MODERN CONCEPTS IN RELATIONSHIP BETWEEN STRUCTURE AND BIOLOGICAL ACTIVITY[1]

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One of the pharmacological triumphs of the nineteenth century was the demonstration that various pure chemicals are effective drugs. The resulting belief that the "virtues" of drugs reside in their chemical properties raised the problem of trying to relate effects which particular drugs might be expected to produce at molecular level with the effects which these drugs are observed to produce at cellular or even higher levels of biological organization (1–5).

Now, although it is evoked by chemicals, pharmacological activity is not something which can be described precisely in chemical terms. The responses with which the pharmacologist is ordinarily concerned take such forms as changes in muscle tone, inhibition of bacterial growth, or relief of pain. He has therefore good reason to fear that relationships between the actions of drugs at molecular level and the responses which he actually measures are too distant to be significant.

Finding that several closely related compounds produce similar pharmacological effects when they are tested under comparable conditions does not necessarily mean that they have similar actions at molecular level, because biological preparations characteristically respond to chemical or to physical stimulation in a limited number of ways. Many examples are known of drugs which produce similar effects by different mechanisms. Of course, it is possible to reduce the risk of making false deductions from structure-activity relationships by working with very simple preparations. Unfortunately, the pharmacologist must be concerned with *all* the chemical processes in which a drug will be involved when it is used therapeutically. Processes occurring at sites of action will be considered first merely because they have attracted more attention than, say, processes affecting the distribution or the clearance of drugs.

Sites of Action

A few types of drug have actions which can be readily explained in terms of their chemical properties; e.g., antacids, absorbents, astringents, demulcents, lubricants, bulk purgatives, osmotic diuretics, plasma expanders, "chemical" antidotes. With most drugs, however, it is far from obvious why the chemical properties of the compound should confer strong pharmacological activity. Such knowledge as we possess has been obtained for the most

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part through the study of structure-activity relationships. These relationships may indicate which (physico-) chemical properties are chiefly responsible for the pharmacological activity of a drug. This knowledge may in turn provide a hint as to the mode of action of the drug at cellular level.

Types of structural specificity.—Acetylcholine, morphine, proflavine, oxophenarsine, and ether form one of the many sets of drugs which could be chosen to illustrate how the importance of structural specificity varies according to the prototype.

The study of acetylcholine analogues shows that the structural requirements for possession of strong muscarinic activity are exacting (6–10). The analogue must possess a small cationic head, it should preferably have an atom similar in position and reactivity to an ester oxygen of acetylcholine, and it must match the acetylcholine molecule closely in size.

For strong morphine-like activity the structural requirements are also exacting, but they have proved much harder to discern (11–14). Not many compounds possess analgesic activity of the morphine type, yet those that do are not remarkably alike chemically—at least so long as one merely compares 2-dimensional representations of their structures. Nevertheless, the study of their conformations (i.e., their most likely 3-dimensional structures) indicates that there are indeed moderately specific requirements for possession of strong morphine-like activity.

With oxophenarsine, on the other hand, the only obvious feature held in common by its pharmacological analogues is an arsenic or similar atom capable of reacting with sulphydryl groups (15, 16). Presumably, because of the strength of the As—S bond, other bonds between drug and tissue receptors are relatively unimportant.

Proflavine, too, has many pharmacological analogues of diverse structure. However, all of those which possess strong bactericidal activity are highly basic and have large, flat molecules (17, 18). The inactivity of chemical analogues which do not ionize freely suggests that antiseptics of the proflavine group combine with tissue anionic groups. Since the cation must be fairly large, it seems that the Coulombic attraction between the drug cation and an anionic receptor must be supplemented by substantial van der Waals forces if the drug is to remain attached to the receptor with sufficient firmness to exert a persistent blocking action (18–23).

Pharmacological analogues of ether differ so much from one to another in chemical structure that it has long been believed that a common basis for their anaesthetic activity must be sought in "physical" as distinct from structural attributes (24, 25, 26). Assuming that such agents as carbon tetrachloride, nitrous oxide, and xenon all act in the same way as ether almost rules out the possibility of the drug acting by forming a hydrogen bond or stronger bond with any tissue component (25, 26). However, there are other ways in which the chemical constitution of anaesthetic agents could determine their pharmacological activity. Even though chemically unreactive, the molecules of the drug might be able to interfere with biochemical actions by

such means as clogging up pathways through cell membranes or by impeding the passage of ions through water in the immediate vicinity of cells by "iceberg" formation (q.v.).

