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PHARMACOLOGICAL RECEPTORS

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TABLE OF CONTENTS

I. Introduction ... 50
II. Access of drug to receptor ... 52
III. The reaction of the drug with the receptor ... 56
IV. The relation between the drug-receptor reaction and the production of an effect. 60
V. The potency of competitive antagonists ... 63
VI. Estimation of the dissociation equilibrium constant of an agonist ... 66
VII. Measurement of the potency of an irreversible competitive blocking agent ... 67
VIII. The shape of the dose-response curve ... 68
IX. Classification of receptors ... 69
X. The absolute concentration of receptors ... 70
XI. Paton's kinetic theory of drug action ... 71
XII. Desensitization, spare receptors, and spare receptor capacity ... 78
XIII. A note on methods ... 81
XIV. The chemical nature of the receptor ... 83
XV. Miscellaneous receptor models ... 84
XVI. Future avenues of research ... 85

List of symbols

(Numbers at right indicate section where symbol is introduced.)

Symbol	Meaning	Section
a, b	Arbitrary constants	VII
A	Rate of drug-receptor combination ($= k_1[D](1 - y)$)	XI
[A], [B]	Concentrations respectively of drugs A and B	II
[AR], [BR]	Concentrations of complexes between drugs A and B respectively and the receptor	II
[A]′, [B]′; [AR]′, [BR]′	Same as for [A], [B], [AR] and [BR], but in a situation where another drug is present	II
α	Ariëns' intrinsic activity	IV
c	Concentration of a drug in units of $K_e = k_2/k_1$ ($c = d$ of Paton and Waud (76); use of concentration c rather than dose d is more appropriate and avoids the expression dd/dt.)	III
[D]	Concentration of a drug	II
$[D]_b$	Concentration of a drug in the biophase	II
$[D]_o$	Concentration of drug outside the tissue (bath concentration)	II
d	Differential coefficient	II
D	Diffusion coefficient	II
"D"	Apparent diffusion coefficient	II
$[D]_a$	Arterial concentration of a drug	II

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$[D]_v$	Venous concentration of a drug	II
$[DR]$	Concentration of drug-receptor complex	III
$[DR]_0$	Initial concentration of drug-receptor complex	III
$[DR]_e$	Concentration of drug-receptor complex at equilibrium	III
e	1. Base natural logarithms	II
	2. A subscript to indicate equilibrium	III
	3. Stephenson's efficacy	IV
E	Effect observed (response)	IV
ϵ	Furchgott's intrinsic efficacy	IV
f, F, g, G	Used in sense "function of"	IV
i	Label	II
I	Label for inhibitor	VI
k_1	Association rate constant of drug-receptor reaction	III
k_2	Dissociation rate constant of drug-receptor reaction	III
k_a	Rate constant for flow of drug into biophase	II
k_b	Rate constant for flow of drug out of biophase into bath	II
k_i	Rate constants for other sites of loss from biophase	II
K_e	General dissociation constant ($= k_2/k_1$) for drug-receptor reaction	III
K_A, K_B	Dissociation constants of the complexes between drugs A and B respectively and the receptor	III
K_{Na}	Dissociation constant of sodium-receptor complex	V
K', K''	Two constants	II
k_B^A	Selectivity constant (for ion exchange equilibrium)	V
log	Logarithm to base 10	V
pA_x	$-\log_{10}$ (Molar concentration of antagonist that gives dose ratio of x)	V
ϕ	Paton's function relating response to receptor activation	XI
$\dot{Q}$	Volume flow (blood here)	II
$[R]$	Concentration of free receptors ($[R]_t - [DR]$)	III
$[R]_t$	Total concentration of receptors	III
S	Stephenson's stimulus	IV
t	Time	II
V	Volume of distribution	II
x	Space coordinate	II
y	($= [DR]/[R]_t$) fractional receptor occupancy by a drug	III
y_I	Fractional receptor occupancy by an inhibitor	VI

(Upper case C and R were not used for concentration and response respectively, so they could be reserved for resistance and capacitance in electrical analogs.)

I. INTRODUCTION

Many drugs exhibit the following four characteristics: (i) they act at low (micromolar) concentrations; (ii) their activity is easily influenced by changes in chemical structure; (iii) they can be antagonized selectively (for example atro-

pine can markedly block the action of acetylcholine on the guinea pig ileum, but leave the activity of histamine practically unaffected); (iv) the activity of the antagonists is also easily influenced by changes in chemical structure.

All these features suggest that a specific chemical reaction takes place between the drug and some specialized site (receptor) in the tissue. This is in contrast to the case of drugs like the general anesthetic agents, which act at relatively high concentrations and which would appear to interact with tissues more by a physicochemical than by a specific chemical reaction (see 78).

The pharmacological investigation of many drugs acting on receptors is straightforward. For example, when the receptor is the active site of an enzyme, the mechanism of action can be investigated by conventional enzymological techniques. In fact it is questionable whether anything is gained by still referring to such an active site as a receptor. Many people do [for example, Ariëns (2)], but in the present review the term receptor will be applied to the site of action of a much more restricted group of drugs. Acetylcholine acting on the smooth muscle of guinea pig ileum is an example of the sort of system to be discussed. The properties of sensitivity and selectivity [(i)–(iv)] discussed above are seen, but also one sees (v) amplification, that is, the administration of a few nanomoles of a drug such as acetylcholine to the tissue can lead to a contraction capable of exerting a force of grams. Furthermore, (vi) the site of action seems to be the cell membrane (see 33) and the mechanism of action seems to involve changes in the permeability of the cell membrane. Finally, (vii) the action has not yet been demonstrated in a system in which the cells are not intact. An adequate description of the mechanism of action of such drugs may thus be expected to be complicated and involve any or all of the properties of the membrane, the mechanisms of excitation-contraction coupling and contraction, the structural arrangement of the cell, and, finally, the interactions among cells.

There is now a considerable body of knowledge about the mechanism of action of this type of drug and it is this particular aspect of the action of drugs on receptors that will be reviewed here.

Many reviews are already available. That by Furchgott (39) is the best starting point. A symposium in this journal (46) as well as Clark's original monograph (27) are both useful. A collection of papers in Advances in Drug Research (3, 17, 30, 40, 61, 75, 84, 103) gives a representative sampling of current activities in the field. Other reviews are also available (2, 13, 48, 60, 63, 90, 95, 101). The second last of these is nominally on structure-activity relationships (which will not be discussed here) but contains a good deal of more general information.

In the present review I shall try to present first a coherent picture of what is known about the action of the drugs involved and then use this picture in the discussion of the development of current thinking.

This review will be restricted to the actions and interactions of drugs at the same receptor. Chemical or physiological interactions (27, chap. 11) will not be discussed. Ariëns *et al.* (2, 6) may be consulted for discussions of interactions at more than one receptor.

As a starting point we may consider a system in which a dose of a drug such

as acetylcholine is administered and a response such as a contraction of a smooth muscle is recorded. Graded doses of acetylcholine give graded responses and, if the appropriate experimental arrangement is used, the same dose gives the same response. We can characterize the system empirically by a dose-response curve, *i.e.*, by saying there is a functional relationship between response obtained and dose administered. Furthermore, this dose-response relationship is time-dependent. A steady state is not reached instantaneously. We want to characterize this relationship of dose, response and time. For purposes of description the sequence of events may be broken into three stages: (i) access of the drug to the receptor; (ii) reaction with the receptor; (iii) production of a response as a result of the reaction with the receptor.

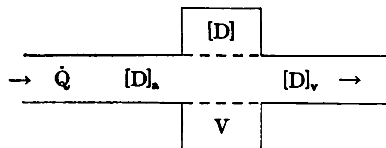
Explicit models will be used extensively to make arguments objective. In important cases assumptions involved will be stated explicitly, and it is hoped that those that are not will be fairly obvious. For example, use of the law of mass action is implicit in the writing of equation 7 (section III). Finally one should note that usual experimental systems in which receptor models are applied are so complex and varied that all assumptions, explicit and implied, must be considered in each new application.

II. ACCESS OF DRUG TO RECEPTOR

Ideally, one would like to have the concentration of a drug rise instantaneously at the receptor after administration and fall instantaneously to zero on washout. In practice, the rise of concentration at the receptor will always lag behind that at the site of administration. Many of the factors involved in the lag in an intact animal are well known and need not be reviewed here. Rescigno and Segre (82) gave examples of the analysis involved.

It will become apparent that an adequate analysis of the mechanism of action of the drugs to be discussed is difficult enough in an isolated preparation. An analysis in a whole animal is at present not a practical experimental task. For this reason most of the work to be described will have been done on isolated organ preparations. For this same reason I shall discuss access limitations only in the two situations relevant to the administration of drugs to isolated organ preparations. These two cases may be called perfusion limitation and diffusion limitation.

Perfusion limitation. Here one might be dealing with administration of a drug to some part of a tissue perfused through its blood supply. If one assumes that the rate of equilibration between the capillary and the extracellular space is considerably greater than the rate at which the drug is brought to the tissue, and that the drug is distributed only through the extracellular space (V), one can use the model:



By inspection:

$$\frac{d[D]}{dt} = \frac{\dot{Q}[D]_a - \dot{Q}[D]_v}{V} = \frac{\dot{Q}}{V}([D]_a - [D]_v) = \frac{\dot{Q}}{V}([D]_a - [D]) \qquad (1)$$

where the final form comes from the assumption of instantaneous equilibration across the capillary membrane.

If the arterial concentration rises instantaneously to $(D)_a$ at $t = 0$, (1) may be solved to give:

$$[D] = [D]_a\left(1 - e^{-\frac{\dot{Q}}{V}t}\right) \qquad (2)$$

Thus access is a simple exponential process whose rate is proportional to the blood flow $\dot{Q}$ and inversely proportional to the volume of distribution V of the drug in the tissue (usually the extracellular space). To get an idea of the actual rates involved, consider a vascular bed that is perfused at a rate that is average for the body. The cardiac output is 6 liters per minute and is distributed to an extracellular space of 12 liters. These values give a measure of the average perfusion rate in the body. The rate constant characterizing the perfusion process would be 0.5 per minute. Thus, perfusion allows one to administer a drug in an easily understood, but rather slow manner.

This model for a perfusion limitation is the simplest; more elaborate schemes are possible and may be necessary in specific cases. Usually, however, there is not enough experimental control to justify going beyond the simplest case, or if an extension is warranted, its nature is obvious. Care is needed; there are enough pitfalls even in simple situations [see Hills' analysis of nonhomogeneous perfusion, (50)].

Diffusion limitation. Here one might be dealing with a thin piece of frog skeletal muscle suspended in an isolated organ bath and the administration of carbaminoylcholine to the bath fluid. Typically, the bath fluid would be vigorously mixed so concentration of the drug in the bath instantaneously rises to its final value. Furthermore, the bath volume is usually so much larger than that of the piece of tissue that concentration of the drug in the bathing fluid may be considered constant. The tissue may be regarded as a plane sheet, in which case the concentration may be expected to follow an equation of the form:

$$\frac{\partial[D]}{\partial t} = {''D''}\frac{\partial^2[D]}{\partial x^2} \qquad (3)$$

where "D" is an apparent diffusion constant and x is distance into the tissue (see 28, equation 1.4). This is Fick's second equation for diffusion in one dimension, and solutions are available (28, 76). In a piece of tissue diffusion is not through free solution, but rather through the extracellular space around cells. Krnjević and Mitchell (58) have shown, however, that for uptake of acetylcholine by rat diaphragm equation 3 still provides a satisfactory model provided

"D" is taken to be roughly one-third of the value for diffusion through free solution.

In the case of a cylindrical piece of tissue such as a vas deferens or guinea pig ileum, Fick's equation may be transformed to cylindrical coordinates. Solutions may again be obtained from the book by Crank (28, p. 67).

