THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND PHARMACOLOGICAL ACTIVITY[1]

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In the broadest sense, all pharmacological studies contribute to the body of knowledge which empirically relates structure and pharmacological action. The methodical compilation of information of this sort is of much value, providing a chemical basis for interpreting the action of known drugs as well as guiding the conception of new ones. It seems to us, however, that the study of structure-activity relationships should attempt to explain as well as correlate; and that such studies are the more notable when they contribute insight into mechanism of action at the molecular level. In this way, the structure-activity relationship becomes a probe with which the chemical nature of the actual biological structures involved in drug action may be explored. The descriptive aspect of molecular biology—the formulation of the three-dimensional structures of biologically important molecules and their active sites—has made striking advances in the past 10 years, and it seemed particularly timely to emphasize in this review some of the attempts that have recently been made to utilize the study of structure-activity relationships in adding to this knowledge.

Most attempts to visualize the receptor in molecular terms are based on the assumption that small molecules whose biological action is specific and structure-dependent display a high degree of molecular complimentarity toward the site at which they act. Viewed in this light, the interaction of a pharmacological agent with its receptor is analogous to the interaction of a substrate with the active site of an enzyme or of a hapten with an antibody. The pharmacodynamic situation is unusually complicated, however, by the presence of manifold physical barriers and sites of loss which intervene between the administered drug and its ultimate site of action. It seems clear that attempts to interpret biological activity in terms of structure in complex biological systems must always take into account drug dynamics—absorption, distribution, metabolic destruction, and excretion—as well as the probability of a low dependence of certain of these phenomena on structural factors. Undoubtedly, the analysis of many structure-activity relationships is limited by a lack of information that allows a distinction to be made between structural effects at the site of action and the dynamic phenomena that control drug concentration at that site.

It has long been recognized that lipid solubility and ionization profoundly affect the ability of small molecules to cross biological membranes

[1] The survey of the literature pertaining to this review was concluded in August 1961.

rapidly and to localize in body tissues. Contemporary reviews of this problem have served to re-emphasize the significance of these properties. Although such physicochemical characteristics are less influenced by structural detail than the factors governing drug-receptor interactions, they are, nevertheless, ultimately dependent on chemical structure. We have therefore deemed it appropriate to discuss the influence of chemical structure on physical properties and drug dynamics in this review.

The wide-ranging approach implicit in any general review of structure-activity research requires that no single aspect of the problem be considered in great depth, nor can all of the original publications related to the subject be included, or even found and read! We have attempted to select examples that illustrate general points about structure-activity research. In keeping with the concept of an annual review, applicable publications from the current literature have been sought out and used for this purpose as often as possible.[2]

Structural Parameters Pertinent to Biological Interactions of Drugs

Size, shape, and electron distribution.—Pharmacologists and medicinal chemists, indeed most people actively associated with drug research, tend to have a well-developed intuition for sensing structural similarities among biologically-active molecules. The receptor theory of drug action implies that a high degree of molecular complimentarity is required for biological specificity, and derives strong support from the frequency with which structural relatives are found to manifest similar biological activities. Gross resemblances in shape and functionality are, in fact, such a consistent guide to drug design that it is the exception among contemporary structure-activity studies that fails to produce useful new examples of this phenomenon. The now classical approach to correlation of biological activity with constitution, which emphasizes part-structure similarities among molecules, is usually referred to rather flexibly as isosterism, a concept whose historical development has recently been traced in an excellent review by Schatz (7). This approach, as applied to compounds of biological significance, is primarily based on an empirical codification of structural elements which, by virtue of steric and electronic similarities, have often proven to be interchangeable. However, past utilization of this correlative device has too often been superficial, failing to capitalize on the possibilities of more profound interpretation offered by the refined state of present-day knowledge of molecular structure. A vivid example of how the effectiveness of isostere analysis can be enhanced by application of modern structural theory has been provided by Belleau in his recognition that adrenergic blocking agents of the dibenamine group are indeed isosteric with phenethylamine (8). This

[2] The general topic of chemical constitution and biological activity has been the subject of a number of recent reviews (1 to 6).

belated observation has subsequently proven to be of immense value in gaining an understanding of the nature of the adrenergic stimulatory receptor (see discussion in the final section). Even in those aspects of isostere analysis which appear to be quite straightforward, such as the estimation of molecular sizes and shapes, it is imperative that correlations with biological activity afford full recognition to subtle structural differences. An example involving the effect of intramolecular interactions on shape is offered by the planar formulas shown for promethazine and promazine (Fig. 1). These formulae hardly encourage the belief that the phenothiazine nucleus in promazine is essentially flat, while in promethazine it is appreciably curved. However, examination of Fisher-Hirschfelder-Taylor models reveals that in promethazine the tendency to achieve maximum resonance stabilization through coplanarity of its benzene rings is counteracted by steric repulsion

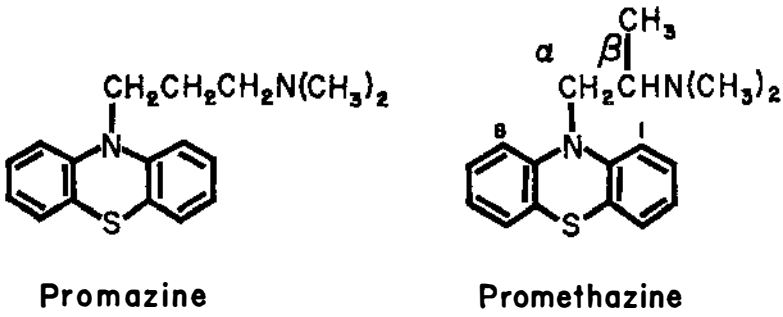


Fig. 1. Planar formulas for promazine and promethazine.

between the methyl grouping on the β-carbon of the side chain and the 1,8-*peri* hydrogens. That this effect is indeed great enough to result in curvature of the tricyclic nucleus has recently been implied by the finding that promethazine occupies an interfacial area of 46 Å^2 under great surface pressures in contrast to the 66 to 70 Å^2 area occupied under similar experimental conditions by a coplanar phenothiazine nucleus such as the one in promazine [Villalonga, Fried & Izquierdo (9)]. That changes in molecular shape of this order of magnitude may have appreciable biological significance is suggested by the consistent emergence of pronounced autonomic blocking properties among phenothiazine derivatives whose side-chains are characterized by β-methyl branching. The introduction of a β-methyl group into the side-chain of the tranquilizer promazine affords trimeprazine, a homologue whose antihistaminic and anticholinergic properties assume proportions of therapeutic significance in contrast to those of the parent tranquilizer molecule (10). Analogous observations by Harms (11) reveal that the skewing effect induced by *o*-alkyl substituents in the diphenhydramine series also markedly increases the anti-acetylcholine activity.

Another case where recognition of subtle factors of shape and function-

ality has led to a more profound understanding of structure-activity relationships involves the stilbesterol family of synthetic estrogens, where it has long been emphasized that maximum potency stems principally from an interoxygen distance of 14.5 Å (12). Recently it has become apparent that considerations of shape and functionality are fully as significant in determining estrogen activity. The relationships involved have been usefully reanalyzed in terms of molecular thickness (13), degree of skewness from coplanarity of the phenyl nuclei (14), and localization of electron distribution as it derives from these structural variations (15).

Non-rigid molecules, such as those containing aliphatic chains, usually present the greatest difficulty in determinations of molecular size and shape. This is a direct result of the large number of widely-differing conformations that such flexible systems can assume in space. Molecular models are of some use here in that they do help to visualize limiting permissible structures. However, they offer relatively little aid in evaluating other important considerations such as the barriers to internal rotation about single bonds (16). Among the more promising approaches to the problem of characterizing the size and shape of aliphatic systems, an outstanding example is the successful correlation by Gill (17) of ganglionic blocking activity with interionic distances in bisquaternary salts through calculation of probability distributions, including in the calculations such factors as the energy barriers to internal rotation about single bonds and the repulsion between two terminal charges of like polarity. As suggested by Ing (18), this approach may be applicable to other drug classes whose structures consist of ionic or polar groups separated by an alkylene chain. Cavallito & Gray (19), in a valuable review of the chemical nature and pharmacological actions of quaternary ammonium compounds, make the suggestion that "chelate-like" ion-pair forms, involving both ammonium cations with a single anionic site, might operate to shorten the probable interionic distances below those calculated by Gill.

A different approach to the analysis of flexible structures, probably of wider applicability, is exemplified by an analysis of the conformation of serotonin and its receptor in the isolated ventricle of the clam, *Venus mercenaria* (20). Thus study exploits the relationship in biological specificity between tryptamine congeners with their flexible aliphatic side-chains and a conformationally more rigid molecule (lysergic acid diethylamide) to develop a detailed three-dimensional map of the conformation of receptor-bound serotonin.

