Understanding synergy

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Geary N. Understanding synergy. Am J Physiol Endocrinol Metab 304: E237–E253, 2013. First published December 4, 2012; doi:10.1152/ajpendo.00308.2012.—Analysis of the interactive effects of combinations of hormones or other manipulations with qualitatively similar individual effects is an important topic in basic and clinical endocrinology as well as other branches of basic and clinical research related to integrative physiology. Functional, as opposed to mechanistic, analyses of interactions rely on the concept of synergy, which can be defined qualitatively as a cooperative action or quantitatively as a supra-additive effect according to some metric for the addition of different dose-effect curves. Unfortunately, dose-effect curve addition is far from straightforward; rather, it requires the development of an axiomatic mathematical theory. I review the mathematical soundness, face validity, and utility of the most frequently used approaches to supra-additive synergy. These criteria highlight serious problems in the two most common synergy approaches, response additivity and Loewe additivity, which is the basis of the isobole and related response surface approaches. I conclude that there is no adequate, generally applicable, supra-additive synergy metric appropriate for endocrinology or any other field of basic and clinical integrative physiology. I recommend that these metrics be abandoned in favor of the simpler definition of synergy as a cooperative, i.e., nonantagonistic, effect. This simple definition avoids mathematical difficulties, is easily applicable, meets regulatory requirements for combination therapy development, and suffices to advance phenomenological basic research to mechanistic studies of interactions and clinical combination therapy research.

cooperative effect synergy; energy homeostasis; food intake; Loewe additivity; response surface methodology

“One and one is what I’m telling you . . .” — Debbie Harry (71)

INTRODUCTION

What is Synergy?

INTERACTION AMONG DIFFERENT ENDOCRINE CONTROLS and among endocrine and nervous controls is a fundamental characteristic of physiological regulation. Interactions are evident at all levels of organization, from genetic to organismic (34, 67, 78, 85, 104, 160). For example, the secretion of most hormones is under both endocrine and neural control, and many endocrine target cells as well as neurons in a variety of brain sites express receptors to multiple hormones (34, 67, 85, 104). At the organismic level, the coordinated actions of nerves and hormones acting in several tissues are vital to many regulatory functions, including blood glucose homeostasis, fluid and mineral balance, and energy homeostasis, which provides many of the examples in this review (7, 54, 68, 83, 85, 110, 151). That many endocrine and metabolic diseases are treated with combination therapies also reflects the importance of interactions in physiological regulation (10, 29, 85).

Administration of combinations of drugs often produces unexpectedly large responses. This is known as synergy. It is often described as “1 + 1 > 2,” or supra-additive synergy. This is an apt description because, as shown below, addition causes the most complications and misunderstandings in synergy analyses. (Note that I couch the discussion in terms of “drugs” for simplicity; what is meant is any quantifiable manipulation, including doses of hormones, neurotransmitters, neuromodulators, pharmaceutical agents, etc., as well as amounts of activity, hours of food deprivation, etc.).

What is the Problem?

Despite the apparent simplicity of the concept of supra-additive synergy, translating it into a specific quantitative methodology with formal validity is a thorny problem for which many solutions have been offered (for a sense of the variety of approaches, see Refs. 12, 64, and 121). Furthermore, few investigators appreciate the prerequisites and limitations of supra-additive synergy methodologies. These problems are exacerbated by the different evolution of the concept in different fields, by the relative paucity of theoretical accounts of synergy that are not forbiddingly quantitative, by the failure of most theorists to clearly distinguish their approaches from those of others, by some widely propagated errors in the existing accounts, and by the momentum of the misdirected literature.

Synergy analyses in energy homeostasis research provide an unfortunate example of inappropriate or incomplete synergy analyses. Review of the energy homeostasis literature identified 44 studies, including four of my own, that used the response additivity approach to supra-additive synergy (2, 8,
Addition is not involved. Figure 1 provides some examples of synergy is defined simply as a response to a drug combination that is greater than the responses to the drugs individually. Review

SYNERGY, LOEWE ADDITIVITY SYNERGY, and OTHER ACTIVITIES leads as explained in RESPONSE ADDITIVITY SYNERGY, it is almost never studies attempted to establish the validity of this approach, and, though nowhere is the concept of synergy more important and more discussed than in anesthesiology (45, 72, 77, 108, 142, 143), one can find recent examples of uncritical application of linear Loewe additivity there as well (e.g., Refs. 38, 73, and 79).

The gravity of incorrect applications of supra-additive synergy metrics can hardly be overstated. For example, as shown in Interdeterminate Loewe Additivity Solutions, uncritical use of linear Loewe additivity can completely reverse the interpretation of combination data, turning what should be antagonism into synergy.

Why is Understanding Synergy Important?

Improving research practice by understanding synergy is important for at least three reasons. First, if synergy were better understood, researchers would make fewer innocent errors in searching for supra-additive synergy. Second, there would be fewer misguided attempts to understand the physiological bases of what are in fact artifactual synergies. Third, there would be fewer misinformed efforts to translate apparently promising but erroneous basic research synergies into clinical research. These improvements in research practice would lead to substantial savings in human, animal, and financial resources.

What is the Solution?

My review of supra-additive synergy in RESPONSE ADDITIVITY SYNERGY, LOEWE ADDITIVITY SYNERGY, and OTHER ACTIVITIES leads me to conclude that there is no metric to detect supra-additive synergy, i.e., 1 + 1 > 2, with adequate formal validity, face validity, and general applicability. Therefore, I recommend adoption of a simpler definition of synergy as a cooperative effect. As I describe in COOPERATIVE EFFECT SYNERGY, such synergy is defined simply as a response to a drug combination that is greater than the responses to the drugs individually. Addition is not involved. Figure 1 provides some examples of cooperative effect synergy and its utility in furthering basic and clinical research.

SUPRA-ADDITIVE SYNERGY IS A FORMAL QUANTITATIVE THEORY

Mathematical Nature of Synergy

As is often emphasized (19, 49, 65, 142), quantitative synergy analyses are formal mathematical exercises, not wet physiology. There are two main reasons. First, there is not sufficient mechanistic knowledge to enable quantitative predictions of the effects of drug combinations. If, for example, two drugs each bound reversibly to a single receptor with known kinetics and if the drugs’ individual effects depended only on the number of receptors bound, then their individual and interactive effects could be computed using the laws of mass action (12, 30, 64, 139, 143, 155). Similarly, interactive effects could be computed if they were shown to result directly from the frequency of action potentials in some accessible population of neurons. However, such quantitative mechanistic knowledge is not yet available in endocrinology or in other branches of integrative physiology. The second reason that synergy is intrinsically mathematical is that physiological measurements cannot be used to test synergy metrics. The theorems derived in Euclidean geometry can be compared with the characteristics of existing objects to determine whether the theory corresponds to our direct experience of physical reality. In contrast, supra-additive synergy metrics lead to mathematical predictions that have no directly observable physiological counterparts. This has the implication that, unlike more mechanistic models, it is difficult or impossible to apply the criteria of construct and predictive validity to synergy. How then to judge synergy metrics? I suggest the criteria of 1) face validity, 2) mathematical soundness, and 3) utility with respect to furthering basic and clinical research.

Although my approach to synergy is quantitative, and many important points can be made only mathematically, the mathematical content of the article is minimal. I present mathematical derivations in the appendices in this article, and I illustrate many points graphically.