Drug acceptors and receptors.—To explain some of the types of structural specificity just referred to is difficult unless we infer that there are "drug receptors" which bear much the same relationship to certain drugs as do locks to the corresponding keys. Some of the best evidence for the existence of drug receptors has been obtained by comparing the effects of stereoisomers (12, 27, 28, 29). It has been found with morphine and numerous other drugs for which optical isomers exist that these isomers may differ strikingly in potency. Since optical isomers have identical properties except insofar as their molecules are mirror images, we are led to suppose that the shape of the drug molecule is important in these cases because part of the drug must fit a structure complementary to it. This idea is implicit in the definition given by Schueler (2): "The drug receptor is in general a pattern R of forces of diverse origin forming a part of some biological system and having roughly the same dimensions as a certain pattern M of forces presented by the drug molecule such that between patterns M and R a relationship of complementarity for interaction exists."

Most pharmacologists would probably agree with Schueler that (*a*) the dimensions of R do not necessarily correspond to those of the drug molecule as a whole; (b) some variation is possible in the chemical groups which make up to the pharmacophore M; and (*c*) the whole range of chemical bonding must be considered when one analyzes a pattern. Perhaps strictly complementary structures are induced in some cases by the initial interaction between drug and receptor (30, 31).

Unfortunately, the term "drug receptor" does not have this connotation for all pharmacologists. Recent symposia (32, 33, 34) have emphasized major differences of opinion. As Schild pointed out at one of these meetings, there are two main schools of thought. Whereas some authorities follow Ehrlich in regarding a receptor primarily as a means of fixing a drug and focus their attention on the chemical means of attachment, others follow Langley in regarding a receptor as something in (or on) a cell which makes it respond to a drug. For yet other pharmacologists, a drug receptor is evidently not just a simple sidechain, such as a thiol or an anionic group, or even so complex a constellation of groups as that envisaged by Belleau (35, 36) as making up an adrenaline receptor or by Beckett (12, 37) as an "analgesic receptor"; it is the whole biochemical mechanism causing the response.

Judging by the views expressed at a Ciba symposium (33) chemical components of living material which can bind drugs but which come into the category of "storage sites" or "sites of loss" (described in later section), as distinct from "sites of action," are not to be regarded as drug receptors. The term *drug acceptor* has been suggested (23; cf. 38, 39, 41) to describe any type of binding site. Thus, all accessible thiol groups would be drug acceptors for an arsenical drug. A *drug receptor* differs from a drug acceptor in being neces-

sarily a site of action; i.e., a focus for the initiation of a pharmacological response. One other qualification seems necessary, if a strong body of opinion is to be respected; namely, that a drug receptor consists not only of a drug acceptor (which provides the minimum pattern of forces necessary for interaction with the drug pharmacophore) but also of all those neighbouring atoms or groups which play a considerable part in binding the drug or in restricting its access to the acceptor.

The need for a clear terminology becomes evident when one considers the different ways in which drugs could affect a receptor.

Drug-receptor interaction.—It is helpful to consider this primarily with respect to acetylcholine and its chemical analogues, because of the variety of ways in which useful effects can be brought about by actions involving acetylcholine receptors. Fewer types of drug-receptor interactions need be considered in elucidating most other structure-activity relationships.

As already mentioned, the structural requirements for possession of strong muscarinic activity are exacting. However, compounds which fail to meet these requirements are not all completely inactive. Some of them resemble acetylcholine in possessing nicotinic activity (6, 7, 42–46). The simplest explanation for the fact that modifying the structure of acetylcholine does not affect muscarinic and nicotinic activity equally is that the receptors at which these actions are initiated are not precisely similar. One type of receptor might be so shaped that access to its 'acceptor' portion is difficult or impossible for some chemical analogues of acetylcholine though not for acetylcholine itself. Another possibility is that differences between acetylcholine receptors reside in their acceptors. Nor can the possibility be ignored that different responses might be initiated by activation of just the one type of receptor. However, the fact that not all actions of acetylcholine are blocked by compounds, such as dibutoline and atropine, which resemble it sufficiently closely in chemical structure to suggest that they have the same pharmacophore (47–50) points to differences in the receptors themselves. This topic is discussed in greater detail elsewhere (pp. 21–50).

In contrast to the *agonists*, which have a direct excitatory action on cholinergic receptors, there are some compounds which produce acetylcholine-like effects mainly or wholly by actions exerted at other sites. The best-known of these *synergists* are the anticholinesterases. Occupation of sites of loss (q.v.) provides another mechanism by which the direct actions of acetylcholine could be simulated. The idea that some pharmacological analogues of acetylcholine might act at least partly by liberating acetylcholine from storage sites through the process of base exchange has also found some support (51, 52).