The usefulness of conformational analysis in determining molecular size and shape has become widely appreciated in chemistry during the last decade (21, 22). The method is based on the recognition that flexible molecules tend to exist in preferred conformations as a direct result of strong repulsion between nonbonded atoms at small interatomic distances. This effect, which in linear chains creates a barrier to internal rotation about single bonds, is particularly amenable to analysis in the case of cyclohexane derivatives. In

cyclohexanes, two types of bonds emanate from the ring carbon atoms. Those parallel to the approximate plane of the ring are called equatorial, and those perpendicular to the plane are termed axial. Bonds of the latter type are particularly subject to steric factors because of their closeness to one another. In particular, 1,3-diaxial substituents larger than hydrogen will suffer appreciable repulsion, thereby enhancing any tendency of a flexible ring to assume an alternative conformation which places the bulkiest substituents in a less crowded equatorial conformation. Since a high predictability for such events has now been established through numerous chemical studies, it has become feasible to utilize this knowledge in analyzing the shape of biologically important molecules, and from this information to obtain accurate measures of critical interatomic distances. Such an approach to the

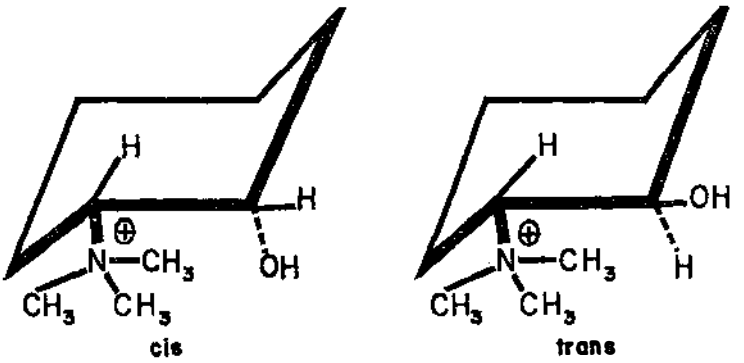


Fig. 2. Favored conformations of *cis*- and *trans*-2-hydroxy cyclohexyltrimethyl-ammonium ions.

use of conformational analysis in developing structure-activity relationships is inherent in the attempts of Friess and his collaborators to map the fine structure of various cholinergic receptor surfaces (23). They have utilized alicyclic analogues of choline and acetylcholine in isomeric pairs which allow accurate measurement of the distance between functional centers (Fig. 2). The resultant correlations of size and configuration with activity as acetylcholinesterase inhibitors or depolarizing agents at the neuromuscular junction have led to some interesting conclusions regarding the nature of the receptor surfaces involved. By use of similar methods, Thomas (24) designed a series of quaternary ammonium spiran compounds of known configuration whose anti-acetylcholinesterase activity could be readily measured. The resultant structure-activity correlation supports the interesting postulate that the electrostatic binding between the anionic site of acetylcholinesterase and quaternary ammonium compounds involves primarily the positive charge on the α-carbon atoms of the inhibitor, rather than the formal positive charge on nitrogen, as commonly believed. Much of

the current interest in muscarine and its analogues, as mapping agents for the cholinergic receptor (25 to 29), stems from the greater predictability of size and shape in these cyclic molecules as compared to acetylcholine itself.[3]

In essence, all major contributions to intermolecular binding strength derive from localizations of electrical charge. Functional groups involving atoms other than carbon or hydrogen usually serve to accomplish this variable distribution of electrons. It follows that a thorough assessment of the functional characteristics of any molecule must be a dominant consideration in approaching structure-activity relationships. In a small but important group of pharmacological agents, some functional groups are so reactive that covalent bond formation is the primary mode of attachment to biological receptors (31). Examples of this type of drug include the β-haloalkylamine adrenergic blocking agents (32), the numerous alkylating species of cancer chemotherapy (33, 34, 35), certain acetylcholinesterase inhibitors that carbamylate or phosphorylate the receptor protein (36, 37), and the classical arsenoxide trypanocides (38). Other functional groups associated with covalent bonding to receptor sites include diazoketones (39), epoxides, and ethylenimines.

More frequently, however, the involvement of molecular functionality is through various types of noncovalent bonding. Under biological conditions the strongest of these is certainly the coulombic attraction of oppositely-charged ionic centers. A number of other less obvious types of bonding are not only permissible in biological situations, but indeed must be invoked to explain satisfactorily much of the specificity of drug-receptor interactions. The hydrogen bond (40, 41) is clearly one of these. This type of interaction involves the partial sharing of a hydrogen atom between two electronegative atoms, Although the energy content of a single hydrogen bond is rather modest (5 to 10 kcal/mole), the sizeable effect of hydrogen bonding on the stability of the protein α-helix and the nucleotide pairing of DNA are examples of the major contribution that can be made when large numbers of bonds are involved. The importance of hydrogen bonds in biological interactions at surfaces is enhanced by the relatively small activation energy involved in their formation. In biological systems, hydrogen bonding usually involves amino, hydroxyl, carboxyl, carboxamide, or sulfhydryl groups.

The chelation of metals by drug molecules involves participation by many of the same functional groups that interact in hydrogen bonding. The importance of this type of bonding in biological interactions has been stressed in recent reviews (42, 43). From a structural point of view, additional emphasis on the importance of metal chelation in modifying covalent bond

[3] Koshland (30) has pointed out that the manner in which drug and receptor combine "may include a dynamic interaction in which the small molecule induces a change in the protein conformation as a necessary part of the specificity
To the extent that such an interaction also results in structural modification of the inducer molecule it would, of course, serve to limit the accuracy of any conformational analysis applied to the drug involved.

strength and in effecting the transport of drugs across membranes is probably warranted.

London-van der Waals dispersion forces are less familar types of interactions which can be induced between similar or identical structural elements of molecules that are electronically inert (44). These bonds derive their energy from the pairing of dipoles momentarily induced by perturbations in the electron clouds of molecules in close proximity. Significant only as short-range forces, the strength of which is inversely proportional to the seventh power of the distance of separation, the obvious importance of these bonds is in determining biological specificity through closeness of fit. This type of bonding is particularly significant in highly conjugated aromatic systems, wherein large oscillations of the electron capsule can be induced by small excitation energies. Indeed, the frequent presence of aromatic rings in drug molecules probably reflects in some measure the excellent London dispersion bonding that can be mediated through such systems. The hydrophobic nature (45) of the structural elements involved is another factor which enhances the significance of London dispersion bonding in biological situations.

Any analysis of molecular interactions must, of course, recognize that repulsive forces are equally important to those of attraction. Repulsion effects derive from nonbonded group interactions which may be either steric or electronic in character, when closeness of approach begins to involve compression of the atomic radii. This type of repulsion, along with the interaction through space of groups bearing a like charge, underlie the common occurrence of poor bonding to receptor sites. Modern organic chemistry has generated numerous examples of the significant influence of steric interactions upon reactivity and stability. Some of these are illustrated in the examples of ionization below.

Among drug molecules, certainly the amino group knows no peer in the remarkable variety of binding involvement which can result from subtle changes in its functionality. Among numerous available examples of this phenomenon, several are provided by the recent recognition that secondary and tertiary amines varying by only a single N-methyl substituent often display markedly different, even opposing, central nervous system properties (46). Particularly noteworthy are the essential lack of stimulatory properties in N,N-dimethylamphetamine as opposed to methamphetamine, and the diametrically opposite effects, presumably exerted at the same receptor site, of imipramine (antidepressant) and desmethylimipramine (depressant) (47). If one considers the various effects brought about by removal of one lower alkyl substituent from nitrogen, the list turns out to be a rather long one. Obviously, methyl group removal brings about a substantial change in the effective bulk around the cationic head of the conjugate acids of these drugs. This factor will be reflected in the strength of electrostatic interaction with anionic sites on the receptor surface, since the smaller secondary amines are much more likely to achieve the closeness of approach required for

complete charge neutralization or ion-pair formation. Other factors profoundly affected by the removal of an alkyl substituent would include the degree of charge dispersion from nitrogen to neighboring carbon atoms, which appears significant in binding (24), and the degree of hydration, with its related effects on base strength, effective bulk, and ability to penetrate lipophilic barriers (48, 49). In addition, secondary amines show an enhanced tendency to form hydrogen bonds intramolecularly with nucleophilic centers, thereby modifying the overall molecular configuration.

By analogy with the requirement for a small cationic head in muscarinic acetylcholine analogues, one might expect that the decrease of effective bulk achieved in going from a tertiary amine to a secondary amine would be primarily responsible for the emergence of stimulatory activity. An analogous explanation of adrenergic stimulatory responses has been offered previously [Belleau (50)].

A new physical technique for gaining insight into the nature of the functional groups which actually participate in bond formation has been provided by Jardetsky (51) and his colleagues. They have recently observed that high-resolution nuclear magnetic resonance spectra can be used to determine the chemical groups preferentially stabilized by molecular complex formation in solution. This method uses the observation that restriction of the thermal motion of a chemical group shortens the relaxation time of the associated nuclear spins and broadens the nuclear magnetic resonance peak of that group. Measurements of this sort applied to protein binding of antibiotics have suggested that the β-lactam carbonyl of penicillin and the guanidino grouping in streptomycin are bonded to serum albumin (52). Similarly, the interaction of epinephrine with adenosine triphosphate in aqueous solution has been visualized as involving the side-chain hydroxyl group through hydrogen bonding along with the expected ionic bond between the ammonium cation and the phosphate anion. The absence of an interaction involving the catechol moiety of epinephrine is perhaps not surprising considering the unphysiological nature of the medium in which the spectral studies were conducted (53).

Although the contribution of functional groups is rarely underestimated in analyzing biological interactions, a less discerning appreciation has been shown for the significance of molecular bonding which arises from localized electron density, not associated with discrete functional substituents. The case for the importance of this phenomenon in determining the biological actions of drug molecules has been persuasively argued by Szent-Györgyi (55). Since molecular diagrams, which are, in effect, detailed "road maps" of electron density for a given molecule, have now been calculated for a variety of biologically important molecules by the Pullmans and others, it has become increasingly feasible to analyze problems ranging from the mechanism of chlorpromazine action (56, 57) to the process of carcinogenesis (58, 59, 60) in terms of the electrical nature of the molecules involved.

