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# THE USE OF DRUG ANTAGONISTS FOR THE IDENTIFICATION AND CLASSIFICATION OF DRUGS

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A sensitive biological method for the identification of drugs has been described by Chang and Gaddum (1933). It consists in estimating the activity of an unknown substance in terms of a standard, quantitatively, in several different pharmacological tests. As a rule the ratio of activity between standard and unknown in different tests is only constant if the two samples are chemically identical, otherwise the ratio of activity in different tests varies. By this method even closely related substances may be differentiated.

Another biological method for the identification and differentiation of drugs consists in testing quantitatively their responses towards antagonists. It has been shown in a previous communication (Schild, 1947) that the effects of histamine and acetylcholine may be thus distinguished, since many antagonists although unspecific at high concentrations will differentiate between these drugs at lower concentrations. The present investigation is concerned with the question whether drugs which are more closely related than histamine and acetylcholine may also be differentiated in this way, or whether antagonists, even though they may be used in an exact and quantitative manner, would fail to discriminate between closely related substances.

If indeed some drugs, although distinguishable chemically or by their relative activity in different pharmacological tests, could not be distinguished by their reactions to antagonists, they might conveniently be grouped into a common pharmacological class. It will be shown that drugs can in fact be found which are related in this way, and that if a certain simple scheme of drug action be accepted a rational classification of active drugs based on their responses to drug antagonists may be attempted.

## METHODS

The experiments were done on the isolated ileum of the guinea-pig, employing the apparatus for assaying antagonists on isolated tissues which has previously been described (Schild, 1947).

*Plan of experiments.*—The object of these experiments was to find out whether drugs could be differentiated by their responses to antagonists. They were accordingly designed to test whether the effects of two given stimulant drugs would be reduced by some antagonist to the same extent or not.

The assays are performed in two stages. In a preliminary experiment doses of the two active drugs producing approximately equal submaximal effects are determined as well as a concentration of the antagonistic drug sufficient to reduce these effects without completely abolishing them. In a final experiment these drugs are administered in the presence and in the absence of the antagonist in a planned sequence, which must be statistically unbiased in order to allow a statistical analysis of the results of each experiment to be made.

A typical experiment is shown in Fig. 1. The order of addition of drugs to the bath is as follows. The doses are administered in sets of four, each set consisting of a random sequence of one dose of each of the active drugs alone and a further dose of each of the active drugs in the presence of the antagonistic drug (after a preliminary period of contact between tissue and antagonist of 2 min.). After each administration of the antagonist several doses of the active drug alone are interjected in order to let the tissue recover, if possible, from the preceding depression.

Persistent depression produced by antagonists is the most serious difficulty in planning an unbiased sequence of doses suitable for statistical analysis. The depressant effect is partly overcome by interposing doses of the active drug alone. More fundamentally, the arrangement of the experiment itself is such that each of the active drugs has an equal chance of being preceded by a depressant injection, and it is thus unbiased with regard to the main point at issue. It is merely a matter of convenience how many " recovery " periods of active drug alone are interposed and it may in some cases be more advantageous to have a fixed small number of such periods rather than to wait until the response has reached a steady state.

A complete experiment consists of several sets (randomized groups) of four doses. The final effect is assessed by averaging the effects obtained in the individual sets. If the two drugs are depressed to the same extent by the antagonist, and $\bar{y}_A$ and $\bar{y}_B$ are the mean effects produced by drugs A and B, and $\bar{y}_{AZ}$ and $\bar{y}_{BZ}$ the mean effects produced by these drugs in the presence of the antagonist Z,

$$\bar{y}_A - \bar{y}_{AZ} = \bar{y}_B - \bar{y}_{BZ}$$

or

$$\bar{y}_A + \bar{y}_{BZ} = \bar{y}_B + \bar{y}_{AZ}$$

The latter equation is tested statistically by an analysis of variance carried out as previously described (Schild, 1942). In practice there is usually some deviation from theoretical equality, but unless this is statistically significant it may be assumed that both drugs have been depressed to the same extent.