The properties of *partial agonists* have come to light mainly through the study of drugs belonging to homologous series. Swan & White (50) have discovered some excellent examples of a transition from muscarinic to atropinic activity resulting from the addition of methylene groups to acetylcholine-like molecules. In between homologues which act almost exclusively as stimulants

and those which act almost exclusively as depressants are found homologues with an obviously dual action. The initial effect of these partial agonists is to excite, but stimulation yields to depression under conditions in which more than a small concentration of the drug is maintained at the site of action. The simplest explanation for this phenomenon is that the drugs which elicit it stimulate by a quantal process: whereas each act of combination between drug and receptor causes stimulation, the continued occupation of the receptor by the drug causes depression (by preventing further acts of combination). According to this view (53), the stronger the binding of a homologue to a receptor, the less pronounced should be its stimulant action and the more pronounced its blocking action. We can thus explain why homologues which would be expected to have an "affinity" for particular receptors differ in what Ariëns (54, 55) calls their "intrinsic activity," and in what Stephenson (56) calls their "efficacy."

Many of the best acetylcholine antagonists are compounds which have not only functional groups corresponding closely to those of the acetylcholine pharmacophore but also large hydrocarbon groups so placed that they are likely to assist rather than impair the binding of the molecule to acetylcholine receptors. These features seem necessary for possession of strong and fairly specific atropine-like activity (47–50). However, less structural specificity is needed for antagonism of nicotinic than of muscarinic actions of acetylcholine.

Studies on the inhibition of cholinesterases have suggested reasons for this.

There is now good evidence that the active sites of cholinesterases "consist of two subsites, an anionic site which binds and orients substituted ammonium ions and an esteratic site containing an essential acidic and basic group, which binds ester (and similar) functions and reacts further in the hydrolytic process" (57, 58). Even simple substituted ammonium ions may have a significant affinity for the anionic site. Nevertheless they are much weaker inhibitors of the enzyme than are compounds, like physostigmine, which have an affinity for both subsites (58, 59, 60). Similarly, many esters have an affinity for the esteratic site but most of them are only feeble anticholinesterases. A compound with an affinity for only one subsite can be a powerful anticholinesterase, however, if it is able to form a strong bond there. Thus, some organic phosphates act as "irreversible anticholinesterases" because they can form a covalent bond at the esteratic site (58, 61).

It is reasonable to suppose that similar phenomena occur at sites other than cholinesterases. Hence we can assume that an effective *direct antagonist* of acetylcholine need not present virtually the same pattern of forces to the acceptor as does acetylcholine: the acceptor could be satisfactorily blockaded by a drug with an affinity for just part of it, provided that the binding is strong enough. For example, a second cationic group in the molecule might help to anchor it by combining with an anionic acceptor other than the anionic subsite of an acetylcholine receptor, as shown schematically in Fig. 1.

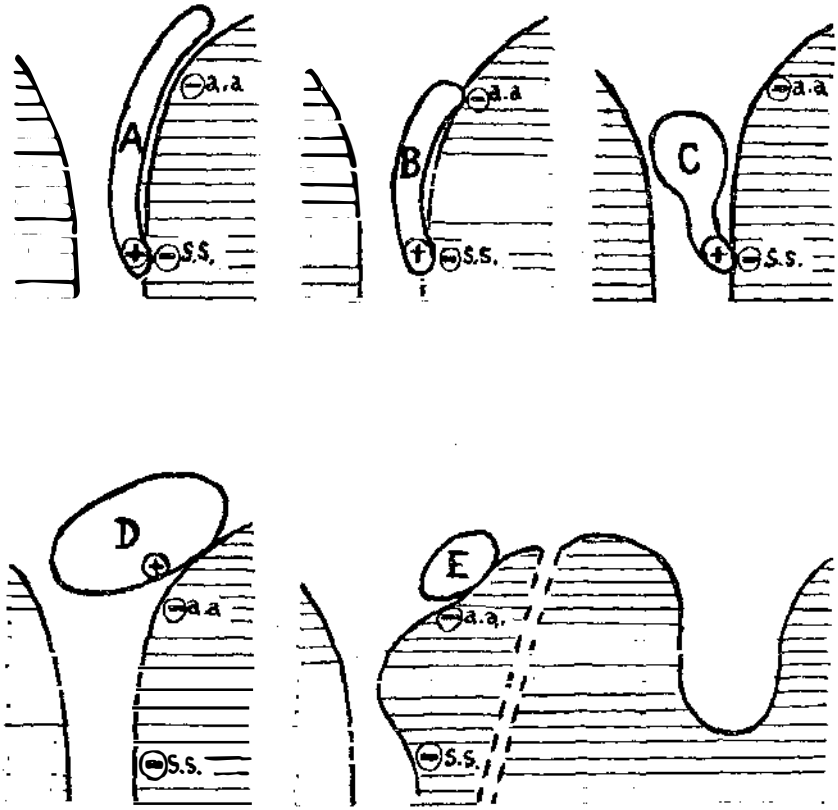

FIG. 1. Different ways in which the action of such a drug as acetylcholine might be antagonized. It is assumed that the receptor lies in a pore or a crevice and that it contains an anionic subsite (s.s.).

