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The Problem of Synergism and Antagonism of Combined Drugs

In honor of Professor Dr. O. Loewi on his 80th birthday

by Professor Dr. S. Loewe

I.

The sweeping consequences of the discovery of the humoral transmission of nerve impulses could not fail to extend even into such a seemingly distant field as that of drug combinations: Before long, the combined effect of physostigmine and acetylcholine, previously on record as a highly superadditive synergism between two similarly acting drugs, was offered an unexpected biochemical explanation by the disclosure of their combined action. Such a denouement was indeed liable to fix thenceforth the attention to the intricate and variegated mechanisms by which drugs may accomplish their cooperation. However, it must also stress the importance of discriminating between a *ction* (in the stricter sense; „Einwirkung“) and *effect* („Auswirkung“), i. e., between the necessarily biochemical events which take place in the earlier phase of the pharmacologic chain process and the physiological events occurring in the aftermath of the biochemical process, often at a considerable distance from the site of action. Not until all the factors which convert action into effect are known can elucidation of the mechanism of action alter the concepts governing analysis and evaluation of drug effect. Indeed, the quantitative problems of combined drug effect still persist unchanged, revolving around the two terms synergism and antagonism, — which are in need of clarification as ever before.

II.

Synergism and antagonism of drug combinations are usually defined as supra- and infra-additive effects of combined drugs. Thus, the meaning of the two words depends on an adequate definition of the basic criterion, the additive behavior („additivism“) of combinations, which unfortunately is as yet lacking. The entire complex is a problem of the relationship between drug dose, D, and intensity of effect, E. This fact will dominate the present analysis.

The clearest view of the dose/effect relationship of combinations is obtained by stripping the problem to its essentials and envisaging a most simplified situation: suitably that of a binary combination of two drugs, A and B, having the same effect in common, each of the two effects following an S-shaped D/E curve, both drugs being equipotent and studied in populations of uniform sensitivity. In this streamlined situation, the analog of the familiar two-dimensional D/E curve of single drugs is a three-dimensional D/E surface extending in the space above a two-dimensional area with the coordinates A and B of the doses of the two drugs, the third coordinate being an E-ordinate of intensity of

effect (figure 1) [5]. By projecting lines of equieffectiveness, at which ordinate planes of appropriately chosen E-values (= „end-points“ of effect) transect the D/E surface, vertically upon the A/B area, one plots a two-dimensional profile map of the D/E surface in the A/B plane. The profile lines of equieffectiveness are

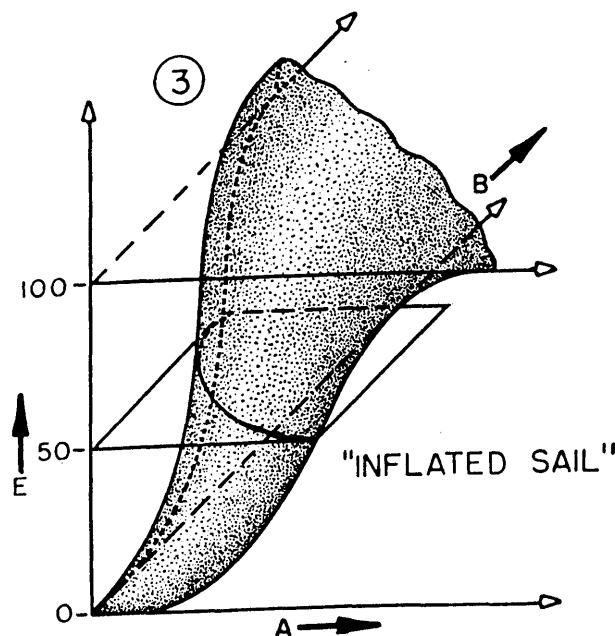
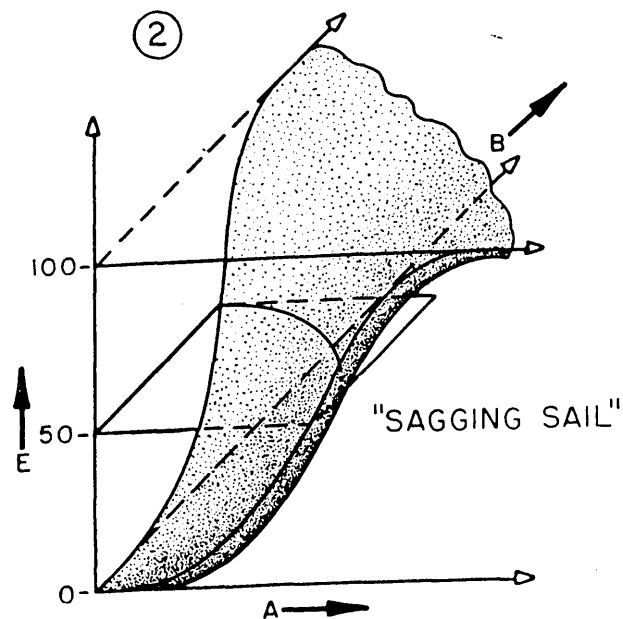
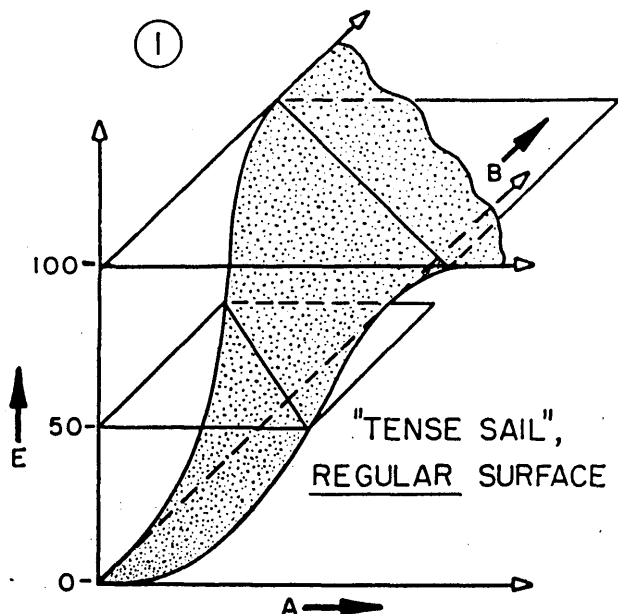


