that, in a graded isobologram of both the heterergic (+) and (−) effects, this reciprocal antagonism results in a "neutralization zone" extending the subthreshold area near the zero point of the combined-dose field more or less radially far out into the field, and that the presence of such a band of ineffective doses most characteristically distinguishes enantiergic from cryptantergic effects (see 93).

Any attempt at further classification of antagonistic phenomena would lead beyond the frame of this brief survey of antagonistic *effects*. Clarification of the processes of antagonistic *action* is, to be sure, the ultimate aim. However, an understanding of the physiological *outcome* is, in general, the prerequisite for the understanding of those more intimate *mechanisms* conditioning the effect phenomena. Discussion of the fortunate circumstances which sometimes allow to circumvent this prerequisite must be dispensed with here. So must also a discussion of the all-too frequent belief that *biostatistics* can open an avenue of approach to problems of combined effect; its role may be stated in one sentence (for details compare 93): In the study of antagonism, as in all comparable problems, biostatistics plays an important part when applied in its due place, namely, when it is employed as the tool to find the behavior of the "normal" individual,—"the probit 5 individual" (93)—within the natural population of test objects with varying drug sensitivity, and a most precarious role when employed in the fallacious belief that the gradation of sensitivity, for instance the percentage scale of individuals exhibiting an endpoint effect, can replace the yardstick of *intensity* of effect,—which, after all, is the only measure of antagonism as a quantitative phenomenon.

A. J. Clark (31, p. 239) who played such a leading part in this field summarized his opinion on the problems of antagonistic *action* in the statement: "Imperfect knowledge",—and that includes: imperfect conceptual clarity,—"appears to be the most probable reason for any apparent simplicity in processes of drug antagonism." If the present review has succeeded in demonstrating that this holds true for antagonistic *effects* as well, it may have served to clarify by illuminating complexities.

DRUG ANTAGONISM AND pA_x

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Clark and Raventos (32) suggested a method of estimating the activity of drug antagonists in terms of "the concentration which altered by a selected proportion, *e.g.* 10-fold, the concentration of an active drug needed to produce a selected effect". The negative decimal logarithm of this (molar) concentration has been termed pA_x where x is the proportion selected (131). Since pA_x is a null measure which involves no change in response it is independent of the method of experimentation and can be determined equally well in perfused and isolated preparations (13). Its usefulness as an empirical measurement

depends on its reproducibility; in general the pA_x values obtained in different laboratories have shown good agreement provided that the same contact time with antagonist was used (85, 120).

The pA_x is a measure which is particularly suitable for determining the activity of antagonists which do not alter the slope of the log-dose-effect curve of the agonist. Difficulties arise with antagonists which affect this slope, since their pA_x values vary with the concentration of agonist. One way of dealing with these is to define the effect of the agonist, *e.g.*, pA_x may be determined when the effect of the agonist is half maximal (133). Another method is based on the finding that antagonists which flatten the slope of the log-dose-effect curve generally also depress its maximum. Their activity can then be expressed in terms of the concentration required to produce a given depression of the maximum, *e.g.*, the concentration which depresses the maximum to one-half. The negative logarithm of this (molar) concentration has been termed the pA_h (13).

Tests for competitive antagonism

Graphical tests of the mass law equation for competitive antagonism usually take one of two forms: (i) The test of Lineweaver and Burk (84) based on plotting the reciprocal of the effect against reciprocal of dose. This kind of plot, though very useful in testing enzyme inhibitors, has only limited applicability to drug antagonism, since it is based on the assumption that the recorded physiological effect is linearly related to the number of active receptors. Although this assumption has frequently been made in the past (30, 79) it is unlikely to have general validity and it is therefore preferable to use tests which do not make use of it. (ii) Tests based on a comparison of equi-active doses. This type of test involves no assumption about the manner in which receptors and physiological effect are related. It assumes only that equal effects involve equal numbers of receptors.