A molecule like A is envisaged as exerting a blocking action because of two factors which would make its attachment to part of the receptor relatively persistent, viz., the Coulombic attraction between its cationic 'head' and the anionic subsite, and the substantial van der Waals attraction between the atoms of its large hydrocarbon 'tail' and those of the cell surface.

The presence of another anionic acceptor (a.a.) would enable a dibasic molecule to form two strong bonds with atoms around the receptor, provided that it could (like B) readily adopt a conformation which enables both cationic groups to come close to anionic acceptors.

The molecules, C and D, are effective blockers because they are bulky and hydrophobic. In contrast to C, molecule D is not assumed to come close to the anionic subsite of the receptor, it could be partly moored by another anionic group.

The type of blocking action represented by E is still more remote from the receptor. This could be inactivated either because it is made inaccessible to the agonist or because an action exerted elsewhere on the cell makes stimulation of the receptor ineffective.

It is difficult to explain otherwise why ganglion-blocking activity and curare-like activity reach sharp peaks in the ascent of homologous series of bis-onium compounds but not in the ascent of corresponding series of mono-onium compounds (6, 62, 63, 65; cf. 66). Admittedly, compounds like chlorisondamine (Ecolid) possess strong ganglion-blocking activity although their two quaternary nitrogens are joined by a much shorter hydrocarbon chain than are those of hexamethonium and other potent bis-onium compounds, the two cationic groups of which are identical (6, 67, 68). One of the two cationic groups of drugs of the chlorisondamine type is much larger than the other. Perhaps the large group augments the activity conferred by the small group by acting as an "umbrella" and repelling molecules of the agonist partly by virtue of its charge (67, 68).

It has been suggested (69–72) that the two main groups of curare-like drugs be called "leptocurares" and "pachycurares" according as to whether their molecules are slender or bulky. It seems significant that the pachycurares do not depolarize skeletal muscle in the course of producing neuromuscular blockade. If the acetylcholine acceptors lay in pores or in crevices (48, 73), access to them could be prevented by a bulky molecule lodged in the orifice as indicated in Figure 1. According to Waser (74), the pores obstructed by the pachycurares might be those through which alkali ions pass.

Still other mechanisms of blockade can be envisaged. Just as it is much easier to jam a lock than to turn it with a substitute for the real key, so it is probably much easier to inactivate a receptor than to activate it. Studies performed with cholinesterases and other enzymes have revealed various mechanisms by which an enzyme can be inhibited by compounds which do not resemble either substrate or product. Thus, the activity of the enzyme might be inhibited by interference with the action of a coenzyme or of a metal activator. No doubt there are various types of compound which can function as *indirect antagonists* of acetylcholine by virtue of actions exerted at sites other than acetylcholine acceptors.

Fortunately, the complexities of drug-receptor interaction are of much less importance in most of the other fields of pharmacology. In chemotherapy, for instance, specific inhibitors of metabolic processes are of vastly greater practical importance than specific stimulants. The concept of the *antimetabolite* (75, 76) is essentially similar to that of the "direct antagonist." Likewise, compounds which cause *therapeutic interference* (18, 77, 78) or which act as antidotes in the same way as nalorphine (79, 80) can be compared to "partial agonists."

Sites of Loss

When Ehrlich began his studies with organic arsenicals, he had good reason to doubt whether he could obtain a drug which was much more toxic to treponemes than to human beings. As Albert (18) has pointed out in his monograph on the subject, the notion of *selective toxicity* is one for which there existed little experimental support before this century. Even

in agriculture, where differences between the crop to be protected and the weed or parasite that one wants to destroy may be pronounced, finding suitable agents for the purpose is a recent accomplishment. Ehrlich's fear that there would be competition for the drug molecules between the parasite's and the host's receptors found experimental support when Eagle (15, 16) showed conclusively that arsenical drugs act by combining with tissue sulphydryl groups, because at least forty different enzymes are known to contain thiol groups. It is likely that arsenical drugs have an affinity for at least some of these different sulphydryl groups under conditions in which they are used therapeutically. The difficulty of obtaining chemotherapeutic agents with a satisfactory *therapeutic index* can be readily explained along these lines.

