Introduction to Chemical Pharmacology

R. B. BARLOW

METHUEN

Introduction to
CHEMICAL PHARMACOLOGY

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WITH A FOREWORD BY

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Foreword

THE subject of this book – namely, the structure-action relationships of drugs, or, to use an old-fashioned phrase, the relation between chemical constitution and physiological action – is one that has fascinated chemists, particularly organic chemists, for many years. It has been a somewhat disappointing subject of study, and some workers have despaired of finding any convincing relationships.

A. J. Clark, in his classical monograph on *General Pharmacology*, concluded that there were 'scarcely any general rules discernible and that every cell-drug system was a law unto itself'. And yet the chemist has an underlying conviction that there must be some chemical explanation of the way in which drugs modify the functions of living organisms; that the fundamental problem of how drugs act is a chemical problem. Of course the chemist is right; we can only think of the drug itself in chemical terms, and, consequently, theories of drug action must also be expressed in chemical terms. A possible viewpoint (it can scarcely rank as a theory) is that drugs modify the functions of living organisms by interfering in some way with the biochemical reactions which are continuously going on in living cells. The difficulty is that the chemist knows so little about the intimate chemical nature and properties of the physiological structures upon which drugs exert their disturbing effects. No doubt the biochemist will ultimately be able to tell him about these things, but meanwhile the chemist ought to pay more attention than he has usually done to what the pharmacologist has to say. One of the most important functions of the pharmacologist is to disentangle the physiological mechanisms by which a given drug action is achieved. Superficially similar physiological effects can be produced by a variety of mechanisms; the chemist is frequently apt to forget this; he is often unaware of the fact that changes in structure, sometimes quite small changes, may alter not only the intensity of drug action but also the mechanism by which some particular physiological result is achieved. The whole problem of the structure-action relationships of drugs involves two separate inquiries: how changes in structure affect the mechanism of action and how they influence the intensity of action, when the mechanism is unchanged. The chemist is apt to think only about how changes in structure affect the intensity of some physiological response. No wonder he is frequently puzzled by the astonishing changes in intensity which small alterations of structure produce. Even if the pharmacologist can provide evidence that a group of related drugs are all acting in the same way, the problem of relating intensity of action to structure is difficult enough, but if changes of structure alter the mechanism of action, the chemist may easily be led astray.

Dr Barlow's books is an attempt – almost the first attempt – to instruct chemists about these matters. Dr Barlow is not only an organic chemist, he

has practical experience of testing the pharmacological activity of compounds which he has made; he can, therefore, speak with authority to chemists about these matters, and it is my hope that his book will encourage chemists, particularly young chemists, to enter this curious and fascinating borderland between chemistry and biology, and to learn more than chemists have usually been willing to learn about the biological side of the subject.

What is obviously needed is a much closer liaison between the chemist and the pharmacologist; but if this liaison is to be achieved, the chemist must try to understand the nature of the problems which face the pharmacologist, and the pharmacologist must try to understand how the chemist thinks about the compounds that he makes. There have been in the history of pharmacology some famous combinations of chemist and pharmacologist – e.g., Crum Brown and Fraser, Reid Hunt and Renshaw, Barger and Dale, &c. These names alone are key names in the development of pharmacology. Pharmacology, which has remained for so many years a rather inferior relation of physiology, can only achieve its proper status as an independent branch of medical science, if it is willing to invite the co-operation of the chemist; but the chemist, fascinated as he usually is by synthetical problems, must, if he is interested in the structure-action relationship of drugs, be prepared to learn something about pharmacology, about the curious ways in which drugs modify the functions of living organisms. The whole history of the study of the structure-action relationships of drugs is befogged by the circumstance that the pharmacologist did not understand what the chemist had in his mind and the chemist did not understand what the pharmacologist was doing. In my opinion the chemist ought to watch the pharmacologist at work on the testing of new compounds; only so can he appreciate the fascination of pharmacology, and understand the curious paradox that although the pharmacologist can detect, and even estimate, amounts of chemical substances well below those which can be detected or estimated by chemical means, he can only get approximate measures of activity, because of the individual variation of living organisms to drugs.

My own belief is that the chemist and the pharmacologist, if they are prepared to understand each other's point of view, can jointly, but not independently, make important advances in a subject which is vital to therapeutics. How drugs act is, as I have said, a chemical problem, but the chemist alone cannot solve it; he can solve it only if he is prepared to work hand-in-glove with the pharmacologist, so that Dr Barlow's book, which attempts to instruct chemists on these difficult problems of drug action, receives my warm recommendation to the reader.