When a drug is administered from a point source, as from the tip of an iontophoretic pipette, diffusion from the source to the receptor may be described by the Fick equation expressed in spherical polar coordinates (28, pp. 5 and 84). Del Castillo and Katz (33) have used solutions of the appropriate equation to describe the rate of action of their iontophoretically applied acetylcholine at the neuromuscular junction and the author (106) has done the same with competitive neuromuscular blocking agents. Methods for handling this radial form of the Fick equation are available (53, 107).

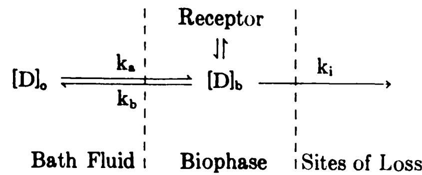
It might be objected that, especially in the case of the neuromuscular junction, the fine geometry of the region of the receptor is too complex to allow a reasonable application of a mathematical model. However, the irregularities of structure are physically so small that the diffusion paths within the crevices of the surface of the cell are short enough not to be rate limiting, *i.e.*, diffusion delay from the tip of a pipette, 10 microns from the endplate region, is much greater than the delay involved in the drug's passing into the grooves of the endplate structure, so that the former delay governs the overall rate. This viewpoint is amply confirmed by the excellent agreement del Castillo and Katz (33) found between their experimental and theoretical results.

More complicated models than the basic Fick equation (equation 3) may be required. If one were dealing with sympathomimetic amines, which are taken up into nerve endings, then one might add a term to equation 3:

$$\frac{\partial[\mathrm{D}]}{\partial \mathrm{t}} = \text{``D''} \frac{\partial^2[\mathrm{D}]}{\partial \mathrm{x}^2} - \mathrm{K}' \frac{[\mathrm{D}]}{[\mathrm{D}] + \mathrm{K}''} \tag{4}$$

where the values of K' and K'' might be estimated from Iversen's (52) uptake studies. The form of the second term on the right hand side of equation 4 was chosen as a convenient model for a saturable process. Paton and Rang (74) have considered a similar model in their analysis of the kinetics of action of drugs on muscarinic receptors. In practice, however, the solution of equation 3 is cumbersome enough. Also, the applicability of equation 3 to the experimental system is usually not exact enough to justify going to more refined models such as equation 4.

The Fick equation 3 can be solved with a computer or a resistance-capacitance analog after conversion to a finite-differences approximation. The resulting solution, or even an analytical solution gives little insight into the underlying mechanisms. Furchgott (38) has used a simplified model to express some general features of an access-limited model. He used a simple rather than partial differential equation to describe rise of concentration at the receptor site. His model is essentially:



The receptor is pictured in equilibrium with a drug solution in a compartment called the "biophase." The biophase in turn is separated from the bath fluid by some form of barrier. For simplicity first-order kinetics is used to describe unidirectional fluxes of the drug across the barrier. In the simplest case, the quantity taken up by receptors is considered negligible in comparison to the total quantity in the biophase. Finally, the possibility of sites of loss within the tissue is introduced by adding n possible outflow paths, again with first-order kinetics. From inspection of the diagram:

$$\frac{d[D]_b}{dt} = k_a[D]_o - k_b[D]_b - \sum_{i=1}^{n} k_i[D]_b \tag{5}$$

Equation 5 may be solved to give:

$$[D]_b = [D]_o \frac{k_a}{k_b + \sum_i k_i}\left(1 - e^{-(k_b+\sum_i k_i)t}\right) \tag{6}$$

Two interesting results appear immediately: the concentration at the receptor at equilibrium may not equal that in the bath, and the rate constant for the approach to equilibrium is independent of k_a.

In his analysis of biophase kinetics Rang (81) has explored the result of relaxation of the assumption that receptor uptake of drug is negligible and so produced what he called a "limited biophase model."

The biophase model as presented above appears as an approximation to the Fick equation, but it also exhibits a property not seen with simple diffusion. If we assume for simplicity that there is no loss in the tissue (all the k_i are zero), then if $k_a \neq k_b$ the concentration in the biophase at equilibrium will not equal that in the bath. This means that the concentration at the receptor site would be unknown if k_a/k_b is unknown. An equilibrium concentration gradient of this sort could not occur with simple diffusion. The asymmetrical barrier ($k_a \neq k_b$) leads to the concentration difference. If the biophase model actually were to describe the experimental situation, then all measurements of equilibrium dissociation constants (see section V) could be in error by an unknown factor as pointed out by Furchgott (38). The presence of an asymmetrical barrier seems so unlikely, however, that measurements of dissociation constants should not be discarded until there is good evidence that a barrier exists.

The biophase model is much more managable analytically than the Fick equation and so can be used to get a rough idea of how an experimental system might be expected to behave. However, the biophase model must be regarded as a form

of shortcut. It cannot be expected *a priori* to describe actual behavior more than roughly. During actual diffusion into a tissue the concentration varies considerably with depth into the tissue (28, fig. 4.1); a model that ignores this must be in error.

Uptake of drugs by receptors and nonspecific uptake sites. As a starting point it is often assumed that the concentration of free drug in equilibrium with the receptors is equal to the amount of drug added divided by the volume of solvent in which it is dissolved; that is, that uptake of drug onto receptors does not significantly changed the concentration of free drug from that which would be present in the absence of such uptake. This is a situation analogous to that of zone A of Straus and Goldstein (93) in the case of substrate-enzyme interactions. However, there is sometimes reason to suspect a situation analogous to that of zone C, where uptake of drug by the receptor does alter significantly the free drug concentration (see 38, 84, 98).

A related situation occurs if there is considerable uptake of drugs by sites other than the receptor. Existence of these nonspecific uptake sites, some of which are called "silent receptors" (30), can alter markedly the drug concentration at the receptor.

Many drugs are particularly difficult to study because of interfering factors other than those discussed in this review. For example, since acetylcholine is rapidly broken down as it diffuses into a tissue, the concentration will be lower deeper in the tissue even at the steady state. Tyramine acts indirectly by releasing norepinephrine from sympathetic nerve endings, and so it too will be especially hard to analyze. As complications such as these are well known and are intensively discussed in many places, they will only be mentioned here for cross-reference. Their relevance to studies of drug-receptor reactions is obvious.

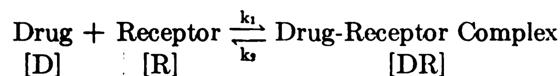
III. THE REACTION OF THE DRUG WITH THE RECEPTOR

In the preceding section the manner in which a drug gets to the region of the receptor was discussed. The receptor may be pictured as being exposed to a drug concentration that rises or falls with a delay described by one of the above models. As a result of this exposure some of the receptor molecules will react with the drug. A model for this stage will now be developed.

For simplicity we shall start by assuming the drug concentration rises instantaneously at the receptor. The conventional model (27) is the reaction[2]:

[2] (a) In the analysis that follows I have tried to present a straightforward unified description of the conventional mathematical models used to describe drug receptor reactions. I have not tried to indicate references for each equation, since this would disturb the line of thought. The overall picture is a synthesis of relationship developed by Clark (27), Gaddum *et al.* (43, 47), Ariëns (1), Stephenson (90), Arunlakshana and Schild (7) and Schild (87), Furchgott (38), and Paton (70). Previous reviewers have discussed the historical development so I focused more on producing a unified picture.

(b) Every author has his own definition of "receptor." I shall define it as that structure in the tissue which behaves as R in the scheme given. This is analogous to defining temperature as that which behaves as T in the Maxwell-Boltzmann distribution.



From inspection of this scheme one may write

$$\frac{d[DR]}{dt} = k_1[D][R] - k_2[DR] \tag{7}$$

[This equation appeared in pharmacological work 4 years before Michaelis and Menten published their model of enzyme kinetics (49).]

Clark (25, 26) considered a more general case than the model given above. He considered the case of n drug molecules reacting with each receptor molecule. This review will be restricted to the case of n = 1.

Introducing $[R]_t - [DR]$ for [R] and rearranging gives

$$\frac{d[DR]}{dt} = k_1[D][R]_t - (k_1[D] + k_2)[DR] \tag{8}$$

Three solutions are relevant:

At equilibrium

$$[DR] = \frac{k_1[D]}{k_1[D] + k_2}[R]_t = \frac{[D]}{[D] + k_2/k_1}[R]_t \tag{9}$$

During approach to equilibrium after addition of the drug

$$[DR] = \frac{[D]}{[D] + k_2/k_1}[R]_t(1 - e^{-(k_1[D]+k_2)t}) = [DR]_e(1 - e^{-(k_1[D]+k_2)t}) \tag{10}$$

On washout of the drug:

$$[DR] = [DR]_0\, e^{-k_2 t} \tag{11}$$

where $[DR]_0$ was the receptor occupancy at t = 0.

The essential features of equation 9 may be seen by changing dimensions of the concentrations involved. First, the absolute concentration of receptors being usually unknown, the equation may be put in a form independent of absolute concentration. To do this, simply express receptor occupancy as fractional receptor occupancy y with

$$y = [DR]/[R]_t \tag{12}$$

then 9 becomes

$$y = \frac{[D]}{[D] + k_2/k_1} \tag{13}$$

Next we may introduce an analytical unit of concentration defined as

$$c = \frac{[D]}{k_2/k_1} = \frac{[D]}{K_e} \tag{14}$$

Equation 13 then reduces to

$$y = \frac{c}{c + 1} \tag{15}$$

This shows that one is dealing simply with a hyperbola or with a logistic function (14). In the form of equation 15 occupancy-concentration curves for all drugs are the same and are given by the curve of figure 1. This functional relationship is the basic model for the stage of the drug-receptor reaction.

The equations 10 and 11 transform to

$$y = y_e(1 - e^{-k_2(c+1)t}) \tag{16}$$

(showing that the rate constant for onset varies with concentration) and to

$$y = y_0\, e^{-k_2 t} \tag{17}$$

(showing an independence of concentration).

Next we may consider the case where two drugs A and B interact at (compete for) one receptor. The equations corresponding to equations 10 and 11 are rather complex, so we shall only consider the equilibrium case. The relevant solution[3] is

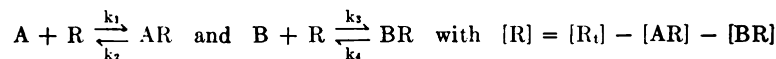
$$[AR]' = \frac{[A]'}{[A]' + K_A[1 + [B]'/K_B]}[R]_t \tag{18}$$

in which the prime indicates that the other drug is present (61).

This form shows that the effect of adding the drug B is equivalent to a change (increase) in K_A. This will lead to a "parallel" shift to the right of the *receptor occupancy*-concentration curve of A. Equation 18 may be transformed to a more fundamental form:

$$y_A' = \frac{c_A'}{c_A' + c_B' + 1} \tag{19}$$

[3] To derive equation 18 start with the reactions:



$$A + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AR \quad \text{and} \quad B + R \underset{k_4}{\overset{k_3}{\rightleftharpoons}} BR \quad \text{with} \quad [R] = [R_t] - [AR] - [BR]$$

At equilibrium the rate of combination of each drug with the receptor will equal the rate of dissociation so:

$$k_1[A]'([R_t] - [AR]' - [BR]') = k_2[AR]'$$

and

$$[k_3][B]'([R_t] - [AR]' - [BR]') = k_4[BR]'$$

Eliminate [BR]′ and rearrange to get equation 18.