Figure 1: Three examples of the dose/effect relationship of two combined drugs A and B. — A and B: Abscissae of the doses of A and B, respectively, delineating the horizontal A/B plane of combined doses of the two drugs. E: Ordinate of intensities of effect (= 100). The S-shaped lines in the vertical A/E and B/E planes are the dose/effect curves of drug A and B, respectively. The dotted surface in the A/B/E space octant represents the dose/effect (D/E) surface of the combination. The horizontal planes at E 50 level (in drawing 1 also at E 100) are shown to transect the D/E surface in lines of equieffectiveness (isoboles). — Drawing 1: D/E surface due to iso-additive behavior. Drawings 2 and 3: D/E surfaces for iso-synergism (3) and iso-antagonism (2), which may also express hetero-additive behavior or heterosynergism or -antagonism. (Further explanation in text).

called isoboles; the profile maps, isobolograms [5, 10]. (For examples see figures 2 and 5.)*

III.

Such view of the D/E relationship of combined drugs should furnish the means of identification of synergism and antagonism. Synergistic effect must be manifested by a deviation of the isobole to SW (see figure 2), antagonistic effect by a deviation to NE from the course

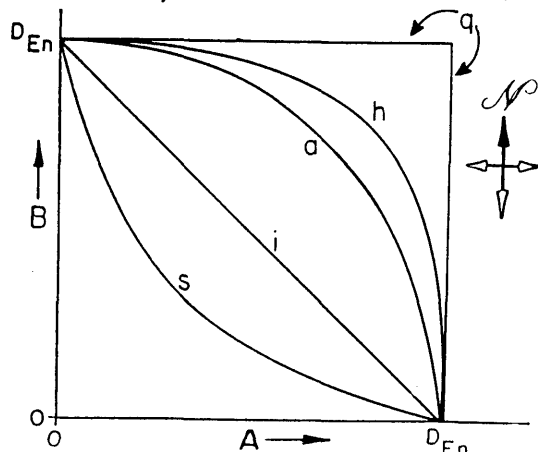


Figure 2: Isobologram (projections from tridimensional presentations as those in Figure 1 upon the A/B dose area), showing 5 isoboles of combined doses having the same intensity of effect, E_n , and being equieffective to the doses D_{En}^A of A and B according to iso-additive (i), hetero-additive (s, a, h, q) or non-additive behavior (s, a, h). (Compass marking at right is entered to facilitate designation of positions and directions in the A/B area).

taken by the isobole in case of additive behavior. However, a certain complication arises, due to the two-fold answer to the preliminary question: What adds what to what?

By many students of the problem, additivism is explained by the example of the halved doses and said to prevail when one half of D_{En}^A , a certain dose of A, combined with one half of D_{En}^B , a dose equieffective to D_{En}^A , evokes the same intensity of effect as D_{En}^A or D_{En}^B . Under this premiss, the course of the additive isobole is defined by the equation

$$D_{En}^A + (D_{En}^B - D_{Em}^B) = D_{En}^C \quad (I)$$

where C is the symbol for the combination of A and B, the subscripts mark that intensity of the effect E, which is correlated to the respective D, and $En > Em$ is the intensity of effect prevailing uniformly all along the course of the isobole concerned.

Others, however, suppose that additivism prevails when the intensity of the combined effect equals the sum of the intensities of effect, which each of the com-

*) It is encouraging to observe that these old presentations of the D/E relationship of combined drugs, both the stereometrical of the D/E space-surface and the geometrical of the isobologram, comply well with the psychologist's requirement for an appropriate „view of the Gestalt of the problem“ (Wertheimer [12]; cf. also Weiland [11]). Complete symmetry, a major criterion of „good Gestalt“ [12], prevails in the specified ideal situation owing to the identity of the two D/E curves (the symmetry axis in the isobologram is the median „ray“ [5, 10] of the A/B quadrant (A:B = 1); the symmetry plane in the stereometric view is the median plane of the space octant, erected on the median ray!); in cases with non-identical, but geometrically similar S-shaped D/E curves, the same symmetry is obtained by establishing an isometrical A/B area, i.e., one in which the dose connotations of equal abscissa distances from zero are reciprocal to the proportionality factor

$$k = D_{En}^A / D_{En}^B$$

(where D^A and D^B refer to doses of the component drugs and the subscripts En indicate that each of the two doses exerts the same endpoint effect); in cases with dissimilar D/E curves, one attains greatest possible symmetry in the same way by building the isometrical A/B area with the aid of the proportionality factor for the two doses exerting the endpoint effect primarily studied. If D^C refers to the combined doses within the A/B area,

$$D_{En}^C \text{ (equieffective to } D_{En}^A \text{ and } D_{En}^B \text{)}$$

designates any dose pair falling on the isobole for E_n . — The expediency afforded by the symmetrical view of the problem will be of decisive assistance in the present study.

ponent doses D^A and D^B evokes when administered alone. In this case, the equation for the additive isobole is

$$D_{Em}^A + D_{(En - Em)}^B = D_{En}^C \quad (II)$$

Type II is indeed additive behavior as it may be expected from a naive approach: Each component of every dose pair simply brings its own inherent effect into the marriage. In type I, the combined drug effect is accomplished in a much more mysterious manner: The one moiety of the combined dose does not add its own effect to that of the other moiety but complements the effect of the latter exactly to that intensity of effect which would be achieved by the sum of the fractional doses if both were fractions of either A or B. Yet, this mysterious type of additivism is invariably encountered in the simplest of all combinations, the sham combination, in which A and B are one and the same drug, — a case in which the additive nature of the cooperation can hardly be questioned.