## Use of a Drug Antagonist for the Identification of an Unknown Substance

Curare causes the release of a histamine-like substance from the perfused gastrocnemius muscle of the dog (Alam *et al.*, 1939). In the present experiment a dog's hind limb was perfused with defibrinated blood by means of a Dale-Schuster pump, the blood being oxygenated through the animal's own lungs ; a solution of 50 mg. crude curare was injected into the artery perfusing the limb and the effluent was collected. The blood was extracted by Code's method and the extract assayed on the guinea-pig's ileum. The apparent concentration

of histamine in the sample was 500 $\mu$g. per l. as against 16 $\mu$g. per l. in the controls.

In order to identify the substance further the effect of a low concentration of benadryl (dimethylaminoethyl benzhydryl ether hydrochloride) on it was compared with the effect of the same concentration of benadryl on histamine. The experiment is illustrated in Fig. 1. A concentration of benadryl of 1 : 150

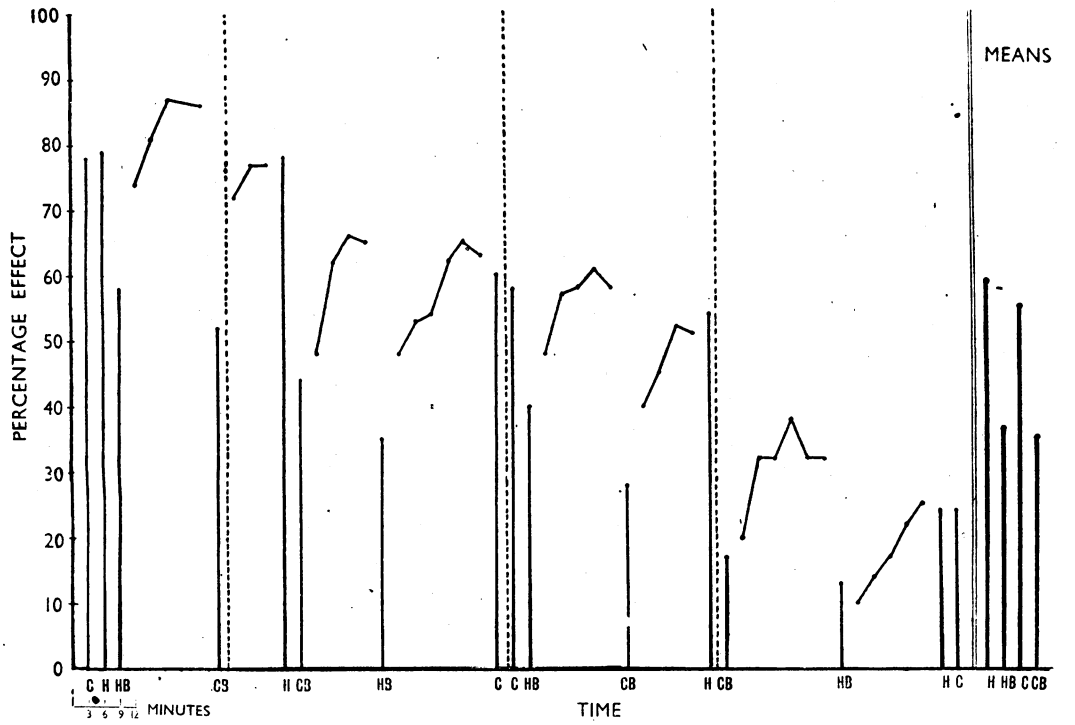



FIG. 1.—Assay to test whether the effects of histamine (H) and an unknown substance (C) released from skeletal muscle by curare are reduced to the same extent by benadryl (B). Sequence of injections explained in the text. Vertical lines represent the effects of test doses of the two drugs given in the presence and in the absence of the antagonist, each effect being repeated 4 times. The irregular curves represent the effects of a constant dose of histamine given in order to allow the preparation to recover from a preceding dose of benadryl. Note incomplete reversal of depression.

million produced an appreciable reduction of the effects of both histamine and the unknown substance. There also occurred a progressive deterioration of the preparation, presumably due to the cumulative effects of the antagonist. In spite of this and the unequal effects produced by individual doses of the antagonist, the mean reduction of effect of the two active drugs is almost identical.

