicrobial agents have been mentioned frequently:

(1) "It is essential to cover the entire microbial spectrum." There can be no doubt that in known mixed infections due to more than one species of bacteria, multiple drugs may be beneficial or even essential. However, indiscriminate administration of mixtures of antibiotics has often taken the place of judicious specific etiologic diagnosis and this should not be condoned. Furthermore, no planned "combined action" is involved, since each drug is presumed to act on those microbes susceptible to it.

Such treatment is comparable to administration to an allergic cardiac patient of an antihistaminic and digitalis simultaneously. Different drugs acting on several organisms simultaneously may also result in antagonism or synergism, but such a system is far too complex for experimental analysis at present and will not be discussed further here.

(2) "Microbial populations of a single species are not homogeneous in their behavior toward drugs." It could be postulated that by giving two drugs, both of which were known to have some effect against the microbial strain, some members of the population would be predominantly affected by one drug, some by the other, resulting in a more complete over-all antimicrobial effect (11, 86). Experimental analysis in a few systems lends little support to this explanation.

(3) "Antimicrobial therapy with a single drug sometimes fails because drug-resistant variants emerge early in therapy." The addition of a second agent may prevent this development of early drug-fastness. Good evidence of the combined action of streptomycin with para-amino-salicylic acid (25, 31, 67, 80) in tuberculosis supports this concept. It is quite possible that in other chronic infections, *e.g.*, of the urinary tract, the same principle can sometimes be invoked (53–56, 68, 75). The ability of aureomycin to inhibit penicillinase formation by staphylococci may fall into the same category and perhaps may have practical significance (16). The inhibition by one drug of the rapid emergence of forms

resistant to a second drug is undoubtedly an important mechanism of combined drug action and may be of considerable clinical importance. The application of this principle is limited by cross resistance among microorganisms (28a). If an agent, *e.g.*, streptomycin, has high antimicrobial activity but permits the emergence of frequent resistant variants, then the addition of another drug with much lower antimicrobial activity and a different pattern of microbial resistance may well permit much longer profitable treatment of the patient with the first agent. On the other hand, such drug mixtures often have no more inhibitory or killing effect on microorganisms than the single more active agent; thus this form of combined action will not be further discussed here.

Because of the many variables that are difficult to control and the several ways in which antibiotic combinations might result in apparent positive summation of drug effects of different mechanisms, an integrated discussion of the many reported clinical claims is not possible. Likewise many laboratory studies purporting to show some definite combined effect should be evaluated in the light of the above considerations. The ultimate validity of any one laboratory method for the assessment of clinical usefulness of antibiotic combinations will have to be established in the future by many additional carefully controlled and correlated studies between laboratory and clinical results. At the present time, only very limited parallelisms can be discerned. Workers in the field of combined antibiotic action must exercise great restraint in transferring results obtained in the laboratory either directly, or by implication, to clinical material.

DYNAMICS OF COMBINED ANTIBIOTIC ACTION

Antagonism can be demonstrated *in vitro* by a decrease either in the inhibitory activity (56, 58, 75, 85) or in the early bactericidal rate (34, 45, 46) of a drug mixture below that of one or both of its components. For the optimal demonstration of the latter form of antagonism *in vitro*, a bacteriostatic amount of the interfering agent must be added to an actively bactericidal quantity of the effective drug, as shown schematically in Figure 2.