Similar ideas have developed in other fields of pharmacology. For example, it now appears that the receptors for such alkaloids as morphine, quinidine and cocaine (81, 82) are much less specific in their structural requirements than was formerly believed. Since the first alkaloids to receive much attention were those isolated from plant products which were used for specific therapeutic purposes, it is not surprising that most of the early studies were along narrow lines and therefore preserved false impressions about specificity of action. So did studies made with compounds into which an alkaloid could be readily transformed, because such chemical operations as alkylation or acetylation are likely to involve the chief functional groups of the molecule. The investigation of synthetic analogues of alkaloids in which these groups are preserved has given a very different impression. They have shown, moreover, that nitrogenous bases have typically a wide spectrum of pharmacological activity (81, 82). Chlorpromazine is far from being the only base which could aptly have been called "Largactil" on account of the large number of its pharmacological actions. Now, if we conclude that specificity of action can seldom be explained satisfactorily by supposing that a high degree of structural complementarity is required to permit interaction between drug and receptor, how else can we explain why certain nitrogenous bases have fairly narrow pharmacological spectra? The width of the pharmacological spectra of numerous other nitrogenous bases suggests an answer. Perhaps some structural features of a good drug are important, not so much because they aid interaction with particular receptors, as because they preclude interaction with numerous other receptors. It could be argued, for instance, that an analgesic of the morphine type might be able to compete with an adrenaline-like molecule only at those receptors which are so shaped that they can accommodate the bulky group which, in all potent analgesics (12, 14, 37) lies outside the plane of the aralkylamine portion of the molecule.

Finding that typical drugs have several sites of action leads us to suppose that they may be taken up also at various sites where they do not produce demonstrable pharmacological effects. This concept of sites of loss has

been ably developed by Veldstra (40). Amongst hitherto puzzling findings which it enables us to explain are certain synergistic effects. Thus it has been observed that the response to a fixed dose of morphine may be enhanced in experimental animals by giving one of a number of bases which are themselves devoid of analgesic activity. If we suppose that these bases are able to compete with morphine for numerous sites of loss, then we can infer that in their presence less of a fixed dose of morphine is taken up by sites of loss, and more by analgesic receptors. The brevity of the anaesthetic effect of a moderate intravenous dose of a thiobarbiturate has been explained along similar lines: it can be attributed mainly to a redistribution of the drug between the central nervous system, which gets a relatively high concentration of the drug initially because of its good blood supply, and other parts of the body which can act as storage depots (83, 84).

The Receptor Environment

Qualitative differences in the pharmacological properties of compounds which are closely related chemically can be largely accounted for, as just indicated, by supposing that the compounds may differ (*a*) in *affinity* for drug receptors which are not all of the one type, and (*b*) in the *efficacy* of their interaction with particular receptors. To account for quantitative differences, we have to consider especially the *availability* of the drug at the sites where its action is required. The amount of drug present at these sites will be less at any time than the amount administered. It may be very much less if the drug does not pass readily through body membranes, if it is highly vulnerable to enzymatic attack, if it is easily excreted, or if it has an affinity for numerous sites of loss.

One aspect of the problem of availability which has not received due attention is the relationship between the concentration of a drug in the immediate vicinity of its receptors and that in the surrounding medium.

Ferguson (85) clarified ideas on the subject when he tackled the problem of explaining quantitatively why compounds like ether, chloroform, and nitrous oxide differ in anaesthetic potency although no structural specificity is involved. He noted that the various "physical" properties with which anaesthetic potency had previously been correlated all depend upon the distribution of the drug between two phases. Now it is clear that the phase in which the drug is administered (e.g., inspired air, the bloodstream) must in some cases at least be different from that in which it acts. Ferguson called the latter the *biophase*, a term which makes no assumption about sites of action other than that they are situated in a different phase from that in which the drugs are administered. In general, the concentration of a drug will not be the same in the two phases. However, at equilibrium the concentration in each phase will be such that the tendency for the drug to escape from each phase is the same. *Chemical potential* is a measure of this tendency to escape. It is directly related to thermodynamic activity, and can be esti-

mated approximately if the degree of saturation can be ascertained for one phase. Ferguson showed by calculation from published data that, for numerous sets of chemically related compounds, there was usually a good correlation between intensity of effect and estimated chemical potential. Similar findings have since been reported by other investigators (86–91). The Ferguson Rule suggests, though it certainly does not prove, that the potency of compounds which produce anaesthesia and allied phenomena ("narcosis") depends on their ability to reach the appropriate biophase. The Ferguson Rule does not explain narcosis at molecular level; it merely indicates why some narcotics are more potent than others.