Attention has been drawn to these two different concepts of additivism as early as 1913, — long before the concept of the D/E surface had been developed or even that of the D/E curve had become common knowledge, — in an ingenious presentation by W. Frei [3]. He coined the terms iso-addition for type I and hetero-addition for type II. Disregard of his work may well be held responsible for much of the confusion created by the coexistence of the two alternatives. The dualism has never been challenged. Both concepts can lay equal claim to the designation additivism. Thus both have to be given equal consideration.

IV.

The question of the course of the additive isobole can now be answered with the aid of equations I and II. Presentation of the answer may be facilitated by the use of the attribute „homodynamic“ for any two or more drug effects which obey congruent or mathematically similar (= proportional) D/E curves and, with a permissible license, also for the respective drugs and combinations with regard to such effects; accordingly the term „heterodynamic“ will apply to effects which obey dissimilar D/E curves*).

A. Iso-additive Isoboles differ in their general course, depending on the D/E curves of the component drugs.

1. For homodynamic combinations, the iso-additive isobole is always linear, because in all these cases D_{Em}^B equals D_{Em}^A , which converts the governing formula into the equation of a straight line, namely, for the linear connection between the equieffective endpoint doses on the A and B axes (herein called „endpoint diagonal“, in preference to the previously [5, 10] used expression „Additionsisobole“). In brief, for homodynamic drug effect, the iso-additive isobole coincides with the endpoint diagonal (e. g., i in figure 2), as is best witnessed by every sham combination. Three out of many implications of this diagonal-isobole iso-additivism are the following:

(a) The D/E surface is mathematically regular, such as that in figure 1, drawing 1.

(b) The D/E surface is symmetrical in reference to the vertical symmetry plane of the space octant.

(c) The isoboles for all endpoints of the common effect are parallel to each other (compare the diagonals in figure 5).

2. For heterodynamic combinations the course of the iso-additive isobole is not so sharply determined by the equation (I), because $D_{Em}^B \neq D_{Em}^A$. Hence the following implications:

(a) The likelihood that the isobole coincides with the endpoint diagonal is minimal; the overwhelming probability is that the isobole deviates from the endpoint

*) The root of the two words (δυναμις) appears to offer an appropriate reference to action in the stricter sense (see section I), as ερροσ appears in reference to effect. One can then justify the use of the expressions „homo-“ and „heterodynamic“ for the purposes defined in the text, by acceding to the general assumption that geometrical similarity of graded D/E curves of drugs points to similarity in their mode of action. The analogous attributes „homergic“ and „heterergic“, — expressing whether the respective endpoint of effect of a combination is common to both component drugs or is encountered only in one of the two, — as well as „anti-dynamic“ (previously [6] used for a similar purpose), etc., — should be appropriate to take the place of complicated explanatory phrases.

diagonal with either SW- (e.g., s in figure 2) or NE-convexity (e.g., a and h in figure 2).

(b) The D/E surface is practically never regular; it may either sag downward or bulge upward, as in the drawings 2 and 3 of figure 1, or may have a still more irregular shape.

(c) Heterodynamic combinations usually have two isoboles for the same endpoint, one deviating to NE, the other to SW from the endpoint diagonal (compare figure 3, which resulted from calculation of a case

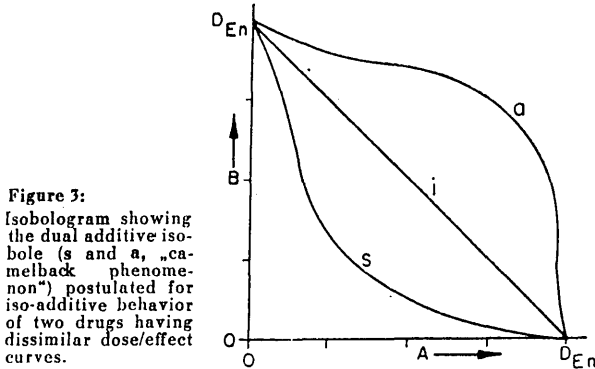


Figure 3: Isobologram showing the dual additive isobole (s and a, "camelback phenomenon") postulated for iso-additive behavior of two drugs having dissimilar dose/effect curves.

with only moderately dissimilar D/E curves). The very banal reason is that the row of equieffective drug pairs, calculated by use of the left-hand summands

$$D_{Em}^A \text{ and } (D_{En}^B - D_{Em}^B),$$

occupies on the A/B plane a location entirely different from that obtained by using

$$D_{Em}^B \text{ and } (D_{En}^A - D_{Em}^A).$$

The dual isoboles express that the D/E surface of these combinations has "camelback" shape, an extra hump being superimposed at endpoint level on the otherwise more or less steadily ascending surface.

B. Hetero-additive Isoboles, according to equation II, have no sharply predetermined course. Hence both homo- and heterodynamic drugs are unlikely to exhibit linear isoboles unless their D/E curves follow a bizarre and/or intrinsically impossible course. Thus, by their shape alone hetero-additive isoboles are with minor drawbacks distinguishable from iso-additive isoboles of homodynamic, but indistinguishable from those of heterodynamic drugs. Three other implications of the equation, however, point to peculiarities of the hetero-additive isoboles of homodynamic drugs having S-shaped D/E curves. Their course varies according to the endpoint level, and their direction may change for the same endpoint isobole according to the ratio of the doses forming the individual drug pair:

1. The isoboles for endpoints up to medium or even higher level ($\leq E_{60}$) always deviate to NE from the respective endpoint diagonal.