Mathematical Notation

The mathematical notation is as follows: a and b denote particular amounts or doses of “drugs” A and B, which might be hormones or any other quantifiable manipulations; E_A and E_B are the dose effect functions of drugs A and B, respectively, although the subscripts are omitted in unambiguous situations, situations; E(a) or E_A(a) and E(b) or E_B(b) are the effects of dose a of A and b of B when applied individually, and E(a + b) is the effect of a and b applied simultaneously. E_ADD(a + b) is the theoretical additive effect of the a + b combination. For clarity, I occasionally denote the effect of a or b alone as E(a + 0) or E(0 + b). I assume that the dose-effect curves of both A and B increase (or decrease) monotonically to a maximum. Other situations bring special problems (4, 5, 26, 27, 157).

RESPONSE ADDITIVITY SYNERGY

According to the response or effect additivity synergy metric, the additive or zero-interactive effect of a drug combination is the sum of the individual effects,

\[ E_{\text{ADD}}(a + b) = E_A(a) + E_B(b), \]

and synergy occurs when the observed E(a + b) is greater than this sum (12, 76, 82, 170). This metric is ad hoc based neither
Fig. 1. Examples of the utility of synergy studies in energy homeostasis. Three examples demonstrate translations of initial basic research findings suggesting synergy among inhibitory controls of eating (A, C, and E) to mechanistic (B and D) and clinical (F) studies. Note that in each case, a simple definition of synergy as cooperative action would suffice as well as supra-additive definitions to motivate followup. A and B: the synergistic effect of gastric loads (GAS) and cholecystokinin agonism (CCK analog) on food intake (141) prompted a neurophysiological study that demonstrated that this interaction occurs at the level of abdominal vagal afferents (data are extracellular recordings of action potentials) (140). i.a., Intrarretinal; VEH, control vehicle injections. *Significantly different from control. C and D: the synergistic effect of peripheral CCK and central leptin (LEP) injections on food intake (41) prompted a molecular genetic investigation of the site of the leptin receptors mediating this effect (113). fak/fak, Koletsky rats, which lack functional leptin receptors; Ad-LEPR-B, adeno virus expressing these receptors; Ad-lacZ, control adenovirus expressing a reporter gene. Ad-LEPR-B and Ad-lacZ were delivered to the arcuate nucleus of the hypothalamus 6 days prior to the effect of intraperitoneal injection of CCK on food intake being tested. In C, *significantly different from VEH/VEH and §significantly different from CCK/VEH. In D: *significantly different from VEH injection in wild-type rats (data not shown) and †significantly different from fak/fak Ad-lacZ. E and F: the synergistic effect of chronic amylin and leptin treatment on body weight in rats susceptible to dietary obesity (DIO Prone) (133) prompted a clinical study that demonstrated a similar phenomenon in obese humans (pramlintide and metreleptin are amylin and leptin agonists, respectively) (133). In E: #significantly different from amylin, \( P < 0.05 \); ##significantly different from pramlintide, \( P < 0.01 \); ###significantly different from pramlintide and metreleptin; \( P < 0.01 \); ####significantly different from pramlintide and metreleptin; \( P < 0.001 \). All parts of the figure reprinted with permission.
on physiology nor on formal axioms. Nevertheless, the notion’s simplicity has intuitive appeal and is used frequently. I listed 44 examples above from the energy homeostasis literature; examples are also easily found in anesthesiaology (e.g., Refs. 38, 73, and 79) and other fields.

The problem with the method is that it usually violates the “principle of sham combinations,” which was introduced by Loewe and Muischnek (93, 94, 96) but independently conceived by others (e.g., see Ref. 69). The principle is simply that a drug cannot synergize with itself (or, equivalently, it must add to itself according to the additivity metric). This seems self-evident. Response additivity violates this principle, because for any drug with a curvilinear dose-effect curve sham combinations indicate synergy in concave-up parts of the dose-effect curve (i.e., 2nd derivative of the dose effect equation > 0) and indicate infraadditivity in concave-down parts (2nd derivative < 0). Figure 2 depicts this graphically. Only if both drugs have linear dose-effect curves with zero intercepts is response additivity synergy valid with respect to this principle. Of course, this is very rarely the case. Rather, most dose-effect curves in pharmacology and physiology are curvilinear (35, 37, 105, 130). Thus, response additivity synergy is generally invalid if one accepts the principle of sham combinations.

Synergy is sometimes claimed if combinations of subthreshold doses of two drugs yield significant effects. This form of response additivity is also invalid. The dose-effect curve of any drug with a threshold is empirically concave-up in the region of the threshold because all subthreshold effects are indistinguishable from zero and thus lie on a horizontal line. Thus, response additivity in the threshold region violates the principle of sham combinations. This is shown schematically in Fig. 2. The mistaken belief that two effects must differ if one is significant and the other is not, which is widespread in neuroscience (117), may contribute to the attraction of “subthreshold response” additivity.

More generally, the principle of sham combinations emphasizes the critical point that, in the context of synergy analyses, addition refers not simply to the addition of two numbers but to the addition of dose response curves. Thus, supra-additive synergy requires the development of a theory of addition of dose-effect curves.

It is also worth noting that factorial analysis of variance of the individual and combination effects of particular doses of drugs A and B [i.e., $E_A(a)$, $E_B(b)$ and $E(a + b)$, respectively] is inappropriate for assessing synergy (28). The problem is that, in factorial analysis of variance, deviations from additivity are indicated by significant interaction effects, i.e., $E_A(a) + E_B(b) \neq E(a + b)$. This is the equivalent of testing $Eq. 1$ or simple response additivity; i.e., it is not a valid approach to synergy if one accepts the principle of sham combinations.

Finally, it is important to note that not all theorists agree on the necessity of fulfilling the principle of sham combinations. This is the case, for example, in a recent response additivity approach developed for the detection of synergy in antimicrobial effects (20, 92, 159) as well as in the Bliss additivity approach (please see Bliss Additivity).

**LOEWE ADDITIVITY SYNERGY**

*Theory and Validity*

Originally, Loewe additivity referred to a graphic way to analyze drug interactions that was introduced by Fraser (51, 52) and developed in detail by Loewe and Muischnek (93–96). Loewe’s most fundamental contribution was to discern the principles underlying the method and recognize that they could lead to a variety of nonlinear forms of drug additivities. The principles are the sham combination principle and the drug dose equivalence principle.

Grabovsky and Tallarida (63), Tallarida (153–156), and Tallarida and Raffa (157) articulated these principles into an axiomatic mathematical model. Loewe additivity is sometimes called the isobolographic method, dose additivity, or, in the toxicology literature, concentration additivity (76, 119, 126). It is also the basis of comparison in most response surface synergy metrics. Despite the method’s popularity, its prerequisites and shortcomings are not widely understood. Therefore, I describe them here in detail.

The principles of sham combinations and drug dose equivalence combine to produce additive predictions as follows: dose $a$ of drug A is equivalent to dose $b_a$ of drug B if $a$ and $b_a$ have equal effects (principle of dose equivalence), and $b_a$ can be added to any other dose $b$ of drug B to give the additive effect of the $(a + b)$ combination (principle of sham combinations). Dose equivalence can be computed on the basis of transforming either from the dose-effect curve of A to that of B ($A \rightarrow B$) or from B to A ($B \rightarrow A$). That is,

$$E_{ADD}(a + b) = E_{B}(b_a + b) = E_{A}(a + a_b),$$

(2)

where $E_B$ is measured on the dose-effect curve of drug B and $E_A$ is measured on the dose-effect curve of drug A. This is shown schematically in Fig. 3.

