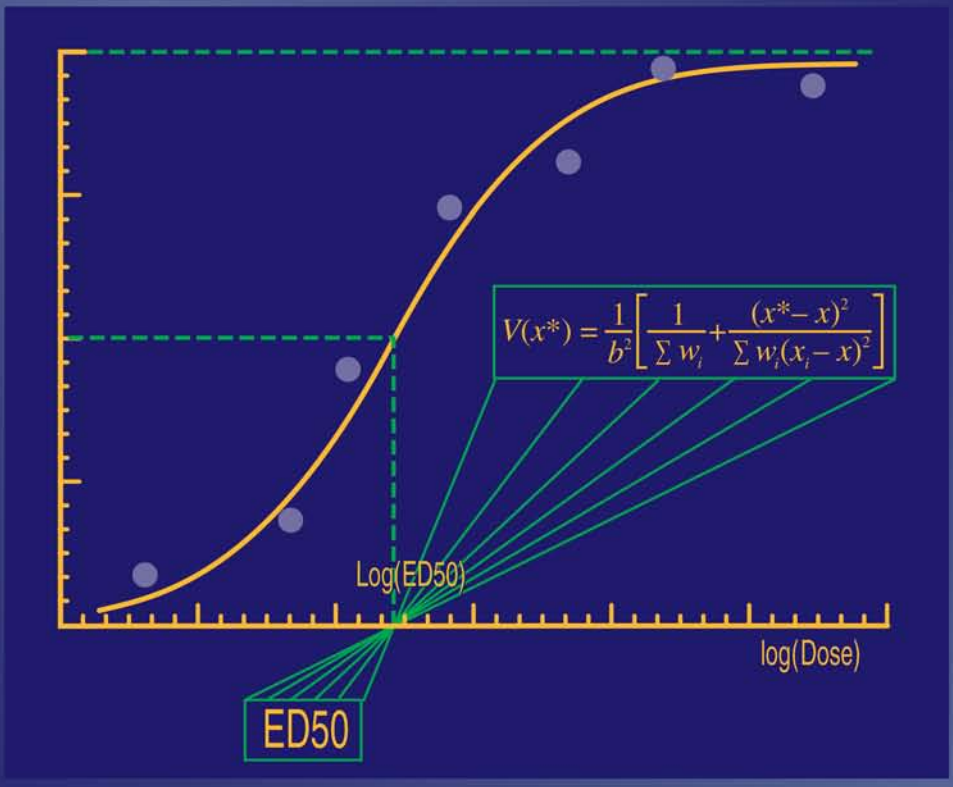
DRUG SYNERGISM and DOSE-EFFECT DATA ANALYSIS



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Preface

The title of this book, *Drug Synergism and Dose-Effect Data Analysis*, could just as well be reversed to, *Dose-Effect Data Analysis and Drug Synergism*. The two topics are inextricably woven and both are covered in this book. I decided on the first title because synergism, as a quantitative topic, has been neglected in mainstream textbooks of pharmacology, though the term and its synonyms, *potentiation* and *super-additivity,* are mentioned frequently. As used here, these terms refer to a phenomenon characterized by drug combinations that produce exaggerated effects. These effects can be the intended effects or the adverse effects of a combination of drugs or other chemicals. In some sense, all pharmacologists, physicians, and most other scientists know what synergism is, yet, it seems, few are familiar with the quantitative methodology that is needed to differentiate synergistic responses from the simply additive responses that are the "expected" effects of drug combinations. The distinction is a quantitative one, and this book deals with the quantitative methodology that is needed to make this distinction. Even when a single drug is administered it enters a system containing myriads of other chemicals and, therefore, interaction with one or more of these compounds is possible. Thus, in a very real sense, this topic has broad applications.