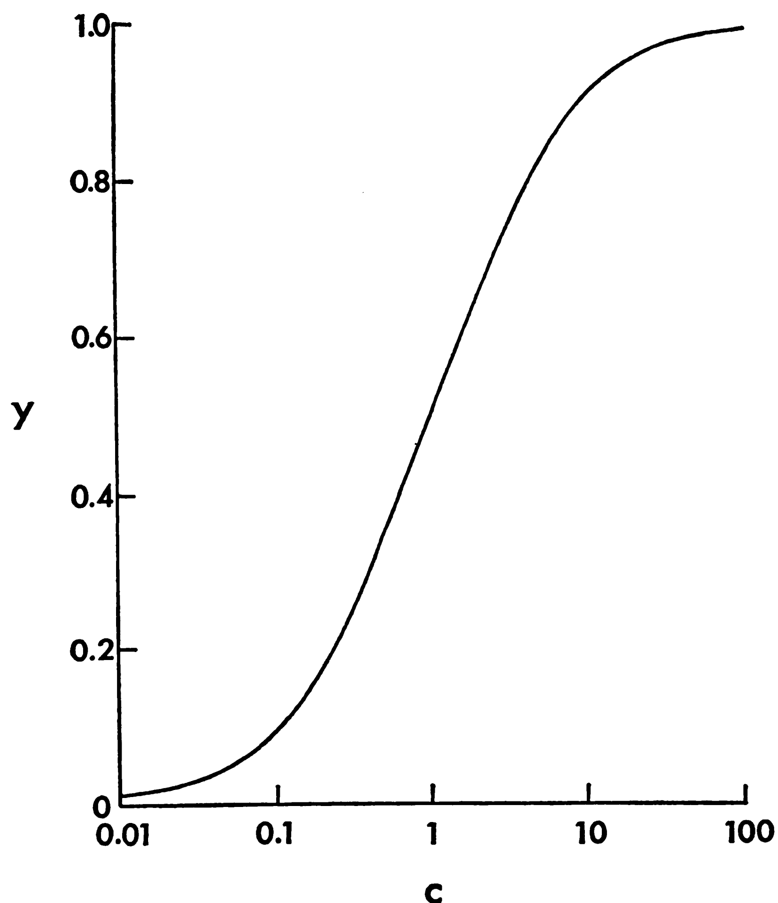


FIG. 1. Receptor occupancy at equilibrium as a function of concentration of the drug. Ordinates: Fractional receptor occupancy, running from 0 to 1. Abscissae: Normalized concentration of drug c (defined as $[D]/K_e$). This curve applies to all drugs acting by a one-to-one reaction with the receptors.

Note that

$$y_A' + y_B' = \frac{c_A' + c_B'}{c_A' + c_B' + 1} \tag{20}$$

This indicates a straightforward addition of receptor occupancies when one works in analytical units of concentration. The mixture of A and B behaves like a single drug at concentration $c'_A + c'_B$. This facilitates analysis of the effects of two or more competitive antagonists [see section V; see also Jenkinson, (56)]. In particular, equation 20 is the basis for analyzing the actions of racemic mixtures.

When the receptor occupancy of one drug is reduced by the addition of a second drug, the second is sometimes said to "displace" the first from some of the receptors. With drugs like acetylcholine and atropine one should not picture this

as an active process, but merely a shift of an equlibrium. It is extremely unlikely that an atropine molecule would actually bump into a bound acetylcholine molecule or *vice versa* and thereby give it enough energy to go over the dissociation activation energy barrier. The energy is much more likely to come from thermal motion of water molecules which are far more prevalent or from intramolecular vibrational energy. What happens is that both types of molecules "spontaneously" leave the receptors and when a receptor so becomes vacant the law of mass action determines the next occupant.

All the analysis of this section so far follows immediately from the assumption of a reaction according to the law of mass action between the drug and the receptor. (The effect of receptor binding of the drug is assumed to be negligible in these basic equations; this restriction will be discussed later.) This part of the pharmacology of receptors seems to be accepted by practically everyone. It is the next stage of the process which is controversial.

Before moving on, the effect of perfusion or diffusion delays in access to the receptor must now be considered. If there is a circulatory delay of the sort described by equation 2 then equation 7 becomes

$$\frac{dy}{dt} = k_1 c_a \left(1 - e^{-\frac{\dot{Q}}{V} t}\right) - k_2 \left(c_a \left(1 - e^{-\frac{\dot{Q}}{V} t}\right) + 1\right) y \qquad (21)$$

[Analytical units of concentration (c, equation 14) have been used and occupancy has been expressed as fractional occupancy for simplicity.]

The variables are no longer separable and in fact there is no analytical function that is a solution of equation 21. A similar problem exists if there is a diffusion limitation obeying a law such as equation 3. Even use of the biophase approximation does not help, since equation 5 is of the same form as equation 2. When the specific behavior of the model must be obtained, analog techniques may be used and solutions are available (53, 76, 106, 107).

IV. THE RELATION BETWEEN THE DRUG-RECEPTOR REACTION AND THE PRODUCTION OF AN EFFECT

The mechanisms involved in the production of an effect as a result of the interaction of a stimulant drug with the receptor have been the center of a considerable degree of controversy. Again I shall avoid the historical approach and begin by presenting what I believe is a reasonable general outlook and then indicate where individual theories fit into the picture. The general model I shall describe is based essentially on that of Paton (72). (I refer here to his general outlook, not his specific kinetic model.)

Empirically one can state that in a reasonably controlled system the effect observed is some function (F) of the concentration (c) of a drug administered, *i.e.*,

$$E = F(c) \qquad (22)$$

More generally one may write

$$E = F(c, t) \qquad (23)$$

to indicate explicitly a dependence on time as well as on concentration. For example, when one administers acetylcholine to a tissue the response rises to a peak and then falls to some lower level. This latter fall cannot be a result of an access lag, since concentration at the receptor must still be rising. Therefore, there is a temporal dependence in the concentration-effect relationship. The dose-response curve is a plane through a dose-response-time surface.

Mackay (61, assumption c) explicity avoided the temporal dependence in equation 23. This must restrict the applicability of his model considerably. Furthermore, as Paton emphasized (70), there is no reason to believe that desensitization (see section XII) does not occur from the moment a drug starts to act. The fact that a response rises to equilibrium monotonically with time could still just mean that subunits of the responding tissue are showing desensitization, but the spatial average increases smoothly to the steady state level.

As indicated in the previous section, part of the functional relationship in equation 22 or 23 is known. That is, the step from concentration to receptor occupancy is described by equation 8 or a variant thereof. The problem of determining the relation between concentration and effect, therefore, reduces to that of determining the relation between receptor activation and effect, and we may write

$$E = G\,(\text{receptor activation, t}) \tag{24}$$

Now a distinction must be made between different models with regard to what is meant by "receptor activation." As a starting point we may consider the classical or occupational model in which receptor activation is assumed to be proportional to the number of receptors occupied, and equations 23 and 24 become

$$E = f\,(y) \tag{25}$$

and

$$E = f\,(y, t) \tag{26}$$

This relationship is quite reasonable given the assumption that stimulation is related to occupancy. It does assume that each receptor molecule contributes to the effect independently, but in the present state of knowledge we are in no position to investigate this possibility. Equation 25 is the minimal requirement that will still allow any interpretation of experiments at the chemical level.

The explicit dependence on time in equation 26 can be of two types. First, it can express the effect of desensitization. This will be discussed in section XII. Second, it can reflect the finite time required for the production of a response. Usually, access, or the drug-receptor reaction, or both, seem rate-limiting, but occasionally the last step in the process is the slowest as in the frog rectus abdominis muscle (69).

The function f in equations 25 and 26 is usually taken to be single-valued and a monotonically increasing function of y. This then allows one to return to a given level of receptor occupancy by a stimulant drug by returning to a given response level. Various simplifications of equation 25 will now be discussed, but

before doing so I shall emphasize that at present any simplification, except in very special cases, is likely to lead to a misleading model of drug action.

Clark (25, 27) originally assumed that the effect was directly proportional to receptors occupied. This is the simplest model. He showed in fact that a large collection of available dose response curves fitted this model. However, this fit must be regarded as completely fortuitous. This represents just the first of a series of cases of excellent but apparently fortuitous agreement with experiments which seem so frequent in the field of receptor studies. Many authors, Stephenson most cogently (90), have shown subsequently that there might be, and in fact are dose-response curves that do not fit the model. Furthermore, it is reasonable *a priori* to expect that the reaction of the drug with the receptor might not be the weakest link in the chain of events leading to the effect, especially if the effect involves the expenditure of a considerable amount of energy, as when the response measured is a contraction. Finally, one can manipulate the nature of the effect observed by the choice of the recording apparatus, so that if the response is proportional to y with one system it will not be so with another.

The next model to appear after Clark's was developed by Ariëns (1). He assumed proportionality between E and y with a proportionality constant α:

$$E = \alpha\,[DR] \tag{27}$$

He called α the intrinsic activity and characterized it as a constant dependent on the choice of drug. Working then from equations 27 and 13 he pointed out that a drug could be characterized by two constants, α and k_2/k_1 or K_e the dissociation constant or reciprocal[4] of the "affinity" of the drug for the receptor. He classified drugs into stimulants, or "agonists" (83), and antagonists on the basis of whether $\alpha > 0$ or $\alpha = 0$, respectively. He also has pointed out the existence of an intermediate class of drugs, Stephenson's (90) "partial agonists," whose α is less than the maximal possible value but not zero. Like Clark, Ariëns has accumulated a vast collection of examples of how this model (equation 27 with 13) fits experimental results (3).

In 1956 Stephenson (90) pointed out most of the features of equation 25 and introduced the notion of spare receptors. He said that maximal receptor occupancy need not be required for maximal response. He also included the possibility that the degree of receptor stimulation could vary from drug to drug, and he pointed out that a linear relation between receptor occupation and response need not necessarily obtain. In his model the function f of equation 25 is split into two parts. The effect E is said to be some function (not necessarily linear) of a property S—the "stimulus" which in turn is a linear function of y, *i.e.*,

$$E = g\,(S) \text{ and } S = ey \tag{28}$$

The drug-dependent proportionality constant e is called the efficacy. It is not

[4] There seems to be no agreement with regard to the use of the dissociation or the association equilibrium constant to characterize the drug-receptor reaction. I have rather arbitrarily used the former (k_2/k_1) here because it has the dimensions of a concentration and in fact is the concentration which would lead to occupancy of half the receptor pool by the drug.

to be equated with Ariëns' intrinsic activity α. The latter does not distinguish between drugs with $\alpha = 1$ even though they vary in efficacy. In equation 28 the f of equation 25 is broken up into a drug-dependent but linear part (e) and a nonlinear but drug independent part (g).

More recently Furchgott (40) has proposed a variation of equation 28

$$E/E_{max} = g(S) \text{ and } S = \epsilon [DR] \tag{29}$$

where ϵ is called the "intrinsic efficacy." It obviously will exhibit most of the characteristics of Stephenson's efficacy. Furchgott's version indicates explicity that the stimulus depends on the absolute concentration of receptors. Stephenson's e equals $[R]_t$ times Furchgott's ϵ and therefore e is directly proportional to the concentration of receptors.

Because dose-response curves are the same shape as substrate concentration-velocity curves of enzymology, plots analogous to Lineweaver-Burk plots have been fitted to dose-response curves. These plots cannot be expected to have more than empirical significance. They cannot be expected to give drug-receptor dissociation constants.

In summary, there seems to be reasonable agreement nowadays that the introduction of assumptions beyond those involved in equation 25 is likely to lead to unsafe grounds (see 3, 39, 70, 84, 90).

As presented in equation 25 the function f is extremely general. However, this very generality dissociates it from the specific underlying physicochemical processes which determine its nature. The nature of the function f is determined by the properties of a number of sequential mechanisms. Therefore, f should be thought of as the product of several to many functions in much the same way that, in operator calculus, one operator can be pictured as the product of others or, in electronics, a tranfer function for a whole system can be built up as the product of the transfer functions of the component parts. Analysis of the nature of the relation between y and effect will involve finding the components of the function f and relating each to the underlying mechanism involved.