The results of an analysis of variance of this experiment are shown in Table I. In the last column the mean square for each item should be compared with the mean square for

error. Two of the mean squares are smaller than the error mean square and thus statistically obviously not significant. One of these is the mean square which assesses the relative reduction of the effects of histamine and the extract by benadryl. Since there is no signifi-

TABLE I

ANALYSIS OF VARIANCE OF BENADRYL DEPRESSION OF HISTAMINE AND HISTAMINE-LIKE SUBSTANCE RELEASED BY CURARE

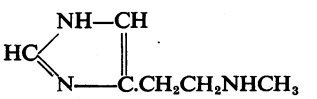
| Source of variation | Sum of squares | Degrees of freedom | Mean square |
|---|---|---|---|
| Between successive "randomized groups" .. .. .. | 4805 | 3 | 1602 |
| Between histamine and extract .. .. .. .. | 25 | 1 | 25 |
| Between active drugs alone and active drugs + benadryl ("slope") .. .. .. .. .. .. | 1764 | 1 | 1764 |
| Between depression of histamine effect by benadryl and depression of extract effect by benadryl .. .. .. | 6 | 1 | 6 |
| Error .. .. .. .. .. .. .. .. | 502 | 9 | 56 |
| Total .. .. .. .. .. .. .. | 7102 | 15 | |

cant difference the reduction of the two effects may be assumed to be equal and the main object of the experiment is achieved. A further incidental result is a lack of statistically significant difference between the effects produced by histamine and the extract, indicating that the activity of the extract might reasonably be assumed to be 500 $\mu$g. per l. as originally assumed. Two of the mean squares are much higher than the mean square for error and statistically significant. One of these is the mean square for "slope" indicating that the concentration of benadryl used produced a significant reduction of effect. The other is the mean square between successive "randomized groups," showing that a real change in sensitivity, in this case a progressive deterioration, occurred in the course of the experiment.

Similar results were obtained in two further experiments.

It was concluded that since benadryl does not discriminate between the two substances they are likely to be related or possibly identical.

## Drugs which are Indistinguishable by their Reaction to Antagonists

N-methylhistamine, 

has been synthetized by Garforth and Pyman (1935). Its pharmacological actions were investigated by Vartiainen (1935) who found it to be twice as active as histamine on the guinea-pig's ileum and about half as active as histamine on the cat's blood pressure. Since histamine and its N-methyl derivative are closely related, as well as clearly distinguishable by their relative activity in different pharmacological tests, they were selected as a representative pair to investigate the effect of antagonists on closely related substances.

In the following experiments the effects of three different antagonists on the action of equiactive concentrations of histamine and N-methylhistamine were

studied. The N-methyl derivative was approximately 2.5 times as active as histamine in terms of molar concentrations. The antagonists used were antergan (N:N-dimethyl-N′-phenyl-N′-benzylethylene diamine hydrochloride; Halpern, 1942), pethidine, and atropine. The results are illustrated in Fig. 2.