The mathematical foundation for studying the effects of chemical combinations was laid in the first half of the twentieth century, mainly through the works of Fisher, Gaddum, Bliss, and Finney. Much of that early work was directed toward the joint action of various toxins, insecticides, and fungicides. Probit analysis, a powerful method for analyzing quantal dose-effect data, grew out of that early work which almost always used models that constrained the (log) dose-effect data of the individual drugs to yield parallel regression lines. That constraint, the intrinsic complexity of the probit method, and the absence of computers in that era probably contributed to the present-day neglect of this old literature and, thus, its exclusion in the curricula

of today's students of pharmacology and toxicology. The current widespread availability of computers, a broadening of the theory, and a general recognition of the importance of combinations in modern pharmacology have restored interest in this subject. This expanded theory and the many old and new calculation algorithms it uses constitute the main subject of this book, and numerous examples illustrate these calculations.

When experiments are planned, the investigator must have some expectation of the kind of data that may result and, hence, a familiarity with the methodology needed to analyze the data. This is an important part of experimental design. In drug experiments these methods must take into account the variability that is expressed in the data collected. Indeed, the abundance of experimental designs, the many ways of measuring effects, and the never-ending appearance of new drugs and chemicals underscore the need to deal with this variability. Hence, much of the material of this book draws on statistics. Statistical methods, and the theory that underlies these statistics, come from observations of dose-response data and the model curves and equations that describe these data. Therefore, many topics in this book deal with dose-effect data, starting with observations from a single drug and expanding the concepts to more than one drug and the effects that result from such combinations.

Our emphasis is always quantitative since the problem of distinguishing a super-additive response from an additive (expected) response is intrinsically quantitative. When synergism is observed, is it dependent on the *doses* of the respective drugs, or on the *ratio of doses* in the combination, or on the *measurement system* that describes the effect? All of these questions must be ultimately answered, even though in most cases the mechanism responsible for the synergism may still remain unknown. But identifying synergism is, in itself, a valuable first step in illuminating the mechanism.

This book's first three chapters deal mainly with dose-response relations, the statistical analysis of the data that come from these relations, and the models that describe them. Linear regression theory is an important part of this analysis. That topic, though well represented in many textbooks, is treated here with the special needs of the pharmacologist in mind. These include calculations of D_{50} (and *ED50*) values and their standard errors, relative potency determinations, and the common transformations of drug data that allow these estimates. In Chapter 4 we put this all together in calculations of synergism for *graded* data. Those calculations allow a distinction between synergism and additivity at one particular effect level. This

idea is broadened in Chapter 5 where we discuss a newer concept, the *composite additive line*, that extends the analysis to other effect levels. Of special importance in pharmacology is the use of probit analysis, a subject that is absent in most statistics books. Probit analysis is a useful and powerful weighted regression technique that is ideally suited to drug experiments that produce binary outcomes (quantal effects) as opposed to effects on a continuous scale. Logit analysis, also applicable to quantal data, is also presented, and the pros and cons of both methods are discussed. Synergism, and the methodology that distinguishes it from simple additivity, has been traditionally tied to the *isobologram*. This historical plot, while useful for graphical display, does not lead to precise statistical conclusions. In that regard we have introduced an alternate graphical method (Chapter 7) that is more useful.

Much of the content of Chapters 7–11 is new. Especially noteworthy is the use of a single compound administered at two different anatomical sites. Site-site synergism represents a novel way of studying drug mechanisms and some of its benefits are discussed in Chapter 9. Also noteworthy is the *response surface* approach. In contrast to the isobolar approach that is tied to one effect level, this method examines interactions over a range of effects and doses.

As previously mentioned computer technology has had an important impact on the analysis of drug data. Some topics, such as probit analysis and nonlinear regression, admittedly require tedious calculations; prior to the widespread availability of computers these calculations taxed the ability and time of most scientists. Today, these calculations are readily performed with the aid of computers. However, the concepts behind these calculations still remain hidden. For that reason we have included material on nonlinear regression that is applicable to dose-response curves (in Chapter 11) and the details of probit analysis (in Chapter 6). With the exception of these two topics, virtually all the other calculations described in this book can be readily performed with the aid of a calculator and the Appendix tables, though many will still want the convenience of the computer. For that reason, a companion software package that performs the calculations is currently in preparation (see page 204). *Illustrations of calculations in the text use fewer figures than those retained by the computer. Accordingly, some intermediate results in the text may differ slightly from computer values due to rounding.*