![Fig. 2. Response additivity violates the principle of sham combinations by producing nonsensical predictions for drugs with curvilinear dose-effect curves. In the example shown, the effect of dose 2 is 1, so according to the principle of sham combinations, the predicted effect of dose 2 plus dose 2 is $1 + 1 = 2$. In fact, due to the concave-up shape of the dose-effect curve in that range, the observed effect of dose 4 is almost 4. Thus, response additivity indicates that low doses of this drug synergize potently with themselves. The same problem occurs when using response additivity to analyze the effects of 2 subthreshold drug doses. If the threshold for a statistically significant effect under the conditions tested is $>3$, then the effect of dose 2 will be nonsignificant, and adding 2 individually nonsignificant doses of 2 will result in a significant effect of $>3$; thus, according to response additivity, drugs will synergize with themselves around the dose range for detecting statistically significant effects.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00308.2012)
Integration of elemental responses. This is exactly how we understand the integrative processing, the representations of diverse stimuli that level of intracellular postreceptor effects or postsynaptic neuronal processing, the representations of diverse stimuli that synergize are transformed, at least in part, into a single representation. This is exactly how we understand the integrative actions of intracellular and interneuronal signaling. It is a logical generalization of the classical Sherringtonian principle of neural signal processing (25, 146) that if two stimuli elicit the same reflex, they must converge into a single final common pathway. From an information-processing perspective, the input signals generated by the two stimuli lose their individual identities at the point of convergence; i.e., the output could be driven by either stimulus, which corresponds to the principle of drug dose equivalence. In addition, the integrated output signal can be increased identically by appropriate increases in either input signal, which corresponds to the principle of sham combinations. A response in the Sherringtonian context is an abstraction referring to an elemental neural reflex, such as the iconic scratch reflex. However, it can be extended to any elemental neuroendocrine response, for example, the secretion of insulin by the pancreatic β-cells or the momentary rate of licking liquid food by a rat. By extension, more integrated responses that are driven in part by the elemental responses, such as blood glucose level or daily food intake, fit the same analysis.

Linear Isoboles are a Rarity

Only in a limited number of situations are isoboles straight lines. The simplest case is if both drugs have linear dose-effect curves with zero y-intercepts and without maxima in the region studied. The isobol for effect level X is the following line

\[ I = a/A_x + b/B_x, \]

shown in Fig. 4 (again, doses A_x and B_x alone each lead to effect level X). The derivation of Eq. 4 from the principles of drug dose equivalence and sham combinations in this simple case is given in APPENDIX 1. As mentioned above, synergy (or supra-additivity) occurs if combinations of doses a and b that fall on the isobole produce an effect greater than X or, alternatively, if combinations of doses a and b that lie below the isobol produce effect X.

Isoboles are also straight lines for dose-effect curves that approximate rectangular hyperbolas with equal maxima. Rectangular hyperbolas have the form

\[ b = B_x \]

\[ a = A_x/2 \]

\[ 1 = a/A_x + b/B_x \]

\[ DOSE of B \]

\[ DOSE of A \]

Fig. 4. The equation and graph (solid line) of the linear isobole. The isobole describes drug combinations that lead to the same effect. Dose A_x of drug A and dose B_x of drug B as well as all (a + b) combinations that fall on the isobole lead to identical effects or display Loewe additivity. The isobole is linear as depicted here only if the relative potency of the 2 drugs is constant. Constant relative potency has the consequence that the effects of (a + b) combinations are infra-additive. If both \( a \) and \( b \) have the same effect as \( A_x \) alone or \( B_x \) alone, the dotted line shows this for \( \alpha = \beta = 0.5 \).
where \( E_A(a) \) is the effect of dose \( a \) of drug \( A \), \( E_{\text{AMax}} \) is its maximal effect, and \( A_{50} \) is its rate or potency constant, which is equal to dose producing the half-maximal effect. If drugs \( A \) and \( B \) have rectangular hyperbolic dose-effect curves with equal maxima (i.e., \( E_{\text{AMax}} = E_{\text{BMax}} \), then the isobole is again linear and described by Eq. 4, as shown in Appendix 2 (156, 157). Figure 5 shows typical rectangular hyperbolic dose-effect curves. Although many physiological dose-effect curves are

\[
E_A(a) = \frac{E_{\text{AMax}} a}{a + A_{50}},
\]

(5)

well described by rectangular hyperbolas (35, 37, 105, 130), the constraint that the two drugs under consideration have the same maximal effects substantially reduces the generality of the linear isobole.

It is possible, of course, to equate different maximum effects by expressing data as percent maximal effect, but this is not advisable in situations where transformation changes the relationship of the variables change to the underlying physiologically relevant variables. For example, if one drug’s maximum weight loss effect is 10 kg and another’s is 20 kg, then 1% maximum response for the former represents only half as much weight loss in kilograms as 1% maximum response for the latter. Synergy analyses in percent transforms in such situations seems senseless.

To my knowledge, the only other dose-effect curves that result in linear isoboles are the probit and logit functions (153), which are commonly used to describe proportions of successes in a given number of cases.

Additivity is often defined as the ability of doses of two drugs to substitute for each other in proportion to their potencies to produce the half-maximal effect (recall that potencies are the doses producing the half-maximal effects \( A_{50} \) and \( B_{50} \)); that is, for predicted additivity, the dose \( b \) of drug \( B \) to add with a particular dose \( a \) of drug \( A \) is

\[
b/B_{50} = (A_{50} - a)/A_{50},
\]

(6)

which rearranges by simple algebra to Eq. 4. Thus, this formulation is not generally valid. Rather, it is valid only for dose-effect curves for which the principles of dose equivalence and sham combination lead to Eq. 4, linear Loewe additivity.

As described in more detail below, isoboles for many, probably most, drug combinations do not follow Eq. 4 but are curvilinear (19, 63, 99, 100, 155, 156, 172). For example, rectangular hyperbolic dose-effect curves with different maxima produce curvilinear isoboles (please see Curvilinear Isoboles). Equation 4, and therefore linear isoboles, results only when the relative potency of drugs \( A \) and \( B \) is constant; i.e., dose-effect curves for which \( a/b \) is a constant for all doses \( a \) of drug \( A \) and \( b \) of drug \( B \) for which the \((a + b) \) combination leads to the same effect. If the relative potency of drugs \( A \) and \( B \) is variable, then the isobole is not linear.

Relative potency is most conveniently tested using log dose-effect curves. Drugs with parallel log dose-effect curves have constant relative potency. Therefore, if the slopes of two log dose-effect curves are not significantly different, it is appropriate to analyze synergy with linear isoboles (or the resulting response surfaces; please Loewe Additivity Response Surfaces). This criterion is rarely, if ever, applied. Furthermore, parallel log dose-effect curves seem to be the exception rather than the rule; for example, I have found no such instances in interaction studies in energy homeostasis research. Even rather small deviations from constant relative potency can lead to curvilinear isobolograms (please see Curvilinear Isoboles) and worse, indeterminate solutions (please see Indeterminate Loewe Additivity Solutions). Without consideration of the individual dose-effect curves, deviations from the linear predictions indicate only that the two drugs are not identical and are not both agonists of a single receptor (139, 155). They do not indicate that they are supra-additive or synergistic.