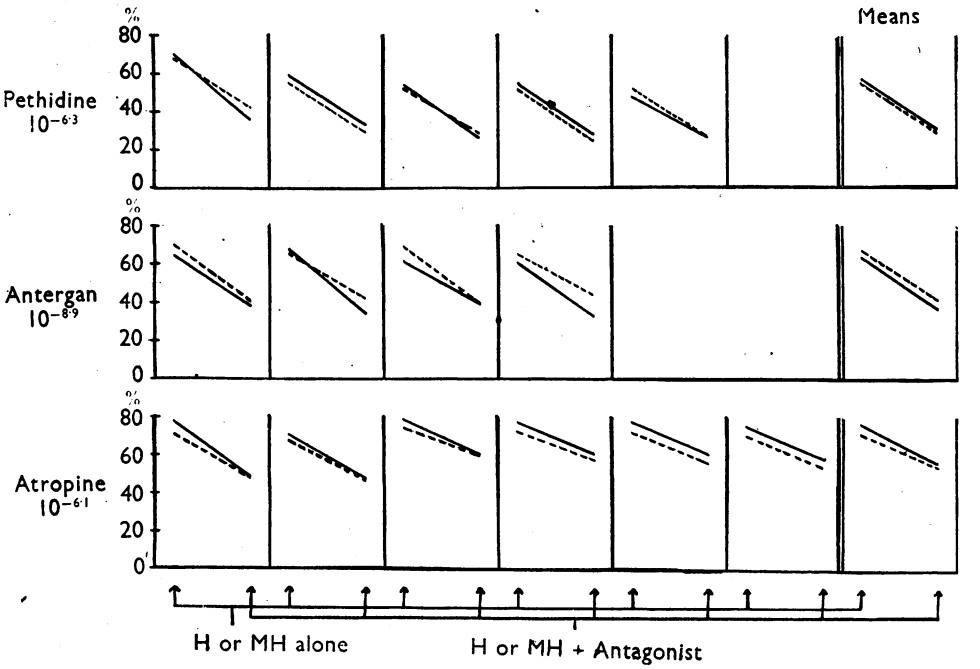



FIG. 2.—Reduction of the effects of histamine (– – – – –) and N-methylhistamine (———) by three different antagonists. The molar concentration of histamine used was approximately 2.5 times that of N-methylhistamine.

Points in the top left-hand corner of each square represent the effects of histamine or N-methylhistamine alone, the lower points the effect of these drugs in the presence of a constant concentration of antagonist. Successive squares in a horizontal row represent effects in successive "randomized groups" and the final square the arithmetical mean of effects, all obtained on the same strip of intestine.

The results were analysed statistically as in the previous example and it could be shown that a given concentration of each antagonist produced, within statistical limits, the same reduction of effect of the two stimulant drugs. It was concluded that none of the three antagonists is capable of discriminating between histamine and N-methylhistamine.

These experiments show that certain drugs react in an analogous manner towards antagonists of very different chemical and pharmacological nature. It is doubtful if any antagonist could be found to discriminate between histamine

and its N-methyl derivative. If the unknown substance discussed in the preceding section had in fact been N-methylhistamine it could not have been distinguished from histamine by means of these antagonists.

## Use of Antagonists for the Classification of Drugs

It might reasonably be assumed that drugs which in a given pharmacological test could not be differentiated by their reaction towards antagonists, or, more precisely, drugs which in the presence of any effective antagonist showed equal reductions of equal effects, would be closely related in their pharmacological action on the particular test object used. Conversely, drugs which could be differentiated by their reactions towards most antagonists except the most unspecific ones might be said to be pharmacologically unrelated.

In thus using the effects of antagonistic drugs as a criterion for classifying active drugs, the following scheme of drug action might perhaps be visualized. It may be assumed that between the first impact of a drug on the tissue and its final effect, muscular contraction in this case, a series of successive processes occur with any of which antagonists can interfere. Two successive stages are indicated in Fig. 3.

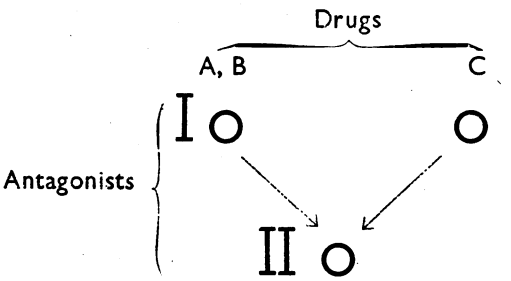



Fig. 3.—Site of action of antagonists. If A and B stand for histamine and N-methylhistamine and C for acetylcholine, it is possible that small concentrations of antagonists may act at one of the primary sites only, whilst larger concentrations may also antagonize a later common reaction.

If two drugs have different primary points of impact their reactions must sooner or later converge into a final common path. An antagonist may interfere with the action of a stimulant drug either before or after a common final path with some other drug is reached. Thus antagonist I reduces only the action of drugs A and B, but not that of C, whilst antagonist II reduces A, B and C. It may further be assumed, at least as a first approximation, that an antagonist acting at a given site would reduce the effect of those drugs with whose pathways it interferes to the same extent. Thus antagonist I would depress A and B equally and antagonist II A and B and possibly C equally.