While our focus is on drug data, the methods presented are equally applicable to a wider class of chemicals, as is evident in the historical development of this subject. The works of many scientists inspired me

to write this book. Most notable are those "giants" of pharmacology and statistics, previously mentioned, who paved the way over 50 years ago. But a special thanks is also due to all those scientists whose works are cited throughout this book, especially Martin Adler, Alan Cowan, Donna Hammond, Frank Porreca, Robert Raffa, Sandra Roerig, and George Wilcox. I am also much indebted to Jeffrey McCary who wrote the companion computer programs and my editor, Bob Stern, who encouraged me to undertake this work and Helena Redshaw who kept things running smoothly. Steve Menke deserves special thanks for his excellent work in production. Finally, I would like to thank my family for excusing me from many family functions, basketball games, and track meets while I worked on this book.

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2000

The Author

Ronald J. Tallarida earned the B.S. and M.S. degrees in physics/mathematics and a Ph.D. in pharmacology. His primary appointment is as Professor of Pharmacology at Temple University School of Medicine, Philadelphia; he also serves as Adjunct Professor of Biomedical Engineering (mathematics) at Drexel University in Philadelphia where he started his professional career. Dr. Tallarida received the Lindback Award for Distinguished Teaching while in the Drexel mathematics department. He is the author of the popular *Pocket Book of Integrals and Mathematics Formulas,* currently in its third edition, also published by CRC/Chapman & Hall.

As an author and researcher, Dr. Tallarida has published over 200 works, including eight books, and is a frequent consultant to both industry and government agencies for his quantitative work in theoretical pharmacology, data analysis, and combination drug studies. His research on drug synergism has been sponsored by the National Institute on Drug Abuse and by several pharmaceutical firms.

Contents

To

Christopher
R.J.
and
Theresa

CHAPTER 1

Combinations of Chemicals

I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts, advanced to that stage of Science, whatever the matter my be.

Lord Kelvin (1824–1907)

1.1 Introduction

A drug or other chemical may produce multiple effects in the system with which it interacts. A system is a set of interconnected components that has some purpose. In biology the system is often an entire organism. But other systems may be considered, such as an organ, a part of an organ, a cell, or a cellular component. An effect is a change in some attribute of the system. If the chemical is a fertilizer an obvious effect is the change in crop yield. If the chemical is a pesticide the effect might be the destruction or inhibition of the invading pest. In a biomedical context the chemicals of most interest in this book are drugs and endogenous compounds, and the effects are changes in the organism or part thereof. Familiar effects of drugs include changes in blood pressure, body temperature, heart rate, pain perception, etc. These are overt effects; other drug effects are intimate and not easily observed, such as the opening or closing of an ion channel in the cell membrane or the release of some other chemical substance from the cell. Drug effects can be desirable or undesirable (adverse effects). The main concern of this book is the study of two or more chemicals present together. Specifically, the interest is in drugs or other chemicals that act together to produce overtly similar effects, e.g., two analgesics or two antihypertensives.

When compounds with similar overt actions are present together, the combined effect may be predictable from knowledge of the individual drug potencies, i.e., there is simple additivity. In contrast, the effect of the combination may be either exaggerated or even attenuated. The exaggerated effect is termed *super-additive* or *synergistic* whereas the blunted effect is termed *sub-additive*. In each of these cases the individual compounds are contributing to the effect, but something occurs with their joint presence that either enhances or diminishes the effect expected from the pair.