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Author's Note to the Second Edition

THIS book has been almost completely re-written. The decision to omit any detailed consideration of the actions of drugs on the central nervous system was taken in order to limit the size of the book and the time which its revision was demanding. It was also felt that, with a few exceptions, there was not really enough known about the mode of action of drugs on the central nervous system to justify their inclusion in an introduction to chemical pharmacology.

The Appendix, however, has been retained because it is thought that this is of real value to chemists who cannot be expected at this stage to look up more detailed works. It is for the biologist to explain the subject in simple terms to the chemist so that the chemist may see where his chemical experience may be relevant. Although the chemist should be aware of his ignorance, it should not be necessary for him to undergo a full formal training in biology before his comments are attended to, otherwise there is the serious danger that a subject such as chemical pharmacology will be explained in terms of the chemistry of yesterday rather than that of today. I appreciate that this criticism may be levelled at sections of this book and would be glad to be informed of errors or criticisms of any sort.

Names of Drugs. Compounds are referred to by their chemical names when these are simple; failing this, by their pharmacopoeial names (indicated by an initial capital letter) and failing this by their approved names or a trade name (indicated by italics).

Optical Isomers. The signs (+) and (−) refer to optical rotation. Where it is possible to assign an absolute configuration I have used the signs R- and S-, following the convention of Cahn, Ingold, and Prelog (*Experientia*, 1956, **12,** 81). This avoids many ambiguities; (−)-adrenaline, for instance, has the L-configuration with reference to glyceraldehyde but the D- configuration with reference to serine whereas the description R- is unambiguous. Even the substance, (+)-tartaric acid, from which all absolute configurations are derived following the work of Bijvoet, Peerdeman, and Van Bommel (*Nature*, 1951, **168,** 271), is similarly ambiguous being L- with respect to glyceraldehyde and D- with respect to serine.

Equipotent Molar Ratios. In order to express the activity of one drug relative to that of another I have used equipotent molar ratios, that is the number of molecules of one drug producing the same effect as one molecule of a standard drug. If the compound is more active than the standard, the ratio will be less than one. This may be confusing to people who feel that high activity should be indicated by a high figure but is more in accordance with what is actually done experimentally; less material is used if the compound is more active.

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Dr C. I. Bliss and the Editors of the *Journal of Pharmacy and Pharmacology* (Table II.1); Professors A. Wilson and H. O. Schild, and Messrs J. and A. Churchill (Table Appendix II.1, taken from '*Clark's Applied Pharmacology*'); Professor F. R. Winton and Dr L. E. Bayliss, and Messrs J. and A. Churchill (Table Appendix II.2, taken from *Human Physiology*); R. P. Stephenson and the Editors of the British Journal of Pharmacology and Chemotherapy (Fig. I.1 and I.2); the late Dr J. W. Trevan, the Editors of the *British Journal of Experimental Pathology and Bacteriology*, and Messrs H. K. Lewis (Fig. III.3); Professor S. Thesleff and the Editors of Acta Anaesthesia Scandinavica (Fig. V.4, V.5, and V.10); Professor W. L. M. Perry and the Editors of the *Journal of Physiology* (Fig. VI.1); Dr N. Ambache and the Editors of the *British Journal of Pharmacology and Chemotherapy* (Fig. VI.8); Dr M. E. Holman and the Editors of the *Journal of Physiology* (Fig. VII.1); Professor K. B. Augustinsson, the Editors of the *Archives of Biochemistry*, and the Academic Press Inc. (Fig. VIII.2 and VIII.6); Professor R. F. Furchgott, the Editors of *Pharmacological Reviews*, and the Williams and Wilkins Co. (Figs. IX.10 and IX.11); Professor M. Nickerson, the Editors of *Nature*, and Messrs Macmillan and Co. (Fig. X.6).

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Approaches to Pharmacology

'Pharmacology may be defined as the study of the manner in which the functions of living organisms can be modified by chemical substances' (A. J. Clark, 1937).

This study can be approached in different ways, depending upon the type and complexity of the tissue selected. The effects of drugs* in man are those most likely to be of practical use. Although experiments in man are almost invariably preceded by experiments in simpler structures, such as small animals, the understanding of the effects of chemical substances on such complex structures as these depends upon a knowledge of the normal working of these structures. At this level pharmacology is an extension of physiology.