As an example of the insight to be gained from detailed considerations of

localized electron density, one can point to the observation that the charge distribution of the indole nucleus has an unexpectedly high electron density about the C_2-C_3 double bond (61). This phenomenon was not predicted from molecular orbital calculations, but its reality and significance have been demonstrated through experiments involving charge-transfer complex formation (62). This finding brings to the fore the attractive possibility that the apparently paradoxical structure-activity relationships found among important electron donor drug families, such as those involving serotonin

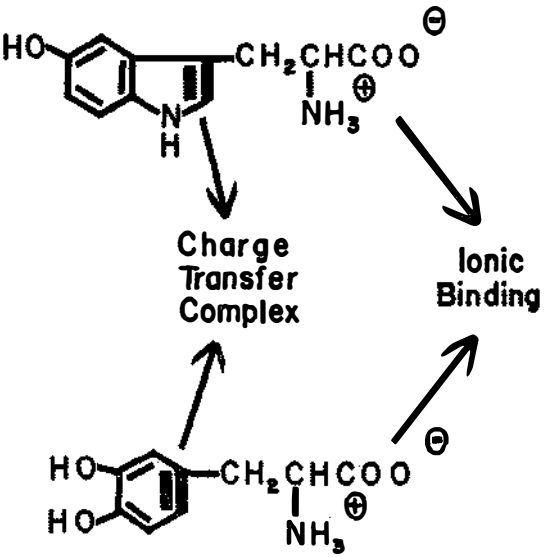


Fig. 3. Binding of 5-hydroxytryptophan and dihydroxyphenylalanine to aromatic amino acid decarboxylase.

and chlorpromazine, may be better interpreted at the level of localized charge density than in terms of more obvious gross structural features. For example, the interchangeability of 5-hydroxytryptophan and dihydroxy phenylalanine as satisfactory substrates for aromatic amino acid decarboxylase (63) seems more readily comprehensible in terms of ring binding via localized electron density than by virtue of any gross functional resemblance of the catechol and indole systems (Fig. 3).

Structure and physical properties.—Certain biological properties of organic compounds have been found to be relatively insensitive to chemical structure, providing certain physicochemical criteria are met. The most generally pertinent of these parameters are the ionization of weak organic acids and bases and the relative affinity of organic compounds for lipids, compared to water. A surprising number of pharmacologically active compounds are organic acids or bases capable of partial ionization in the physiological pH

range. This behavior is of great importance because the ionized member of the pair, by virtue of its electrical charge, possesses physical and chemical properties which differ strikingly from those of its uncharged conjugate form. The role of ionization as a factor in drug absorption and distribution will be mentioned below. In addition to this highly important consideration, however, there are numerous other biological effects of drugs which can be most simply interpreted as a function of ionization properties. In a recent review. Albert (5) cites more than 20 examples, drawn chiefly from the study of drug effects on microorganisms, in which it has been possible to deduce from ionization behavior and the effects of pH variation that either the unionized or ionized form of the compounds in question is responsible for the biological activity measured. Additional cases are cited in an earlier review by the same author (64). Examples of direct effects of ionization on pharmacological activity in higher animals have also been reported. In a recent study of the action of procaine on the turtle heart *in vitro*, Baird & Hardman (65) deduced that the stimulation threshold and prolongation of conduction time are directly related to the concentration of the cationic form of the drug; negative inotropic activity appeared to be closely correlated with the concentration of unionized procaine. Consistent with these findings, a quaternary derivative of procaine, procaine ethochloride, was found to resemble procaine in its actions on threshold and conduction time, but lacked the negative inotropic effects entirely. In another experiment with turtle heart tissue, Hardman, Moore & Lum (66) showed that the negative inotropic activity induced by pentobarbital is directly proportional to the concentration of unionized drug in the bath fluid. Since the activity of the heart remained constant over a wide pH range, the authors concluded that the receptor, if ionizable, is probably intracellular. In this case, the concentration of unionized drug is believed to influence activity by its mass action effect on diffusion to an intracellular site of action, presumably through a membrane with lipoidal properties. As evidenced by many subsequent examples, the nondiffusibility of the ionized form of drugs through biological membranes is probably the most general mechanism by which ionization influences activity.

Biers & Stevenson (67) have attempted to assess the relationship of ionization to activity of meperidine-like analgesics, basing their deductions, in this case, on relative pharmacological effects *in vivo*. In a series of cyanoalkylene analogues of meperidine, whose structures seem reasonably compatible with currently recognized size and shape criteria for analgesic activity, but whose ionization varied over a 10,000-fold range, it was found that only the two members of the series whose pKa's fell closest to that of meperidine were biologically active as analgesics. (Table I) Presumably, the less basic members of the series do not ionize adequately to permit binding to the analgesic receptor (68).

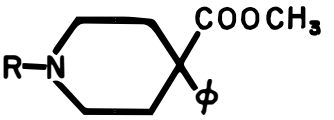
Although not reported as often as it should be, the ionization constant is a relatively easily measured property of organic compounds. Possibly be-

cause of this fact, the role of structure as a determinant of ionization appears not to be widely appreciated. Actually, because the ionization reaction serves as a convenient model for reactivity in general, few other properties of organic compounds have been subjected to more intensive theoretical analysis or offer a greater wealth of data for application to new cases. Among the practical results of this work is the demonstration that the ionization of organic acids and bases is modified in predictable fashion by a variety of electronic and steric influences (69). An awareness of these relationships can be highly suggestive in the interpretation of pharmacological phenomena, and is fundamental to the design of new compounds to meet any predetermined ionization criterion.

Some of the more important structural effects on ionization are encountered sufficiently regularly in compounds of pharmacological interest to merit their brief mention here: (*a*) Electron-withdrawing substituents increase the ionization of acids and suppress the ionization of bases. Electron-releasing substituents have the opposite effect. Positively charged substituents and positively charged dipoles are electron-withdrawing. These, as well as other substituents whose electronic properties cannot be so readily deduced by inspection, include: NO_2, CF_3, CN, COOH and its covalent derivatives, halogen, SO_2, SO, S, O, and ethylenic and aromatic structure elements. Commonly encountered electron-releasing groups are the negative poles and dipoles, alkyl groups, COO^-, and $-O^-$. Some very simple but clear-cut examples that illustrate the striking effects that electromeric substituents can exert on ionization are seen in the series of acids CH_3COOH, NO_2CH_2COOH, COO^-CH_2COOH, whose relative acid strengths are 1:1200:0.1, and in the series of amines, NH_3, CH_3NH_2, $CF_2HCH_2NH_2$, whose relative base strengths are 1:2500:0.02. Electronic influences on ionization are very commonly encountered and hence are the most useful in interpretation. The cyanoalkylene analogues of meperidine mentioned earlier serve to illustrate the dramatic effects of a powerful electron-withdrawing substituent

TABLE I

FORMULAS AND pK_a'S OF A SERIES OF MEPERIDINE ANALOGUES (BIERS & STEVENSON)



R	pK_a
CH_3— (meperidine)	8.7
$CNCH_2$—	3.1
$CNCH_2CH_2$—	6.2
$CNCH_2CH_2CH_2$—	8.5

on ionization, as well as the progressive dissipation of such effects as the substituent is moved away from the ionizing center (67) (see Table I).

(*b*) Resonance is a specialized electronic effect which assumes major importance in conjugated systems, such as aromatic compounds. Alkoxyl, hydroxyl, and amino substituents are normally electron-withdrawing, but this effect is reveresd by resonance effects when these substituents are located *ortho* or *para* to the substituent being influenced by them in an aromatic ring. The normal electron-withdrawing properties of the nitro, cyano, and carbonyl functions are markedly accentuated by their participation in resonance, which results in a further net withdrawal of electrons: thus, the relative acidities of benzoic acid, *p*-nitrobenzoic acid and *p*-methoxy-benzoic acid are 1:6:0.5.[4]

(*c*) Steric effects on ionization are more varied and more difficult to predict from structure than are electronic effects. Very bulky substituents adjacent to the carboxyl group in aliphatic acids reduce acidity, primarily by interfering with the approach of water, which stabilizes the ionized form; e.g., acetic acid, di-isopropyl acetic acid, 1:0.1. Similar bulk effects might be expected to reduce the ionization of bases as well. However, bulky substituents *ortho* to the carboxyl group of aromatic acids, even when electron releasing, increase acidity, because they prevent the carboxyl from assuming its most favorable conformation for resonance stabilization in the free acid form, an effect which is less important in the ionized form. For example, benzoic acid: *p*-T-butyl-benzoic acid: *o*-T-butyl benzoic acid ionize in the ratio 1:0.6 (electron release!): 5.5. Adjacent substituents capable of hydrogen bonding often stabilize the ionized form of acids, and increase acidity: the relative acidities of benzoic acid, *p*-hydroxybenzoic acid, *o*-hydroxybenzoic acid are 1:0.5 (resonance!): 17. A number of other special steric effects on ionization have been defined (69). Some examples of significant steric effects on ionization in compounds of medicinal interest are mentioned in subsequent discussion.

Unfortunately, the theoretical treatment of the influence of structure on lipophilicity, and other physical properties of drugs which are of interest to the pharmacologist and medicinal chemist, is much less well developed. Completely ionized functional groups, because of their attendant solvent cloud, are least lipid soluble, followed by functions which hydrogen bond strongly. Bulky hydrocarbon substituents, the larger halogens, and sulfide sulfur are usually associated with a good degree of lipid solubility. Steric

[4] The electronic effects exerted by *m*- and *p*-substituents in di- and polysubstituted benzene derivatives are quite reproducible, and have been subjected to quantitative treatment of general applicability (70). By applying this approach to the sulfonylurea antidiabetic drugs, Morozowich (71) was able to demonstrate a linear relationship between pKa and the appropriate substituent constants. The applicability of the technique has been demonstrated by the fact that the pKa of any new *m*- or *p*-substituted sulfonylurea can be calculated, provided the constants for the aromatic substituents are among the many available in the literature (70).

considerations are also pertinent. As in the case of steric effects on ionization, bulky adjacent substituents would be expected to block water away from polar functional groups, masking their hydrophilic properties. A general survey of the relationship of functionality to distribution coefficient has been made (72). An interesting and suggestive recent attempt to place the partition coefficient of compounds of pharmacological interest on a more quantitative basis is noteworthy. In a series of barbiturate hypnotics, Lamb & Harris (73) were able to define the distribution coefficient surprisingly accurately in terms of (*a*) the number of carbon atoms in the barbiturate side-chains as a measure of the mass of the hydrocarbon substituents and (*b*) the Taft polar constants of the substituents as a measure of their inductive effect on the hydrophilic portion of the molecule.[5]

In addition to the key role played by lipid solubility in absorption and distribution, it should be remembered that the biological activity of a large group of important drugs—the general anesthetics—can be related directly to this physical property, or to some more sophisticated refinement of it (5, 75). A survey and reappraisal of the general central nervous system depressants has recently been published by Pauling (76), who postulates that the intimate mechanism of the pharmacological effect of such compounds may be mediated via formation of minute hydrate crystals of the clathrate type in the brain cells. Hence, the general anesthetics may involve a greater degree of meaningful structural specificity in their action than is implied by any of the earlier interpretations of the phenomenon. Perhaps because of the practical value of the depressants as central nervous system drugs, there is a tendency to overlook the fact that a great variety of other tissues and physiological functions can be influenced by a miscellany of organic compounds whose common denominator is conformity to a relationship between activity and lipid solubility. In a recent *in vitro* study, a series of homologous primary alcohols were compared using four biological end-points, three of which involved effects on mammalian tissues: i.e., smooth muscle contractility under stimulation by acetylcholine, and oxygen donsumption and histamine release by lung tissue (77). In all three cases, the log concentration-action curves were straight lines, which were, in general, parallel for any one tissue system. As lipid solubility increased with increasing chain length of the alcohols, the concentration required for effect decreased logarithmically.