2. The NE convexity decreases, the more the endpoint studied approaches maximum intensity of effect (E_{100}) and may even be reversed into SW convexity at doses above D_{E100} if the conditions allow for "supramaximal" effect.

3. At endpoint doses in a certain range to both sides of D_{E100} , the isoboles may have a winding course. For obvious reasons, the reversal of the convexity starts at mixtures of the two drugs in very small and very high ($D^A : D^B$) ratios, i. e., at those parts of the isobole closest to the A and B axes; in these sections, then, the isobole may not significantly deviate from the endpoint diagonal or may already have assumed SW convexity, whereas in its middle section, near the ($D^A : D^B = 1$) symmetry axis of the A/B plane, it may still have NE convexity.

The first two of these implications are illustrated in figure 4 with regard to their expression in the D/E surface and in figure 5, a profile map of a similar space surface, with regard to their expression in the isobologram.

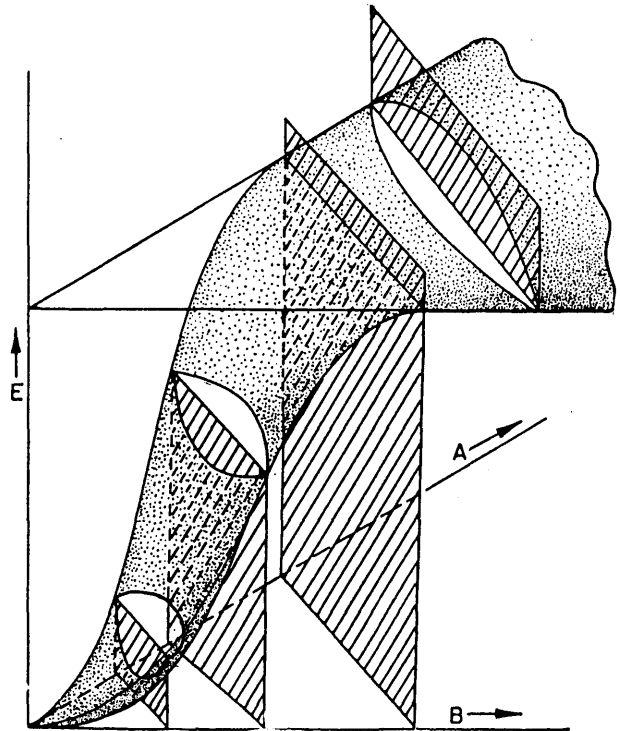


Figure 4: Hetero-additive dose/effect surface of combination of two drugs A and B, having similar dose/effect curves. — Vertical planes are erected in four endpoint diagonals of the horizontal A/B area, to illustrate by their transections through the dose/effect surface how the sagging of the "sail" decreases with increasing dosage and may even be reversed to bulging (uppermost right) at supramaximal doses.

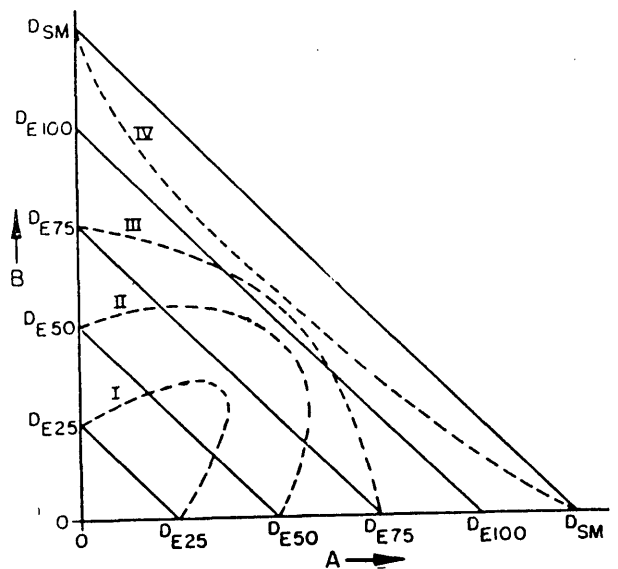


Figure 5: Isobologram showing isoboles (dotted lines) for five different endpoints of hetero-additive effect of a combination of two drugs with similar dose/effect curves. — The straight lines within the A/B area are the respective endpoint diagonals. — D_{SM} = supramaximal dose. — The graph is a profile map of the dose/effect surface presented in figure 4. Note the decreasing NE-convexity of isoboles I, II and III, and the reversed (SW-) convexity of isobole IV. At E_{100} , isobole and endpoint diagonal coincide.

C. Quantal Effects. The difference between the two types of additive behavior of combinations is particularly emphasized when the experimental procedure is designed to employ "quantal" evaluation of effect. In these cases, only one single endpoint is taken into consideration, and the D/E relationship is determined only on the basis of a "yes-or-no" answer to the dose stimulus, i. e., according to whether or not the requirement of the endpoint effect is fulfilled. In the ideal, uniformly sensitive population, D/E curves plotted on this basis have bayonet shape expressing an abrupt progression from $E = 0$ to E_n at a single dose level (D_{En}). In case of iso-additivism, the isobole for combined quantal

effect must coincide with the endpoint diagonal, as is obvious in any quantal effect of any sham combination, and separates a triangular SW area of ineffective doses from the entire NE area of effective doses. Hetero-additivism results in a quite different, unique course of the quantal isobole. Since the effect of any component dose lower than the endpoint dose is zero, no dose pair can exert a combined effect > 0 unless at least one of the component doses equals or exceeds D_{En} . The result is an L-shaped isobole running along the upper and right sides of the D_{En} -square (q in figure 2), a result postulated by equation II in which, for quantal effects, any dose $< D_{En}$ is $D_{Em} = D_E$, zero, and $D_{(En-Em)} = D_{(En-0)} = D_{En}$.*).