The study of the effects of chemical substances on organisms which are parasites in man may also lead to discoveries of practical value in the treatment of diseases caused by such parasites. Here pharmacology may be regarded as an extension of parasitology, particularly of bacteriology.

At a more fundamental level, the effects of drugs on any tissue depend upon their actions on the cells of which that tissue is composed. This demands a knowledge of the chemical processes occurring in the cells, and of the ways in which these processes are related to those occurring in other cells. Here, then, pharmacology is an extension of biochemistry.

Finally, all the effects of drugs are the consequence of the interactions of molecules of drug with molecules of the tissues, however complex, being studied. At this level, therefore, pharmacology is an extension of chemistry in all its aspects, organic, inorganic, and physical.

Pharmacology, therefore, draws upon physiology, bacteriology, biochemistry, and chemistry for essential basic information and most pharmacological laboratories contain workers from all these subjects. The medical or therapeutic approach to pharmacology, however, is usually the dominant one because of its practical value and the need to train medical students.

* In this book the word 'drug' is used to describe any molecular species which may be of interest to the investigator; it does not refer only to substances which are known to be active.

Several books have been written on this aspect of pharmacology: this book is an attempt to approach pharmacology at its most fundamental level, to discuss it as an extension of chemistry.

Justification for a Chemical Approach to Pharmacology

The biggest difficulties in the way of regarding pharmacology as an extension of chemistry are the complexity of the processes occurring in living tissues and the impossibility of obtaining a precision in biological experiments comparable with that attainable in chemical experiments. A great deal can be done by studying the actions of drugs on small pieces of tissue, or even on single cells, rather than on whole animals, and by a statistical treatment of the results. Nevertheless, even simplified biological systems are far more complex than those met with in most types of physicochemical problem. In the latter at least the identity of the molecular species involved is usually known, but in biological problems this is seldom true.

Furthermore, interest in pharmacology, particularly in the extensive research work carried out in industry, lies chiefly in the production of compounds of potential therapeutic value. Ideally such drugs could be discovered by a systematic study of the relationships between chemical structure and actions on simple isolated tissues, or even single cells. A knowledge of such relationships between structure and action at all the important sites in the body should enable one to predict accurately the effect of a drug on the body as a whole. In point of fact, with the present limited knowledge of biochemistry and pharmacology, it is not really possible to attempt this with any likelihood of success. Most useful drugs have been discovered empirically as the result of 'screening' a large number of compounds in animals to see if they are useful and finding out why afterwards.

A chemical approach to pharmacology can, therefore, be criticized on the grounds that it is based on over-simplifications and is anyway unlikely to lead to results of any immediate practical value. There is, however, much information about the apparent pharmacological properties of a lot of compounds. In the circumstances it is natural to attempt to correlate chemical structure with biological activity even though such correlations can only be of any value if the compounds all act by the same mechanism. There is, moreover, a certain amount of fundamental information about the mode of action of some drugs.

A chemical approach to pharmacology can therefore be defended on the grounds that it is a rational approach to the subject which will not always be based on over-simplifications, even if this is often so at present. Further it may provide a logical attack on the problems of discovering new drugs. Although it is true that many useful drugs have, in the past, been discovered empirically, that empirical approach has not usually been entirely haphazard, but has had some rational plan, even though the theories on which it has been based have often been shown to be quite wrong.

Validity of Physical and Chemical Laws in Biological Systems

Before a chemical approach to pharmacology can be attempted it is necessary to show that the fundamental laws of physics and chemistry apply to atoms and molecules in systems composed of living matter.

Clark (1933, 1937) has discussed one of the most striking features of pharmacology, the smallness of the amounts of a really active drug which may affect a really sensitive tissue. Hunt (1918) recorded that detectable changes in the blood-pressure of a cat were produced by as little as 0·000,002 μg of acetylcholine per kg body weight. This corresponds to 10^{-14} of a Mole, but is still equivalent to a very large number of molecules, 6×10^9, and these can be distributed over a very considerable area before the numbers become so small that the second law of thermodynamics is endangered. The figures obtained by Hunt are exceptional. Clark and White (1927) found the minimum effective dose to be 0·004 μg/kg in a similar experiment, and this order of magnitude seems more likely to be trustworthy.