This interference apparently takes place only when the drugs act on organisms capable of multiplication and does not occur in an environment unsuitable for growth. For this reason, and also because the drugs most readily antagonized, *i.e.*, penicillin and streptomycin, act optimally on multiplying microorganisms, interference has been attributed to the bacteriostatic character of drugs like aureomycin and chloramphenicol. It was postulated that these drugs greatly diminished multiplication of microorganisms under their influence and thus might make them less susceptible to the action of penicillin or streptomycin (47, 49). Bacteriostasis *per se*, however, cannot be held responsible, for if it is induced by minute amounts of the "effective" drug there is no interference with the action of subsequent, larger doses of the same drug on the now "static" population (13, 45). Furthermore, there is no antagonism between bacteriostatic amounts of one member of a synergistic drug pair and any concentration of the other member (45). In rare, highly selected circumstances, penicillin can antagonize aureomycin or terramycin *in vitro* when the latter are rapidly bac-

tericidal (32). It seems, therefore, that antagonism must be based on a specific interference by one drug with those conditions or metabolic processes that are essential for optimal action of another drug, rather than on mere inhibition of multiplication. One reason for attributing significance to the early bactericidal rate *in vitro* and its reduction by antibiotic antagonism is the correlation between such *in-vitro* results and the reproduction of the phenomenon *in vivo* (2, 6, 12, 47, 49, 83, 84).

Antibiotic antagonism is sharply limited by time-dose relationship *in vitro* and *in vivo*. The interfering agent must act either before or simultaneously with the effective drug (6, 34, 49, 60, 84). This resembles the examples cited by Woolley (98) where a metabolite must be administered prior to the analog if antagonism is to occur. Consequently, multiple dose treatment schedules in experimental infections make the demonstration of antagonism difficult (2, 60, 83), since absorption and excretion of the drugs, with ever-changing blood and

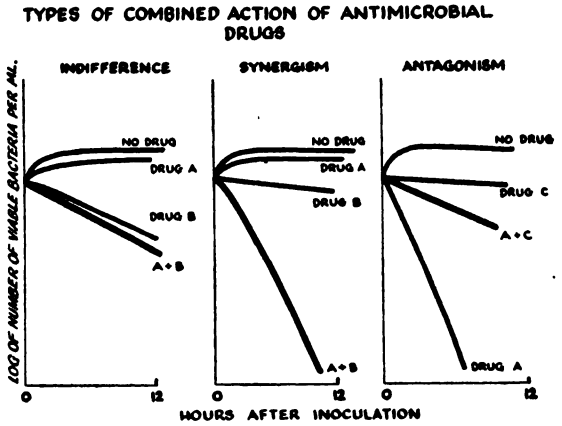


FIG. 2

tissue levels, make it unlikely that the sequence of drug effects necessary for antagonism will be maintained.

Antagonism is most marked with concentrations of the interfering agents that are barely biologically active. The phenomenon is either obscured or suppressed by a large excess of either one of the participating agents *in vivo* (84). Since in clinical practice a large excess of drug dosage is the rule, the likelihood of the observation of antibiotic antagonism is restricted. It must be re-emphasized that in the only instance where antibiotic antagonism has been unequivocally established in human disease (pneumococcal meningitis), the circumstances were well suited to its occurrence (59): a combination of one very effective bactericidal drug (penicillin) with a barely static agent (aureomycin), both present in critical concentration, but without any excess, at the site of bacterial proliferation (central nervous system, meninges), in a disease where rapid killing of microorganisms may be essential for cure. In other reports where the possibility

of antagonism was strongly considered (15, 61), the circumstances were likewise compatible with its occurrence. In the majority of human infections, however, the regimen of antimicrobial therapy is such that it virtually precludes antibiotic antagonism. The strict time-dose relationships essential for the experimental demonstration of antagonism make its occurrence as the outcome of clinical antibiotic treatment most unlikely. It is not known whether antagonism might result in quantitative reduction of antibiotic effectiveness at the site of bacterial proliferation without disturbing the curative end result.

Parenthetically, it should be noted that several sulfonamides incorporated into a mixture may also give rise to antagonism among its components (78, 95). The clinical significance of these observations with sulfonamides is likewise unknown thus far.