V.

With the preceding analysis, the immediate objective of this study, to furnish the lacking definition of additivism, is attained and the variety of appearances of additive behavior outlined. Therewith the definition of synergism and antagonism is placed upon a solid basis. The usefulness of these clarifications depends primarily on the predictability of which of the two principles of additivism applies to the individual combination.

The originator of the two concepts of additivism suggested that combinations of drugs „from the same pharmacological class“ obey the principle of iso-, those from different classes, that of hetero-additivism [3]. These two types of combinations, though only vaguely delineated, may well be considered to coincide with the two classes of homo- and heterodynamic combinations, respectively. Even with this specification, that conjectural answer does not hold without major corrections.

According to A 2 (c) of the preceding section, isoadditivism could apply to heterodynamic combinations only if the occurrence of the „camelback phenomenon“ were demonstrated**. If demonstrable, it would cause a new complication, because then synergism in reference to one of the dual isoboles may be antagonism in reference to the other. The overwhelmingly greater probability is that heterodynamic drugs are indeed relegated to hetero-additive behavior. Homodynamic drugs are then the only ones capable of obeying the rules of iso-additivism; however, according to B 1 of the preceding section and contrary to W. Frei's assumption, they can also follow the rules of hetero-additivism.

According to item C of the preceding section, isoboles established by way of the quantal effect procedure offer a more favorable situation. Here, not only isoadditive behavior of homodynamic drugs is at once expressed by the diagonal course of the isobole, but likewise there is only one expression, and a very characteristic one, of hetero-additive behavior, namely, the L-shaped isobole. These particularities gain added importance in view of the wide use of the quantal effect procedure. In fact, every determination of an endpoint dose, in single as well as in combined drugs, employs some procedure of quantal effect evaluation; in the ideal, uniformly sensitive population the minimum single-drug dose correlated with the „endpoint quantum“ of effect is always derived by interpolation between doses with too low („no“) and those with too high („yes“) effects, and the equieffectiveness of drug pairs along an isobole of their combination is established by the aid of analogous inequality comparisons. Thus, iso- and hetero-additivism should be readily recognizable and clearly distinguishable in quantal effect studies. However, transitions from hetero- to iso-additivism cannot be discounted. A frequently studied endpoint phenomenon, necessarily dealt with by the quantal effect procedure, namely, lethal effect, is an instructive example. Ultimately, even extremely dissimilar drugs may mutually facilitate their quite heterodynamic effects. At adequately high doses, the „independent action“ [1, 2] is no further independent and, accordingly, the L-shape is obtunded and the isobole may be indistinguishable from any other NE-convex isobole. Moreover, even under the relatively uncomplicated condition of quantal effect procedure there remain difficulties of classification, pointed out, for instance, by the example of the lethal effect of the combination of acetphenetidine and barbital [9], which is hardly homodynamic and yet obeys a virtually diagonal isobole.

Without going into further details, one can state that, whereas there are certain criteria to distinguish between iso- and hetero-additive behavior of a combination, the further alternative of homo- versus heterodynamic effects poses complicating problems. Thus it is prerequisite for any classification of combinations according to this alternative, to ascertain the complete D/E curves of the component drugs. Mere establishing of the isoboles might be of great assistance if the possibility of non-additive behavior could be excluded.

*) Accordingly the equation for iso-addition (I), although at first sight it may appear to become meaningless in the quantal procedure, is applicable at least for homodynamic effects, when the left side is written

$$D^A + [D_{En}^B - (1/k \times D^A) B]$$

where the subtrahend is changed in accordance with the postulate for iso-addition of homodynamic effects, namely, that

$$D_{Em}^B \text{ is equieffective to } 1/k \times D_{Em}^A.$$

**) In certain respects, the coalitive effects encountered in quite a number of drug combinations [7] might be found comparable to the camelback phenomenon; however, further evidence will be needed before they can be employed as proof for the existence of the here derived phenomenon.

VI.

According to the preceding analysis, the coexistence of the two sets of alternatives — iso- vs. hetero-additivism and additive vs. non-additive behavior — would raise enormous, virtually insuperable obstacles to the identification of synergism and antagonism in a combination, since in the great majority of cases non-additive isoboles are indistinguishable from additive isoboles. It is therefore imperative to ask whether all four concepts are backed by reality and, hence, all six resultant possibilities have to be considered.

As to the two types of additivism, it is pointed out above that neither of them can be accepted as a unitarian view of the additive potentialities of combinations. Indeed, only the reality of iso-additivism has ever been questioned, and the only argument proffered — namely that no two drugs are as identical as the components of the undoubtedly iso-additive sham combination — is by no means conclusive. Even if iso-additivism were limited to homodynamic effects, its prerequisite would not be overall-identity, but only similarity of the components with regard to the one effect under consideration; the possibility that two drugs fulfill this prerequisite cannot be dismissed.

It is therefore the tenability of the second set of concepts, synergism and antagonism, which has to be thoroughly scrutinized. The difficulties encountered by many in their attempt to define synergism, antagonism and additive behavior of drug combinations are often manifested in that they delineate additive from non-additive behavior according to expectability. Such demarcation line is subjective and liable to vary with the state of knowledge. If all the factors were known qualitatively and quantitatively, which concur in the processes determining the cooperative effects of drugs, these effects were always additive, that is, predictable by way of mere addition of all the positive and negative factors in play, ergo: expectable. The terms supra- and infra-additive, pivotal in the definition of synergism and antagonism, then express nothing but the assumption that in instances unexpectedly high or low intensities of effect can result from the combined action of drugs. To attribute reality to synergism and antagonism, as they are defined in the application of these words to combined drug effects, means to expect the unexpected. That would seem to relegate the two terms from the realm of science to that of epistemology.