Whether the pair of compounds consists of drugs, fertilizers, pesticides, or any other chemical types that act similarly, the methods of analysis presented here will apply. Our focus is on the relation between concentrations and effects and the methodology that distinguishes between additive and non-additive interactions, but, in some cases, this distinction may also help us better understand the intimate actions of the compounds. Several methods of analysis for distinguishing between simple additivity and the other non-additive outcomes will be discussed. These involve the use of quantitative information regarding the dose (or concentration) and the magnitude of the effect. The data contributing to this information are analyzed in a variety of different ways, very often from graphs of the relation between concentration and effect or from suitable mathematical transformations of these quantities. Accordingly, the dose-response relation is a key topic that is applied throughout this book.

Drug effects are often highly variable and the variability exhibited in this kind of data necessitates the use of statistical methodology. Thus, much of the material we discuss will consist of dose-effect curves and the statistical analysis of these curves, often with the aim of distinguishing simple additivity from sub-additivity and synergism for compounds acting together. Synergism is especially important in clinical situations with drugs, for it allows the use of smaller amounts of the constituent drugs. An adverse effect may also synergize, a phenomenon of special importance in clinical situations. The detection of synergism may also be useful in illuminating mechanisms of drug action and in the development of new theories. The same applies to synergistic combinations of other classes of chemicals. Although observational results are the primary material of pharmacology, the use of theory allows a correlation of these results, places them into the regularities of experience that we call principles, and uses these principles to predict the results of new experiments.

1.2 Independent joint action of drugs

If the dose-response relation is known for each of two chemicals used individually, how can the expected response for some combination of the two be calculated? This is a key question that was first systematically addressed by Bliss (1939) and subsequently expanded by Finney (1942) in connection with insecticides. An important consideration is whether the two chemicals act independently. Bliss referred to three types of joint action that he termed *independent joint action, similar joint action* and *synergistic action*. An important concept contained in the first two of these is the idea of independent action. Similar and independent action are useful for our future discussion of drug combinations. By this we mean that each drug produces overtly similar effects (for example, each lowers blood pressure) such that all or part of one component may be substituted for the other in some proportion that is based on the dose-response relations of the two.

For example, an antihypertensive drug that lowers blood pressure by blocking angiotensin II receptors and one that exerts its antihypertensive effect through diuresis would fit this definition of similar independent joint action. Their individual potencies allow a calculation of how much of one is equivalent to the other in the production of this effect, a calculation that is discussed in the next section. In contrast, two antihypertensive drugs that have general *beta* adrenoceptor block as components of their action would not fit this definition because of competition of the two for the common *beta* receptor.

In general, if two overtly similar drugs (either two antagonists or two agonists) act on the same cellular receptor, their actions are not independent because the effect of their combination depends on the bound concentrations of the two (and their intrinsic activities if they are agonists). One could not substitute an amount of one for the other in a combination based solely on their individual dose-response relations because a change in the concentration of one affects the bound concentrations of both. (Competition is discussed in Chapter 9.) The importance of independent action is further illustrated in our discussion of additivity as it is commonly defined in pharmacology.

1.3 Additivity

Drugs or other chemicals that produce overtly similar effects will generally do so with different doses. The dose-response relation of each

agent provides this information and allows one to focus on a specific magnitude of the effect. For example, two drugs that are each capable of increasing the heart rate may differ in the respective doses needed to increase the rate. To distinguish these quantitatively one can choose an effect level, for example a rate increase of 10 beats per minute. The first drug might achieve this with a dose of 100 mg whereas the second requires only 25 mg. These are indicators of drug *potency*. The drug that requires the lower dose is said to have a greater potency than the other. The dose ratio, in this case 100/25 = 4, called the *relative potency*, is a convenient indicator of this quantitative attribute of the drug pair. This same relative potency may or may not apply to all levels of effect for these two drugs, a concept that is discussed in some detail in Chapter 2. For now we will assume a constant relative potency, i.e., one drug is four times more potent than the other at *all levels of effect* achieved by each drug. Further, we now introduce notations that will be convenient in this and in subsequent discussions.