There are two stages to the test. First, a series of log-dose-effect curves are plotted, one without antagonist and the others with different concentrations of antagonist. If these curves are parallel, they afford presumptive evidence of competitive antagonism (13). Next the constants of the competitive equation are determined. The equation may contain several affinity constants (144), but in practice it is generally sufficient to consider a single affinity constant for the antagonist. The equation given by Gaddum (53) for this case may be written as follows:

$$\frac{y}{1-y} = K_1 A = \frac{K_1 A x}{K_2 B^n + 1} \qquad (I)$$

where y is the fraction of active receptors (receptors combined with the agonist) and K_1, K_2 and n are constants. A and Ax are the concentrations of agonist causing the response corresponding to y in the absence and presence respectively of the antagonist in concentration B. Eliminating K_1A, and taking logarithms

$$\log (x - 1) = n \log B + \log K_2,$$

and since by definition $pA_x = -\log B$ (133)

$$\log (x - 1) = \log K_2 - npA_x \qquad \text{(II)}$$

A plot of $\log (x - 1)$ against pA_x thus gives a straight line with slope $-n$. The line intersects the pA_x axis at a point corresponding to pA_2. When $n = 1$, $pA_2 = \log K_2$, and $pA_2 - pA_{10} = 0.95$.

Equation (II) provides a useful test of competitive antagonism which is independent of y. This relation is readily verified experimentally but it is worth pointing out certain recurrent sources of error which may invalidate the results. 1) Depression by a maximal dose: large doses of the agonist often produce a long lasting depression of the effect of subsequent doses. This results in a shift of the concentration-action curve which may be wrongly attributed to the antagonist. 2) A change in the receptor/effect relation. This may manifest itself by a gradual alteration in sensitivity to the agonist or a change in the slope of the regression line in the course of an experiment. If this change occurs after the antagonist has been administered it cannot be detected and constitutes a source of error. 3) Failure of the antagonist to reach equilibrium: antagonists often take a long time to produce their full effects and low concentrations are frequently slower to reach equilibrium than high concentrations; consequently the $pA_2 - pA_{10}$ difference may be underestimated.

The rate at which equilibrium is reached varies with the experimental preparation used, and with the concentration and nature of the antagonist. Certain antagonists do not reach equilibrium within a measurable time; these "non-equilibrium" antagonists are discussed by Dr. Nickerson.

In testing competitive antagonists a wide range of concentrations can be explored. This greatly adds to the significance and reliability of the result. Atropine is a competitive antagonist of acetylcholine (with $n = 1$) over a one thousand-fold range of concentrations (13, 29). Atropine is probably also a competitive antagonist of histamine, but over a narrower range and only in higher concentrations. The affinity of atropine for acetylcholine receptors is about one thousand times greater than its affinity for histamine receptors.

The use of antagonists for the classification of drugs (132). The concept of specific receptors with which drugs and antagonists react reversibly is a convenient working hypothesis which besides accounting for the effects of certain antagonists also provides a rational basis for the classification of drugs. A natural consequence of the receptor theory is that drugs should be classified according to the receptors on which they act. Each tissue probably has a limited complement of receptors and an important aim of pharmacological research is to identify these receptors. Receptors are best identified by antagonists. If two drugs act on the same receptors and obey the same mass law they can be expected to give rise to the same pA_x when tested with a competitive antagonist. Drugs which produce the same pA_x values with antagonists can thus be classed together. There seems no doubt that drugs in fact exist which, although they may vary greatly in activity, produce the same pA_x with antagonists. For example, the activities of histamine, pyridylethylamine and pyrazolethyla-

mine on the guinea-pig ileum vary in the ratio of approximately 1:30:1,000 but their pA_x values with antihistamines are the same (13).

The use of antagonists for the comparison of receptors. If a competitive antagonist produces the same pA_x in different preparations it can be assumed that it reacts with closely similar receptors. Antagonists can thus be used for the comparison of receptors in different tissues and species. The pA_x values obtained in different preparations are sometimes remarkably alike. For example, similar pA_x values are found when antagonists of histamine and acetylcholine are tested on the guinea-pig ileum and the guinea-pig lung (13); even the nerve-free plain muscle of the amnion of the chick gives pA_2 values which are similar to those obtained in mammalian preparations (39a).

Agonists are as a rule more variable than antagonists; *e.g.*, the guinea-pig ileum is much more sensitive to histamine than the tracheal chain but the pA_x values of antagonists in the two preparations are the same (13). This is not surprising in view of the greater complexity of action of the agonist. Stephenson (139) and Ariëns (4) have suggested that the activity of an agonist depends on at least two factors: its affinity for receptors and the contribution made by the drug-receptor complex to the physiological effect; whereas the activity of a (competitive) antagonist depends only on its affinity for receptors.