The misunderstanding of the applicability of linear isoboles stems in large part from influential reviews by Berenbaum (11, 20849, 20850, 20851). EMAX, but a larger EMAX, so that the midranges of their log dose-effect curves are not parallel and their relative potencies vary; for small effects, drug \( Y \) is nearly as potent as drug \( Z \), but for larger effects, drug \( Y \) is much less potent than drug \( Z \).

**Fig. 5.** Examples of rectangular hyperbolic dose-effect curves, showing that constant relative potency is associated with parallel log dose-effect curves. A: formulas for 4 rectangular hyperbolic dose-effect curves, drugs W–Z. The formulas have the form \( E = E_{\text{MAX}} D/(D + D_{50}) \), where \( E_{\text{MAX}} \) is the maximum effect, \( D \) is the dose, and \( D_{50} \) is a rate or potency constant that equals the dose yielding half-maximal effect. B: the 4 dose-effect curves in untransformed coordinates. C: the same dose-effect curves with dose on a log scale. Note that for drugs \( W, X \), and \( Y, E_{\text{MAX}} = 100 \), the rate constants vary by factors of 10, and the nearly linear midranges of their log dose-effect curves are parallel and separated by 1 log unit. This means that their relative potencies are constant; the effect of any dose of drug \( W \) is also the effect of dose 10W of drug \( X \) and dose 100W of drug \( Z \). Note also that drug \( Z \) has the same rate constant as drug \( Y \), but a larger \( E_{\text{MAX}} \), so that the midranges of their log dose-effect curves are not parallel and their relative potencies vary; for small effects, drug \( Y \) is nearly as potent as drug \( Z \), but for larger effects, drug \( Y \) is much less potent than drug \( Z \).
12) that included derivations supposedly establishing that Eq. 4 is true regardless of the form of the drugs’ dose-effect curves. This derivation is incorrect (please see Appendix 3). The proof is in fact limited to dose-effect curves whose relative potency is constant. Either this was not recognized or its implications were not understood, and the linear-isobole (and linear response surface method described in the next section) quickly became the gold standard for synergy studies (21, 55).

Although he did not compute additive effects mathematically, Loewe (94) understood and stated clearly that linear isoboles are valid only if the dose-effect curves of the drugs considered have constant relative potency and that this is rarely the case (“...the overwhelming probability is that the isobole deviates from the endpoint diagonal with either SW- or NE-convexity” and that “...heterodynamic isoboles usually have two isoboles for the same endpoint, one SW- or NE-convexity” and that “...the overwhelming probability is that...”).

Although as stated, heterodynamic isoboles are valid only if the dose-effect curves of the drugs considered have constant relative potency and that this is rarely the case (“...the overwhelming probability is that the isobole deviates from the endpoint diagonal with either SW- or NE-convexity” and that “...heterodynamic isoboles usually have two isoboles for the same endpoint, one SW- or NE-convexity” and that “...the overwhelming probability is that...”).

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Loewe addition frequently leads to such indeterminate regions. Furthermore, the region of indeterminacy is often large. In a series of studies of combinations of various antiepileptic drugs in rat models, Luszczki and colleagues (98–101) and Wojda et al. (172) found that A → B and B → A transformations of the data resulted in indeterminate areas that filled ~25–75% of the total dose space [i.e., the area bounded by the 4 points (0, 0), (Ax, 0), (0, Bx), and (Ax, Bx)]. Lorenzo and Sánchez-Marín (97) concluded that the gravity and frequency of this problem suffice to invalidate Loewe additivity as a general solution to synergy.

Combinations of two drugs with linear log dose-effect curves that have different slopes also lead to indeterminate outcomes. The energy homeostasis literature exemplifies this. The 11 Loewe additivity energy homeostasis studies also appeared to differ by >20% (9, 50, 91, 131, 134, 136, 161, 169), suggesting that correct analyses would also change the interpretation of these studies.

**Boundary Conditions**

Boundary conditions pose additional problems for Loewe additivity. If dose equivalence is computed graphically, there is no exact transform for subthreshold doses. A reasonable solution is to use linear interpolation between zero and the smallest dose with a measureable effect. If dose equivalence is computed mathematically, below-threshold doses are not a problem.

More difficult is when doses of drug A lead to effects exceeding the maximum effect of drug B (i.e., drug B is a partial agonist). Such doses of drug A cannot be transformed to equivalent doses of drug B either graphically or mathematically, leaving the upper bound of Loewe additivity undefined. The problem of different maxima is common. One example is provided by leptin. As shown in Fig. 1, C and E, exogenous leptin alone often has only modest effects on food intake and body weight in rats but apparently large interactive effects when combined with CCK or additivity.

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The interpretation different outcomes of A → B or B → A transformations is problematic. Tallarida (155) argues that, because each solution represents additivity, the region contained between them is a region of additivity. But each solution represents additivity for only one of the two equally valid transformations and represents infra- or supra-additivity for the other. Thus, it seems more reasonable to interpret both solutions and the area between them as indeterminate outcomes.

This counterintuitive result can be described as a situation in which \( E_A(a) = E_B(b) \) and \( E_B(b) = E_A(a) \) but \( E_A(a + a_0) \neq E_B(b + b) \). Synergy is then restricted to the area below both solutions and antagonism to the area above both.

Loewe addition frequently leads to such indeterminate regions. Furthermore, the region of indeterminacy is often large. In a series of studies of combinations of various antiepileptic drugs in rat models, Luszczki and colleagues (98–101) and Wojda et al. (172) found that A → B and B → A transformations of the data resulted in indeterminate areas that filled ~25–75% of the total dose space [i.e., the area bounded by the 4 points (0, 0), (Ax, 0), (0, Bx), and (Ax, Bx)]. Lorenzo and Sánchez-Marín (97) concluded that the gravity and frequency of this problem suffice to invalidate Loewe additivity as a general solution to synergy.

Combinations of two drugs with linear log dose-effect curves that have different slopes also lead to indeterminate outcomes. The energy homeostasis literature exemplifies this. The 11 Loewe additivity energy homeostasis studies that I know of all used a linear approach (9, 13, 50, 84, 91, 131, 134, 136, 161, 169). An example is shown in Fig. 9. Note that the slopes of the log dose-effect curves of cholecystokinin (CCK) alone and amylin alone differed by ~25%. Correct calculation of Loewe additivity indicated that this difference is sufficient to change the interpretation of some of the CCK-amylin combination effects from synergy, as these authors concluded, to antagonism. The individual log dose-effect curves in eight of the 10 remaining Loewe

---

**Fig. 7.** Loewe additivity often leads to indeterminate isobolar solutions. The dotted lines are equally valid alternate solutions for the isobole for the effect of 30% \( E_{\text{MAX}} \) (yielded by doses \( Ax \) and \( Bx \)) for drugs A and B with dose-effect curves given by the Hill equation, Eq. 6, with the same \( E_{\text{MAX}} \) but different exponents (i.e., with relative potencies that change as effect level changes). The upper dotted line results from transforming doses of drug A to their equivalent doses of drug B, and the lower dotted line results from transforming doses of drug B to their equivalent doses of drug A. The area between the lines is supra-additive according to the A → B transformation solution and infra-additive according to the B → A transformation. Please see text for further details. From Ref. 155, used with permission.