As Furchgott (40) pointed out, the relationship between receptor stimulation and response can be expected to vary considerably among tissues while drug-receptor association constants should be relatively consistent, since they represent a definite chemical parameter. Perhaps the macromolecule that is the receptor may show a few variations in structure as do isoenzymes, but it seems unlikely that a whole spectrum of receptor types should occur.

This completes a survey of the general models used to describe the relationship between the dose of a drug and the effect it produces. I shall now discuss a series of topics illustrating the use of these general concepts as tools for the investigation of receptor properties.

V. THE POTENCY OF COMPETITIVE ANTAGONISTS

(dose ratio, pAx, k_2/k_1)

A competitive antagonist may be regarded as a drug whose effect is receptor occlusion. When it combines with the receptor no observable change is seen.

However, when an agonist is added in the presence of the antagonist, the agonist is found to have less effect because it has access to less than the full receptor pool.

The concentration-effect curve for an antagonist thus reduces to the concentration-receptor occupancy curve, *i.e.*, to equation 13. The behavior of the antagonist at the receptor can be completely characterized by the constant k_2/k_1, and so measurement of this constant has been of considerable interest. Also, as indicated in the comments on equation 20, knowledge of the relevant dissociation constants will allow prediction of the effect of a mixture of competitive antagonists.

The study of competitive antagonists is based on Gaddum's (47) concept of the dose ratio, that is, the ratio of the concentration of agonist required to produce a given response in the presence of the competitive antagonist to that required in its absence. That a comparison of dose ratio rather than response ratio is appropriate follows from the considerations that led to the use of equation 25 as a model for the effect-agonist receptor occupancy relationship. When one assumes that the effect is a function of y and possibly of t (see section XII), but not of the concentration of antagonist, then one can match receptor occupancy by matching effects, *i.e.*, one can use a null method with regard to effect. Nothing need be known about the function f except that it be single-valued and independent of concentration of antagonist, and also of time, preferably. If one gives an agonist in the presence of the competitive antagonist, receptor occupancy by the agonist will be given by

$$y' = \frac{[A']}{[A]' + k_2/k_1(1 + [B]'/K_B)} \qquad (30)$$

which is a modified form of equation 18 with $[A]'$ for the concentration of agonist, $[B]'$ for the concentration of antagonist and k_2/k_1 ($= K_A$) and K_B their respective equilibrium dissociation constants. K_B is to be determined.

Matching the responses in the presence and absence of the antagonist amounts to equating the right-hand sides of equations 30 and 13. Rearranging gives

$$\frac{[A]'}{[D]} - 1 = \text{dose ratio} - 1 = \frac{[B]'}{K_B} \qquad (31)$$

Since the concentration of antagonist used is known, and the dose ratio may be determined experimentally, K_B may be obtained. Schild (87) has introduced the notation pA_x as an empirical index of the negative logarithm (to base 10) of the concentration of antagonist that produces a dose ratio of x. Arunlakshana and Schild (7) went one step further and showed how in the presence of a competitive antagonism the pA_x values should take a particular form. They transformed equation 31 to

$$\log(\text{dose ratio} - 1) = \log [B]' - \log K_B \qquad (32)$$

so that the pA_2 (or alternatively, the equivalent: K_B) may be obtained directly from the intercept with the log $[B]'$ axis of the plot of log (dose ratio -1) against

log [B]'. This method has a built-in check on applicability of the model; the slope of the plot should be unity. This approach is the fundamental way to estimate the potency of a competitive antagonist.

Jenkinson (56) has considered the possibility that an ion-exchange reaction describes the drug-receptor reaction [see also Ing and Wright (51)]. If this is the case the K_B in equation 31 is multiplied by a factor $(1 + c_m)$ (see equation 30). (The c_m here is the concentration of an ion m which reacts with the site in a one-to-one fashion.) The slope of a plot of equation 32 will still be unity, but the intercept will no longer give an unbiased estimate of K_B. K_B will appear larger than it is. To remove the bias or to show that none exists, one would have to study the antagonism at different concentrations of the ion involved. Jenkinson found, in fact, that lowering the sodium concentration decreased K_B for tubocurarine. He replaced sodium by sucrose and, therefore, as he pointed out, altered ionic strength as well as c_{Na} ($=[Na]/K_{Na}$). In fact, the change in ionic strength would be expected to produce about the observed change in K_B (lower ionic strength $\rightarrow$ increased affinity $\rightarrow$ decreased K_B). Jenkinson also found a decrease in his estimate of K_B when calcium or magnesium concentrations were raised. In these cases the change in ionic strength was negligible. A more extensive quantitative investigation will be necessary before a satisfactory interpretation of this will be possible, but Jenkinson has produced enough results to make one want to reinvestigate all estimates of equilibrium constants to date.

Taylor (95) discussed this same point and recommended use of a selectivity constant ($k_B^A = [AR]'/[A]'//[BR]'/[B]'$) to characterize the situation. This constant equals K_B/K_A as may be seen from equation 19. (A and B here would refer to the ion involved and the antagonist, respectively.)

Measurements of the potency of competitive antagonists can be interpreted at several levels: (i) in terms of the empirical measure the pA_x (87); (ii) in terms of an "apparent" K_B ("apparent" because of access uncertainties; see for example, reference 38); or (iii) as a way to measure K_B. Often only the empirical interpretation is appropriate. In other cases a more chemical one may be possible. No fixed rules can be given for choosing which interpretation is appropriate.

Ideally one wishes to measure fundamental physicochemical parameters such as entropy or free energy changes accompanying the drug-receptor reaction. In our present state of knowledge this is difficult, and one usually realizes that the ideal goal has not been reached. It is still better to aim at a dissociation constant even though it is "apparent" (38) or dependent upon the concentration of a third reactant (56, 95) than simply to measure an empirical value such as a pD'_x (4). For example, if an apparent dissociation constant has been measured in what proves to be an ion-exchange situation, the dissociation constant will still be of use in the description of events to be expected from an ion-exchange mechanism since it would fit naturally into the new scheme (see 95). An empirical measurement will have much less utility. One has seen a similar situation frequently during the development of the physical sciences. For example, all of Newtonian mechanics fits, with minimal adjustment, into relativistic mechanics, and classical mechanics is now regarded as the limiting case of quantum me-

chanics as one considers dimensions large on the molecular scale. The advent of a refined model supplemented but did not invalidate the more limited version. On the other hand, the more empirical earth, fire, air, and water did not fare so well in the newer chemistry.

Potency and selectivity. The *a priori* probability of getting a potent agonist or antagonist acting at any given receptor by simply selecting a chemical at random is very small. The probability would be expected to get smaller as the potency increases. If the structures of different receptors are unrelated, as, for practical purposes, they seem generally to be, the probability of getting a drug active at two receptors will be the product of the probabilities of getting one which is active at each of the two receptors and this product will be very small indeed. It is for this reason that high potency in a drug is most useful. The potency may imply selectivity. The absolute amount of material that must be administered clinically is rarely of consequence; it is usually the amount relative to that amount which produces the first undesirable effect which is a relevant figure of merit. Pfeiffer has observed (79) that among optical isomers of high potency, the difference in the activity of the isomers is greater than among those of low potency. This observation would be expected from the probabilistic viewpoint just discussed.

VI. ESTIMATION OF THE DISSOCIATION EQUILIBRIUM CONSTANT OF AN AGONIST

This relatively new development will be discussed at this point since it seems logical to discuss the dissociation constant of an agonist after that of the antagonist. When Ariëns (1) introduced the distinction between intrinsic activity and affinity, he suggested that the dose producing a 50% response could be taken as a measure of the affinity (k_1/k_2) of the drug for the receptor. Stephenson (90) pointed out that this was not a very sound argument. Furthermore, he devised an ingenious experiment (90, pp. 384–385) which, in principle, could yield a reasonable measurement of the affinity constant (k_1/k_2) of a weak partial agonist. The method put severe demands upon the experimental technique required for accurate estimation and so was not followed up. Until recently, however, this was the only sound approach to appear.

In the last 4 years, several groups have returned to the problem (40, 48a, 61, 91, 105) and all use essentially the same procedure. The principle involves the use of an irreversible competitive antagonist. Such an agent can be pictured as removing a fraction of the receptor pool permanently. Administration of an agonist will then lead to a receptor occupancy given by

$$y' = \frac{[D]'}{[D]' + k_2/k_1} (1 - y_I) \tag{33}$$

when y_I is the fraction of the receptor pool blocked by the inhibitor. Equation 33 is just equation 13 scaled down in proportion to the number of receptors still left to combine with the agonists.

By matching responses before and after blockade of receptors by the irrevers-

ible agent, we will be matching the occupancies for the agonist. As with equation 31, the right-hand sides of equations 13 and 33 may, therefore, be equated and rearranged to give:

$$\frac{1}{[D]} = \frac{y_I}{1 - y_I} \cdot \frac{k_1}{k_2} + \frac{1}{1 - y_I} \cdot \frac{1}{[D]'} \tag{34}$$

Thus, a plot of the reciprocal of the control dose for a given response against the reciprocal of the dose required to give the response after the irreversible antagonist will give the k_1/k_2 of the agonist as

$$\frac{k_1}{k_2} = \frac{\left(\text{intercept on } \frac{1}{[D]} \text{ axis}\right)}{(\text{slope} - 1)} \tag{35}$$

This approach has been studied very extensively by Furchgott (40) and Furchgott and Bursztyn (41), who have sought optimal conditions for maximal precision. He and Bursztyn (41), using muscarinic agonists on isolated stomach muscle of the rabbit. also produced a clever test of the applicability of the theory. With equation 35, they first estimated the dissociation constant for the drug-receptor complex of a partial agonist from the dose-response curves before and after irreversible block of a large fraction of the receptor pool. Next, they irreversibly blocked a still larger fraction of the receptors, so that the muscle now gave no detectable response to the partial agonist, but still responded to a strong agonist. With the muscle in this state they were now able to use the partial agonist as a competitive antagonist of the strong agonist and obtain an independent estimate of the dissociation constant of the partial agonist by use of equation 31. The two estimates agreed. This agreement, combined with the linearity obtained when appropriate experimental results are plotted in accordance with equation 35, provides strong experimental support for the validity of the approach.

Since it is thus possible to measure k_2/k_1 of the agonist receptor complex, the problem of finding the relationship between concentration and effect can be considered to be reduced to that of finding the relationship between receptor occupancy and effect.

If, for a competitive reversible antagonist, the equation analogous to equation 34 is obtained (from equations 13 and 31; see reference 108), it is found that a plot of $1/[D]$ against $1/[D]'$ will go through the origin, and the slope will be the equilibrium dose ratio. Equilibrium dose ratios may thus be obtained for use in equation 32 without an arbitrary choice of response level at which to measure the shift of a dose-response curve. All the information in the dose-response curves would be used. However, in practice the dose ratio can usually be obtained with minimal error directly from dose-response curves by simple inspection.

VII. MEASUREMENT OF THE POTENCY OF AN IRREVERSIBLE COMPETITIVE BLOCKING AGENT

When a drug like dibenamine combines irreversibly with the receptor, the use of equation 13 becomes inappropriate, since k_2 is vanishingly small. Ariëns and

van Rossum (4) have suggested using the negative logarithm of the molar concentration of antagonist that reduces the maxium of the dose-response curve to one-half of its original value. They call the resulting value a pD'_2. Although they introduced this index in connection with "noncompetitive" antagonists, the pD'_x has been used as a measure of the potency of irreversible competitive agents as well. This is not a practice to be recommended, as the result obtained depends on the nature of the relationship between dose and effect, *i.e.*, on the function F of equation 22.