Non-competitive antagonists. The term "unsurmountable antagonist" (55) serves as a useful descriptive term for antagonists which produce a progressive depression of the maximum of the concentration-action curve. The term "non-competitive antagonist" is more properly reserved for a special type of mass-action antagonism in which agonist and antagonist act on different sites. The simplest equation for non-competitive antagonists is as follows (27, 134):

$$y = \frac{K_1 A}{K_1 A + 1} = \frac{K_1 Ax}{K_1 Ax + 1} \frac{1}{K'_2 B + 1} \qquad \text{(III)}$$

This equation gives a series of log-dose effect curves with a common origin and progressively declining slopes and maxima. More elaborate equations can be derived by assuming that the antagonist acts both competitively and non-competitively or that an antagonist molecule interacts with several receptors or *vice versa* (this gives rise to an exponential term). A detailed discussion of various types of interaction is given in the article by Dr. Ariëns.

A notable difference between the equations for competitive and non-competitive antagonism is that the former can be verified without reference to y—the fraction of receptors combined with the agonist—whereas the latter cannot. This makes the tests for non-competitive antagonism more complicated and less reliable. For example, it follows from equation (III) that when $y = 0.5$ the difference $pA_2 - pA_{10} = 0.39$, but when $y < 0.5$ this difference is larger. If the difference $pA_2 - pA_{10}$ of a non-parallel-line antagonist, determined when the effect of agonist is half maximal, is found to be larger than 0.39 it may be difficult to decide whether a half maximal response has taken place with less than 50% of receptors combined with the agonist or whether equation (III) is inapplicable. If only a very small fraction of receptors is occupied by the agonist

the $pA_2 - pA_{10}$ differences of non-competitive antagonists approach those of competitive antagonists and the distinction between the two types of antagonism becomes altogether blurred. It can sometimes be assumed on the basis of the concentration-action curve of the agonist that the effect is proportional to y. In that case for a non-competitive antagonist $pA_h = \log K'_2$; this relation is obviously less likely to be experimentally realized than the corresponding competitive relation $pA_2 = \log K_2$.

The pA_h can be considered as primarily an empirical measurement. Many drugs which depress the maximum of the concentration-action curve are probably not true non-competitive antagonists but unspecific depressants. When the pA_h of these drugs is measured it is found to be closely correlated with depression of oxygen consumption (106).

Conclusion. The pA_x and pA_h are empirical measures of the activity of drug antagonists. In some special cases they have theoretical significance since they may correspond to the mass equation constants of competitive and non-competitive antagonists.

NONEQUILIBRIUM DRUG ANTAGONISM[1, 2]

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Pharmacological antagonists to endogenous or exogenous chemical stimuli classically have been categorized as competitive or noncompetitive. Competitive antagonists are believed to react with the same groupings or configurations on or in cells with which the agonist combines to produce its characteristic effect, the specific receptors[3]. Noncompetitive antagonists may act at any other point,

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[2] There is no general agreement on the appropriate term to be applied to the type of pharmacological action discussed herein, *i.e.*, the blockade by a drug which forms a stable bond with specific receptors, and as a result is not in mass-action equilibrium with the agonist. It is the opinion of the author that "nonequilibrium blockade" is the most suitable. This designation has been used to distinguish the action of members of the Dibenamine series from that of other adrenergic blocking agents (115), and appears to most closely describe the action in question. The terms "irreversible competitive blockade" (49) and "unsurmountable blockade" (55) also have been applied to this type of action. However, the blockade is not strictly irreversible, and the term unsurmountable is appropriate only when the antagonist is used in sufficiently large doses to prevent a maximal response even in the presence of massive doses of agonist.

[3] The concept that drugs produce their effects by combining with specific receptors (or receptive substances) in cells originated with Langley (82), and has been very fruitful in the development of pharmacology. It has been attacked from time to time, but it is difficult to deny that chemical agents must combine or react with something in order to produce an effect. It is also clear that receptors may be specific for certain compounds or groups of compounds because it is possible to block responses to one substance or group without