Occasionally, pharmacological studies appear which involve lipid-soluble compounds whose diversity suggests an insensitivity of the receptor to structural effects similar to that which characterizes the general anesthetics. For instance, a number of steroid compounds were recently reported to block reversibly the action of pilocarpine, and a variety of other antagonists, on smooth muscle *in vitro*. The antagonists exhibit profound structural varia-

[5] The Taft constants (74) are means of expressing the inductive effects of organic functional groups in terms that are applicable to aliphatic compounds and *ortho*-substituted aromatic derivatives. Their application is similar to the aromatic substituent constants referred to in Footnote 4.

tions which do not coincide with any of the known endocrine activities of steroids (78). The order of increasing activity in the series does appear to us to parallel quite closely the order of increasing lipid solubility, as estimated by paper-gram mobility. It will be interesting to learn from subsequent work whether physicochemical effects, or some new structurally determined activity of steroids, controls this novel pharmacological antagonism.

Because they can be readily compared *in vitro*, and because one likely site of their action is at the nerve membrane, local anesthetics have been the subject of perennial investigations of the relationship of activity to physical properties. Within a given series, local anesthetic activity may be found to parallel such diverse physical effects as the ability to decrease surface tension, ability to coagulate colloids, adsorbability on charcoal or other adsorbants, and partition into, or solubility in, lipoid-like solvents (79, 80). Interaction with most chemical models, as well as penetration into the nerve *in vivo*, is limited to the unionized species of amine local anesthetics (80).

A number of attempts have been made to define physical systems that best mimic events at the nerve membrane. Skou (81, 82) investigated several such systems, eventually concluding that a monolayer of nerve tissue lipoids served as the best *in vitro* model for estimating local anesthetic activity at the nerve fiber. There was a close parallelism between the ability of local anesthetics to penetrate such monolayers and their blocking potency. The possible general applicability of this kind of approach is suggested by the recent work of Shane & Gershfeld on the cardiac effect of veratrum alkaloids (83), in which a monolayer of stearic acid was used as a model system in an attempt to demonstrate pharmacological differences in a group of veratrum alkaloids. It was possible to distinguish between the veratrinic membrane "destabilizers," veratridine and cevadine, and the membrane "stabilizer," veratramine. The concentrations used were comparable to those active pharmacologically, and certain characteristic antagonisms demonstrable in living systems were also antagonistic to the effects on the stearic acid membrane.

Structure and Drug Dynamics

Absorption and distribution of drugs.—Contemporary reappraisal of the phenomena of drug absorption and distribution has underscored the importance of physical properties as determinants of these important dynamic properties of drugs (84). The biological basis of this view is the relatively recent demonstration, by physical means, that some biological membranes consist of a lipoidal core covered by layers of unfolded protein or mucoprotein (85, 86, 87). That biological membranes, in fact, function as lipoidal barriers was actually deduced from the diffusion properties of organic compounds many years ago (88). Most contemporary studies in the field assume that the rate of diffusion of organic compounds across biological membranes can be correlated with their lipid solubility, unless some special transport mechanism is involved. In the case of weak organic acids and bases, diffusion

appears to be limited to the unionized form. The effective concentration available for diffusion is therefore determined by the pK of the drug, the pH of the biophases involved, and the lipid solubility of the unionized species. Since a number of recent reviews (84, 85, 89, 90), including a chapter in Volume I of this series (91) have been devoted to discussion of the mechanisms believed to be involved in drug absorption and distribution, and include much experimental data which underlies the pH-partition hypothesis (92), this topic will not be discussed in detail in this review. However, several specialized aspects of particular importance to structure-activity studies will be considered briefly.

Perhaps most important, it must be said that the practical objective of the structure-activity approach, the quantitative estimation of relative absorption and distribution characteristics of organic compounds from a consideration of structure, is not usually possible. This limitation of knowledge requires that absorption-distribution properties be measured if they are to be taken into account in the analysis of drug action *in vivo*. Nelson (93) and Wagner (94) have recently surveyed the status of methodology for measuring the kinetic properties of drugs in higher animals and in man, as well as some of the results of such studies.

Kinetic analyses of oral absorption under conditions that mimic the therapeutic situation have shown that many factors other than distribution coefficient and ionization constant, notably solubility in water, rate of dissolution, physical form, even state of subdivision of the administered compound, may be important, if not actually rate-determining in the absorption process (94). Dissolution rate is clearly of major importance when drugs are administered orally as crystalline solids. The rates of oral absorption of a diverse series of theophylline salts, aspirin, penicillin, and tetracycline were found to be correlated with dissolution rate (95, 96, 97). Nelson (94) found that the sodium salt of tolbutamide dissolved in dilute acid approximately 10,000 times faster than tolbutamide itself. This dramatic difference could readily be demonstrated to influence the onset and duration of the pharmacological response—fall of blood sugar level—in man. Since tolbutamide is rapidly metabolized and excreted in man, its duration of action after oral administration is dependent on this relatively slow rate of absorption (98).

Change of physical state is not, of course, a factor in diffusion across membranes after a drug has entered the circulation. The equilibrium between plasma drug levels and brain (99), and rate of drug entry into cerebrospinal fluid (100, 101, 102), have been successfully predicted by application of the pH-partition hypothesis. Differences in pH between tissue compartments, as is the case with brain, gastrointestinal tract, and the renal tubules, and tissue binding, especially to plasma protein, influence the effective concentration of drug available for diffusion in each tissue compartment and must be taken into account in the calculations (99). Complex formation with macromolecular species prevents diffusion across biological membranes; complex formation with small molecules, e.g., chelation, may actually facilitate

diffusion. Participation of bisquaternary ammonium compounds in a cyclic complex has been suggested (19) as an explanation of the ability of some of these highly polar compounds to traverse the blood-brain barrier (103, 104).

The influence of lipid solubility on diffusion is directly relatable to events at equilibrium, rather than to rates.This must be taken into account when interpreting biological events that occur at a time of rapidly changing blood and tissue levels. In a recent kinetic analysis of thiobarbiturate anesthesia (105, 106), the short duration of action of these drugs was related to rapid entry and exit from the central nervous system during the initial abrupt fall in blood level after intravenous administration. Subsequent distribution of drug into fat occurs much more slowly, and does not appear to be directly involved in limiting the action of the drug.

Increasing appreciation of the influence of chemical structure on the ability of drugs to pass biological membranes has stimulated numerous attempts to synthesize drugs to meet specialized absorption-distribution requirements. Hansson & Schmiterlöw (107) have studied the quaternary derivative of promethazine, a drug designed as a nonsedative antihistaminic agent, on the presumption that the quaternary ammonium structure would prevent passage across the blood-brain barrier. No central effects were, in fact, observed in animals and man. In addition, distribution analysis demonstrated that no appreciable amount of the quaternary derivative entered the brain. Promethazine is, however, much more efficiently absorbed from the gut. An ingeneous application of the known property of haloalkylamino compounds to cyclize to quaternary ammonium compounds *in vivo* was made the basis of an attempt to generate bisquaternary ganglion blocking agents after oral administration of non-quaternary precursors (108). The authors concluded that it should be possible to synthesize compounds that cyclize slowly enough in the gut to be well absorbed, but which cyclize rapidly enough *in vivo* to produce useful pharmacologic effects. The extremely weak basicity and good organic solubility of the salts of the local anesthetic, oxetazaine, have been suggested in explanation of the potent local anesthetic activity of this compound at low pH's (109, 110). The influence of aromatic substituents on partition coefficient and tissue penetration of arylboronic acids was made the basis for a program directed to the synthesis of the optimal boron derivative for efficient penetration of brain tumor tissue, with minimal localization in normal brain tissue (111). In this series, as was expected, the more lipid-soluble compounds readily entered the brain; the less lipid-soluble compounds tended to concentrate preferentially in tumor tissue.