The approach of the previous section can yield a sounder measure (108). The slope of the relation between 1/[D] and 1/[D]′ in equation 34 is $1/1 - y_1$. Thus, the receptor occlusion by the irreversible competitive antagonist can be obtained directly as

$$y_I = \frac{\text{slope} - 1}{\text{slope}} \tag{36}$$

All the precautions (see reference 41) associated with measurement of k_2/k_1 of an agonist are still applicable here.

Gill and Rang (48a) estimated activity of an irreversible competitive blocking agent by measuring its association rate constant. However, with this approach, kinetic factors require consideration, so that a measurement made at equilibrium will be easier to interpret until all kinetic factors in drug action are fully understood. Gill and Rang, for example, discarded access as the rate-limiting process by pointing out that a biophase model would give a linear onset of receptor blockade. But, as mentioned in section II, the biophase model cannot be used as a quantitative approximation to the Fick diffusion equation. It is not easy to rule out an access limitation convincingly.

A note of caution. Note that modifications of equation 13 can take two forms only[5] (or a combination thereof):

$$y = \frac{[D]}{[D] + ak_2/k_1} \quad \text{or} \quad y = \frac{[D]}{[D] + k_2/k_1}b \tag{37}$$

For example, as in equations 30 and 33 respectively.

Therefore, if an experiment is found to fit one or the other, the pharmacologist should keep in mind that there are not many alternatives. This viewpoint is relevant to all the three preceeding sections. Hence, it is important in the application of the methods described there to try to include a check for internal consistency, such as Furchgott's measuring k_2/k_1 by two approaches (41) or Schild's plotting log (dose ratio −1) against log [antagonist] to check that the slope is unity (7).

VIII. THE SHAPE OF THE DOSE-RESPONSE CURVE

Many people (6, 27, *etc.*) have attempted to explain the shape of dose-response curves. However, because the function f of equation 26 is, in general, of unknown nature *one cannot safely attach any fundamental significance to the shape of dose-*

[5] Assuming linear operations. One can of course contrive many more complicated maneuvers.

response curves. The explanation of the shape of a dose-response curve which I find most satisfactory was given to me, somewhat tongue-in-cheek, by P. B. Dews: if you give no drug you get no response, if you give a lot of drug you hit a ceiling (tissues cannot produce infinite responses). Draw a line between these two points. Round the corners (asthetics). Finally, use a logarithmic dose scale (*cf.* Weber-Fechner law, or the nature of chemical potentials). You now have a sigmoid dose-response curve. The point to be made is that it is easy to get a sigmoid curve. Do not make too much of it unless you have more to go on than its shape.

These comments are not intended to imply that one cannot use dose-response curves empirically. For example, dose-response curves are useful for the empirical distinction between drugs with regard to differences in *potency* (ED50) or *effectiveness* (maximal response obtainable, sometimes called *power*).

Also, one can make reasonable qualitative predictions of changes in shape of dose-response curves. For example, when norepinephrine is given to an isolated atrium and force of contraction is measured as the response, cocaine will increase the slope of the dose-response curve. This can be explained (16) by recognizing that cocaine blocks the loss of the administered norepinephrine into nerve terminals so that after cocaine it is no longer necessary to raise the bath concentration so high to activate maximally the deepest parts of the tissue. Trendelenburg (99) gave an example of how the existence of a saturable uptake mechanism might influence the slope of a dose-response curve.

IX. CLASSIFICATION OF RECEPTORS

There are three ways to investigate whether two drugs act on the same receptor. (i) Barsoum and Gaddum (11) used specific desensitization. If a large dose of an agonist A can be shown to abolish the response to a subsequent dose of B but not of C, then B but not C would appear to act at the same site as A. Unfortunately, a partial agonist might be affected by desensitization more than a strong agonist, so this method must be used carefully. (ii) Schild's pA_x (7, 87) is the most convenient tool for receptor identification. Two drugs acting at the same receptor should be blocked to the same extent by a competitive antagonist acting at that receptor. If two different pA_2's are obtained, the drugs act at different sites. If the same pA_2 is obtained the evidence is strongly suggestive, but not proof-positive that they act at the same site. (iii) Furchgott (37) suggested a third way to try to differentiate receptors. To illustrate, suppose we wish to see if serotonin acts on receptors for norepinephrine. In the presence of a large "protecting" concentration of norepinephrine, dibenamine is added. This will combine irreversibly with many sites, but will not be able to get at many of the norepinephrine receptors because they are occupied by the norepinephrine. Next the dibenamine, and then the norepinephrine are washed out. Sensitivity to serotonin is tested. If it still were active, we should conclude that its receptors had been protected by norepinephrine and were, therefore, the norepinephrine receptors. The technique of receptor protection must be used with careful regard to the quantitative aspects (39, 104) if the argument is to be convincing.

In classification of receptors, competitive antagonists are much more useful

than agonists since there is no involvement of steps beyond receptor occupation. The action of competitive antagonists is just receptor occlusion. Classically, pharmacologists have classified receptors on the basis of agonists—"nicotinic" or "muscarinic," "alpha" or "beta" adrenergic—but identification and nomenclature is much more natural in terms of the relevant antagonist (hexamethonium, tubocurarine, hyoscine, propranolol, dibenamine or phentolamine, *etc.*).

X. THE ABSOLUTE CONCENTRATION OF RECEPTORS

Most of the analysis above has been phrased in terms of fractional receptor occupancy (y) rather than absolute concentration of drug-receptor complexes [DR] because absolute concentration of receptors is generally unknown. In one system, however, we now have a direct measure. Paton and Rang (74) studied uptake of radioactive atropine by strips of longitudinal muscle from guinea pig ileum. They found the uptake curve behaved as if three compartments were involved, but most important, they found a saturable compartment with a k_2/k_1 in excellent agreement with that estimated from the pharmacological effect of atropine. This compartment (binding site) had a capacity of 180 p moles of atropine per g tissue. Lachesine (another muscarinic blocking agent) blocked atropine uptake competitively and again the k_2/k_1 for lachesine estimated from uptake studies agreed with that obtained from pharmacological study of its antagonism to acetylcholine.

The uptake of atropine was not affected by cocaine. Cocaine might, from its structural similarity to atropine, be expected to compete at nonspecific binding sites, but not at the receptor itself since cocaine's pharmacological action is different from that of atropine. This again implies the uptake is by the receptor itself.

The capacity of the uptake site for methylatropinium was roughly half that for atropine, so perhaps 90 p moles per g would be a better estimate for receptor concentration. The order of magnitude is still probably correct.

Working from 180 p moles per g Paton and Rang calculated that there are 1.6×10^5 receptor molecules per cell; this would cover about 1/5000 of the cell surface.

May *et al.* (64) gave 1.15×10^{12} receptors per milligram of tissue dry weight as an upper limit for concentration of α receptors in aortic strips. They got this limit from binding studies with labelled irreversible competitive blocking agents.

Waser (103) has attempted to count receptors by autoradiography. He got a clear picture of motor end plate regions with tubocurarine and estimated that there are about 4×10^6 molecules bound per end plate. This is near the 1.6×10^5 receptors per smooth muscle cell that Paton and Rang (74) estimated. Since he did not present results suitable for application of the Langmuir adsorption isotherm, it is hard to estimate an affinity constant from his results. He attempted to do so from a plot of percent neuromuscular paralysis against concentration of toxiferine, but this ignores the margin of safety of neuromuscular transmission (77). If we assume that 50% blockade of transmission occurs at about $y = 0.8$ to 0.9 (see 77, fig. 9) then working back from Waser's concentration of 0.18 μg/ml at 50% block we can estimate (equation 13) k_2/k_1 as 2.0 to 4.5×10^{-8} g/ml for toxiferine. If toxiferine is about 20 times more potent than tubocurarine (102),

then tubocurarine would be predicted to have a k_2/k_1 of 4.5 to 9×10^{-7} g/ml, which is close to the 3×10^{-7} g/ml reported by Jenkinson (56). Admittedly, Jenkinson's estimate was in another species, the frog, but since I have obtained an almost identical value in perfused rabbit legs, I suspect that species variation is not great here. Waser's estimate of receptor density, therefore, seems reasonable. The only disturbing aspect of his work is the higher uptake of an agonist (decamethonium). However, this uptake is not saturable, nor does it produce the sharp picture tubocurarine does. Perhaps the simpler decamethonium molecule is more subject to nonspecific uptake or enters through a depolarized endplate (see reference 95). Waser (103) noted that only high doses of acetylcholine will prevent tubocurarine binding. This is quite in line with the notion of spare receptor capacity (see below).

Potter (80) attempted a similar study with beta adrenergic receptors of guinea pig atria. He used propranolol as his competitive antagonist. Unfortunately, this agent is rather fat-soluble so there was a considerable nonspecific background clearance. No uptake that could be related to receptors could be seen superimposed. His experiments can only give an upper limit to receptor density. He estimated such a limit and got a value similar to the concentration Paton and Rang reported for muscarinic receptors.

XI. PATON'S KINETIC THEORY OF DRUG ACTION

I have undertaken to write this review for two reasons. First, I felt there was a need for a discussion of the overall picture of the models used in the analysis of the pharmacology of receptors. Secondly, the time seemed ripe for an intensive discussion of the relative properties of the classical and the rate theories of drug action. This review so far has dealt with the first objective. We may turn now to the second.

Gaddum (43) observed a discrepancy between the rates of action of adrenaline and ergotamine. The effect of the antagonist was much slower than that of the agonist. Furchgott (38) also noticed the discrepancy and invoked the biophase model to provide an explanation. Next, Paton became intrigued by the slower action of antagonists and by the observation that their rate of action varied inversely as their potency. Furthermore, he was interested in the fact that many stimulant drugs produce an effect that rises to a peak and then falls with time even though the drug is still in the bath. Although some of these cases of a falling response seem nonspecific and similar to the phenomenon described by Cantoni and Eastman (23), others, particularly one illustrated by Paton and Perry (73, fig. 9) and those described by Barsoum and Gaddum (11), are not easily explained. Paton (70) suggested that the three phenomena, (i) slow action of antagonists, (ii) relation of rate of action of antagonists to their potency, and (iii) fading responses of agonists, might all be explained by postulating that it was not the number of receptors occupied that governed the response elicited, but rather the rate of drug-receptor combination. He replaced equation 25 by

$$E = \phi\,(A) = \phi\,(k_1[D](1 - y)) \tag{38}$$

Implicit in the model was the notion that the observed slow rates of action of

competitive antagonists were expressions of the rates of the antagonist-receptor reaction. By measuring these rates one might be able to measure k_1 and k_2. Furthermore, the ratio of k_2/k_1 estimated from the kinetics of action of the antagonist could be compared with the estimate of k_2/k_1 obtained from the equilibrium dose ration (see equation 31). Paton compared the two results and found good agreement. Since this agreement is probably the strongest argument for the model, the experimental method will be described more detail.

Contractions of a piece of guinea pig ileum exposed to acetylcholine were recorded. After a control dose-response curve, a series of roughly half-maximal responses was elicited and then equilibration with atropine was begun. At the same time the test dose of acetylcholine was doubled. As the effect of atropine came on, the response to the 2-fold acetylcholine dose fell and as soon as it fell below the control series of responses the dose ratio was increased to three. This process was repeated until a steady state was reached. The result was a series of contractions of roughly the same height from which a series of dose ratios *versus* time could be obtained. Since the method was a null method, knowledge of the nature of the dose-effect relationship was not invoked. Paton estimated receptor occupancy by atropine (y') as (dose ratio -1)/dose ratio. This equation is applicable provided the agonist does not occupy an appreciable fraction of the receptor pool or if full equilibrium among agonist, antagonist, and receptor is reached. It is not applicable if atropine does not leave the receptor rapidly and acetylcholine occupies a large fraction of the receptor pool (see 70). Paton ruled out this last possibility by checking that (dose ratio -1) was a linear function of [antagonist]. From the experimental results then, he could derive a series of y' *versus* time values. Equation 16 indicates that a plot of log ($y_e' - y_t'$) against time should be linear with a slope $k_1[D] + k_2$. Finally, from an analogous procedure during washout of the atropine and a plot of log y' *versus* time (see equation 11) he could estimate k_2. The rates of onset and offset can thus be used to measure k_1 and k_2. He did this sort of experiment with hyoscine and atropine and with the antihistamine pyrilamine (mepyramine) and in all cases the ratio of k_2/k_1 from the kinetic method was in line with that obtained from an equilibrium dose ratio.