**Fig. 8.** Graphic application of the dose equivalence principle yielding indeterminate Loewe additive solutions. A: dose 1 of drug A is equivalent to dose 3.2 of drug B (dashed lines), and dose 1 of drug B is equivalent to dose 0.2 of drug A (dotted lines), as explained in Fig. 3 and in the text. B: different additive predictions are generated for combination of dose 1 of drug A and dose 1 of drug B when the drug A-equivalent dose of drug B is used (dashed lines, 1.2 units of drug A yields effect 2.5) than when the drug B-equivalent dose of drug A is used (dotted lines, 3.3 units of drug B yields effect 2.8).
cholecystokinin (CCK; /H17040 presumably also have sufficed to motivate this followup. weight loss in dietary obese rats (162). A cooperative effect synergy analysis which also poses a problem for the computation of Loewe additivity. The CCK). Note also that the maximum effect of CCK exceeded that of amylin, or indeterminate (e.g., combination of 0.77 nmol/kg amylin with 0.88 nmol/kg CCK), others were infra-additive rather than synergistic (e.g., combination of 0.26 nmol/kg amylin with 2.62 nmol/kg CCK). As discussed in /H11011 dose estimations; this analysis suggested that almost all amylin-CCK dose combinations resulted in synergy. However, note that the amylin and CCK log dose-effect curves are nonparallel; the slopes differ (Δα) by −25%. Thus, the observed combination effects were synergistic was assessed with linear isoboles and response surfaces based on the isoeffective dose estimations; this analysis suggested that almost all amylin-CCK dose combinations resulted in synergy. However, note that the amylin and CCK log dose-effect curves are nonparallel; the slopes differ (Δα) by −25%. Thus, analysis of the combination data with linear isoboles and response surfaces was inappropriate. Graph from Ref. 13, reproduced with permission. B: graphic estimation of Loewe additivity predictions for dose combinations near the curves’ linear portions indicates that additive solutions were indeterminate for considerable effect range. For example, for combination of 0.26 nmol/kg amylin with 0.88 nmol/kg CCK, Loewe additivity was indeterminate for −30 to 42% decrease, and for combination of 0.77 nmol/kg amylin with 0.88 nmol/kg CCK, Loewe additivity was indeterminate from −42 to 56%. As a result, although some dose combinations indeed appeared synergistic (e.g., combinations of 0.77 nmol/kg amylin with 0.09 nmol/kg CCK and 0.26 nmol/kg amylin with 0.88 nmol/kg CCK), others were infra-additive rather than synergistic (e.g., combination of 0.26 nmol/kg amylin with 2.62 nmol/kg CCK) or indeterminate (e.g., combination of 0.77 nmol/kg amylin with 0.88 nmol/kg CCK). Note also that the maximum effect of CCK exceeded that of amylin, which also poses a problem for the computation of Loewe additivity. The effects described in this article prompted a study suggesting that 7-day infusions of amylin-CCK combinations elicited a synergistic increase in weight loss in dietary obese rats (162). A cooperative effect synergy analysis would also indicate that most dose combinations were synergistic and would presumably also have sufficed to motivate this followup.

amylin. As discussed in Linear Isoboles are a Rarity, normalizing to equate maxima, for example, by percent maximum transformations, is an inadequate solution. Another possibility is to develop an alternative supra-additivity metric. Howard and Webster (76), for example, recently proposed a “generalized concentration addition” method that was based on the inverse functions of the drugs’ dose-effect curves \( f^{-1}(x) \) is the inverse of function \( f(x) \) if \( f^{-1}[f(x)] = x \). Although this clever mathematical ploy avoids the partial agonist problem, it abandons the axiomatic foundation of Loewe additivity.

A conceptually similar situation is when in one drug is completely inactive. Much of the synergy literature in clinical anesthesiology is designed to meet just this problem (e.g., Refs. 45, 74, 77, 102, 108, 142, and 143). The basic approach of contemporary anesthesiology is to capitalize on the potent synergistic effects of combinations of a hypnotic drug with an analgesic drug that alone has little or no hypnotic potency; i.e., it is a partial agonist (e.g., Refs. 74 and 102). Loewe additivity cannot handle these situations because there are no equivalent doses. In response, anesthesiologists have developed a variety of alternative additivity metrics (45, 74, 77, 102, 108, 142, 143), frequently not emphasizing that these are no longer true Loewe additivity analyses. Tallarida (153) suggested a solution based on an ad hoc formula to generate pseudoequivalent doses. Another is to abandon supra-additive synergy, as described more in Response Surface Modeling and Cooperative Effect Synergy.

Loewe Additivity Response Surfaces

The response surface approach extends the analysis of synergism from a single-effect level to a range of effect levels. In simple cases, the surface is a three-dimensional contour describing the Loewe additive effects of continuous ranges of doses derived mathematically from the principles of sham addition and dose equivalence in a manner analogous to the derivation of isoboles (153). Doses of drugs A and B are plotted on the x- and z-axes, and the expected E(a + b) is plotted on the y-axis. The intersection of the response surface with a horizontal plane at height z = X is isobole for effect level X. If the dose-effect curves of drugs A and B are linear with zero z-intercepts, rectangular hyperbolas with equal maxima, or parallel logits or probits, these intersections will be linear. An example is shown in Fig. 10.

Synergy can be assessed in two ways. If the observed E(a + b) is added to the surface plot, then synergy is indicated by points above the surface and infra-additivity by points below the surface. Alternatively, the observed data can be fit to a surface using second- or higher-order polynomials (57, 64, 108, 153). The observed contour is then compared with the additive predictions. Dose combinations under areas of the observed contour that lie above the additive prediction are synergistic.

Response surfaces are more complex, and indeed are not surfaces in the usual sense at all, if the drugs’ dose-effect curves lead to indeterminate solutions, as discussed in Indeterminate Loewe Additivity Solutions. In such cases, single-dose combinations will approach as a limit the additive solutions of many effect levels, not just one. This can be envisioned with reference to Fig. 7, which shows the additive solutions for effect X, which is obtained with dose 60 of drug A alone and dose 22 of drug B alone. If one now imagines the additive solutions for effect 0.8X given by dose 50 of drug A alone and dose 18 of drug B alone, it should be clear that the lower additivity solution for X and the upper additivity solution for 0.8X will intersect; i.e., the dose combinations represented by

---

**Figure 9.** An example of indeterminate Loewe additivity solutions and erroneous interpretations arising from improper application of Loewe additivity. A: the acute eating-inhibitory effects of intraperitoneal injections of amylin (○) and cholecystokinin (CCK; □). The authors smoothed the raw data with 4-param-eter logistic fits, which they used to interpolate isoeffective doses of amylin and CCK. Whether the observed combination effects (△) were synergistic was assessed with linear isoboles and response surfaces based on the isoeffective dose estimations; this analysis suggested that almost all amylin-CCK dose combinations resulted in synergy. However, note that the amylin and CCK log dose-effect curves are nonparallel; the slopes differ (Δα) by −25%. Thus, analysis of the combination data with linear isoboles and response surfaces was inappropriate. Graph from Ref. 13, reproduced with permission. B: graphic estimation of Loewe additivity predictions for dose combinations near the curves’ linear portions indicates that additive solutions were indeterminate for considerable effect range. For example, for combination of 0.26 nmol/kg amylin with 0.88 nmol/kg CCK, Loewe additivity was indeterminate from −30 to 42% decrease, and for combination of 0.77 nmol/kg amylin with 0.88 nmol/kg CCK, Loewe additivity was indeterminate from −42 to 56%. As a result, although some dose combinations indeed appeared synergistic (e.g., combinations of 0.77 nmol/kg amylin with 0.09 nmol/kg CCK and 0.26 nmol/kg amylin with 0.88 nmol/kg CCK), others were infra-additive rather than synergistic (e.g., combination of 0.26 nmol/kg amylin with 2.62 nmol/kg CCK) or indeterminate (e.g., combination of 0.77 nmol/kg amylin with 0.88 nmol/kg CCK). Note also that the maximum effect of CCK exceeded that of amylin, which also poses a problem for the computation of Loewe additivity. The effects described in this article prompted a study suggesting that 7-day infusions of amylin-CCK combinations elicited a synergistic increase in weight loss in dietary obese rats (162). A cooperative effect synergy analysis would also indicate that most dose combinations were synergistic and would presumably also have sufficed to motivate this followup.
Interviews are closely related to Loewe additivity.