VII.

Barring „synergism“ and „antagonism“, as terms without logical background, and so preventing them from being at the mercy of conceptually unsolicitous users would return the attention to the real problems and aims of the quantitation of combined drug effects. Biometrical treatment and evaluation of combined drug effects are on solid ground when they concentrate on an exhaustive pharmacologic characterization of the individual combination by the aid of such adequately defined concepts as the D/E curves of the components and the experimental isoboles which express the relation between component doses and combined effect. For the major scope of such quantitations it is anyway not enough to limit the investigation to one single effect. Much more important objectives are the comparative study of various effects of the same combination and the determination of the ratios between their intensities, i. e., of the degree of selectivity [8] of an individual effect. One of the major interests in combined drugs is to know whether these ratios — which for practical purposes are margins of safety or therapeutic indices — assume a larger or smaller value in the combination than in the components.

For the pursuit of all these objectives, the here presented view of the combination problem should afford a useful basis. To be sure, the limitations and simplifications, which were introduced for the special purpose of this study must be abandoned. A major limitation was the restriction of the considerations to homodynamic and homergic effects, effects which the combined drugs have in common. To expand the discussion to the other — antidynamic, heterergic, etc. — types of the

large variety of combined effects would exceed the frame of this study. That variety has been emphasized, classified and exemplified elsewhere [4, 6, 7, 9, 10].

Suffice it to exemplify the dispensability of the concepts of synergism and antagonism even in particularly complicated situations. An example which seems to be most suggestive of non-additive interpretations is that of seemingly heterergic effects, i.e., those effects of a combination which are exhibited by only one of the component drugs, say, drug A. Heterergic effect is obviously expressed by a rectilinear isobole running from D_{EN}^A parallel to the dose abscissa of drug B. Truly heterergic effects cannot be treated on the basis of an isometric isobologram, because $D_{EN}^B = \infty$. However, if the isobole of such a one-sided effect is found to deviate either to N or to S from the rectilinear course of the truly heterergic isobole without meeting the B-axis within the A/B-area studied, this alone cannot possibly justify a conclusion of non-additive behavior. Rather a deviation toward N must be ascribed to antidynamic effect, an S-deviation to homergic or homodynamic effect, unless the assumption of a heterergic nature of the combined effect has been given reasonable support by extensive study of the entire spectrum of effects of B and of the D/E curves in question. A single-endpoint study by a quantal effect procedure, which throws no light on the D/E relationship, is certainly inadequate.

The simplification most helpful in the general considerations of this paper, but which may seem to remove them farthest from biological reality with its abundance of phenomena of variation, was the restriction of all considerations to the assumption of a population of „ideal individuals“, i.e., of uniform sensitivity throughout the population studied. However, from the beginning (l. c. [5], p. 187) it has been emphasized that these factors of variation expand every point, line and surface of the simplified view into a circle, band or wall of scatter. That does not diminish the validity of the simplified view, nor does it hamper its applicability to the realities of the experiment. Quite to the contrary: Under the conditions of natural variability and under the rules of frequency distribution which govern it, the „ideal individual“ simply becomes „the median individual“, the individual of median sensitivity to the drug(s). In reality, the median individual determines the elements — points, lines, surfaces — of our structural view. It is the median individual, to which the ED_{50} refers (a term, by the way, in which the symbol E, without change of its meaning, can be spelled „endpoint-effective“ instead of „effective“).

It is here, in the evaluation of the ED_{50} , where biostatistical mathematics add their share to the „Gestalt-mathematics“ of the general view. The ED_{50} is ascertained by interpolation with the aid of an accumulative curve of the „distribution of tolerances“ [2]* in the population studied, Finney's „D/P curve“**. This curve, which indeed also has an abscissa of drug dose as has the D/E curve, differs sharply from a D/E curve in the dimension of the ordinate. Its ordinate values < 50 and > 50 (sc. per cent of population, exhibiting the endpoint effect) and the coordinated abscissa values — ED_{50} , ED_{10} , ED_{90} , etc., — serve merely to find the ED_{50} , but should not be misinterpreted as indicators of intensity of effect, as it is so often done. As will be explained in detail elsewhere, it is important to keep in mind that with every D/E curve an infinitely large family of D/P curves is affiliated, that a different D/P curve is coordinated to each of the endpoints on a D/E curve, and that the respective ED_{50} value is the only point which each of these D/P curves has in common with the D/E curve. All other points of the D/P curves serve only to determine, so to speak, the density of the band of variation extending to both sides of the D/E curve. Obviously, the density of this band is not a yardstick of intensity of effect, and hence determination of the D/P relationship cannot take the place of the determination of the D/E relationship in the treatment of problems of the effect of either single or combined drugs.

Although, according to his study, the terms synergism and antagonism and analogous terms for supra- and infra-additive effects of combined drugs have no definable place in the treatment of combination problems and should be eliminated from the field because of the menace of confusion, the greater probability is that they will live on as so many other undefined and undefinable „terms“. If that cannot be avoided, one should try to make them serve a useful purpose by giving them a precise, though only conventional definition. As has been shown, the only sharply delineated and recognizable appearance of additive behavior is that of greatly similar drugs, illustrated by the example of the sham combination composed of identical components. This type of additivism, characterized by the

coincidence of its isobole with the endpoint diagonal, offers an unambiguous standard of reference for the evaluation of drugs from the viewpoint of whether or not their combined effect promises a certain limited advantage or disadvantage over the use of either one or the other of the component drugs alone, be it for therapeutic or for toxicological purposes. Effects following an isobole deviating from the endpoint diagonal into the lower-dose moiety of the A/B area („to SW“) may be called synergistic, those deviating in the opposite direction („NE“), antagonistic. It is certain that this pragmatic use of the two words makes them instruments of really helpful information to the physician and the toxicologist, whereas, for instance, interpretation of a slight SW-deviation of an isobole of combined lethal effect of two heterodynamic insecticides from the L-course as „synergism“, on the strength of whatsoever definition of the word, may most unfortunately mislead the user of such a combination, who would obtain the same effect more economically with perhaps only half the amount of the „synergistic“ mixture if he would employ one of the two components alone. It will be recalled that the here proposed „re“-definition of the two terms is by no means new; they have always been used in this sense not only by this writer but by many other investigators.