Interestingly, the recognition of the role of lipid solubility and ionization in determining the distribution behavior of drugs does not appear to be often honored by the actual citation of these important physical parameters among the data normally reported for compounds of biological interest. The routine determination of solubility in water or buffer, partition between water and a lipid-like solvent, and ionization constant of compounds whose

pharmacological activity is being compared would be of value in interpretation, and might ultimately uncover additional mechanisms which function in controlling the dynamic properties of drugs *in vivo.*

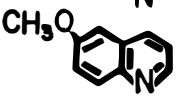
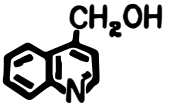
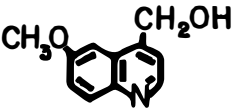
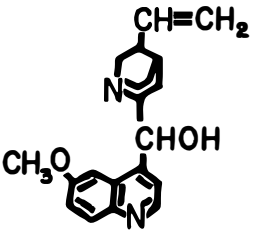
Localization of drugs in tissues.—It is not surprising that the accumulation of drugs in adipose tissue parallels their solubility in lipid-like solvents (84). Body fat appears to function as a relatively homogeneous, randomly structured substance, which extracts lipophilic molecules from body fluid indiscriminately, in a manner not different in principle from distribution between immiscible solvents. The ordered tertiary structure of protein, and the heterogeneous nature of its surface functionality, bestows more stringent structural requirements for interaction with small molecules. The extensive body of information relating to the binding of drugs by serum albumin shows that this is so (112, 113). Drug adsorption on protein is thus more directly dependent on chemical structure than are drug absorption and distribution, or drug localization in lipoidal tissue.

Some of the characteristics of drug interactions with serum albumin apply to protein binding in general, and serve to distinguish this phenomenon from the dynamic properties of drugs considered earlier. Although non-ionic molecules form protein complexes, the more typical strongly bound drug is ionized at pH 7.4. Both anionic and cationic substances are adsorbed, without respect to the net charge on the protein.[6] It has been observed that structural features which increase lipid solubility often enhance protein binding (84, 112). These effects result from the summation of energies yielded by London dispersion bonding, hydrogen bond formation in solvent upon removal of a hydrophobic solute, and from shielding of polar substituents whose solvation favors dissociation of the complex. They also illustrate the relatively low order of structural specificity of adventitious binding to tissue protein. The analogy to fat localization, or solvent partition, breaks down, however, when the size or shape of the substituents is varied sufficiently to exceed the steric and spatial limitations of the binding sites, at which time the substantial forces of steric repulsion begin to reduce the binding energy. The very real influence of steric factors is well illustrated by the progressive limitation of permissible sites for binding as complexity increases in a group of compounds which are part-structures of quinidine (Table II) (114). In this series, the eight binding sites available to quinoline can no longer accommodate this nucleus when confronted with the steric requirements imposed by the characteristic functional substituents of quinidine. These substituents, however, contribute substantially to the energy of binding at sites that will accommodate them. It is interesting that the contribution of the ring nitrogen, aromatic methoxyl, and side-chain hydroxyl functions are essentially constant and additive. The authors interpret the virtually identical bonding energies conferred by these substituents as evidence of participation in

[6] Although serum albumin bears a net negative charge at pH 7.4, its affinity for organic anions in general exceeds that for cations (113).

TABLE II

RELATIONSHIP OF STRUCTURE TO ALBUMIN BINDING IN A GROUP OF COMPOUNDS STRUCTURALLY RELATED TO QUINIDINE

	Molar Ratio of Drug Bound to Albumin	Free Energy of Binding (Kg-cal/mole)	Association Constant
	approx. 6 7	2.9	approx. 0.15×10^3
	8.1	3.5	0.6×10^3
	6.1	4.4	1.7×10^3
	3.9	4.1	1.1×10^3
	2.9	5.0	5.0×10^3
	1	5.3	7.7×10^3

hydrogen bond formation with the receptor. A critical dependence of binding on dimensional relationships, reminiscent of other drug-receptor phenomena, has also been demonstrated in protein binding (113).

Although the binding of drugs to other body proteins is presumed to be comparable in principle to binding to serum albumin, experimental difficulties have limited the detailed study of protein localization of drugs almost entirely to the circulating proteins of blood. Of these, serum albumin, because of its abundance, small size, and consequent great surface area, is the most prominent, and has been shown to influence pharmacological effects of drugs in numerous instances. Classic examples, such as that offered by the trypanoside suramin (115), show that sufficiently strong bonding to albumin can maintain a pool of bound, circulating drug for many months. Such

extremely stable binding is a rarity. However, the significant influence of serum binding on drug distribution and persistence in the body is evident in the case of a number of drugs of contemporary interest. Johnson, West & Masters (116) found chlorpropamide to be 80 per cent bound to serum in man. The level of drug in the blood correlated well with the total vascular albumin. As a result of binding, serum drug concentration is maintained at levels two to six times that of edema fluid. It is tempting to consider that the greater clinical potency and longer half-life of chlorpropamide in man, compared to that of tolbutamide, may be explained in part on the basis of the protective action of binding to serum protein (117). However, although protein binding reduces the effective concentration of drug for participation in diffusion across membranes, it should be remembered that serum binding is a reversible phenomenon, and even intense binding will not conserve a drug in the presence of metabolic, excretory, or transport mechanisms whose affinities exceed that of the serum site. The drug sulfinpyrazone is 50 per cent cleared from blood in two hours by efficient tubular secretion, despite the fact that the drug is almost completely bound to serum (118).

Serum binding may also limit the therapeutically effective drug concentration; in the case of the sulfonamide antibacterial drugs, that fraction of drug which is bound to protein has been shown to be devoid of antibacterial action (119), a phenomenon found to exist among other antibiotics and antiinfective drugs. Florini & Buyske (120) suggest that the high activity of some of the synthetic corticosteroids is due to reduced affinity for the specific hydrocortisone-binding protein, transcortin (121). Powerful activity-enhancing substituents, such as 9-fluoro and 16-methyl, were found to markedly and additively reduce binding affinity. The hormones insulin, thyroxine, and steroids other than hydrocortisone (122, 123) appear to be transported in part as complexes with plasma protein. Since examples of displacement of a protein-bound organic compound by another compound of stronger affinity are known (119, 124), the action of some drugs which mimic hormones might be mediated by displacement of a bound hormone from its protein depot. Aspirin and phenylbutazone do not appear to be able to displace hydrocortisone from protein binding sites (120), but sulfonylurea antidiabetic agents have been recently reported to liberate insulin from its complex with plasma protein (125), in addition to their established ability to release insulin from pancreatic β-cells (126). The diverse group of sympathomimetic amines which competitively interfere with the storage of nor epinephrine and epinephrine at tissue-binding sites (127, 128), represent perhaps the best possible example of a specialized tissue localization effect that might be considered a receptor interaction.

Notable, and in some cases, pharmacologically significant localization of drugs in tissues has been reported in cases where the nature of the concentrating mechanism cannot be readily subjected to experimental investigation. It has been observed that strongly basic drugs as a class tend to leave the circulation rapidly, localizing predominantly in parenchymous tissues (129).

The quaternary sympathetic blocking agent bretylium tends to accumulate preferentially in sympathetic ganglia and postganglionic sympathetic nerve fibers, and this has been suggested in explanation of the selective action of the drug (130).

Special transport mechanisms.—Like protein binding, the adventitious participation of drugs in special physiological transport mechanisms appears to involve some degree of structural specificity. With the exception of the renal mechanisms, specialized transport has not been demonstrated to be of great significance to the dynamics of drugs, although other examples are known such as the transport of close analogues of natural metabolites across the intestinal wall (131). As indicated earlier, current work on the mechanisms of drug absorption and distribution has emphasized the pre-eminent role of *trans*-membrane diffusion in the passage of most drugs from site of administration to sites of action, excretion, or inactivation. In contrast to this general pattern, intensive current research appears to be rapidly increasing the already large number of drugs and extraneous organic molecules which interact with the secretory transport mechanisms of the renal tubules (132 to 135).

The mechanisms for tubular secretion of organic compounds appear to be at least two in number; a mechanism with specificity for organic acids (132), and a mechanism with specificity for strong organic bases (135). The study of the structural requirements for transport via these mechanisms is of twofold significance: structural effects which selectively influence transport in a drug family may influence degree and duration of drug action; in turn, analysis of the structural requirements for transport may grant insight into the intimate mechanism of the transport process itself.

Tubular secretion is of practical significance as a factor in the dynamics of a number of acidic and strongly basic drugs. Numerically speaking, the acid-secreting mechanism appears to be the more important. In addition to penicillin and aromatic carboxylate radio opaque agents, pyrazolidinedione anti-inflammatory agents in general (118), *p*-aminosalicylic acid (136), and the thiazide diuretics (137) are secreted by the tubular mechanism The organic base-secreting mechanism has been shown to transport the anticholinergic mepiphenadol, tolazoline, hexamethonium, and more recently, the non-quaternary ganglion blocking agent, mecamylamine (135). The recent reviews cited summarize a number of points which are pertinent to the role of these mechanisms in drug clearance. The quantitative significance of tubular secretion in limiting drug action is difficult to assess. Drugs secreted by the tubules may be subsequently resorbed by diffusion or by active processes. Resorption by diffusion seems to be a particularly general event, and in numerous cases, quite obscures the effects of secretion on net clearance (138, 139, 140). The diffusion process is influenced by lipid solubility and ionization, as described earlier for other diffusion phenomena involving biological membranes, and can be frequently unmasked by inducing changes in pH of the renal filtrate. As illustrated by the family of phenylbutazone

analogues to be discussed subsequently, and by a group of homologous sulfonamides (141), structural modification may influence net renal dynamics in a very complex fashion. Increased lipid solubility may increase serum binding, thus minimizing the amount of drug filtered at the glomerulus, while simultaneously modifying affinity for the transport mechanism and reabsorption by back-diffusion from the tubular filtrate. It has been noted in a general survey of the thiazide diuretics (137) that activity is related inversely to the renal clearance ratio, suggesting that the higher activity and longer duration of some of the more potent analogues may be in part explained by their low rate of renal clearance. Although the high tubular excretion rate of the earlier thiazides, which may be as much as four times greater than the glomerular filtration rate, is clearly significant in limiting the action of these drugs, it does not seem possible to ascertain readily from available data which factor—secretion rate, tubular reabsorption, or tissue localization—is predominant in determining the low clearance ratio of the more potent thiazides such as trichlormethiazide, benzhydroflumethiazide (137) or polythiazide (142).