The dynamics of *positive summation* of antibiotic action are less clear than those of antagonism because there are no universally acceptable definitions to separate additive from synergistic action. There is little agreement between the various *in-vitro* methods for determining positive summation. In particular, it has been pointed out repeatedly (12, 71, 75, 85) that, in methods mainly measuring inhibition of growth, the proportion of drugs in the mixture greatly influences the result. It also has been mentioned that combinations resulting in antagonism in terms of diminished early bactericidal rate not infrequently appear to give positive summation later (33, 91). It may well be that growth inhibition tests (Method Ia) reliably reflect positive summation as it may apply to treatment of some human diseases. Yet positive summation in such tests merely indicates prevention of visible growth possibly attributable to any one or more of the following factors: (a) increased rate of killing; (b) increased bacteriostatic effect; (c) selective influence of each drug on a separate portion of the bacterial population; (d) delay by one drug of the emergence of variants resistant to the other drug. One or more of these possible phenomena may be important in the clinical application of drug mixtures, but few definite correlations have been attempted. On the other hand, a more concrete, though limited, body of evidence exists for the dynamics of synergism evidenced by increased early bactericidal activity. For this reason and because of some correlation with clinical facts, this criterion will be used in the present discussion.

The subsequent generalizations apply to findings in studies which employ the following definition of synergism (as stated on page 178, but here referring more specifically to *in-vitro* studies): "The addition of one drug to another results in a marked increase in bactericidal rate within the first 8–24 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" (Fig. 2). Such synergism extends over a wide range of concentrations of each member of the drug pair and is not significantly influenced by the proportion in the mixture (32, 45). Only one member of the pair need exhibit inhibitory activity alone, the other may be ineffective in the concentration entering into synergism, though having some influence in a hundred-fold or thousand-fold larger amount (45). An outstanding characteristic of this type of positive

summation is the rapid increase in bactericidal rate at any time that the second member of a synergistic pair is added to the first, provided that the first still has some activity of its own (41). The simultaneous presence of the two drugs is essential (66), although they need not be added at the same time. When they are applied in sequence (with the first removed before the second one is added), no synergism occurs.

This type of *in-vitro* positive summation of antibiotic action correlates with results in certain infections of mice (12, 60, 83) and agrees well with the results of treatment in subacute bacterial endocarditis in man. This disease, fatal if untreated, requires bactericidal antimicrobial agents for cure. Certain patients are infected with enterococci, drug-resistant staphylococci, or other bacteria which fail to respond to the usual therapy with single drugs, even though the organisms may be inhibited *in vitro*. However, bactericidal tests (Method Ib) can detect antibiotic combinations that subsequently cure the patients. A number of clinical reports have stressed this correlation (1, 5, 15, 24, 28, 48, 52, 73, 74, 87, 92). These cures are particularly convincing evidence of synergistic action, if, as is not infrequently the case, the patient had failed to respond to large quantities of single drugs before the infection was eradicated by smaller amounts in a suitable combination.

Other human diseases in which bactericidal methods might be applied to the evaluation of combined antibiotic action are meningitis (29, 59, 61) and brucellosis. In the latter, a large series of experimental and clinical studies suggests that drug combinations (*e.g.*, streptomycin with aureomycin) which exert a lethal effect on the causative microorganisms might effect a cure, whereas single drugs are likely to fail (36, 62, 88, 99). While the intracellular habitat of the parasite complicates the experimental situation, it is likely that, here too, suitable antibiotic combinations result in positive summation of the drug effects (36, 62, 65).

What, then, is a suitable combination? Is it possible to designate certain drug pairs as being "synergistic," others as "antagonistic?" It must be stated emphatically that uniformly "synergistic" or "antagonistic" drug pairs do not exist (41). Certain drug pairs may exhibit synergism when acting on one microorganism, antagonism when acting on another (43). The behavior of the microorganism in question toward the constituent members of the pair determines the result of combined antibiotic action. Not even different bacterial strains of the same species behave in an identical manner (43). While the individual behavior of microorganisms precludes the prediction of the result of combined antibiotic action in a given case, an attempt has been made to integrate the available information into a scheme of combined action (43).