Another feature of the kinetic model is that potency of a competitive antagonist should be greater when the agent acts slowly. This is because a high turnover rate for the agonist is impossible if the antagonist does readily leave the receptor, *i.e.*, if k_2 is small. For a given k_1, a lower dissociation constant means a higher affinity for the receptor and hence, a more potent antagonist. Paton demonstrated this relationship in the series of alkyltrimethyammonium compounds n-C_n H_{2n+1} N Me_3. The more potent they were as blocking agents the more slowly were they washed out.

The other main feature of the model was the phenomeon that Paton called "fade," that is, the fall of a response to an agonist even though the concentration of the agonist is not falling. Fade might be expected intuitively from the model, since one can picture drug molecules rushing into the receptors rapidly at first when all the receptors are free and then settling down to some lower equilibrium turnover rate. Paton has compared the classical occupational model to an organ

where the tone is emitted as long as the key is depressed. The kinetic model then becomes analogous to a piano where the sound comes out only when the key is first depressed and subsequently no more sound is heard. Unfortunately, Paton's fade mechanism is not the only reason a response may fall with time (section XII), so a convincing quantitative demonstration of fade is hard to produce. Paton (70) described some suggestive experimental results, but most authors seem unconvinced. Paton and Waud (76) have subsequently indicated that fade should be hard to see anyway because (i) diffusion is usually rate-limiting, (ii) when fade is most marked (high [D]) it is also most rapid and therefore most likely to be missed, and (iii) most agonists are probably active at low concentrations (*i.e.*, $c \ll 1$, y small), at which fade is slight. A convincing demonstration of the phenomenon would be a strong argument for the kinetic model, but is probably not possible. Rejection or acceptance of the model will probably not involve the existence or absence of fade.

More recently, Rang (81) has described a series of experiments on the kinetics of action of mixtures of slow- and fast-acting competitive antagonists. If one assumes that atropine acts slowly only because it diffuses slowly into the tissue, *i.e.*, if one assumes the classical model, then if the receptors are "protected" (37) with a short-acting competitive blocking agent during equilibration with atropine, the atropine should immediately come to its equilibrium level of receptor occlusion on the washout of the fast-acting antagonist. Rang found, however, that atropine regained the receptors slowly and at a rate in excellent quantitative agreement with that to be expected if the drug-receptor reaction were rate-limiting.

It has also been suggested (75, 77, 81, 90) that the maximum of the dose response curve to a partial agonist (which requires a large fraction of the receptor pool to produce a full effect) should be depressed by a competitive blocking-agent if the probability that the latter leaves the receptors is so low that not enough receptors become free during the period of exposure to the partial agonist. The quantitative aspects of this situation have been worked out and the kinetic model seems to agree with experimental observation (81).

One might suppose that if atropine has a very low dissociation constant even acetylcholine will be unable to produce a maximal response during a brief exposure, since there will not be enough time for atropine to leave. But, as Paton and Rang (75) noted, even if atropine's dissociation rate constant is small, an appreciable fraction of receptors can still become available in a brief time.

Paton and Rang (74) measured the kinetics of uptake and washout of atropine in strips of smooth muscle from guinea pig ileum. Uptake and washout took place (i) at approximately the same rate, (ii) at a rate independent of concentration, and (iii) at a rate slower than that to be expected from the kinetics of the pharmacological effect. They discussed several possibile explanations for these discrepancies and concluded that the "receptors may transfer drug molecules to a further system of binding sites so that the overall uptake bears only an indirect relationship to receptor occupancy." The necessity of invoking such a complicated notion tends to offset the original simplicity of the kinetic model.

Several attempts have been made to examine drug kinetics in other systems.

Paton (71) examined adrenergic drugs and found no great support for a kinetic model. This particular system also exhibited a tendency for the effect of a competitive blocking agent to reach a maximum and then wear off somewhat, although the agent was still present. So this preparation will probably require taming before it will yield useful information on the present problem.

Since the rates of action of drugs at the neuromuscular junction seem to be determined by the rate of access to the receptor and not by the rate of a drug-receptor reaction (33, 106), no support for the kinetic theory can be found there.

Thron and Waud (98) investigated the rate of action of atropine on the muscarinic receptors of guinea pig atrium and again found that access was rate-limiting. Furthermore, they reinvestigated the guinea pig ileum and found the rate of action of atropine after removal of a short-acting blocking agent to be highly variable. Sometimes it acted at a rate similar to that found by Rang (81), but occasionally it acted very rapidly. Finally, they found that the rate of action of atropine could be accelerated 4-fold by pretreating the ileum with dibenamine so as to reduce the sensitivity to carbachol 10-fold. The sensitivity to atropine (in terms of a pA_x) was not altered by this treatment with dibenamine.

These results, coupled with the fact that the kinetic model seemed demonstrable only in guinea pig ileum, led Thron and the author to search for an access-limited model that would explain the kinetics seen in the ileum. Simple diffusion was not slow enough (28). The biophase model could hardly by expected to provide an exact description (see section II) and Rang (81) showed by direct calculation that it was not adequate. The most desirable explanation would involve the introduction of no unknown barriers or other structures, so they suggested that access be regarded as free diffusion through the extracellular space. The receptors would be on the surface of the cell (see 33, fig. 12) in contact with the extracellular fluid of the small space between cells. The drug would diffuse from one of these spaces to the next and so forth into the depth of the tissue. The model so far would represent free diffusion around the cells. However, if the receptors take up an appreciable amount of the drug, as the measurements of Paton and Rang suggest, the diffusing drug would fill a "virtual" extracellular space larger than the real space and consequently the rate of approach to equilibrium would be slower. This model will explain qualitatively all the features explained by the kinetic receptor model. As the capacity of the receptor uptake site is limited, it can be saturated. The amount of drug required to saturate it, and so to make the effect of receptor uptake negligible, will be a fixed *absolute* amount, and so will be a smaller *fraction* of the amount of drug administered for a weak antagonist (a drug used at high concentration) than for a potent antagonist. Thus, the observation that potent antagonists are slower than weaker ones receives an explanation. Furthermore, agonists, which seem to occupy a small proportion of the receptor pool, will not be taken up appreciably by the receptors and so will act more rapidly than antagonists. Partial agonists, which occupy a large fraction of the receptor pool, are, however, given at relatively higher concentrations and so would behave kinetically like weak antagonists; they would act reasonably rapidly.

Rang's experiments with short-acting antagonists (81) also fall in line. The tissue does not really get equilibrated with atropine, since the major uptake site for atropine, the receptor, is occupied by the short-acting antagonist. Conversely, if a short-acting antagonist is added on top of atropine, the concentration of atropine in the extracellular space will rise because large quantities of atropine leave the receptor to flood the extracellular space. Thus, the receptor will be in equilibrium with a high concentration of atropine until the excess diffuses out of the tissue. The observation of variation (98) in the rate of action of atropine after removal of the short-acting blocking agent can be attributed to variation in the geometry of individual pieces of ileum. It is easier to picture a continuous series of shapes and sizes of extracellular spaces than a continuous spectrum of receptor types.

Thron and Waud's acceleration of atropine's action with dibenamine is also easily explained. Dibenamine prevents a large fraction of the receptor pool from taking up atropine and so the atropine fills a smaller virtual space and acts faster.

Thron and Waud also pointed out that the effect of pacemaker shifts must be considered in a complete analysis of the kinetics of drug action. This was particularly apparent when working with atria, but is also relevant with ileum, since pacemaker activity seems present there (19). For example, suppose atropine is added to a spontaneously beating right atrium exposed to carbachol. The atropine will rapidly block the action of carbachol on superficial cells of the sinoatrial nodal region, and cause the pacemaker to shift out to the surface of this region. Thus, the action of atropine will appear more rapid than it otherwise would.

A similar explanation might be applied to the discrepancy between Paton and Rang's (74) kinetics of uptake and kinetics of pharmacological action of atropine. (In the ileum there need be no inherent pacemaker before the addition of the agonist. However, the agonist may create a pacemaker.) Paton and Rang used 20-second exposures for their test doses of agonist. This might produce a good response because a pacemaker is produced at the surface. The cells that are studied pharmacologically are only those on the surface while those studied with uptake measurements will include the whole tissue. The uptake will, therefore, be slower than the pharmacological action. However, without direct experimental evidence, one cannot say whether or not pacemaker activity is significant in longitudinal muscle of guinea pig ileum.

What then is the present status of the kinetic theory of drug action? In its favor are the following points:

1. It is simpler analytically than the occupational model. Differences in efficacy amount to differences in k_1 and k_2. A third drug-dependent constant is not needed.

2. It provides the first neat explanation of the differences in rates of action between agonists and antagonists and explains why the rates of action of the latter can be related inversely to potency.

3. There is excellent quantitative consistency in the kinetics of action of

antagonists. In fact, there are few examples in biology of such extensive testing of a model quantitatively with such good agreement. Since most of the experimental tests were made after the theory was advanced, the model is definitely more than *ad hoc*.

4. There is no other way known to explain a drug-specific fading response.

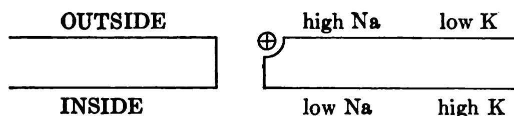
5. The kinetic theory provides an explanation for a decrease in slope of the dose-response curve to an agonist in the presence of a competitive antagonist without invoking a second receptor site (see 77, 81).

6. It provides the hope that k_1 and k_2 might be measured directly as well as k_2/k_1. This is not a criterion for acceptance of the theory, but rather an outlook on drug action that had been neglected previously.

The counterarguments may be listed as:

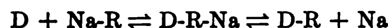
1. Although the kinetic model is simpler mathematically, it is more constrained chemically than the classical model. There is no chemical precedent. It is even difficult to find examples of ionic bonds which last longer than a few milliseconds. There are a few cases of a small molecule tucking itself into a cavity, in a large one, so that it is much less likely to be dislodged by solvent thermal motion, but such examples are rare. On the other hand, the drugs involved have high affinities compared to most reagent systems used for chemical studies, so the lack of chemical precedent is only a weak objection. With the kinetic model it is more difficult to see how the individual molecular contributions add; whereas with an occupational model one can easily picture an overall summation of molecular effects even though any given receptor may be occupied only part of the time.