**The Greco and Minto Models**

Greco and colleagues (64, 66) and Minto et al. (108) proposed intervention models for drugs whose dose-effect curves fit the Hill equation, Eq. 9, which was discussed in *Indeterminate Loewe Additivity Solutions*. Greco and colleagues’ (64, 66) starting point was to accept the assertion by Berenbaum (11, 12) that the linear isobole, Eq. 4, is generally valid. They then used it and a form of Eq. 9 for inhibitory effects to generate an interaction index that expressed the difference between the observed combination effects and the linear isobole

\[
1 = \frac{a}{(Ax Ex^{1/p})} + \frac{b}{(Bx Ex^{1/q})} + \alpha ab/(Ax Bx Ex^{(1/2p+1/2q)})
\]

where \(Ex\) is the effect of \(Ax\) and of \(Bx\) normalized to the 0-dose effect \(E_{CON}\) [i.e., \(Ex = E/(E_{CON} - E)\)], \(p\) and \(q\) are the slope parameters for drugs \(A\) and \(B\), respectively, and \(\alpha\) is the interaction index. Note that the two left terms of Eq. 10 are similar to Eq. 4. Synergy is indicated by \(\alpha > 0\), and additivity is indicated by \(\alpha = 0\), i.e., if the right term of Eq. 10 drops out. Several modified forms of the original model have been proposed (16, 24, 74).

The quite different approach by Minto et al. (108) is based on the dose-effect curves of combinations of fixed-dose ratios of \(A\) and \(B\), i.e., combinations for which \(a/b\) is constant. The dose ratios, called \(\theta\), were expressed with respect to the drugs’ half-maximal effects, i.e., \(a/A_{50}\) and \(b/B_{50}\). That is,

\[
\theta = (a/A_{50})/(a/A_{50} + b/B_{50})
\]

where \(\theta\) ranges from 0, a mixture of all drug \(B\), to 1, a mixture of all drug \(A\). Each \(\theta\) value was treated as a new drug whose dose-effect curve is a new Hill equation. Minto et al. (108) then derived fourth-order polynomial equations to estimate the three Hill equation parameters (i.e., the maximal effect, half-maximal dose, and slope parameter) as functions of \(\theta\). These polynomials involved five coefficients, \(\beta_0 - \beta_4\), but \(\beta_0\) and \(\beta_1\) could be expressed in terms of \(\theta\) and eliminated. Finally, the type of interaction is reflected by values of the remaining three \(\beta\)-coefficients. If all three \(\beta = 0\), the drugs are additive; if \(\beta_2 \neq 0\) and \(\beta_3 \neq \beta_4 = 0\), the drugs synergize; if two or three \(\beta > 0\), the drugs have complex interactions, for example, interactions including both synergy and antagonism.

Both Greco and colleagues (64, 66) and Minto et al. (108) accepted Berenbaum’s assertion (11, 12) of the generality of Eq. 4 and, therefore, that the criterion for additivity is the linear isobole. Unfortunately, as discussed above (Linear Isoboles are a Rarity; also see Appendix 3), Berenbaum’s assertion is incorrect, and true Loewe additivity isosoles generated for Hill equation dose-effect curves using the principles of dose equivalence and sham addition are to a great extent indeterminate (*Indeterminate Loewe Additivity Solutions*). Thus, rather than being mathematically valid applications of the axioms of Loewe additivity, both the Greco and colleagues (64, 66) and Minto et al. (108) algorithms are ad hoc supra-additive synergy...
metrics. Few of the many discussions of these models mention this problem (1, 16, 24, 74, 81, 89, 102, 106, 107, 139, 165). However, it should be noted that this criticism applies only to the interpretation in terms of synergy, not to the goodness of fit of the Hill equation response surfaces to the experimental data. Thus, both models could be used in general response surface analyses of combination effects, as described in COOPERATIVE EFFECT SYNERGY (see, for example, Ref. 74).

The Chou Model

Chou (30) and Chou and Talalay (31, 32) described a synergy model based on the derivation of a generalized equation representing the “unified general theory for the Michaelis-Menten, Hill, Henderson-Hasselbalch, and Scatchard equations” that they derived:

\[ f_a = 1 / (1 + (D_m / D)^m) \]

where \( f_a \) is the fractional response, \( D \) is dose, \( D_m \) is the median effective dose, and \( m \) is a shape parameter (\( m < 1 \) for hyperbolic dose-response curves and \( m \geq 1 \) for sigmoidal dose-response curves). This equation was used to generate equations for the sum of the effects of two or more drugs, which in turn was used in conjunction with Eq. 4, the linear isobologram, to generate an expression for deviations from Loewe additivity. Lee and Kong (88) recently developed a method to estimate the confidence intervals of the Chou interaction index.

There are several problems with Chou and Talalay’s approach for integrative physiology. First, the dose-response curves are transformed to fractional or percent maximum response, which, as discussed in Linear Isoboles are a Rarity, is not appropriate when the maximal effects differ. Second, although Chou and Talalay do not emphasize it, the method is limited to drugs with constant relative potencies. The calculations do not apply to “mutually nonexclusive drugs,” i.e., those whose relative potency is not constant. Therefore, Chou (30) simply posits that “nonexclusivity” indicates synergy (in Chou’s words, the author “integrated the nonexclusive condition as an intrinsic contribution to the synergistic effect”). From the perspective of formal theory, this is an indefensible ad hoc maneuver. Third, Greco et al. (64) pointed out a number of mathematical weaknesses in Chou’s derivations.

Bliss Additivity

Bliss (14) additivity is an axiomatic supra-additive synergy model based on the principle that drug effects are outcomes of probabilistic processes so that zero interaction is equivalent to probabilistic independence. Thus, combination effects are quantified as joint probabilities according to the familiar rule

\[ P(E_a \text{ or } E_b) = P(E_a) + P(E_b) - P(E_a \text{ and } E_b), \]

in which \( P \) represents the probability of observing the effect indicated (\( E_a \), etc.). The most common criticism of Bliss additivity is that, except in the case of exponential dose-effect curves (11, 12, 152), it violates the principle of sham combinations; i.e., combinations of a drug with itself lead to synergy or antagonism. Another criticism from the perspective of integrative physiology is that interaction seems to be the antithesis of independence.

Bliss additivity is often used in the analysis of synergy in antimicrobiology, toxicology, radiology medicine, and other fields (20, 39, 60, 64, 123, 124, 152, 174, 177). According to Yeh et al. (176), an advantage of Bliss additivity is that it is exactly analogous to the definition of epistasis used in molecular biology. The additivity algorithm used by the GraphPad statistical package is also based on Bliss additivity (see http://www.graphpad.com/faq/viewfaq.cfm?faq=991; GraphPad Software, La Jolla, CA).