For drug A, the lower potency drug, its dose when it acts alone is denoted by the italicized symbol, A; for drug B, the corresponding quantity is denoted B. The relative potency R is then A/B, a value greater than one. We now consider the situation in which both drugs are present together. In this situation lower case symbols are used, i.e., we denote by a and b the doses of the respective constituents when given as a combination. Because these drugs are assumed to have a constant relative potency (R) the combination (a, b) can be expressed as an equivalent quantity of either drug. If drug A is the reference drug then the combination dose satisfies the relation

$$a + Rb = A. \tag{1.1}$$

In words, Equation 1.1 means that one can use respective amounts a and b calculated from the above in order to achieve the effect of dose A of drug A acting alone. Implicit in Equation 1.1 is the concept of independent joint action, i.e., the presence of B is like the addition of a more concentrated form of A. The same combination (a, b) can also be expressed in terms of an equivalent of drug B and is given by the equation

$$a/R + b = B. \tag{1.2}$$

Here the less potent drug (A) acts like a dilute version of the other and adds to B. The relations expressed by Equations 1.1 and 1.2 mean that the doses in the combination contribute to the effect in accord

with the individual drug potencies, a situation that is termed *additive*. Rearrangement of these gives a more familiar form:

$$a/A + b/B = 1. \tag{1.3}$$

In each of the above equations, the doses A and B are equieffective doses of the individual agents when each is present alone, R is the ratio A/B, and the quantities a and b are the respective doses in the combination that give the effect level achieved by dose A alone or dose B alone. When the relative potency R is the same at all effect levels the first two forms are convenient; however, when R varies with the effect level, the more explicit relation of Equation 1.3 is convenient because it uses the values of A and B that apply to that effect. Equieffective dose pairs are termed *isoboles*; thus, (A, 0), (0, B) and the pair (a, b) given by the above relations are isoboles. Additivity as defined here is a most important concept. Departure from additivity means that some kind of interaction occurs when both substances are present together. Hence, calculating quantities that are additive is the basis for determining these departures when actual pairs are studied. Nonadditive pairs may be a useful first step in illuminating mechanisms.

1.4 Isobologram

Equation 1.3 provides a simple graph of equieffective dose pairs (a, b). If A and B are known to be the respective doses that give a specified effect, e.g., 50% of the maximum effect, when each agent acts alone then these are constants that are used to identify the doses a and b in a combination that produces this same effect. These combination doses must satisfy Equation 1.3. For example, if A = 500 mg and B = 100 mg, then the equation, $a/500 + b/100 = 1$, gives additive dose combinations such as (100, 80), (250, 50), etc. The totality of pairs (a, b) graph as the straight line shown in Figure 1.1. This *line of additivity* has Cartesian coordinates that represent all possible combinations that are equivalent in producing the effect of either 500 mg of drug A or 100 mg of drug B. A graph of this kind is useful for displaying the results of actual tests with combinations. Such testing may reveal departures from additivity. Suppose, for example, that the combination a = 100 mg, b = 50 mg produced the specified effect level. This point (100, 50) lies below the line of additivity as shown in Figure 1.2 as point P, meaning that lesser quantities of drugs A and B are needed in the combination. Some interaction has taken place,

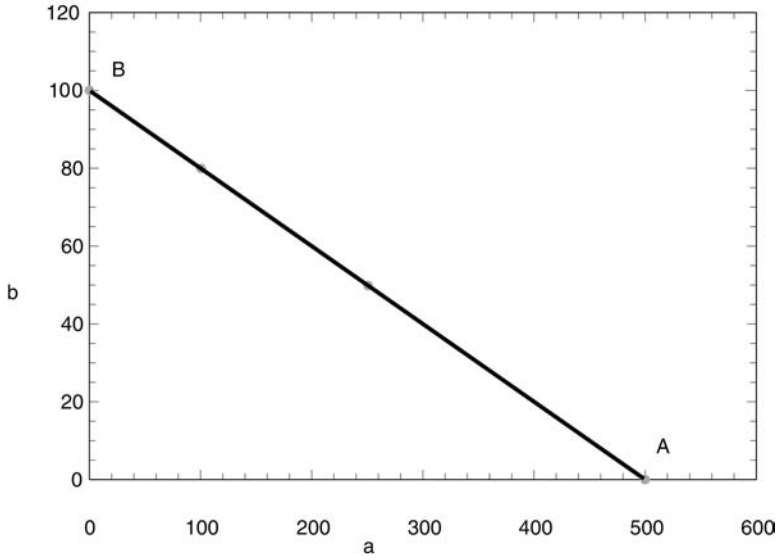

Figure 1.1. Line of additivity of the isobologram. Intercepts are doses of each when present alone.