The role of chemical structure in influencing renal transport has been studied intensively to deduce the nature of the mechanism involved in the transport processes. In recent publications from several laboratories, a large number of acidic organic compounds have been examined to determine susceptibility to transport by, or blockade of transport in, the tubular acid-secreting mechanism. The possibility that this process might be mediated via an actual covalent derivative of the carboxyl group of the acids, although operationally attractive, appears to be eliminated by studies which show that labeled O^{18} is not lost from the carboxyl group of *p*-aminohippuric acid during transport (143, 144). A less direct, but compelling argument against the formation of a covalent bond between the transport mechanism and the acidic function is the fact that a variety of non-carboxylic organic acids are effectively transported. The acidic sulfonamide fraction of thiazide diuretics, and the pyrazolidinedione enolate of phenylbutazone and its analogues appear, on chemical grounds, unlikely to participate in covalent bonding with the more obvious anionic substituents of the receptor. Attempts to explore the geometric limitations of the transport mechanism for acid secretion have been made through a study of benzoic and hippuric acid analogues *in vivo*, and in isolated kidney tissue (145 to 148). Interpretation of the results of these experiments is rendered difficult by the fact that transport and subsequent back-diffusion mimic failure of transport, as in the case of *p*-nitrohippuric acid (149). Antagonism of *p*-aminohippurate transport appears to be a less ambiguous tool for investigating the transport mechanism. Essig & Taggart (148) compared the effectiveness of a series of mono-substituted hippurate derivatives in blocking *p*-aminohippuric acid transport in rabbit kidney slices. Relative inhibitory effectiveness was found to be unrelated to the electronic properties of the substituents, but roughly paralleled their size. In similar experiments, Despopoulos compared the ability of series of oxy-

purines and pyrimidines to block *p*-aminohippuric acid transport. The effect of structural modification on inhibition of transport appeared to support a three-point interaction between the oxygen substituents of the inhibitor and the receptor, possibly through hydrogen bonding. On the basis of these and earlier studies, the receptor has been depicted as a cationic center, with appropriately spaced adjacent hydrogen-bond donors to allow three-point attachment (150, 151). Although not actually measured in the oxypyrimidine series, the order of inhibitory potency in this group appears to us to be inversely related to polarity. Such a relationship, if confirmed, would seem more readily interpretable in terms of contribution of non-polar substituents to binding affinity at a protein or protein-like receptor than to distribution effects, in view of the relative insensitivity of inhibitory index to pK changes in this series. The inhibitory activity of phenylbutazone analogues also fails to correlate with pK or uricosuric activity (152).

The secretory mechanism for organic bases similarly demonstrates increasing affinity for cations as the bulk of hydrocarbon substitution is increased about the cationic head (153, 154). Among other interpretations, Peters (135) has pointed out that this trend may connote increasing ability to bind to a protein which may serve as the intracellular carrier or receptor in the tubular transport system. Comparison of the blocking potentialities of more complex organic bases has demonstrated differences between isomeric compounds, suggesting that the receptor may also have characteristic steric requirements (155), which should be useful in the exploration of its chemical and structural makeup.

Metabolic transformations.—Metabolic inactivation is often the predominating event in the termination of drug action. Recognition of this fact has resulted in a widespread appreciation of the value of the study of the fate of therapeutically important drugs. Unfortunately, the comparative study of metabolic parameters in inactive members of a family of related drugs is rarely undertaken. The correlation of rate and course of metabolic transformation with chemical constitution in structural analogues is exceptional. One practical limitation to the study of comparative drug metabolism —the inherent technical difficulties—appears to be somewhat diminished by the potentialities of isotopic techniques and the availability of isolated liver microsomal preparations whose metabolic activities account for many types of drug metabolic phenomena *in vivo* (156).

Some very real problems which limit generalizations relating to structure and drug metabolism must not be overlooked, however. There are marked strain, species, and, in some cases, sex differences in mammalian drug-metabolizing enzyme systems (156, 157, 158). These differences may be reflected in the rate of metabolism, the course of metabolism, or both. In some species, at least, the metabolic machinery can be depressed by exogenous inhibitors, the number of which appears to be increasing rapidly (159 to 162), and may be under the modulating influence of endogenous inhibitors as well (163, 164). The activities of the enzyme system can sometimes be increased

by chemical compounds, including substances normally attacked by the enzymes (165 to 168). Finally, the chemical potential of these enzymes is great; a vast variety of substrates is known to be attacked. This lack of specificity portends more severe difficulties in determining the influence of structure on metabolic phenomena than those encountered with other enzyme systems.

Contemporary studies, *in vitro*, have been fruitful in establishing some generalizations pertinent to the oxidative drug-metabolizing enzymes of liver microsomes (156, 169). Gaudette & Brodie (170) demonstrated in a large series of organic alkylamines of miscellaneous structure type that only the lipid-soluble members of the group were dealkylated. The lipid-insoluble compounds were not attacked. The same relationship was found to obtain by McMahon (171) in a series of closely related amines. In the latter series, the rate of demethylation, *in vitro*, was proportional to the partition coefficients of the compounds between heptane and aqueous phases. A good relationship between rate of metabolism and polarity has also been found to apply in a series of lipid-soluble aliphatic tertiary amines, by application of the usual pK-polarity consideration (172).

The dependence on lipid solubility and pK has suggested that the microsomal enzymes are shielded by a lipoidal membrane, which limits rate of metabolism to the rate of *trans*-membrane diffusion of the substrate (156, 169). However, in a series of homologous alkylated aminoantipyrine derivatives (170) and homologous dialkyl carbamates (173), the rate of dealkylation was related inversely to the size of the alkyl groups (and hence to lipid solubility), which suggests the possibility of steric factors operating at the enzyme active site. Comparisons of this sort are rendered ambiguous by the possible intervention of more than one enzyme. However, the microsomal and soluble reductases of liver which function in steroid catabolism show a substantial degree of structural specificity (174, 175).

There appears to be no unambiguous pattern of electronic effects on rate of microsomal oxidation. Electron-withdrawing substituents appear to facilitate oxidative attacks, however (176, 177). This and other findings (178, 179), appear unfavorable to the commonly held view that the attacking species in microsomal hydroxylation is cationic (180).

The complex factors which influence rate of metabolic degradation suggest that chemical features which require a qualitative change of metabolic course are likely to be of greater practical importance to drug design. The rapid and essentially complete metabolic oxidation of the antidiabetic drug tolbutamide [to the biologically inactive *p*-carboxy analogue (126)] cannot occur in chlorpropamide, whose aromatic chloro substituent is immune to oxidative attack (245). The potency and duration of action of chlorpropamide are more marked in man than in other species which inactivate the drug by alternate routes (245). Smith & Williams (246) demonstrated an apparently analogous relationship between the *p*-chloro and *p*-methyl derivatives of the phenylthiourea. The toxic effects of these compounds appear to be

entirely ascribable to the hydrogen sulfide generated by degradation of the thiocarbonyl group *in vivo*. The *p*-methyl analogue generated less hydrogen sulfide and proved less toxic than its chloro congener, apparently because a large part of the methyl drug was degraded by oxidation of the methyl group to the relatively nontoxic carboxyl derivative. Relative rates of metabolism may be of practical, predictive value in the synthetic corticosteroids, whose biological activity is fairly consistently enhanced by substituents which interfere with metabolic degradation (247, 248, 249).

Drugs which are carboxyl derivatives are potential substrates for numerous esterases and amidases, which are often the major factor in the disposition of compounds of this type. Structural parameters which determine the rate of ester hydrolysis by plasma esterase have been worked out in detail (181, 182). Possibly because the plasma esterases are known to have a relatively low order of substrate specificity, structural features which exert steric or electronic effects on the carboxyl function influence hydrolysis rate in a consistent way (182). Increased steric bulk at the α-carbon of either the alcohol or the acid moiety reduces the hydrolysis rate. Electron-withdrawing groups at the *p*-position of esters of aromatic acids markedly accelerate the rate of hydrolysis, presumably by increasing the susceptibility of the ester carbonyl to nucleophilic attack. As might be expected in view of their relative stability to nonenzymic hydrolysis, analogous amides are essentially nonhydrolyzed under these conditions; the metabolic stability of procaine amide compared to procaine *in vivo* represents a practical application of this relationship of structure to susceptibility to enzymic hydrolysis (183). Another example is the long-acting local anesthetic, lidocaine, whose stability to hydrolytic cleavage has been ascribed to the severe steric hindrance generated by the 2,6-*ortho-methyl* functions (184).

Metabolic transformation that occurs before drug action constitutes a peculiarly significant aspect of drug metabolism, since structure-activity correlation that is based on the "pro-drug" structure is clearly not pertinent to events at the site of action. A surprisingly large number of recent examples of this phenomenon have been reported. In some of them, recognition of the metabolic transformation is fundamental to an understanding of the action of the drug.

The dealkylation of substituted amides is a fairly general metabolic transformation of drugs and has been found to yield significant quantities of therapeutically-active metabolite from the anticonvulsant mesantoin (185) and other N-substituted hydantoin and barbituric acid derivatives (186). N-alkyl aromatic sulfonamides are also subject to dealkylation *in vivo* to yield biologically active products. The N-alkyl derivatives of acetazolamide, which are inactive as carbonic anhydrase inhibitors *in vitro*, are active *in vivo*. This has been hypothesized to be the result of metabolic dealkylation to acetazolamide itself (187). Lund & Kobinger (188) suggested that the analogous phenomenon might apply to the thiazide diuretics, and this has been subsequently studied in detail by Wiseman *et al.* (189), using N-methyl derivatives of polythiazide. In this series, the rat readily dealkylates a methyl

group from the 7-sulfonamido function, but appears to be incapable of demethylating the sulfonamide nitrogen of the thiadiazine ring. It seems likely that a comparable metabolic effect underlies the action of 3,7-dimethylhydrochlorthiazide, which has been reported to be diuretic *in vivo*, although completely inactive as a carbonic anhydrase inhibitor *in vitro* (190).