Bacterial toxins, however, are much more active and the results here cast some doubt about the validity of adopting a chemical approach to their action. Botulinus Toxin type A has a molecular weight of 900,000 and the 'minimum lethal dose' for a mouse is $3{\cdot}2 \times 10^{-11}$ g (Van Heyningen, 1950). This is $3{\cdot}6 \times 10^{-17}$ Mole which should contain 2×10^7 molecules. It has been calculated that 250 g of this material, if suitably administered, would suffice to kill the entire population of the world. Botulinus Toxin type D is even more toxic. Wentzel, Sterne, and Polson (1950) have obtained a purified material, of molecular weight about 1,000,000, which, when tested in mice, was found to contain 4×10^{12} 'minimum lethal doses' per mg of protein nitrogen. For a nitrogen content of 15 per cent this gives a 'minimum lethal dose' of approximately

$$\frac{100}{15} \times \frac{1}{4 \times 10^{12}} \text{ mg}$$

$$= \frac{100}{15} \times \frac{1}{4 \times 10^{12}} \times \frac{1}{10^9} \text{ Mole}$$

$$= \frac{100}{15} \times \frac{1}{4 \times 10^{12}} \times \frac{6 \times 10^{23}}{10^9} = 1{,}000 \text{ molecules}$$

The claims of homeopaths are of a different order altogether. Clark (1933) cites results of König (1927) reported to be obtained with concentrations of silver nitrate and lead nitrate as dilute as 1 in 10^{60}, and points out that this corresponds to about one molecule in a sphere with a circumference about equal to the orbit of the planet Venus. Faith in such results clearly entails abandoning the laws of physics and chemistry, but otherwise, with possible reservations about bacterial toxins, it seems resonable to assume that these are valid in the conditions prevailing in biological systems.

The Mode of Action of Drugs on Cells

Although effective doses of even the most active substances contain large numbers of molecules, their action may be highly selective and confined to a very small area. Clark (1933) estimated the percentage of the area of the cells of certain tissues which could be covered with drug molecules. On the frog heart, for instance, a dose of 0·02 μg acetylcholine/g tissue caused a 50 per cent reduction in the rate of beating. This corresponds to 10^{14} molecules (approximately). The dimensions of frog ventricle cells have been given as 131 × 9 microns and the surface area as 1,900 square microns ($= 1{,}900 \times 10^{-8}$ cm^2) and volume 2,600 cubic microns ($= 2{,}600 \times 10^{-12}$ cm^3). The number of cells per cm^3 or per g tissue is therefore $10^{12} \times 1/2{,}600 = 3{\cdot}3 \times 10^8$, and the total surface area approximately $3{\cdot}3 \times 10^8 \times 1{,}900 \times 10^{-8} = 6{,}000$ cm^2. This means that the number of molecules which are available for each cell is $10^{14}/3{\cdot}3 \times 10^8 = 3 \times 10^5$. Clark assumed that each molecule could cover an area of 100 Å^2, and so the total area covered by 3×10^5 molecules would be $3 \times 10^5 \times 100 \times 10^{-8} = 0{\cdot}3$ square microns, and this is only 0·3/1,900 of the area of the surface of a cell.

Similar calculations showed that the drugs ouabain, acetylcholine, atropine, adrenaline, and histamine cannot possibly cover more than a fraction of the area of the cells of the tissues concerned (frog heart or rat uterus), whereas other compounds, such as caffeine and normal aliphatic alcohols (in the range heptyl to dodecyl), only produced effects when given in amounts which would suffice to form a monomolecular layer over the whole area of the cells.

Similar calculations reveal the extremely selective uptake of bacterial toxins. Botulinus Toxin is known to act on nerve-cells and the mouse contains approximately $2{\cdot}5 \times 10^6$ such cells: this indicates an average of 8 molecules of Botulinus Toxin Type A per cell and 1 molecule of Type D Toxin per 2,500 cells. It would seem necessary to investigate further the site of action of these substances. If only certain particular nerve-cells need be affected to produce death, it is still possible that the numbers of molecules involved are sufficient to discuss their action in normal chemical terms.

Although calculations of this sort can only be approximate, they indicate the likelihood of two types of drug action, one involving a large part, if not all, of the cell surface and the other involving only a very small part indeed. The picture of a drug producing its effects by an action over a large part of the cell surface is consistent with the idea that it is acting by some physical or physicochemical process. It may be having an effect on interfacial tension at the cell surface, dissolving preferentially in certain parts of the cell, having an action on cell colloids or affecting the membrane which surrounds the cell and which selectively gives passage to various ions. Such a hypothesis was put forward at the end of the nineteenth century by Overton and Meyer to account for their results in research on anaesthetics.