either between the drugs or the systems on which they jointly act, and therefore quantities less than those predicted by additivity are needed. This is called a super-additive or *synergistic* combination. In contrast, some combinations may require doses that are greater than the additive amounts of Equation 1.3 in which case the point representing the combination will lie above the line of additivity as shown in Figure 1.2 as point Q. This phenomenon means sub-additivity, i.e., the constituents are somewhat antagonistic for some reason. This graph, consisting of the additive line and the actual dose pairs needed to attain the specific effect level is called an *isobologram*. It was introduced by Loewe who conducted a number of studies of combinations that used this kind of graph. (See Loewe, 1927, 1928, 1953, 1957.) These non-additive cases are expressed as inequality relations that contrast with Equation 1.3 as follows:

$$a/A + b/B < 1. \tag{1.4}$$

$$a/A + b/B > 1. \tag{1.5}$$

Relation 1.4 indicates synergism or super-additivity whereas Relation 1.5 means sub-additivity.

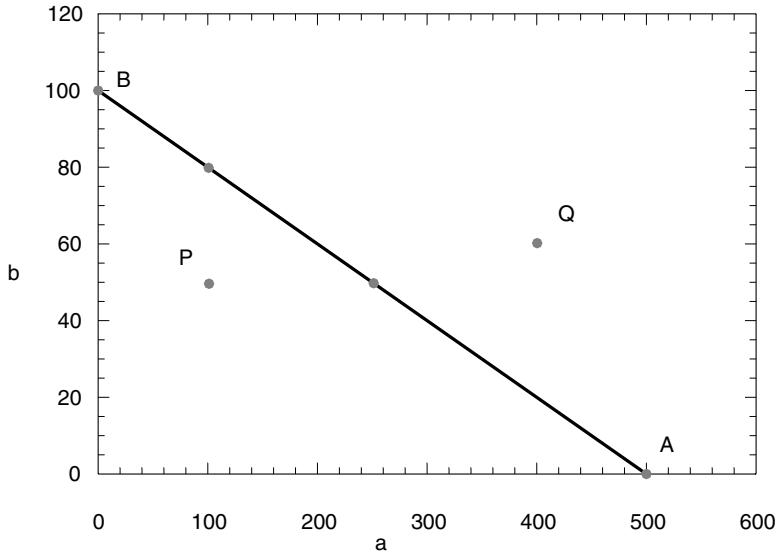

Figure 1.2. Isobologram showing line of additivity and dose combination P that is synergistic and dose combination Q that is sub-additive.

Testing two drugs together may reveal many synergistic combinations, and, thus, their graphical representation suggests a smooth curve that is concave upward as shown in Figure 1.3 (curve I) or a curve that is concave downward, indicative of sub-additive combinations, shown as curve II in the figure. Curves, or sets of discrete points (doses) that give the same effect, are termed *isoboles*; these are curves of constant effect and have termini (axial points) that indicate the individual doses, *A* of drug A and *B* of drug B when each is present alone. Although smooth curves such as these indicate either synergism or sub-additivity over all dose combinations, there is no reason why such patterns must occur when actual combinations of chemicals are tested. In other words, some dose pairs may be synergistic while others are additive, or even sub-additive. Accordingly, the isoboles of Figure 1.3 should be regarded only as models that could describe the combined action of two active drugs.

An interesting case is that in which one of the drugs (drug A) is inactive when given alone. Here the isobole of additivity is a horizontal line (Figure 1.4) so that synergism and sub-additivity are indicated by dose pairs giving points P and Q below and above this line, respectively.