N-demethylation of the tertiary amino group of the antidepressant imipramine appears to be the most likely explanation for the characteristic lag in onset of therapeutic activity of this drug (47, 191, 192, 193). The hydrazide antidepressant drugs also appear to involve a metabolic hydrolysis prior to, or at the time of, drug action (194, 195).

The biological effects of numerous carboxyl derivatives are dependent entirely, or in part, on hydrolytic transformation *in vivo*. Methyl salicylate is rapidly hydrolyzed in rat and dog to saliclyic acid (196). Hydrolysis of heroin proceeds rapidly both *in vivo* and *in vitro* to yield monoacetyl morphine and morphine itself; monoacetyl morphine, but not heroin, appears in brain tissue (197, 198). Glazko *et al.* (199) discovered that several chloramphenicol esters were actually hydrolyzed in the gut before absorption. Only chloramphenicol itself appeared in the circulation after the oral administration of these compounds.

Antimetabolites and other drugs which adventitiously fit into the normal enzymatic processes of intermediary metabolism frequently undergo one or more metabolic transformations prior to the appearance of the pharmacologically active form of the drug. Anticancer purine and pyrimidine analogues are quite generally metabolized along normal pathways to nucleotides, nucleosides, or nucleoside phosphates, which represent the pharmacologically active form of these drugs (200). Beta-oxidation of aliphatic acids (201) and transamination (202) have also been found capable of generating pharmacodynamic activity after administration of appropriate precursors.

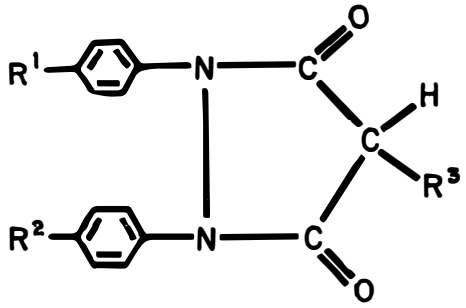
In view of the well-known species differences in the metabolic disposition of drugs, it is to be expected that failure of drug action may occasionally be ascribable to failure of some metabolic transformation that is obligatory to the generation of active principle from an administered pro-drug. The 11-ketone-11-β-ol interconversion of the 11-oxygenated glucocorticosteroids has been suggested to be an obligatory step in the generation of hormonal activity in 11-keto corticosteroids (203). Failure of the interconversion has been evoked to explain the unexpected inactivity of 2-methyl cortisone (203). Cortisone itself has recently been reported to be biologically inactive in the fowl (204). It is tempting to speculate that this may be the result of inability of this species to perform the metabolic reduction at C-11.

Pyrazolidinedione antirheumatic drugs.—The biological activity of certain classes of drugs of current interest appears to be almost entirely understandable in terms of their dynamic properties *in vivo*. Discussion of one such family, the pyrazolidinedione antirheumatic drugs, has been included to illustrate the application of the several dynamic factors just discussed in a practical drug research program.

In a series of publications (84, 118, 205 to 221), extending over the past 10

TABLE III

Physical and Pharmacological (Man) Properties of Phenylbutazone Analogues



Compounds	R_1	R_2	R_3	pK_a	Plasma Half-Life	Solubility mg/ml (pH 7.4)	Plasma Binding (24 hr., 7.4)	Oral Absorption	Disposition (% of dose recovered unchanged in 24 hr. urine)	Anti-Rheumatic Activity	Uricosuric* Potency	References
1	HO	H	$-C(CH_3)_3$	7.0	1 hour	—	—	—	‡		—	(216)
2	H	H	$-CH(CH_3)_2$	5.5	72	-	at least 95%	—	<1		>1200	(118, 207)
3	CH_3	CH_3	$-CH_2CH_2CH_2CH_3$	4.9	24	0.12	at least 95%	I	—		>1200	(84, 207, 214)
4	Cl	Cl	$-CH_2CH_2CH_2CH_3$	4.8	20	0.09	—	I	<1		1000	(84, 118)
5	HO	H	$-CH_2CH_2CH_2CH_3$	4.7	72	10	98%	C	<2	+	800–1000	(84, 118 205)
6	H	H	$-CH_2CH_2CH_2CH_3$	4.5	72	2.2	98%	C	<1	+	800–1000	(84, 118, 205, 218)
7	H	H	$-CH_2CH_2CHOHCH_3$	4.0	8(10)	—	93%	I	8	-	150–300	(118, 205, 214)
8	H	H	$-CH_2CH_2S\emptyset$	3.9	3	1.6	—	C	<3		150–300	(84, 118)
9	H	H	$-CH_2CH_2CH_2\emptyset$			0.14	—	I	—		—	(84)
10	CH_3SO_2	H	$-CH_2CH_2CH_2CH_3$	3.4	24	—	—	—	-		—	(213)
11	NO_2	H	$-CH_2CH_2CH_2CH_3$	3.2	24	—	—	C	<2	+	30–100	(118, 209)
12	H	H	$-CH_2CH_2SO\ \emptyset$	2.8	3	—	at least 95%	—	43	-	30–70	(118, 207, 209)
13	CH_3SO_2	CH_3SO_2	$-CH_2CH_2CH_2CH_3$	2.6	1	—	at least 95%	—	40		100–150	(118, 207)
14	H	H	$-CH_2CH_2SO_2\emptyset$	2.7	1–3	—	at least 95%	—	35		—	(118, 207)
15	HO	H	$-COCH_2CH_2CH_3$	2.3	8	—	—	C	41	–	—	(118, 215)
16	HO	H	$-COCH_2\emptyset$	2.0	3	—	—	—	—		30–70	(118)
17	H	H	$-COCH_2CH_2CH_3$	2.0	1–3	0.12	at least 95%	I	25–50		—	(84, 207)

years, Brodie, Burns, Gutman, Dayton, and their colleagues have collected physical, chemical, and biological data pertinent to a number of these compounds. The work is remarkable because almost all of the biological information was obtained from studies in man, and because key parameters such as efficiency of oral absorption, plasma half-life, and rates of metabolism and excretion have been measured in many closely related compounds. The data and references to the pertinent original publications are summarized in Table III. Inspection of the data discloses some of the generalizations developed by the authors in the course of their study of this series.

Gross correlation of the desired biological activity, antirheumatic properties in man, with chemical structure reveals the interesting fact that the close chemical similarity of all of the compounds does not confer universal biological activity. Examination of the inactive compounds leads, in a number of cases, to surprising but convincing explanations of this fact. For example, the highly acidic compounds 12 to 17, several of which are not anti-inflammatory, were found as a group to have half-lives in man of only a few hours, compared to a half-life of about three days for phenylbutazone. The inability of these compounds to maintain high, long-lasting blood levels seems to explain adequately the reported failure of several of them as analgesic anti-inflammatory agents in man. A search for an explanation for the dramatically rapid clearance of these analogues resulted in the discovery that they are excreted largely unchanged by a tubular mechanism. This mechanism is operative also in the case of the less acidic analogue (118); however, these are less ionized at acidic pH, and relatively lipid-soluble, and are therefore effectively resorbed from the renal filtrate. Rapid tubular secretion was found to be directly correlated with acidity in this group of compounds, as was clinical uricosuric activity (210).

Compounds whose pKa is three or greater are in general slowly cleared, but not all of them have long action which can now be associated with clinical antirheumatic activity. Compounds 7 and 8, appear to be examples of structure types which are particularly prone to metabolic degradation. The sulfide group of compound 8 is metabolically oxidized to the sulfoxide (compound12) and this latter, more acidic derivative is rapidly cleared by the kidneys. The hydroxyl group of compound 7 also appears to be a potential site for ready metabolic attack, although this compound is in part excreted unchanged. Compound 1 is metabolized and cleared with great rapidity. This compound is clearly differentiated from the group in that its acid strength is almost 1000-fold weaker than that of phenylbutazone. Unlike its analogues, which are almost completely ionized at the pH of blood, compound 1 is 45 per cent unionized at pH 7.4 and undoubtedly diffuses efficiently to sites where metabolic degradation occurs.

Yet another factor eliminates some of the remaining compounds from therapeutic consideration. Compounds 3 and 9, although they appear to meet the structural and physical-chemical criteria thus far associated with activity, were found ineffective on oral administration. This unexpected

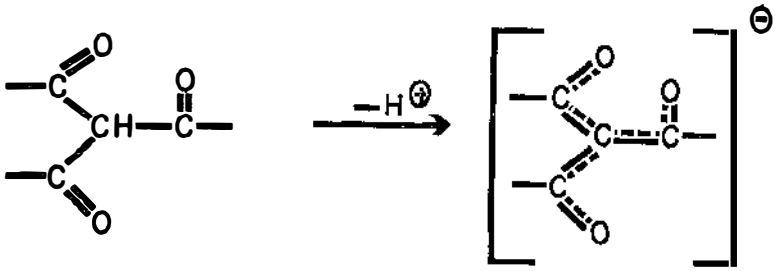


Fig. 4.

Influence of new carbonyl group on acidity of phenylbutazone analogues.

failure was related to slow and incomplete oral absorption, which could be associated with very poor solubility in water. Examination of a larger group of compounds yielded the empirical correlation that compounds soluble at the level of about 1.5 mg/ml or greater in pH 7.4 buffer are rapidly and efficiently absorbed; compounds soluble at the level of 0.1 mg/ml or less were found to be inadequately absorbed for therapeutic purposes.