Common antibiotics have been placed into two groups: I. penicillin, streptomycin, bacitracin, neomycin; II. aureomycin, chloramphenicol, terramycin. Polymyxin B could not be definitely placed, whereas the sulfonamides seem to fit into group II. Members of group I are frequently synergistic with each other, occasionally indifferent, never antagonistic. Members of group II are neither synergistic with nor antagonistic to each other, but simple additive effects are

often observed, which presumably also could be obtained by an increase in the dose of a single drug. When a member of group I is added to one of group II, the combined effect is a function of the microorganisms' behavior, and is not predictable *a priori*. When the microorganism is highly sensitive to the group I drug, antagonism often may be demonstrated with small amounts of the group II agent. With bacteria that are moderately or highly resistant to the group I drug, synergism sometimes can be obtained by the addition of the group II agent, within the limits of drug concentrations obtainable *in vivo*. This positive summation of drug action is usually of smaller magnitude than customarily obtained between group I agents. This scheme is entirely empirical, but is in agreement with all conclusive experimental and clinical data available (71a). Obviously it will require modification as more information accumulates.

It is of interest to establish whether the proportion of the constituents in a given combination of antibiotics acting in a standardized experimental system determines the end result, *i.e.*, synergism or antagonism. While investigators working with methods that utilize growth inhibition as the endpoint have observed that the effects are linked to the proportions of each drug in the mixtures (71, 75), a conversion from antagonism to synergism has not as yet been observed within a single bactericidal system. Recent exhaustive *in-vitro* tests by Gunnison *et al.* (32) have extended the knowledge of the behavior of I + II combinations in a wide range of drug concentrations. It was demonstrated, that within a synergistic system, increases in individual drug concentrations or changes in proportions resulted in shifts from ineffectiveness, to simple addition, to synergism, but never to antagonism. In an antagonistic system, similar changes in the constituents resulted in shifts from ineffectiveness to frequent additive effects in a zone of varying width, then to antagonism and rarely to mutual antagonism between the participating drugs, but never to synergism as defined above. The zone of addition between ineffectiveness of the combination and antagonism was usually narrow and sometimes not demonstrable to all. While no complete *in-vivo* studies have been performed to substantiate these findings, the observation (60, 83) that either additive or antagonistic effects may be obtained in multiple-dose combined therapy of experimental infections in animals is in agreement with the *in-vitro* results.

SPECULATIONS ON THE MODE OF COMBINED ANTIBIOTIC ACTION

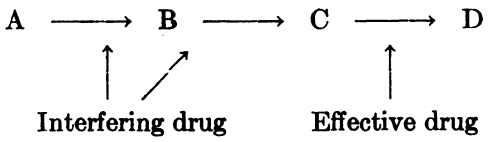
It may be permissible to speculate on the mechanisms of antibiotic synergism and antagonism in spite of the fact that the exact mode of action of not a single antibiotic is known as yet (14). Thatcher (89) has summarized the hypotheses on the physiological basis of synergism. The thoughts expressed here pertain only to the situation where two drugs simultaneously affect an entire microbial population, and do not consider the possibilities discussed earlier for other forms of positive summation, *e.g.*, selective influence of each drug on different members of a microbial population.

1. It might be postulated that the two drugs interact with one another chemically or physically and that the product has an effect different from that

of its components. But whereas certain antibiotics can form salts with one another, the action of these compounds is not strikingly different from the action of their constituent parts. Some of the antibiotic mixtures which resulted in antagonism were examined by ultraviolet absorption spectroscopy (49) but failed to give evidence of chemical or physical combination. In a further attempt to differentiate between chemical or physical combination and biological influence as the cause of combined antibiotic effects, biologically inactive but chemically similar materials were introduced into combinations in place of their biologically active counterparts. Aureomycin inactivated by gentle heating, at alkaline pH, or the biologically inactive isomer from the d-base of chloramphenicol was incapable of participating in combined antibiotic action. It does not appear likely that chemical or physical interaction between drugs is responsible for positive or negative summation of action.