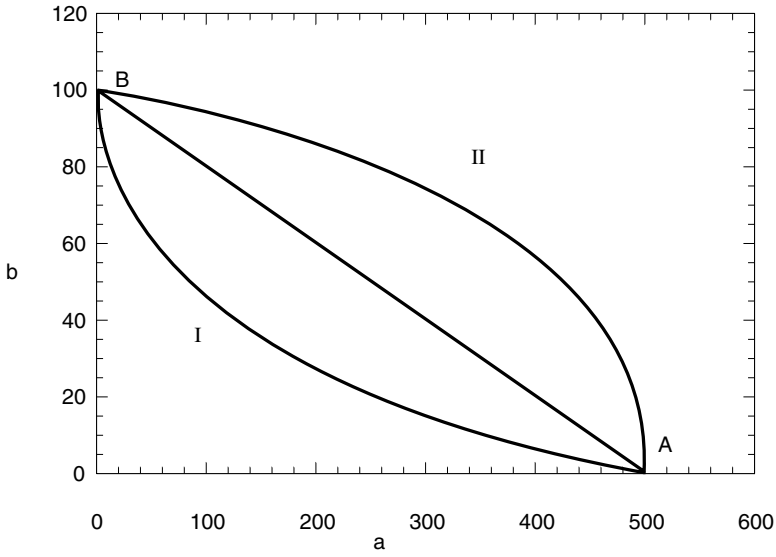

Figure 1.3. Isobologram showing line of additivity and curves for combinations that are synergistic (curve I) and sub-additive (curve II).

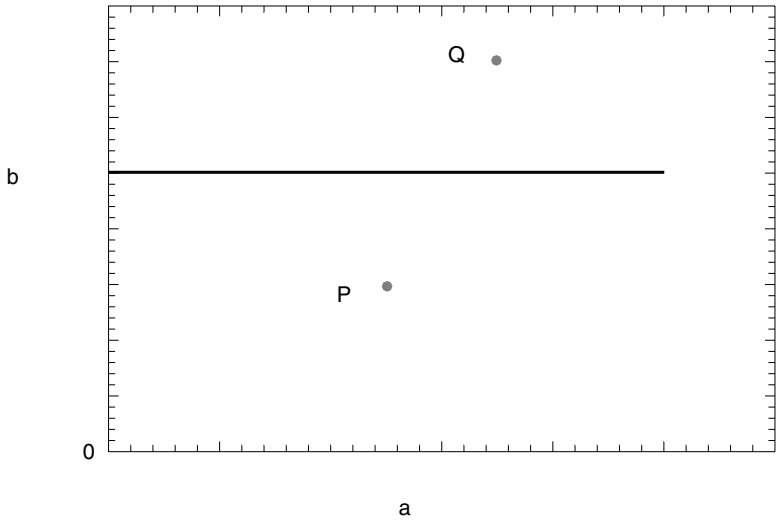

Figure 1.4. Isobologram when one drug (A) is inactive. The active drug (B) produces the desired effect with dose b and this effect is independent of the dose of A in a theoretically additive combination. If actual dose combinations, indicated by points P and Q, produce the specified effect, these are synergistic and sub-additive, respectively.

1.5 Chloral hydrate and ethyl alcohol

The isobologram seems to have attracted little attention until it was used in a well-publicized study of the combined action of chloral hydrate and alcohol by Gessner and Cabana (1970). Both agents are hypnotics; that is, they are capable of inducing sleep, and this study was aimed at answering the question of whether the combination of the two was synergistic. The experiment was carried out in mice that received intraperitoneal doses of the individual drugs and combinations. An indicator of hypnosis was the loss of the righting reflex and that could be quantitated for each dose or dose combination as the proportion of animals that displayed this endpoint. The effect level used was hypnosis in 50% of the mice tested ($p = 0.5$). The dose of either drug (acting alone) that gives this level is the *ED50*. For ethanol (horizontal axis) the *ED50* was found to be 2666 mg/kg, and for chloral hydrate the value was 244 mg/kg (vertical axis). These are the respective mean values obtained from analysis of the individual dose-effect curves of the agents. For our current purpose, we will postpone discussions of dose-effect data analysis and the methods that gave these estimates of the means and thus concentrate only on the display of data points shown on the isobologram of Figure 1.5.