**COOPERATIVE EFFECT SYNERGY**

**Definition and Advantages**

The simplest quantitative concept of synergy is that it reflects an increase in effect over the agents alone. The quantitative definition is that dose \( a \) of drug \( A \) and dose \( b \) of drug \( B \) synergize if \( E(a + b) > E(a) \) and \( E(a + b) > E(b) \) (recall that drugs \( A \) and \( B \) are drugs with qualitatively similar overt effects). The analogous definition of antagonism is that doses \( a \) and \( b \) are antagonists if \( E(a + b) < E(a) \) or \( E(a + b) < E(b) \) for example, inverse agonists fit this definition). Drug combinations with intermediate effects may be termed functionally neutral. Cooperative effect synergy is also called superior yield, highest single agent, or therapeutic synergy (3, 18, 90, 120, 152a).

Cooperative effect synergy has much to recommend it. The lack of any addition metric is a major advantage, as it obviates the need for an axiomatic mathematical theory for the addition of dose-effect curves and the complications that come along with it, as described above. This also enables synergy to be assessed for particular doses without characterization of the drugs’ dose-effect curves. Thus, simple experiments suffice to identify interesting results that encourage further mechanistic or translational research, as exemplified in Fig. 1.

Another major advantage of cooperative effect synergy is that it meets the criteria recently adopted by the US Food and Drug Administration (FDA) for evaluation of combination therapies; i.e., “two or more drugs may be combined in a single dosage . . . to enhance the safety or effectiveness of the principal active component” [Code of Federal Regulations of the USA, Title 21, Volume 5, Section 300.50(a)(1), April 1, 2011]. This is an important shift away from the previous regulatory guideline that combination therapies demonstrate some form of supra-additive synergy (for discussions related to the evolution of the FDA’s stance, see Refs. 118, 125, 164, and 173). This change should influence the designs of both basic discovery and clinical research.

**Response Surface Modeling and Cooperative Effect Synergy**

Response surface analysis is a powerful general method to analyze multivariate data (22, 114) that can be profitably applied to cooperative action synergy. In drug discovery and other fields, cooperative effect synergy based on response surface analysis is considered an excellent strategy for high-throughput drug discovery (1, 3, 18, 90, 120, 178).

Response surface analysis is based on modeling the topology of response magnitudes. This is usually done empirically, i.e., with no assumptions about the shape of the surface. Second-order polynomial equations, which contain linear, quadratic, and interaction terms, usually suffice to provide good fits to the data. If several dose combinations are tested, even small amounts of data \( (n = 5 \) or so per dose combination) usually
permit rough estimation of the equation parameters. This enables predictions of dosages for maximum effects or for particular effect levels, for example, using algorithms designed to determine “paths of steepest ascent” toward the maximum or desired response (i.e., lines normal to the fitted surface contours directed toward the desired end point). Additional experiments targeted to restricted ranges of combinations or computer simulations can then be performed to better characterize the response surface. Such methods have been extended to quite complexly contoured surfaces. Multiple responses can be studied simultaneously, for example, to optimize “therapeutic windows,” i.e., identify dose combinations with the largest difference between therapeutic and undesired responses (90).

An important advantage of response surface analysis of cooperative effect synergy is that it need not make assumptions about the dose-effect curves of the individual drugs. Thus, drug combinations involving drugs with variable relative potency or drugs with different maximal effects or one drug that alone has no measurable effect do not pose modeling problems.

Response surfaces with theoretical bases can also be used. For example, one study of opioid-sedative anesthetic interactions fit the data to a compound logistic dose-effect curve (102) and another to a hierarchical function that modeled the ascending neural mechanisms of analgesia and hypnosis (74). The former study also exemplifies the predictive use of response surface analysis. That is, Manyam et al. (102) first computed an area on the response surface that identified sedative-hypnotic dose combinations that were optimal for anesthesia and then used the drugs’ individual clearance times and a stepwise iterative simulation procedure to identify the dose combinations that were predicted to lead to the most rapid recovery from anesthesia.

CONCLUSION

Functional analyses of the effects of combinations of drugs or other manipulations that invoke a notion of additivity are mathematical models that must be developed and validated mathematically. Unfortunately, this is rarely recognized, much less done. As a result, much of the theoretical and empirical synergy literature propagates fundamental misunderstandings of Loewe additivity. True Loewe additivity is a formally valid axiomatic mathematical theory, with face validity as a model of integrative physiological processes. Unfortunately, however, it does not solve the synergy problem. Rather, in most situations involving integrative physiology, including applications in energy homeostasis, endocrinology, and anesthesiology, the shapes of the dose-response curves complicate the application of Loewe additivity and prevent clear additivity solutions. Other supra-additive synergy models are no more satisfactory (Table 1).

The elusive nature of good quantitative synergy solutions has long been a concern. In 1953, Loewe (94) wrote, “Although, according to this study, the terms synergism and antagonism . . . 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.’” In 1996, the view of Greco et al. (65) was that “good synergy assessment and interpretation will never be simple” and likened the search for such a “proper and easy” solution to Dorothy’s escape from Oz in that we are waiting “for some wizard to tell us the secret.” And in 2012, Shafer (142), in discussing eight synergy models in anesthesia, concluded that, although useful, “all models are wrong.”

Supra-additive synergy approaches also fail to provide unique clues as to mechanism. Yates (175) characterized nine characteristics of a good mathematical model of physiological processes. Neither Loewe additivity nor the other supra-additivity approaches described here seem to fulfill any of them and, therefore, seem unlikely to be guides to understanding the mechanisms of integrative responses.

For these reasons, I conclude that the disadvantages and difficulties of supra-additive synergy models far outweigh their benefits and recommend that they be abandoned.

Nevertheless, synergy is an important physiological and clinical issue worthy of pursuit. I believe that the simple definition of synergy as cooperative, i.e., nonantagonistic, action is adequate both for basic discovery research and clinical research. The designs and analyses are far simpler than those of supra-additive synergy and avoid the many problems described above. Cooperative effect synergy suffices to identify good candidates for further mechanistic or clinical research (Fig. 1). The definition meets regulatory requirements for establishing combination therapies. Finally, it has proven productive in other fields.

**APPENDIX 1: LOEWE ADDITIVITY EQUATION FOR LINEAR DOSE-EFFECT CURVES**

The simplest application of the theory of Loewe additivity is to generate an additive formula for linear dose-effect curves with zero intercepts and with maxima ignored. In this case, the effects E(a) and E(b) for doses a and b of two drugs (or other manipulations), drugs A and B, are given by:

\[ E(a) = n_A a \]  
\[ E(b) = n_B b \]

\[ (AI.1) \]

where \( n_A \) and \( n_B \) are the slopes of the two lines. The equations can be used to generate equivalent doses of drugs A and B. If \( E(a) = E(b) \), then

\[ n_A a = n_B b \]  
\[ a = \frac{n_B}{n_A} b \]  
\[ (AI.2) \]

This equation also indicates that the relative potency of drug A with respect to drug B is \( n_B/n_A \). Note that, unlike the situation in **Appendix 3, I**