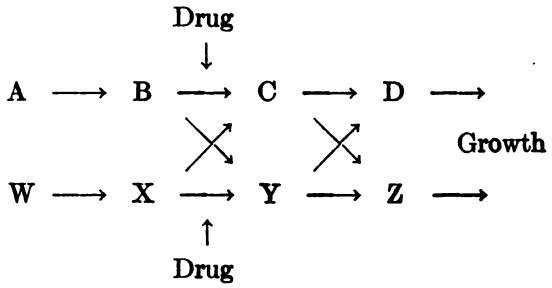
2. It might be postulated that one drug alters the surface of microorganisms so that penetration by a second drug is either easier (synergism) or more difficult (antagonism). Direct evidence on this hypothesis as yet is lacking. Earlier work with the uptake of radioactive penicillin by bacteria (76) led Rowley *et al.* to the belief that the amount of penicillin taken up by the bacteria is directly related to the bactericidal rate. Detailed observations, however, indicated that neither streptomycin nor chloramphenicol had any measurable effect upon the uptake of penicillin by bacterial cells (72). Another approach to the same hypothesis was carried out by Miles *et al.* (66). If synergism and antagonism resulted from modifications of the bacterial surface, it might be assumed that this alteration would persist for some time. This possibility was tested by exposing bacteria to one member of a known synergistic pair, then removing the first drug by washing, and adding the second. Synergism was not observed unless both agents were present simultaneously. These results militate against the idea of a predominant surface action of antibiotic combinations.

3. Antibiotics might exert their action through interference with some metabolic pathways which participate in protein synthesis (26). A hypothesis for the mode of action of antibiotic antagonism could then be adapted from Eagle (18) as follows:



A given microorganism utilizes a metabolic pathway, A, B, C, D. The "effective" drug can block it between C and D, with accumulation of C which is toxic and results in rapid death. The blocking of earlier steps in this pathway by the interfering agent results in diminished synthesis of C and thus interferes with optimal action of the "effective" drug. If the interfering drug is present in sufficient concentration to block the pathway efficiently, then it becomes the over-all rate limiting agent in the metabolic sequence and makes the observation of antagonism impossible. This hypothesis fits the experimental facts. It is of

interest that thirty years ago Browning and Gulbransen (13) attributed interference among trypanocidal substances to "combination of both chemical substances with the same haptophore groups of the trypanosomes' protoplasm."



Antibiotic synergism could be explained by the simultaneous blocking of the multiple interacting pathways essential for growth. If one pathway (A, B, C, D) were blocked by one drug, the organism might be temporarily restrained, but might soon utilize other pathways (W, X, Y, Z) with greater efficiency and thus bypass the block. If, however, two or more metabolic pathways were blocked, the organism might die. To fit such a hypothesis only one of the two synergistic drugs would need to manifest antimicrobial activity when acting alone, *i.e.*, one of the agents might block the pathway utilized more efficiently by the microorganism; the other might effect a pathway not ordinarily used and would thus appear to have little, if any, effect alone. This is in agreement with experimental findings discussed earlier. These hypotheses are of necessity entirely speculative and no direct evidence can be adduced to support them at this time.

SUMMARY

The outline drawn here of combined antibiotic action is, of necessity, tentative and incomplete. Only a framework of the complex structure is discernible at this time. Far too few facts are available to guide the physician, anxious to use drug combinations quickly and to best advantage. We might agree with the warning expressed about another "new" remedy almost 60 years ago (63): "We are not unaware of how much caution is necessary in judging a new remedy. We must beware of hasty conclusions. We have exposed the facts and doctors may be anxious to use this serum. This has not yet reached the point of efficiency we hope for. At any rate let doctors not forget the necessity of a bacteriological diagnosis without which they expose themselves to serious mistakes." The author of this warning statement was concerned with anti-streptococcus serum, a remedy now long forgotten. His words of caution are a timely warning in the field of combined antibiotic therapy.

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