Acceptance of Ferguson's views has been hindered by erroneous impressions about the composition of biophases. These are not necessarily restricted to the interior of cells. The limits of a phase are determined by thermodynamic criteria as distinct from chemical composition. Featherstone & Muehlbaecher (26) have rightly emphasized in their review that lengthy arguments about whether anesthesia occurs in lipid, water, or protein are nugatory. As they point out: "The modern concept of cellular membranes is based largely on monomolecular layers of cholesterol and phospholipids interspersed between areas of protein and water. If an anaesthetic molecule occupies the area between a lipid monolayer and a hydrated protein molecule, is it 'dissolved' in lipid or is it 'interacting' with protein? At the level of monomolecular layers, such discussions become absurd. Nowhere in the body, with the possible exception of depot fat and glycogen storage granules, does any biological material exist in pure form."

It has long been recognized that such ions as Na^+ and H^+ do not exist in aqueous solution as the bare ions implied by their conventional symbols but as hydrated ions. The inner sheath of water molecules is strongly oriented by such ions. However, the outer sheath has a much less definite structure; water molecules in it are distributed even more randomly than they would be in a pure aqueous phase. It has been deduced more recently from thermodynamic data that various unionized molecules also can affect the degree of randomness in orientation of nearby water molecules. Some solutes decrease the degree of randomness—in other words, they impart structure to nearby water molecules. These form a so-called "iceberg" or even a clathrate (26, 92, 93). Proteins, too, are believed to take on an icelike lattice in aqueous solution. Klotz (94) envisages the nonpolar side chains in proteins inducing "a crystalline, cagelike, arrangement of hydration water, with the added possibilities of long-range cooperative effects due to the presence of many such side-chains bound to the frame of the protein molecule."

Now, it follows from the Ferguson Rule that the compounds to which it applies are equipotent in a biophase. Mullins (25) has concluded that narcosis to a given degree occurs when a constant fraction of the total volume of some nonaqueous phase in the cell is occupied by narcotic molecules. He supposes that, if these molecules are small enough, they can easily fit into a lattice and occlude a certain amount of free space. The partial occlusion of

channels in a membrane might, by decreasing permeability to agents of physiological importance, depress cell function. We can imagine that, just as bystanders in a narrow street slow busy pedestrians, so the molecules of a narcotic might interfere with biochemical reactions by clogging intramolecular channels.

Rang (90) agrees that it is difficult to formulate any other mechanism by which the mere presence of inert molecules in a cell can depress its activity, although his results did not agree well with predictions made according to Mullins' hypothesis. Perhaps, as he acknowledges, the predictions were based on doubtful assumptions. Miller (92) and Pauling (93) think it significant that water molecules in the vicinity of a narcotic molecule are likely to be in a more ordered state than elsewhere because they believe that the resulting "icebergs" (Miller) or "clathrates" (Pauling) will have some sort of clogging effect.

Since Mullins' hypothesis implies that ability to produce narcosis should be restricted to molecules which are small enough to fit into a lattice, it is of interest that Wulf & Featherstone (95) have noted a correlation between anaesthetic potency and molecular volume (as estimated for volatile anaesthetics from the magnitude of the van der Waals correction factors). Unfortunately, as Feathersone & Muehlbaecher (26) point out in their review, such properties as molecular volume, polarizability, lipid solubility, and adsorptability at an interface are interdependent, all being reflexions of the intermolecular van der Waals forces. To get crucial evidence, we must adopt a different approach. Rang (90) suggests using a monolayer of orientated protein molecules as a model. It has already been demonstrated by Gershfeld & Shanes (96) that monolayers of stearic acid are affected by drugs at pharmacological concentrations and "exhibit responses and antagonisms that are sufficiently distinctive to suggest interactions related to biological effects." Again, it might be profitable to compare the molecular volumes of typical depressants with those of closely related compounds which act as convulsants. The existence of compounds which appear to act as central stimulants amongst such groups as the barbiturates, glutarimides, and ureas has not been given the attention it deserves.

It is reasonable to suppose that the Ferguson Rule operates whether the site of action of a drug is within a cell or on the cell surface. Because of their relative inability to form hydrogen bonds with water molecules, nonpolar substituents tend to be driven from the aqueous phase and to combine with the nonpolar groups of surface macromolecules (20, 21, 22, 57). The composite van der Waals forces (which involve the drug and nearby solvent molecules as well as the drug and the surface) can be substantial providing that the drug molecule is both large and able to make close contact with the surface—for these forces operate effectively only over very short distances.