**Table 1. Comparison of several synergy metrics**

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<tr>
<td>Cooperative effect</td>
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<td>Yes</td>
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N/A, not applicable. Supra-additive indicates that synergy is assessed against an additive or zero-interactive prediction. Face validity is based on whether the metric distinguishes a drug from itself (the principle of sham combinations) and other considerations described in the text. Formal validity is indicated if the metric generates additive predictions based on an axiomatic mathematical theory of dose-response curve addition; this is not applicable for cooperative effect synergy because it does not involve addition. Loewe additivity is not recommended because it is often complex, often leads to indeterminable solutions for many or most drug combinations, and cannot be applied to some boundary conditions. Please see text for further details.
Eqs. A1.1 and A1.2 are true at all effect levels, 2) the relative potency of drugs A and B is derived, not asserted, and 3) \( n_B/n_A \) is constant. Next, consider an effect level X that is given by doses Ax and Bx alone. What combinations of dose a of drug A and dose b of drug B also give effect X? For any such (a + b) pair, the dose a and the drug A-equivalent dose of drug B must add to Ax because Ax has the desired effect X. Substituting the formula in Eq. A1.2 for equivalent doses, this yields

\[
a + \left( \frac{n_B}{n_A} \right) b = Ax. \tag{A1.3}
\]

Dividing by Ax gives

\[
a/AX + \left( \frac{n_B}{n_A} \right) b/AX = 1. \tag{A1.4}
\]

Finally, again from Eq. A1.2 above, the dose of drug B that is equivalent to Ax is \( (n_B/n_A)Bx \). Substituting this into Eq. A1.4 gives

\[
a/AX + \left( \frac{n_B}{n_A} \right) b/((n_B/n_A)Bx) = 1, \tag{A1.5}
\]

in which the potency ratio \( n_B/n_A \) cancels, leaving

\[
a/AX + b/Bx = 1, \tag{A1.6}
\]

the linear-isobole equation (Eq. 4 in the main text). Thus, for the particular situation of two linear dose-effect curves with zero intercepts, the additive prediction for dose combinations yielding a constant effect that is generated by the dose-equivalence principle is the linear isobole equation.

**APPENDIX 2: LOEWE ADDITIVITY EQUATION FOR RECTANGULAR-HYPERBOLIC DOSE-EFFECT CURVES WITH IDENTICAL MAXIMUM EFFECTS**

This proof was presented by Tallarida (156) and Tallarida and Raffa (157). If both drugs A and B have rectangular hyperbolic dose-effect curves, the effects E(a) and E(b) of doses a and b of drugs A and B are

\[
E(a) = E_{\text{M_Max}}a/(a + A_{50}) \quad \text{and} \quad E(b) = E_{\text{M_Max}}b/(b + B_{50}), \tag{A2.1}
\]

where \( E_{\text{M_Max}} \) and \( E_{\text{M_Max}} \) are constants describing the maximal effects of drugs A and B and \( A_{50} \) and \( B_{50} \) are rate constants, or potencies or half-maximal effects, of drugs A and B. If the maximal effects are equal, \( E_{\text{M_Max}} = E_{\text{M_Max}} = E_{\text{M_Max}} \). If the effects of doses a and b are equal, then

\[
E_{\text{M_Max}}a/(a + A_{50}) = E_{\text{M_Max}}b/(b + B_{50}). \tag{A2.2}
\]

Dividing by \( E_{\text{M_Max}} \) gives

\[
a/(a + A_{50}) = b/(b + B_{50}). \tag{A2.3}
\]

Multiplying by \( (a + A_{50}) \) \((b + B_{50})\) gives

\[
ab + aB_{50} = ab + bA_{50}. \tag{A2.4}
\]

Subtracting ab from each side and dividing by \( B_{50} \) gives

\[
a = bA_{50}/B_{50}. \tag{A2.5}
\]

which is the dose of b that is the dose equivalent to a. Note that, as in the linear example above, \( A_{50}/B_{50} \) describes the two drugs’ relative potencies and is constant. Finally, for an effect level X that is given by doses Ax and Bx alone, what combinations of dose a of drug A and dose b of drug B also give effect X? Again, for any such pair, dose a and the drug A-equivalent dose of drug B must add to Ax because Ax has the desired effect X. Expressing dose b as its drug A-equivalent dose, given in the formula in Eq. A2.5, yields

\[
a + b(A_{50} + B_{50}) = Ax. \tag{A2.6}
\]

Except for the names of the constants expressing relative potency, this is identical to Eq. A1.3 and again can be transformed to the linear-isobole equation (i.e., Eqs. 4 and A1.4). Thus, for two hyperbolic dose-effect curves with equal maxima, the Loewe additive prediction for dose combinations yielding a constant effect is the linear-isobole equation.

**APPENDIX 3. BERENBAUM’S “GENERAL VALIDATION” OF THE ISOCOBE METHOD BASED ON THE SHAM-COMBINATION PRINCIPLE**

Berenbaum (12) claims that the following derivation is a general validation of the linear-isobole synergy metric because “Its derivation took no account, either explicitly or implicitly, of the shapes of dose-response curves of the agents . . . .” In fact, as shown below, the derivation does take account of the shapes of dose-response curves by implicitly assuming that the two agents have a constant relative potency. Therefore, its validity is limited to that situation.

The notation is that \( E(a) \) and \( E(b) \) are the individual effects of various doses \( a \) and \( b \) of drugs A and B, respectively, and effect level \( X \) is given by doses \( Ax \) of A and \( Bx \) of B. Berenbaum (12) begins by considering a sham dose of drug A, called dose \( A' \) that has the same effect as \( Bx \), namely \( X \). Because dose \( A' \) is isoeffective with dose \( Bx \), then the sham dose \( A' \) must equal the dose \( Bx \) times the relative potency of \( Ax \) and \( Bx \) at effect level \( X \)

\[
A' = (Ax/Bx)Bx. \tag{A3.1}
\]

This is straightforward application of the principle of dose equivalence, although Berenbaum (12) did not recognize that as a general principle. Next consider that \( a + b \) dose combination also yield effect \( X \), i.e., doses that have an additive effect according to the linear-isobole method. Berenbaum (12) wishes to construct an equivalent dose \( a' \) of drug A that can be substituted for dose \( b \). He writes, “Now, the sham combination is, in fact, a of A plus (Ax/Bx) b of A’” (italics added). That is, Berenbaum substitutes dose \( a' \) for drug A’ and dose \( b \) for technique in Eq. A3.1 to generate the dose \( a' \) with an effect equivalent to that of \( b \) units of B,

\[
a' = (Ax/Bx)b. \tag{A3.2}
\]

This substitution is incorrect. There is no reason that \( (Ax/Bx)b \) of A should have the same effect as \( b \). Recall that \( Ax/Bx \) is the relative potency of A and B at effect level \( X \). No information is available about the relative magnitudes of A and B that lead to other effect levels. For example, \( 0.5 \) Ax need not have the same effect as \( 0.5 \) Bx. Thus, Eq. A3.2 is invalid, and no equivalent dose of drug B in terms of drug A can be generated.

Berenbaum (12) completes the derivation by equating the effect of the sham combination with that of \( Af \)

\[
E(a \text{ of } A + (Ax/Bx)b \text{ of } A) = E(Ax). \tag{A3.3}
\]

Because all the dose terms are in units of \( A \), the doses on each side of the equation must be equal; thus,

\[
a + (Ax/Bx)b = Ax. \tag{A3.4}
\]

Finally, dividing by \( Ax \) would yield the linear-isobole equation (Eq. 4).

\[
a/Ax + b/Bx = 1. \tag{A3.5}
\]

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