Paton (70) has suggested some sort of ion exchange mechanism, but without a quantitative model. One can put such an ion exchange site at the mouth of a pore in a cell membrane



and imagine that ions cannot get through the pore if the site is occupied. Normally a sodium or potassium ion would occupy the site. If now a drug molecule comes along and actively[6] displaces the sodium or potassium ion, the potassium ion has roughly an equal probability of going in or out and the sodium ion is likely to go

[6] By "actively" I mean that the presence of drug molecules increases the probability that the sodium or potassium ion will leave. For example, the reaction might be of the nature:



$$D + Na\text{-}R \rightleftharpoons D\text{-}R\text{-}Na \rightleftharpoons D\text{-}R + Na$$

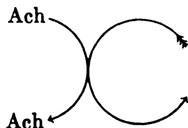
rather than

$$Na\text{-}R \rightleftharpoons Na + R$$

$$R + D \rightleftharpoons D\text{-}R$$

in. When the drug dissociates the site will be reoccupied by a sodium or a potassium ion. If it is a sodium ion it will be more likely to have come from outside while if a potassium ion, from inside. The overall result will thus be a net increase in the sodium flux inward and the potassium flux outward. This is of course what acetylcholine action seems to involve at the neuromuscular junction; chloride permeability is unaffected (94). But the model is awkward in that the drug must actively displace the ion bound to the negative site. The existence of a drug-ion-site intermediate complex seems rather unlikely. In general, it seems unwise to invoke an active displacement with molecules the size of most drugs. It is more likely that the first ion to get on the receptor leaves spontaneously and is replaced by the second. It is not actively driven off.

The kinetic model seems to imply a specific class of reaction:



That is, a cyclic process coupled to the reaction of acetylcholine with the receptor. One turn of the cycle requires one acetylcholine-receptor reaction. Alternatively, one could view the acetylcholine-receptor reaction and dissociation as analogous to an escapement mechanism in a watch, allowing a blocked cyclic process to proceed in discrete steps.

Belleau (13) considered that there is no possible physical model which could give Paton's kinetic model. But Belleau seemed to be discussing a rather limited version of the kinetic theory. He seemed to feel that the agonist would only bump into the receptor, but not react with it. Paton explicitly pictured a reaction.

Burgen (21) has estimated the rate of drug-receptor association to be expected if diffusion were rate-limiting. He used the equation of Smoluchowski (89), which is really a steady-state solution developed to deal with the coalescence of colloidal particles. The sort of reaction Paton pictures would involve a jumping on and off the receptor. It seems, however, from the calculations of Collins and Kimball (31), that Smoluchowski's solution does not give results too different from the transient solution; so Burgen's estimate is probably still of the right order. His rate constant (k_1) of 2.5×10^9 $sec^{-1}M^{-1}$ is about 1000 times that estimated by Paton for atropine. However, if this is viewed as a charge of free energy of activation, it becomes 4 kilocalories per mole, which is not a huge discrepancy.

2. Thron and Waud's (98) explanation of the relation to be expected between the rates of action of agonists and antagonists is a reasonable alternative to the kinetic model.

3. Thron and Waud's model gives a qualitative explanation of how the rates of action of antagonists should behave, but nobody has produced a quantitative agreement. The access model will be very cumbersome to solve. The excellent quantitative aspects of the kinetic model have just not been matched by any other model.

It is difficult, however, with the kinetic model to explain Thron and Waud's acceleration of the rate of action of atropine by dibenamine. One has to suggest that dibenamine by some unknown mechanism increases k_1 and k_2, but not their ratio.

Finally, the difference in rate of action of atropine and its rate of accumulation in tissue can be explained only by adding hypotheses if one uses the kinetic model whereas it fits readily with the viewpoint that access is rate-limiting.

4. Although fade cannot be explained by models other than the kinetic model, one need not discard the other models; for the phenomenon of fade has not been very convincingly demonstrated, nor, for the reasons outlined above, is it likely to be. I do not feel that the phenomenon of fade is crucial one way or the other.

5. The decrease in slope of a dose-response curve to a partial agonist in the presence of a competitive antagonist can also be explained with Thron and Waud's access-limited model. The administration of a high dose of the partial agonist simply leads to a shift of the equilibrium so that atropine leaves the receptors, but thereby increases the concentration of atropine in the small extracellular space. Therefore, the atropine appears more potent than at first expected. This will depress the dose-response curve. Thron (cited in reference 77) has in fact shown that in the limit the biophase model can give behavior expected from the kinetic model.

6. At best k_1 and k_2 seem measurable only in guinea pig ileum. Elsewhere, they always seem experimentally inaccessible.

This rather long section may be summarized by saying that the kinetic model of drug action agrees extraordinarily well with experiment. A similar degree of quantitative agreement has not been produced by any other model. Nevertheless, the absence of experimental support in systems other than the ileum, and the few discrepancies in the ileum itself lead me to feel that access to the receptor and not the drug-receptor reaction is the rate-limiting step in drug kinetics. Nevertheless, the effort involved in trying to decide this has led to a considerable reorganization of our views on drug kinetics.

A summary of kinetic factors. The rate of action of drugs may be limited by any of four stages: (i) access to the receptor; (ii) conversion of the drug from an inactive to an active form; (iii) rate of combination with the receptor; (iv) rate of production of the response. The first factor seems the most common rate-limiting step. The second is important with drugs like dibenamine that undergo a preliminary molecular rearrangement to form the active ethyleneimmonium form (100). The third appears when one considers Paton's kinetic model. The classical example of the fourth case is the slowly-contracting frog rectus abdominis muscle.

XII. DESENSITIZATION, SPARE RECEPTORS, AND SPARE RECEPTOR CAPACITY

In the equation 26 the effect of an agonist was treated as an explicit function of time as well as of occupancy y (or of rate of drug-receptor combination if one includes the possibility of a kinetic model of drug action). We return now to this aspect of drug action.

It is common knowledge that many preparations, typically the guinea pig ileum, do not give a sustained response even though the concentration of agonist is not falling. Barsoum and Gaddum (11) described one phenomenon of this sort. In the fowl rectal caecum the response to histamine was abolished by soaking the tissue in histamine, but the response to barium, acetylcholine, or adrenaline was only slightly diminished. Cantoni and Eastman (23) have reported the other such phenomenon. They found that in guinea pig ileum a large concentration of acetylcholine rendered the tissue temporarily insensitive to a subsequent dose of either acetylcholine or histamine. A large dose of histamine had the same effect. The phenomenon of Barsoum and Gaddum (11) may be called "specific desensitization" and that of Cantoni and Eastman "nonspecific desensitization" (45). I know no explanation for the former. Paton's kinetic model with its "fade" is no help, since the rates involved are of the wrong magnitude (see 95). Specific desensitization can be distinguished from nonspecific only if drugs acting on two different receptors exist for the system in question. If only one class of agent is available, as for example at the neuromuscular junction, one cannot distinguish the two possibilities rigorously. Elaborate receptor models have been developed (57) to explain desensitization at the neuromuscular junction without any evidence to rule out a nonspecific type of desensitization. A nonspecific desensitization is somewhat easier to explain and seems a more general phenomenon and so will be discussed (i) in connection with the idea of spare receptors and (ii) with regard to possible explanations.

Nonspecific desensitization can probably be regarded as a form of "fatigue" in the simple sense. It seems reasonable that a piece of smooth muscle might not be able to maintain a sustained contraction even though receptor stimulation does not decrease. The existence of desensitization thus is equivalent to there being some stage between receptor and effect that is the weakest link in the whole process. It must be past the receptor, since otherwise acetylcholine would not produce desensitization to histamine. Then, if desensitization is occurring, the receptor stage is not the weak link in the chain. The phenomenon of "spare receptors"[7] becomes an expression of the existence of desensitization (70). Paton (70) preferred the term "spare receptor capacity" to imply that one is dealing not so much with an excess of receptors as with a limitation elsewhere. Although this term was introduced in connection with the kinetic theory, in which one could in principle increase receptor activation indefinitely by increasing agonist concentration and thereby increasing turnover at the receptor, the change of viewpoint is useful with the classical approach as well. It focuses attention on the postreceptor stages rather than on the receptors, which are not responsible for the phenomenon. As Paton (70) has pointed out, a nonspecific desensitization probably occurs from the moment an agonist starts to act. For this reason, the use of the time course of a response to measure "fade" is very difficult.

[7] The term spare receptors means simply that the agonist need only react with a small fraction (say 1%) of the receptors to produce a full response. It does not imply there are two reception pools which can be identified before addition of the agonist—a small pool to react with the agonist and a larger one kept in reserve.

Nickerson (68) tried to demonstrate the existence of spare receptors by showing that a maximal response could still be obtained after an exposure to an irreversible competitive blocking-agent such that an appreciable degree of block was obtained (dose ratio about 30). He interpreted this to mean that only a small fraction of the receptor pool was necessary for the production of a maximal response. However, he administered dibenamine for only 10 minutes. Suppose not all the cells in the preparation are needed to lift the lever maximally. It is then quite possible that he blocked superficial cells, but not deep ones and that he was examining not spare receptors, but spare cells. As already noted in connection with equations 22 and 28, the existence of spare receptors seems reasonable *a priori*. However, a direct experimental demonstration is still needed. A similar objection might be raised when irreversible competitive blocking agents are used to determine the equilibrium dissociation constant of an agonist. Furchgott and Bursztyn (41) have, however, introduced an internal check in this case by using a partial agonist and thereby getting an independent estimate. So, too, did Mackay (61).

May *et al.* (64) have attempted to show experimentally that there are no "spare receptors." They reported similar rates for the recovery of the response to both agonists and partial agonists after washout of a short-acting, irreversible, competitive antagonist. But this situation would be described by the model of equation 33 with y_I of the form y_I^0 exp-kt. This rate constant should be applicable to all agonists or partial agonists, as they observed. There is no reason, assuming spare receptors or not, to expect a difference between the rates of recovery of agonists and partial agonists.

Many explanations have appeared for desensitization, and, by implication, for spare receptors. None seems particularly convincing. The first explanation, that of Straub (92), was that the agonist accumulated inside the cell. He postulated that the action of the agonist was related to its flux across the cell membrane, which in turn was related to the driving force; hence he called his model a "Potentialsvergiftung" theory. After a while the agonist accumulated inside the cell, and the gradient disappeared and with it the effect. It was difficult to see the relationship between the transmembrane flux and the response, a quantitative model never appeared, and this theory quietly faded away. Barsoum and Gaddum (11) proposed no explanation for their observations; they were interested primarily in using specific desensitization to get selectivity in an assay. Cantoni and Eastman (23) suggested that the energy supply of the muscle might be run down by a large contraction. This seems possible and should be regarded as one factor present whenever a contractile response is studied. However, there are many drug effects where another explanation may be necessary, particularly when the effect is an electrical response of a membrane. Desensitization at the neuromuscular junction falls in this category. Katz and Thesleff (57) have studied this case and proposed an elaborate cyclic receptor process to explain events. They implicitly assumed that they were considering a receptor phenomenon; yet everything they saw could result from changes in events following receptor activation. I expect that

their results will become explicable at one of these later stages, so that the cumbersome receptor model will not be necessary.

Nastuk (67) also suggested a change at the receptor level. He did at least imply some suggestion of specificity with respect to choice of drug. He referred to experiments (66, cited in 67) (the actual results are not available apparently) showing more rapid kinetics of desensitization with phenyltrimethylammonium than with tetramethylammonium ions. His specific explanation is a form of receptor saturation that somehow leads to a decrease in permeability of the membrane. Both he and I are at a loss to explain the slow rates observed. Bloom and Goldman (17) explained desensitization with a model in which the receptor is an enzyme. But, since receptors in general are probably not enzymes, this model is of limited use.

Taylor (95) suggested that desensitization may be related to uptake of drugs by the cell (without implying a Potentialsvergiftung mechanism of action). However, the observation by del Castillo and Katz (33, fig. 16) that intracellular application of acetylcholine was without effect on the response to extracellularly applied acetylcholine argues against Taylor's suggestion (see also 67).

I suspect that there will be an appreciable contribution to desensitization from a running down of the ionic gradients that exist across the membrane. The ionic fluxes brought about by drug action are such as to abolish the concentration gradients driving them. One can expect an effect of unstirred layers (see 32) both inside and outside the membrane. A quantitative evaluation of the magnitude of the effect to be expected will be awkward, but should be possible.