Summary:

1. This study endeavors to examine the characteristics of the dose/effect relationship of combined drugs when the combined effect is due to additive behavior.

2. The analysis indicates that additivism is dual in nature, that one cannot predict which of the two types of additivism governs the behavior of an individual drug pair, and that rarely, if ever, do the dose/effect relations of a combination indicate whether the combined effect is the result of additive or of non-additive behavior.

3. The conclusion is reached that „synergism“ and „antagonism“, which can be recognized only as supra- and infra-additive deviations from a firmly established additive dose/effect relationship, are imaginary magnitudes devoid of a basis of reference and of practical applicability.

4. It is also pointed out that the question of non-additive behavior of combinations cannot be answered through a biostatistical approach and that the problem is not solved by confounding the relationship between dose and effect with the relationship between dose and individual tolerance or susceptibility.

5. The same view of the combination problem, which helped in deciding the special issue of this study, is shown to furnish the proper basis for the treatment of all other questions of combined drug effect, once synergism and antagonism are duly relegated from consideration, and to delineate clearly the circumscribed field in which biostatistical procedures become an important technical expedient.

Zusammenfassung:

1. Es wird untersucht, welches die Merkmale der Beziehung zwischen Dosis und Wirkung einer Pharmaka-Kombination sind, wenn die Kombinationswirkung additivem Verhalten zuzuschreiben ist.

2. Es zeigt sich, daß es zwei Arten von Additivismus gibt, daß beide erwartet werden müssen, daß die Zugehörigkeit eines Pharmakonpaares zu einer oder der anderen der beiden Additionsformen nicht vorausbestimmt werden kann und daß auch die Dosis-Wirkungs-Beziehung einer Kombination höchstens in Ausnahmefällen verrät, ob die Kombinationswirkung das Ergebnis additiven oder nicht-additiven Verhaltens ist.

3. Der Schluß liegt nahe, daß Synergismus und Antagonismus, die ja nur als über- und unteradditive Abweichungen von einer sichergestellten additiven Dosis-Wirkungs-Beziehung erkennbar sind, imaginäre Größen ohne Bezugsgröße und ohne praktische Anwendbarkeit sind.

4. Es wird ferner gezeigt, daß die Frage des nicht-additiven Verhaltens von Arzneimittel-Kombinationen auch nicht auf biostatistischem Wege entschieden werden kann und daß das Problem nicht dadurch einer Lösung nähergebracht wird, daß man die Beziehung zwischen

*) Finney [2] employs the word „tolerance“ in the reciprocal sense of the word „sensitivity“ used in this paper.

**) The D/P curve is sometimes called „dose/response curve“, an expression which also should not be confounded with „dose/effect curve“.

Dosis und Wirkung mit derjenigen zwischen Dosis und individueller Toleranz oder Empfindlichkeit verwechselt.

5. Schließlich wird gezeigt, daß die gleiche Betrachtungsweise des Kombinationsproblems, die bei der Lösung der engeren Aufgabe dieser Untersuchung half, auch die gegebene Basis für die Behandlung aller anderen Fragen der Mischwirkung liefert, wofür nur erst Synergismus und Antagonismus nach Gebühr von der Betrachtung ausgeschlossen sind, und daß sie auch der Biostatistik den genau umschriebenen Platz zuweist, an dem sie zum wertvollen technischen Hilfsmittel wird.

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Vom Alkohol zum Acetylcholin

Herrn Professor Dr. Otto Loewi zum 80. Geburtstag gewidmet

Von E. Racker, M. D.