It follows that if two drugs such as A and B act by the same mechanism, their effects will be reduced to the same extent by antagonists, independently of whether the latter exert their action by competing for a primary site or by

interfering with some other reaction involved in the contractile process. Such drugs might well be classed together into a primary pharmacological class.

Antagonists may of course act at more than one site. A possible explanation for the dual action of drugs such as atropine and neo-antergan (Schild, 1947), which antagonize both histamine and acetylcholine at relatively high concentrations, but only one of the two at low concentrations, would be that at low concentrations the antagonists act at the level of I only and at high concentrations at the level of II as well as of I. Another way of explaining the dual action would be to assume that at high concentrations neo-antergan begins to affect the primary site for acetylcholine and atropine to affect the primary site for histamine. At any rate it is clear that at higher concentrations these antagonists must act on at least two sites.

The scheme is capable of expansion to include the action of further groups of drugs and their antagonists. A scheme of this kind cannot, however, account for special types of antagonism, such as a chemical combination between active drug and antagonist.

## Discussion

One of the earliest attempts at classifying drugs by their reaction to antagonists was the classification of substances contracting plain muscle into musculotropic and neurotropic according to their reaction to atropine. Various objections have been raised from time to time to this conception.

It was pointed out by Magnus (1905) that using the criterion of atropine to localize the site of action of drugs in the intestine implied firstly that atropine had only one site of action in the wall of the intestine and secondly that the effect of atropine could not be reversed by some other drug acting on the same site. Both these assumptions were unlikely to be true. It was indeed shown by Magnus himself that atropine had more than one site of action in the intestine and that it would antagonize at different concentrations drugs belonging to quite different groups such as pilocarpine and barium, and it had been shown by Langley and others that there existed a quantitative antagonism between atropine and pilocarpine. This line of criticism does not necessarily invalidate the use of drug antagonists for localizing the site of action of drugs; rather it points to the necessity of refining these methods by using drug antagonists in a more quantitative way. In practice the setting apart of a group of "muscarinic" drugs (Dale, 1914), exceptionally sensitive to atropine, has been extremely fruitful, and although the localization implied in the term "neurotropic" cannot be maintained any longer, these drugs must still be regarded as forming a group apart, likely to have a common mechanism of action.

From another point of view the old classification has been criticized by Winder *et al.* (1946). These workers came to the conclusion that a subdivision into two groups was inadequate, since in addition to the acetylcholine group, a histamine and a barium group of plain muscle stimulants could be clearly dis-

tinguished by their differential reactions towards antagonists. There is, however, no special reason for confining plain muscle stimulant drugs to three types only and it would seem reasonable to look for further types to be differentiated by their reactions towards antagonists. This is precisely what the present classification proposes to do, since drugs which react quantitatively alike to antagonists are placed together, and drugs which can be differentiated by their quantitative response to antagonists are separated.

The first distinguishing feature of the proposed classification is that it relies upon a quantitative discrimination, the second that it relies upon the response to several antagonists rather than to a single one ; only those drugs are assigned to a primary pharmacological class (in relation to a given tissue) which respond in a quantitatively identical manner to every effective antagonist. This method of classification has the merit of being sharply defined, but it may ultimately prove to have been too rigidly conceived. In a more general way, however, quantitative similarity in behaviour to antagonists is bound to denote some pharmacological relationship between drugs, and the recognition of these relationships may eventually lead to a better understanding of the mechanism of action of drugs.

## Summary

1. A method is described for evaluating statistically whether the effects of two drugs are reduced equally by antagonists.

2. If equal effects are produced on the guinea-pig's ileum by histamine and N-methylhistamine, they are antagonized to the same extent, quantitatively, by effective concentrations of three different antagonists. Similarly the effects of histamine and a histamine-like substance released from striated muscle by curare are equally depressed by an antagonistic drug. It is concluded that antagonists probably cannot be used to discriminate between closely related drugs.

3. A scheme of drug action is proposed which can serve as a basis for a classification of active drugs by means of drug antagonists.

I am indebted to Dr. A. L. Morrison of the Roche Research Department for preparing N : N$^{1}$-dimethyl-N′-phenyl-N′-benzylethylene-diamine, and to Dr. Harold King for a supply of N-methylhistamine.

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