There is no reason to believe that desensitization is just one process. Even in one system there may be more than one mechanism. For example, at the neuromuscular junction the fast desensitization described by Katz and Thesleff (57) is probably the result of a different mechanism from that responsible for the slower desensitization studied by Jenden *et al.* (55), Thesleff (96) and Nastuk *et al.* (67).

XIII. A NOTE ON METHODS

While a useful theory should not be limited to one experimental system, even a general model must not be misinterpreted nor misapplied. In the receptor field as in others there are "tricks of the trade" some of which are worth explicit mention.

The most important factor seems to be choice of tissue for study. Isolated organ systems seem essential. It is hard enough to produce quantitative results with an isolated muscle without complicating the system by introducing uncontrolled effects from other organs, *etc.* The particular choice of system seems to be determined by the stability one can obtain. For example, the British School likes the guinea pig ileum, Furchgott has tamed the aortic strip, Blinks (15) uses isolated atria effectively, *etc.* Certainly it is important to work with any tissue extensively enough to know as many of its properties as possible. There is an advantage to keeping the preparations as simple as possible. Paton and Rang (74) used thin strips of longitudinal muscle removed from the guinea pig ileum.

This gives a preparation where diffusional delays are much reduced. The introduction by del Castillo and Katz (34) of the motor endplate as a pharmacological test system allows one to eliminate the stages of excitation-contraction coupling and of contraction, since the response recorded can be the depolarization of the membrane. One can then also examine one cell and avoid cell-to-cell variations. I prefer (106) to work with just a few cells of such a preparation so that connective-tissue barriers are minimal.

Not only is choice of tissue important, but also good experimental control. Reproducibility of results is essential to getting results accurate enough to be useful in distinguishing different mechanisms. Boura *et al.* (18) and Schild (86) revolutionized techniques by automating drug administration. This reduced tremendously the variation due to irregularities in manual administration and made possible a much higher precision in experiments.

While we are on techniques, a word on levers is in order. When Paton (70) introduced his rate theory he prefaced the paper with an aside on what lever he used to record the contractions of guinea pig ileum. Unfortunately, this digression has led to a considerable amount of distraction. For example, Ariëns (2, p. 193), confusing a specific with a nonspecific desensitization, suggested that fade may be simulated by a heavy lever, and implied that fade is an artifact produced by choice of lever. The arguments for a kinetic model of drug action have nothing to do with choice of lever. Paton's original point, and I find it well taken, is that use of a light isotonic lever will produce a steep dose-response curve. This is why the light isotonic lever is preferred in biological assays. The steep curve results because little contraction will occur until a force great enough to lift the lever develops. Then, because the lever is isotonic, little additional force is required to take it to the top of its travel. But if you are studying the relationship between contraction and effect it is preferable to work over as wide a range of concentrations as possible. This can be done by measuring force rather than shortening, since the former has no limit whereas a muscle can only shorten so much. An isometric strain-gage transducer would be ideal. Lacking this, Paton resorted to an auxotonic lever.

Dose-response curves can be obtained several ways. The easiest is to increase drug concentration cumulatively and note the response at each step. Or the drug can be given in increasing doses with a washout after each dose. Or the drug can be given in single doses with some arbitrary standard response(s) interposed so that each response is preceded by a standard response (10). Or finally, one may randomize the order of doses (85). In many cases any method will do. In general, however, the more information one wants to extract from a dose-response curve the more sensitive the experiment will be to the manner in which the dose-response curve is done. For example, nonspecific desensitization can be expected to be greater by the time the top of a cumulative dose-response curve is reached, than if the maximal dose were given to a fresh preparation. Schild's (85) careful use of randomization of dose administration is worth copying.

When comparing responses to drugs in two situations (*e.g.*, before and after a competitive antagonist) one could compare (i) responses to the same dose or (ii)

doses to produce the same response. It should be clear from the foregoing discussion that the second type of comparison is by far the more meaningful (47, 70, 87, 90).

Paton (70) has been particularly careful in this regard and during his kinetic measurements not only has aimed at a null response, but has tried to keep all responses about the same height so that shifting levels of desensitization cannot influence the result.

XIV. THE CHEMICAL NATURE OF THE RECEPTOR

The structure of receptors is unknown. A considerable volume of literature deals with estimates based on the nature of drugs which are active. This has been reviewed extensively by many people and need not be taken up again here [Gill's review (48) appeals best to me]. It seems likely that receptors will turn out to be compounds of high molecular weight, probably protein, and probably rather similar to enzymes or antibodies. Direct chemical methods are not yet applicable. The nearest approaches have been studies of model systems, indirect chemical approaches, and attempts at receptor isolation.

Model systems. Burgen *et al.* (22) have demonstrated directly with nuclear magnetic resonance techniques that quaternary ammonium compounds bind at the quaternary end to antibodies specific for quaternary compounds. Enzymes are also used as models for receptors and a considerable amount of the fund of knowledge built up about enzymes can be carried over to receptors. There is, however, no reason to believe that generally receptors are enzymes. In particular, it is hard to see how tetramethylammonium ion could stimulate muscarinic receptors if it had to be split first. The nature of binding to enzymes and to receptors is probably similar, as is general structure. In these respects, enzymes are probably reasonable models for receptors. Del Castillo *et al.* (35) have developed a protein-coated lipid film membrane that changes its impedance with addition of drugs. This seems a promising system for study of possible mechanisms involved in the translation of receptor activation into an observable response. Beckett *et al.* (12) described another model. They used micelles of alkyl betaines as structures with polar and nonpolar regions, and studied the effect of exposure to drug molecules on the refractive index of the betaine preparation.

Indirect chemical approaches. The measurement of dissociation constants of agonists and antagonists fits in this category. This has been described above. Burgen (20) has gone one step further and has estimated the magnitude of the ionic contribution to binding of quaternary compounds to muscarinic receptors. He preferred to estimate relative affinity constants from dose-response curves rather than from experiments with irreversible blocking agents. However, since he was interested only in relative values, and since he had parallel dose-response curves, his approach is reasonable. He concluded that the cationic head of the drug comes about 3.3 Å from the negative charge of the receptor site.

Schild (88) has attempted to characterize muscarinic receptors in smooth muscle by techniques that have been found useful in enzymology (29). He suggested that an imidazole group may be an active part of the receptor.

Receptor isolation. Chagas (24) and Ehrenpreis (36) claimed to have isolated receptor material, but in both cases the substance obtained did not behave appropriately in the long run. Paton and Rang (74) in their study of absolute concentration of receptors in tissue obtained such a low density of receptors that, as they pointed out, receptor isolation is going to be a very difficult job.

The general approach, outlined years ago by Gaddum (44), will be similar to that used to get at the active site of enzymes. One can protect the receptor with a specific competitive blocking agent, while reacting a covalently-bonding nonradioactive compound with nonreceptor uptake sites. Then these two drugs could be washed out and the tissue exposed to a radioactively-labeled solution of the irreversible blocking agent. This would put a covalently-bound label on the receptor to aid in tracking it through subsequent tissue fractionation procedures. This is just the principle; the actual experiment will require very careful attention to quantitative aspects and a convincing isolation of a receptor preparation will be very difficult [see difficulties described by Moran *et al.* (65)].

Even identification of the product as receptor will be difficult. One could compare binding constants with those obtained from pharmacological studies, but the fragment isolated will have to be big enough to possess all the binding properties of the receptor *in situ*. Since the receptor normally may lie in a rather steep electrical gradient, it will be difficult to examine an isolated material in an environment that is physiological. The most interesting property of the receptor, the ability to lead to a grossly observable effect, will in all probability not be demonstrable, since it depends on integrity of a structure that is huge on the molecular scale.

XV. MISCELLANEOUS RECEPTOR MODELS

I have tried to outline the mainstream of thought on receptors. Occasionally, alternative models have been proposed. I mentioned two in section XII (Potentialsvergiftung, and Katz and Thesleff's model to explain desensitization). Another was proposed by Janku and Mandl (54). They approached the drug-receptor reaction from a statistical point of view and derived an expression, which they regarded as a generalization, which included both the occupational and kinetic models as special cases. The model has no chemical basis and so seems little more than an interesting academic exercise. Mackay (59) proposed what he called a "flux-carrier hypothesis" of drug action. This was an elaborate specific model. Recent experiments by Paton and Rang (74) seem to have ruled it out.

Gaddum (42) suggested that the shape of the dose-response curve was an expression of a log-normal distribution of sensitivities of responding units in the tissue. This model does not get at the reason for the distribution, and so just rephrases the problem. It is of use in some special cases, however. For example, the height of twitch elicited in a skeletal muscle by maximal nerve stimulation will involve the statistical element contributed by a variation in electrical threshold of individual muscle fibers. The nature of the reaction of a drug like tubocurarine with the receptors is then not the only determinant of the degree of neuromuscular block to be expected.

Paton and Rang (75, p. 78) have distinguished between two types of kinetic theory. They point out that the agonist may dissociate fast and therefore stimulate, or alternatively, may dissociate fast because it stimulates. That is, the action of the agonist on the receptor might lead to a change in the receptor so that the agonist is much more likely to dissociate. They call this second case the "dissociation theory." This model will account for the differences in the rates of action of agonists and antagonists but will not require that partial agonists give a fading response because stimulation will be proportional to receptor occupancy which rises monotonically with time. As with the original kinetic theory different chemical models become of interest. The authors discussed this point.

Ariëns *et al.* (5) have considered the possibility of a threshold of receptor activation below which no response is seen.

Ariëns (3) pointed out that some antagonists resemble the agonist (*e.g.*, propranolol) whereas others, like atropine, are considerably different chemically. Therefore, the receptor for atropine will not be identical with that for acetylcholine. This observation carries with it the possibility of finding an inhibitor of an inhibitor.

Belleau (13) preferred to equate receptors with enzymes. I see no *a priori* reason to do so.

In general, the attitude I have taken in this review has been to focus on models that: (i) seem reasonable from a chemical, physiological, and anatomical point of view; (ii) are the simplest possible in terms of principles involved, although often the exact mathematical description is otherwise; and (iii) are not just specific examples, often experimentally untestable, of more general theories.

I have also restricted the discussions to the interactions of drugs at one receptor. It is hard enough to sort things out in the simplest of cases. I believe one is rarely in a position to justify considerations of more complex cases. For similar reasons I find it unwise glibly to invoke such phenomena as allosteric effects as *ad hoc* explanations of experimental observations. Certainly, the extra degrees of freedom obtained by adding extra facets to the receptor model will make it easier to get a model compatible with experimental results, but such schemes have little predictive value.

There is a tendency nowadays to emphasize the mathematical apparatus used to describe receptors. In fact, discussions of the specific algebra involved have appeared (*e.g.*, reference 60), with the implication that there is a special mathematics applicable to receptor studies. There is not. Certainly, one would find it difficult to describe models without mathematics, but the analysis is secondary. The particular description used in this review is not unique, but was chosen because it has been convenient. Another person in another context might use a different approach. One hopes, however, that both of us would be asking the same biological or chemical questions.

XVI. FUTURE AVENUES OF RESEARCH

Besides continuation of several present lines such as estimation of affinity constants of agonist-receptor reactions and extension of systems studied to include structures in the brain, the major problems to be attacked seem to be

isolation and chemical characterization of the receptor, elucidation of the nature of the processes in the stages between receptor activation and effect, and a related problem—the characterization of desensitization. Specific desensitization also remains to be explained.

In general, one can expect a trend from the measurement of empirical parameters like pA_x, to the measurement of more fundamental ones like free energy changes or actual chemical structure of the receptor. This last goal is still quite a way off. At present, we still do not even know the structure of many important drugs. Therefore, an empirical description of events can be expected to be useful for quite some time in much the same way that the Hodgkin-Huxley model of the action potential serves in nerve physiology.

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