Allgemein wird angenommen, daß die angenehmen Empfindungen nach Einnahme bekömmlicher Dosen Äthylalkohols den narkotischen Eigenschaften des Äthanolz zuzuschreiben seien, während die klinischen Nachwirkungen wenigstens teilweise auf seine Oxydationsprodukte zurückgeführt werden müßten. Es ist bezeichnend, daß die Bemühungen des menschlichen Geistes nicht darauf gerichtet worden sind, die Anhäufung dieser toxischen Oxydationsprodukte zu verhindern, sondern darauf, sie zu vergrößern. Die außerordentlich unangenehmen Begleiterscheinungen, die „Antabus“ (Tetraaethylthiuramidisulfid = TETD) nach der Einnahme von Alkohol hervorruft, haben es zu einer weit verbreiteten Waffe im Kampf gegen den Alkoholismus werden lassen [1]. Viele der von diesem Arzneimittel hervorgerufenen Symptome sind dem Acetaldehyd, der ersten Oxydationsstufe des Alkohols, zugeschrieben worden. Nach kombinierter Zufuhr von Alkohol und TETD wurde ein höherer Acetaldehyd-Bloodspiegel gefunden als nach der alleinigen Einnahme von Alkohol [1]. Schon sehr kurze Zeit nach Bekanntwerden der ersten klinischen Berichte über die Anwendung von TETD wurden in mehreren Laboratorien, ganz unabhängig voneinander, Untersuchungen angestellt, um die Wirkungsweise dieses Arzneimittels auf einer enzymatischen Ebene zu klären. Da bekanntlich mehrere Enzyme die Aldehydoxydation katalysieren, überrascht es nicht, wenn dabei von verschiedenen Untersuchern von einander abweichende Wege beschrieben wurden. So untersuchte Kjølgaard [2] die Wirkung von TETD auf die Aldehydoxydase, Graham [3] und Racker [4] auf die Aldehyddehydrogenase, Richert u. Mitarb. [5] auf die Xanthinoxidase, und Nygaard und Sumner [6] auf die Triosephosphatdehydrogenase. Nach diesen Untersuchungen hat TETD eine hemmende Wirkung auf fast alle die Aldehyde beeinflussenden Enzyme, und zwar in einigen Fällen in so niedrigen Konzentrationen (10^{-5} bis 10^{-6} molar), daß an eine physiologische Bedeutung gedacht werden kann. In unserem Laboratorium wurde bei einer Reihe weiterer Enzyme (Fumarase, Milchsäuredehydrogenase und Gärungsalkoholdehydrogenase) eine Hemmung durch derartig niedrige Konzentrationen nicht beobachtet. Da bei der Oxydation von Ketosäuren und Aldehyden die meisten Enzyme S-H-Bindungen enthaltende Coenzyme — wie das Coenzym A (CoA) oder das Glutathion [7] — benötigen, war die Feststellung interessant, daß ein die S-S-Bindung enthaltendes Arzneimittel diese stark hemmende Wirkung ausübt. Überdies scheinen reduzierende Substanzen wie Glutathion oder Cystein die hemmende Wirkung des TETD zu verhindern [3, 6], was möglicherweise durch die Reduktion der S-S-Bindung des Arzneimittels bedingt ist. Andererseits muß betont werden, daß nach unseren Beobachtungen die durch Jodessigsäure leicht zu vergiftende Gärungsalkoholdehydrogenase gegenüber dem hemmenden Einfluß von niedrigen TETD-Konzentrationen resistent war.

Ein anderes Arzneimittel, das Chloralhydrat, sehr bekannt bei Klinikern und Mitgliedern der Unterwelt

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wegen seines Einflusses auf den Alkoholstoffwechsel, wurde in unserem Laboratorium ebenfalls untersucht. Auch dieses Arzneimittel hemmt die Aldehyddehydrogenase (durch Verdrängung vom Substrat), jedoch hemmt es ebenfalls die Alkoholoxydation. Diese Ergebnisse können dazu beitragen, die Verstärkung der Narkosewirkung bei gleichzeitiger Verabfolgung von Alkohol und Chloralhydrat zu erklären.

Die letzten Jahre haben unser Verständnis für den Stoffwechsel des Alkohols und seiner Oxydationsprodukte beachtlich gefördert. Die augenblicklich bekannten Stoffwechsellvorgänge können zusammenfassend wie folgt dargestellt werden:

1. Äthanol wird zu Acetaldehyd oxydiert

Dieser Prozeß wird durch eine nukleotid-gebundene Alkoholdehydrogenase, die weit verbreitet in Tieren, Pflanzen und Mikroorganismen vorkommt, katalysiert. Bei Gegenwart von Wasserstoffsuperoxyd oxydiert auch die Katalase den Alkohol zu Acetaldehyd. Diese Reaktion findet *in vitro* statt [8], ihre physiologische Bedeutung ist jedoch angezweifelt worden [9, 10].

2. Die Oxydation von Acetaldehyd zu Essigsäure

Diese Reaktion wird durch verschiedene bekannte Enzyme katalysiert, die in tierischen Organen, besonders in der Leber, vorkommen. Eines davon ist ein Flavoprotein, das verschiedene Aldehyde oxydiert, ein anderes ist die Xanthinoxidase, die auch Purine oxydiert, und ein drittes ist eine nukleotid-gebundene Dehydrogenase, welche viele Aldehyde einschließlich des Betinaldehyds oxydiert. Durch die kürzlich gemachten, interessanten Entdeckungen von Kendall u. Ramanaathan scheint noch ein weiterer Mechanismus der Aldehydoxydation möglich zu sein [10]. Sie fanden, daß Enzympräparate aus der Leber, die bekanntlich sowohl Alkoholdehydrogenase als auch Aldehyddehydrogenase enthalten, Ester bilden, wenn ein Aldehyd zusammen mit einem Alkohol hinzugefügt wird. In der nächsten Stufe wird der Ester zu der freien Säure hydrolysiert. Die ganze Reaktion kann als eine Oxydation des Aldehyds mit Alkohol als Katalysator angesehen werden. Da durch Jodacetat (das hemmend auf das Aldehydenzym, nicht aber auf das Alkoholenzym in der Leber wirkt) keine Hemmung des Reaktionsablaufes erfolgte, wurde die Reaktion der Tätigkeit der Alkoholdehydrogenase zugeschrieben. In unserem Laboratorium wurde eine ziemlich ähnliche Reaktion mit kristallisierter Gärungsalkoholdehydrogenase beobachtet, welche die Reduktion von bestimmten Thioestern des Glutathions katalysierte.

3. Essigsäure wird zu CO₂ und Wasser oxydiert

Während in einigen Mikroorganismen der Acetaldehyd direkt zu Acetyl-CoA oxydiert wird, wurde diese Reaktion in Säugetierorganen nicht gefunden. Hier gibt es jedoch Enzyme, die das Acetat mit Hilfe der Adenosintriphosphorsäure (ATP) aktivieren. Es wird angenommen, daß bei diesem Prozeß, der von Coenzym A abhängt [12], eine Esterbindung zwischen Pyrophosphat und der S-H-Gruppe des CoA gebildet wird, und daß diese Bindung durch Essigsäure gespal-