The picture of a drug producing its effects by an action only at a very small part of the cell surface is consistent with the idea that there are active spots, called receptors, on the cell surface and that the drug acts by forming a

complex with these. This idea was suggested by Langley (1878, 1905), but is generally associated with the work of Ehrlich who used it as a basis for his research in chemotherapy (see, for instance, Albert, 1960).

Investigation of the Mode of Action of a Drug

It should, theoretically, be possible to decide whether a drug is acting by a physicochemical mechanism or at receptors by making calculations similar to those of Clark. The necessary information about cell structure, however, is not always available and a decision about the type of action of a drug is frequently made instead by studying the activity of compounds chemically related to it.

If there is no very obvious relationship between chemical structure and activity it is reasonable to suppose that the drugs are acting by a physicochemical process. In these circumstances chemical structure will only be important in so far as it affects physicochemical properties.

In the study of most drugs it is usual to find that there is a marked variation of activity with chemical structure. In these circumstances it is reasonable to suppose that the drugs are acting on receptors because these receptors will themselves have a definite chemical structure in two or three dimensions and the action of the drug must depend upon its ability to fit.

Physicochemical properties, however, may be extremely important in determining the rate of transport of a drug to its site of action on, or in, the cell and it is not always possible to distinguish clearly between drugs which are acting by a physicochemical mechanism and those which rely upon their physicochemical properties for transport but may act ultimately at receptors.

The Drug-Receptor Complex and the Kinetics of Pharmacological Reactions

The combination of a drug with a receptor can be compared with the adsorption of a molecule at a catalyst surface. An expression similar to the Langmuir adsorption isotherm relates the concentration of the drug, (A), with the proportion, y, of receptors occupied in the reaction:

$$\text{Drug} + \text{Receptor} \rightleftharpoons \text{Complex} \rightarrow \text{Breakdown} \rightarrow \text{Response}$$

The rate of formation of the complex will be $k_1(A)(1 - y)$, and the rate of dissociation will be $k_2(y)$, where k_1 and k_2 are constants.

At equilibrium, $k_1(A)(1 - y) = k_2(y)$ and hence, $K(A) = \frac{y}{1 - y}$, where K, the affinity constant $= \frac{k_1}{k_2}$. When 50 per cent of the receptors are occupied, $(A) = \frac{1}{K}$.

Note that K is an affinity constant; some workers (e.g. Ariëns, 1954) use the dissociation constant, which is the reciprocal of this and can be compared with the Michaelis–Menten constant used by enzymologists (see page 20).

If the biological response is directly related to the proportion of receptors occupied, the graph of dose against response will be a hyperbola. In view of experimental errors, however, the mathematical relationship between dose

and response cannot, with confidence, be deduced from the shape of the curve. Clark (1933) showed that the differences between the hyperbola,

$$KA = \frac{y}{1-y}, \ (K = 1),$$

the exponential curve, $Ky = \log (bA + 1)$, $(K = 0{\cdot}0166, b = 5{\cdot}3)$,

and the parabola, $KA^n = y$, $(K = 49, n = 0{\cdot}5)$, were much smaller than the errors in the most accurate biological experiments which had been performed at that time. The second formula represents the empirical Weber–Fechner 'Law' and the third an adsorption isotherm of the Freundlich type.

Although the formation of the drug-receptor complex is generally regarded as being governed by the Langmuir Adsorption Isotherm, the pharmacologist usually plots the logarithm of the dose against the effect and finds that this graph is linear over a considerable range. This process is much more convenient than plotting the dose against

$$\frac{\text{Percentage effect}}{100 - \text{Percentage effect}},$$

though the logarithm of this is the 'logit' function, which is available in tables. The graph of the logarithm of the dose against the logit of the response might, therefore, be expected to be linear, but the expression

$$\frac{\text{Percentage effect}}{100 - \text{Percentage effect}}$$

will only be the same as $\frac{y}{1-y}$ if the biological response is directly proportional to the proportion of receptors occupied. This was assumed by Clark (1937), although he himself says the assumption seems improbable, and such attempts as have been made to test the validity of the assumption (see below) indicate that it is not justified. If it were true, the concentration of a drug which produced half the maximal response would be equal to the reciprocal of the affinity constant; this value has, in fact, sometimes been used as an index of the affinity of the drug for the receptors.