With drugs which contain pharmacophores no less than with those which exert a structurally nonspecific action, we would expect molecules consisting mainly of groups which have much less affinity for water molecules than

these have for one another to be extruded from a bulk aqueous phase and to accumulate at a surface. Studies with homologous series are informative. Ing (97) has pointed out that, although the pharmacological properties of homologous series change in a bewildering variety of ways as the series are ascended, several main types of change may be discerned. The simplest is that in which molar potency increases as $1:n:n^2:n^3\ldots$. In other words, the molar concentration needed to produce a given intensity of effect is decreased by a factor, n. The value for n varies over a remarkably narrow range for different homologous series; it is usually about 3. This is what we would expect if the geometrical increase in potency up to the cut-off is due to an increasingly favourable distribution of the homologue between the phase in which it is administered and the biophase (23, 98). The existence of a *cut-off*, the position of which within a given series depends upon the sensitivity of the test objects, provides further support for Ferguson's theory (87, 97). As Ferguson himself noted, the geometrical increase in potency observed when certain homologous series are ascended is usually distinctly less than the decrease in solubility in the aqueous phase. Hence compounds will eventually be reached which are not sufficiently soluble in the aqueous phase for them to be transported in adequate amount to particular biophases. At which homologue the cut-off occurs will depend upon the sensitivity of the test object, for the less sensitive the test object the greater will be the drug concentration needed in the bulk phase to ensure an adequate concentration in the biophase.

Absorption and Clearance

Studies dealing with the mechanisms by which drugs cross body membranes are reviewed elsewhere (pp. 69–84). Of more direct concern to this review are the chemical features of drugs which are well absorbed.

Many of the compounds which readily penetrate body membranes of different types are lipid-soluble. Amongst lipid-soluble compounds of similar structure, the most rapidly absorbed are those with the greatest lipid-to-water partition coefficients. Their speed of penetration is directly proportional to the concentration gradient, as would be expected of a simple diffusion process (98).

Some lipid-insoluble compounds can cross body membranes. In a recent review of the problem, Schanker (98) has concluded, like most others before him, that the existing data can best be explained by supposing that typical body membranes behave not only as lipid barriers but also as fine sieves. The *passive transfer* of lipid-insoluble compounds across membranes does not readily occur unless the compounds have small molecules.

It is of special interest, since many of the drugs in common use are either weak bases or weak acids, that ionization of a molecule usually impairs its ability to penetrate membranes. Brodie, Shore and their Bethesda colleagues (56–98, 99, 100) have obtained much evidence to support the idea that the absorbability of a drug depends in part upon the proportion of

molecules which are unionized at the pH of the medium in contact with the membrane.

The passage of certain lipid-insoluble drugs across membranes cannot be explained at present without postulating *specialized transport*, notably when the drug moves across the membrane against an electrochemical potential gradient (98, 101) Particular interest has been taken in mechanisms for transferring such compounds from plasma to urine (98, 102, 103). It seems that some "carriers" at least resemble drug receptors in that they have an affinity for compounds of a certain type. The value of this idea is shown by the development of drugs, like probenecid (102), which can be used to interfere with the specialized transport of other compounds having an affinity for the same carrier.

Drug metabolism.—As already stressed, little of a drug will reach the sites where its action is wanted if it has an affinity for numerous sites of loss or if it cannot easily penetrate body membranes. The availability of a drug will be curtailed also if the compound is rapidly cleared from the body. Although some drugs are eliminated largely or entirely by renal excretion (e.g., hexamethonium, mandelic acid) or by excretion through lungs (e.g., volatile anaesthetics), most of them are inactivated chiefly by biochemical modification (104, 105).

As drug metabolism is the subject of a separate review (pp. 85–114), only some of its features need concern us here. It is fortunate that few chemical pathways—and not many more enzymes—seem to be involved (104), because that makes it easier to anticipate what will be the metabolic fate of a new compound. Despite species differences and the liability of numerous compounds to be vulnerable to more than one type of enzymatic attack, existing knowledge has sometimes been exploited successfully in the development of new drugs.

Procainamide is a good example. This compound was synthesized and tested in the hope that it would have cardiac actions which are similar to, but more persistent than, those of procaine. The basis for this hope, which was soon vindicated (106), was the knowledge that amides are much less susceptible than the corresponding esters to enzymatic hydrolysis. Similarly, a choline ester like methacholine has a more persistent action than acetylcholine because it is much less vulnerable to cholinesterases. On the other hand, the very vulnerability of succinylcholine to cholinesterases is advantageous therapeutically in that the effect of this drug is seldom required for long.