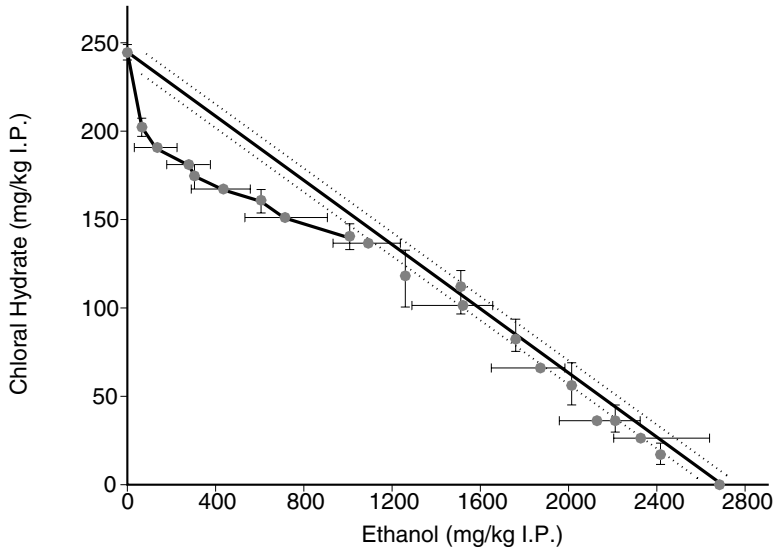

Figure 1.5. Isobologram for the hypnotic effect of a combination of ethyl alcohol and chloral hydrate. (From Gessner and Cabana, A study of the hypnotic and of the toxic effects of chloral hydrate and ethanol, *J. Pharmacol. Exp. Ther.*, 1970, with permission.)

This figure shows a solid line having vertical intercept 244 and horizontal intercept 2666, these being the individual drug *ED50* values. This is the line of *additivity*. Individual dose pairs that gave 50% effects are also plotted, and these points show either horizontal or vertical error bars whose meaning is related to the way these were obtained. In some cases the chloral hydrate dose was fixed, and the amount of ethanol used concurrently to produce the 50% response was estimated (from regression analysis). Accordingly, this estimate of the ethanol mean dose has statistical confidence limits that are displayed as horizontal bars through the points. In cases in which ethanol was fixed and chloral hydrate varied until the 50% response was attained, we get estimates of the latter's dose and the confidence limits of this mean are indicated by vertical bars.

The main idea here is that some of the data points appear to be well off the line of additivity, while others are close to the line and have error bars that intersect it. As a purely visual conclusion this means that some combinations are synergistic whereas others are simply additive. In other words, synergism is not only a property of the drug pair but also depends on the relative amounts in the combination tested. Another observation is that a plot of this kind may not be adequate for a rigorous conclusion since terms like "on" and "off" the line are loose constructs, as is the location of the "line" itself, since its vertical and horizontal intercepts (the individual *ED50s*) are also estimates and, thus, have error.

In this same article the authors report the results of toxicity experiments with the same two drugs. In those tests the incidence of fatality was determined; thus, the important determination is the dose (or dose combination) that is lethal in 50% of the animals. The isobologram in this case was based on *LD50* values and therefore is different from the isobologram for hypnosis. In the lethality isobologram (not shown here), there was synergism for only one of the dose pairs tested (highest ratio of chloral hydrate), simple additivity in combinations containing lower proportions of chloral hydrate, and apparent sub-additivity in combinations containing larger amounts of alcohol. This finding points out that the isobologram for one endpoint is not necessarily the same as that for some other endpoint.

1.6 The need for statistics

The distinction between additive and nonadditive actions uses dose values that produce a specified level of effect. Up to now our discus-