Efficacy

In experiments with acetylcholine and tetramethylammonium, Clark and Raventos (1937) showed that although acetylcholine was about 1,000 times as active as tetramethylammonium, the effects of equiactive doses of the drugs were antagonized to the same extent by an antagonist (Table I.1). From these results there emerged the idea of the all-or-none nature of the drug-receptor complex, i.e. that the complex between a drug and a receptor was either completely effective or completely ineffective. If it were effective, the compound would be an agonist; if it were ineffective, the compound would be an antagonist. In either situation the activity of the drug (as an agonist or as an antagonist) would depend only on its adsorbability or affinity constant, *K*.

This idea was supported by the observation that the effects of acetylcholine and tetramethylammonium were additive. If the effects of x acetylcholine were the same as y tetramethylammonium (y would be about 1,000 times x), then these were also produced by $x/2$ acetylcholine plus $y/2$ tetramethylammonium together. If the complex produced by one drug were less effective than the complex produced by the other, it would occupy receptors which could more profitably be occupied by the other drug, so the effects would not be additive.

Although these conclusions are perfectly valid for acetylcholine and tetramethylammonium on the tissues listed in Table I.1, they cannot be

TABLE I.1

Antagonism of the Actions of Acetylcholine and tetramethylammonium

	Preparation					
	Rat intestine		Frog auricle		Frog rectus	
Antagonist \ Agonist	Ach	$Me_4\overset{+}{N}$	Ach	$Me_4\overset{+}{N}$	Ach	$Me_4\overset{+}{N}$
Atropine . . .	−8·1	−7·9	−8·3	−7·7	−4·2	−3·8
n-Oct$\overset{+}{N}Me_3$ . .	−5·5	−5·0	−5·0	−4·6	−4·1	−4·0
n-$Bu_4\overset{+}{N}$ . . .	−3·6	−3·7	—	—	−3·2	−3·0
'Curarine' . . .	—	—	−5·1	−4·5	−6·8	−6·5

Figures indicate the logarithm of the molecular concentration of antagonist which necessitated multiplying the concentration of agonist by 10 in order to keep the biological effect constant; these are, in effect, values of $-pA_{10}$ (page 43). The value for an antagonist on a particular tissue is considered to be independent of the nature of the agonist; compare, for example, the values for atropine and acetylcholine (Ach) with those for atropine and tetramethylammonium, −8·1 and −7·9 on rat intestine, −8·3 and −7·7 on the frog auricle, and −4·2 and −3·8 on the frog rectus.

Clark and Raventos (1937).

extended universally. From further experiments, Ariëns (1954) and Stephenson (1956) have suggested that the activity of a drug depends not only on its affinity (adsorbability) but on another property termed 'intrinsic activity' (Ariëns) or 'efficacy' (Stephenson), this factor being a measure of the effectiveness of the drug-receptor complex.

Ariëns (1954) considers the situation in which the response depends directly upon the intrinsic activity, α, and the proportion of receptors occupied, y. In the experiments of Clark and Raventos α for the antagonists is zero, whereas α for tetramethylammonium is the same as that for acetylcholine, which is taken as unity. There are, however, substances with an intermediate intrinsic activity, which are called dualists (Ariëns) or partial agonists (Stephenson). Their low intrinsic activity is revealed by their ability to antagonize compounds of higher intrinsic activity. They fail to act additively,

presumably because they occupy receptors which could be more profitably occupied by the drug with the higher intrinsic activity. Furthermore, such drugs, however high the concentration tested, do not produce the maximal response of which the tissue is capable, for if the response varies with αy, and the maximal response, R_{Max}, is obtained when $y = 1$ and $\alpha = 1$, the response R to a dualist (intrinsic activity α) obtained with any concentration sufficient to saturate the receptors ($y = 1$) will be $R/R_{Max} = \alpha$. Ariëns therefore estimates intrinsic activity by determining this fraction experimentally.

In his papers (e.g. Ariëns, 1954; Ariëns and De Groot, 1954; Ariëns and van Rossum, 1957) will also be found estimates of the affinity constant, K, based on the determination of the concentration which causes half the maximal response (in the case of a dualist, half the maximal response of which the drug is capable). The logarithm of the reciprocal of this concentration is called pD_2, following a convention originally proposed for antagonists by Miller, Becker, and Tainter (1958; see page 193, D stands for dilution).