One stratagem which is being used with increasing success is *drug biotransformation*—also known as *drug latentiation*. Harper (107) defines this as "the chemical modification of a biologically active compound to form a new compound which upon *in vivo* enzymatic attack will liberate the parent compound." The chemical alterations of the active agent are such that giving the modified compound results in a more useful distribution of the active agent in the body than could be achieved by giving the modified drug itself;

and the modified drug of this type can be called a *pro-drug*, a term introduced by Albert (18) to define a substance which is converted after its administration into the actual substance which combines with the receptors. Several compounds have had extensive clinical use before it was realized that they are actually pro-drugs; e.g., castor oil, senna, chloral hydrate, phenacetin, oxophenarsine, proguanil. However, a few pro-drugs have been introduced as such; e.g., hexamine, certain steroid esters, succinyl-sulphathiazole, diethyldithiolisophthalate (107).

Drug biotransformation is well illustrated by the use of the α-carboxylate of 5-hydroxytryptamine or of dopamine in experiments in which one wants to get a high concentration of the amine within the central nervous system. Whereas the blood-brain-barrier is almost impermeable to the amine, it can be readily passed by the amino acid. Enzymatic decarboxylation of the amino acid in the brain yields the amine. Harper (107) suggests several ways in which suitable transport forms for drugs might be found. Thus, if the active compound possesses a hydroxyl group, this might be phosphorylated or esterified with an organic acid.

Certain compounds owe their usefulness as pro-drugs to the fact that they can be subjected in the kidney, though not in the blood or tissue fluids, to a pH sufficiently low to liberate at least some of the active agent. Hexamine is a classic example: the rationale of giving it is that enough formaldehyde may be formed from it in the kidneys to have a useful antibacterial effect. Diuretic organo-mercurials may be pro-drugs. Weiner, Levy & Mudge (108) have recently obtained strong experimental support for the view that the diuretic response to certain organic mercurials is due to the intrarenal release of mercuric ions. Only a minute fraction of the pro-drug need be broken down to provide an adequate concentration of mercuric ions. None of the acid stable organo-mercurials tested by Weiner et al. were found to be diuretics, and all the active diuretics were found to be acid-labile.

Vigorous attempts are now being made to find suitable pro-drugs for biological alkylating agents (109, 110). The search for drugs to treat cancer may be regarded—perhaps wrongly—as a problem in selective toxicity. One hopes to discover compounds which can kill tumour cells when they are given in amounts which do not injure other rapidly proliferating cells. It is vital to obtain a complete kill, since the body's defence mechanisms cannot dispose of surviving cancer cells in the way that they can dispose of the small proportion of microorganisms likely to survive drug therapy. One approach to the problem is to try to improve upon drugs which are known to be partly selective in their toxicity. Biological alkylating agents come into this category. These are compounds, such as β-haloethylamines and epoxides, which react with certain chemicals of physiological importance through displacing a hydrogen atom by an organic radical. Their functional groups are so reactive that covalent bond formation is the primary mode of attachment to biological receptors. It seems that biological alkylating agents prevent the multiplication of cells by reacting with deoxyribonucleic acid

(109). Thus alkylating agents with two suitably spaced functional groups might be able to form a bridge between the guanine residues in two opposing strands of DNA and so prevent their separation (111). The use of prodrugs might enable suitable alkylating agents to reach this site without too much of them being lost by participation in unwanted actions. Several investigators are now trying to find compounds which do not become transformed into alkylating agents until they reach tumour tissue.

Drug Design

Since it is often far more important to judge what new compounds will do to patients than what they will do to "receptors," the pharmacologist faces a dilemma. Intensity and specificity of action, acute and chronic toxicity, and convenience of administration: these and yet other properties may all have to be assessed. The more nearly his experiments mirror the clinical situation, the more likely are they to yield results of immediate worth—and the less likely to provide data which throw any light on structure-activity relationships. No one will dispute the urgency of the problem of discovering good new drugs, and many must have a sneaking admiration for the frank empiricist who, like General Nathan Hale, believes in "getting there firstest with the mostest." It is indisputable that submitting a multitude of compounds to a battery of tests does occasionally lead to the discovery of valuable drugs. Unfortunately, this approach seldom provides much insight into structure-activity relationships. Most of the published data are of extremely limited application. It is only by giving attention to comparatively simple problems that we shall discover broad concepts. Admittedly, progress to date has been disappointing, but for this we must blame the ineptness of many of our experiments no less than the complexity of biological material of any kind. To get useful answers, we must frame reasonable questions.

We can scarcely hope to solve the general problem of deciding on the basis of its chemical structure whether a new compound will be a good drug; but we may before long be able to decide in many cases whether the compound is capable of activating (or deactivating) a particular receptor, whether it will have an unspecific action, whether much of the compound will reach its site of action when it is taken by mouth, whether it will be readily metabolized, and so on. Progress along these lines would make the search for new drugs far less the grossly inefficient and expensive task that it is at present.

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