This treatment involved two assumptions, that the response is directly proportional to the fraction of the total receptor population occupied, and that no agonist can have an intrinsic activity greater than unity. Stephenson avoids making these assumptions by introducing another term, the biological stimulus, S, where $S = ey$, e being the efficacy, which can have any positive value from zero upwards, and y being the proportion of receptors occupied. No assumption is made about the relationship between S and the biological response, though it is supposed that equal values of S will give equal biological responses: as an arbitrary standard, a 50 per cent response is regarded as being produced when $S = 1$. Stephenson further suggests that it may be incorrect to suppose that a maximal response is only produced when all the receptors are occupied. He postulates that there may be 'spare' receptors and that, in consequence, a drug of high efficacy may give a maximal response when only a small proportion of the total receptor population is occupied.

Following from the convention, $S = 1$ for a 50 per cent maximal response, a partial agonist which can only produce a 50 per cent maximal response, even when all the receptors are occupied, has an efficacy of 1, for $S = ey$ (and $y = 1$).

From results with butyltrimethylammonium (on the guinea-pig ileum) Stephenson was able to obtain experimentally some idea of how the response was related to the biological stimulus for this particular drug and tissue. If the proportion of receptors occupied by butyltrimethylammonium is only a small fraction of the total, this proportion, y, will vary directly with the concentration of the drug, for,

$$K(A) = \frac{y}{1-y} \quad \text{and} \quad 1 - y \rightarrow 1$$

and $S = ey$, so S will vary directly with (A). The relationship between response and stimulus can then be obtained by plotting response against dose, where the dose interval between no response and 50 per cent response is taken as

1 unit (Fig. I.1). This curve is not linear. From it and from the equations $S = ey = \frac{eK(A)}{1 + K(A)}$ Stephenson was able to construct a series of graphs showing the variation of percentage response with concentration of drug for different values of e but with the same value of K. These (Fig. I.2) are quite different from a similar family of curves calculated on the assumptions of Ariëns (Fig. I.3), one of the chief differences being that in the latter, once the drug is capable of producing a maximal response, activity can only

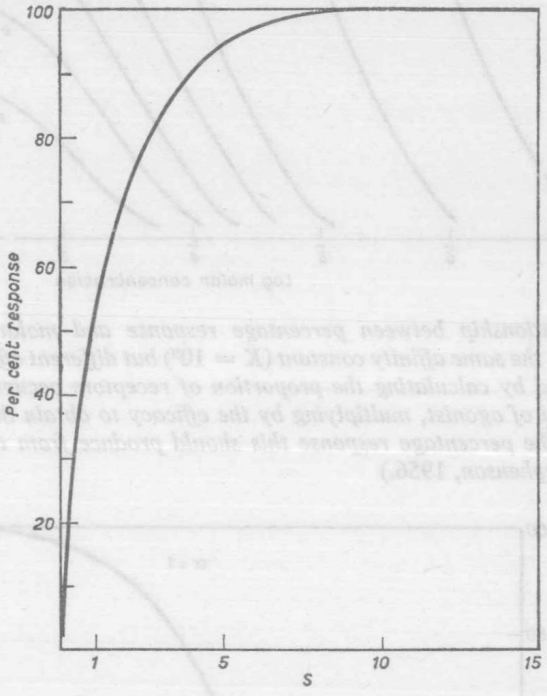

FIG. I.1. *Relationship between stimulus, S, and percentage response for* n*-butyltrimethylammonium on the guinea-pig ileum. This is obtained by plotting the percentage response against the dose when the latter is expressed as a multiple of the difference between the amount producing no response and the amount producing half the maximal response.* (*Stephenson*, 1956.)

increase if affinity increases: in the former it can increase because efficacy rises without an increase in affinity.

If the postulate of the existence of spare receptors is correct (and this seems likely to be so) the affinity constant K cannot be obtained by measuring the concentration of agonist which produces 50 per cent of the maximal response. It can, however, be arrived at in certain circumstances. For an active agonist the expression

$$S = \frac{eK(A)}{1 + K(A)}$$

reduces to $S = eKA$, because KA will be small (page 8). Now let an experiment be performed in which the response (I) to a concentration (P) of a