Members of this series of compounds illustrate well the variety of ways in which chemical structure is capable of influencing ionization. The ionization of compounds with substituents in the aromatic rings is clearly influenced by the electronic effects of these substituents: the electron-releasing effects of aromatic methyl and hydroxyl functions (compounds 3 and 5) result in derivatives that are more weakly acidic than phenylbutazone. The electron-withdrawing substituents, methyl-sulfone (compound 10), nitro (compound 11), and di-*p*-methylsulfone (compound 13), induce progressively increased acidity. The highly acidic derivatives 15, 16, and 17 illustrate the powerful influence on acidity exerted by an adjacent new carbonyl group which stabilizes the ionized form through resonance. (See Figure 4).

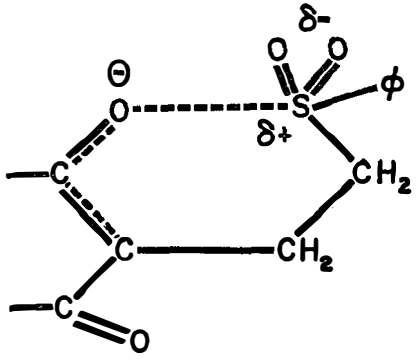


Fig. 5. Cyclic form of R^3 sulfoxide-substituted phenylbutazone analogue (compound 12).

The enhancement of acidity induced by the side-chain sulfoxide and sulfone functions in compounds 12 and 14 can probably be explained in terms of inductive effects, although they are isolated from the ionizing function by a saturated alkyl chain. In addition, these derivatives are probably capable of interacting with and stabilizing the ionized form by formation of a sterically acceptable cyclic structure by ion-dipole interaction (Fig. 5). Perhaps the most interesting illustration of nonelectronic effects of structure on ionization is illustrated by compound 1. The tertiary butyl substituent is seen to reduce acidity about 300-fold, compared to its normal butyl isomer (compound 5). The electron-releasing (hyperconjugative) effects and the steric effect of the bulky *t*-butyl function in preventing solvation of the ionic form are underlying factors in reducing acidity. Possibly more importantly, however, construction of an accurate molecular model of compound 1 demonstrates that the spatial requirements of the tertiary butyl group must crowd the oxygen substituents of the pyrazolidinedione ring in order to form the planar ionic form. Since the energy requirements for crowding atoms are great, it is easy to understand why the unionized form is favored.

A general characteristic of the pyrazolidinedione antirheumatic drugs is their intense and selective binding to plasma protein. Phenylbutazone is therapeutically effective although it is at least 98 per cent serum bound; it has been estimated that approximately 30 per cent of a 400 mg dose is localized on serum protein, which constitutes a minor fraction of body weight. The suggestion has been made that the long duration and very slow rate of metabolic degradation that characterize phenylbutazone in man are the result of protective binding by serum. Phenylbutazone is less than 90 per cent bound to dog serum, and is also more rapidly metabolized in this species. The rapid clearance of the acidic analogues, compounds 13, 14, and 17, is striking in view of the fact that all of them, like phenylbutazone, are nearly completely bound to serum protein. This finding suggests that the affinity of these compounds for the tubular acid-secreting mechanism exceeds their affinity for plasma protein.

Structure and Actions at the Receptor

There is perhaps no more intriguing problem to the medicinal chemist and the pharmacologist than the analysis of the mechanism of drug action at the molecular level. Although this problem received serious attention as early as half a century ago when Ehrlich first formulated the concept of specific receptor sites for drug action, it is only during the last 15 years that our theoretical knowledge and experimental techniques have been adequate to permit some significant understanding of the nature of drug-receptor interactions. Thus, it is hardly surprising that the pessimism long prevalent in this field is only now being widely displaced by a bold confidence that major breakthroughs are close at hand in our search for knowledge about drug receptors. It seems inevitable that as more powerful tools of physical measurement are brought to bear on this problem, new and effective ways to approach drug mechanism studies will emerge, especially at the macromolec-

ular level. However, in spite of the rapid strides being made in molecular biology, the available information on receptor proteins is still so primitive as to require continued utilization of isosteric and homologous series of small molecules as the primary probing devices in studying the nature of receptor sites.

A most revealing measure of the potentialities inherent in present-day research in this field may be derived from the series of brilliant studies on the nature of acetylcholinesterase by Nachmansohn (223) and Wilson (224). In particular, application of the concept of molecular complementarity to the development of nerve gas antidotes stands as a peerless example of the utilization of knowledge about receptor sites in the design of drugs [Wilson (225)]. The impressive flow of research reports from this group is so studded with methodology applicable to the investigation of drug-receptor interactions as to be worthy of careful study. However, the need for discussion of this work here is minimized by the existence of excellent reviews by both Nachmansohn (223) and Wilson (224) which cover all but the most recent work by this group. An important development which has occurred subsequent to these reviews involves the isolation from the electric eel of a protein alleged to be the acetylcholine receptor [Ehrenpreis (226, 227)]. This would support the concept of Nachmansohn that such a protein must exist in the conducting membranes of nerve and muscle as well as at synaptic junctions. Identification of this protein was based largely on the demonstration of a striking parallelism between strength of binding to various pharmacologic agents and the ability of these agents to affect electrical activity of the ioslated single electroplax (228). It should be noted, however, that in his latest analysis of the interactions of this protein with small molecules, Ehrenpreis has drastically revised the view held earlier as to the significance of the isolated material. He now feels

"that the isolated drug-binding protein is a component of the active membrane of the electroplax, may or may not also be present in the synaptic region, but is distinct from the true acetylcholine receptor. That the protein may have some features in common with the acetylcholine receptor is a possibility, especially with regard to the interaction with curare" (229).

An equally important program on receptor site characterization is the continuing study of the adrenergic receptor by Belleau (50, 230 to 236). In view of the effectiveness with which this author has employed modern structural theory in exploring the nature of drug-receptor site interactions, it seems appropriate to devote most of this final section to a review of these recent studies, emphasizing the concepts and techniques of potentially broad applicability in this field of research.

The active sites at which the adrenergic amines exert their stimulatory and inhibitory actions probably constitute the most complex pharmacodynamic receptor analyzed to date with any degree of success. Recent interest in this problem originated with the fundamental observation that the ethyleneiminium ions generated *in vivo* from members of the dibenamine group of

adrenergic blocking agents are truly isosteric with the phenethylamine backbone characteristic of the important adrenergic amines [Belleau (230)]. This relationship of agonist and antagonist structures had previously escaped recognition through failure to appreciate the true electrical nature of the ethyleneiminium ion (Fig. 6).

Belleau clarified the matter by pointing out that this type of ion contains a partial positive charge on carbon induced by the formal positive charge of the neighboring nitrogen atom. Since this carbon atom is also known to be the site of covalent bond formation in subsequent reactions with a nucleophilic (or electron donating) species, it follows that the distance between the reactive cationic center and the aromatic ring is three interatomic distances, just as is the case with the pertinent adrenergic amines.

On the basis of this critical observation, it was possible to formulate a compelling hypothesis based on structural theory which pointed out that a

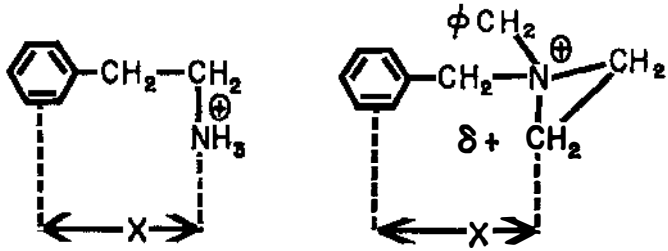


Fig. 6. Isosteric species of ethyleneiminium ions.

phosphate grouping is the logical anionic binding site of the adrenergic receptor, and that the duality of reversible blockade followed by non-equilibrium antagonism, which is characteristic of the β-haloalkylamine family, is a direct reflection of the chemical properties of the parent drug and its ethyleneiminium ion derivative [Belleau (230)]. The hypothesis also offered a structural basis for understanding a variety of factors, such as the importance of the aromatic ring to binding of the antagonist molecule. This has led to an analysis of structure-activity relationships among dibenamine analogues so incisive as to have predictive value.

In a subsequent pair of publications dealing with structurally perplexing subgroups of β-haloalkylamine adrenergic blocking agents, conformational analysis is used to elucidate the interaction of flexible drug molecules with their receptor sites [Belleau (231, 232)]. The manner in which phenoxyethylamine derivatives such as dibenzyline fit the phenethylamine pattern of the receptor site is ascribed to an electrostatic 1,5-interaction which facilitates folding of the molecule to conform with the required phenethylamine pattern by lowering the energy barrier to eclipsing interactions (Fig. 7). On the other hand, the absence of blocking activity in β-haloalkylamine structures which would lead to spiro ethyleneiminium ions, was postulated to be attributable

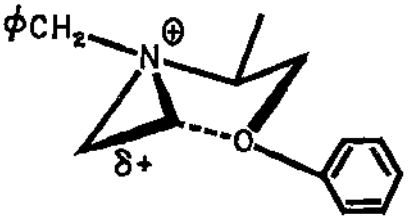


Fig. 7. Folding of phenoxybenzamine (Dibenzyline) molecule to conform with phenylethylamine pattern.

to the suppressant effect of ring strain on the rate of ethyleneiminium ion formation as well as to the unfavorable conformational rigidity of the resultant spiro ion (Fig. 8). Enlargement of the six-membered ring, which reduced or eliminated these factors, led to a return of effective blocking action as anticipated.

Studies with still another group of alkylating agents have afforded further support for the basic supposition that the reactive blocking species must be a carbonium ion structurally isosteric with phenethylamine. The prototype molecule of this series, N,N-dimethyl-β-chloro-phenethylamine, is characterized by very marked adrenergic blocking activity, more rapid in onset and shorter in duration than that observed with dibenamine. The equal amounts of blocking activity shown by the separate D- and L-forms of N,N-dimethyl-β-chloro-phenethylamine, whose optical activity is structurally analogous to that of epinephrine, was interpreted to mean that loss of optical integrity through formation of an open carbonium ion at the active center must have preceded interaction with the receptor site. The recognition that the p-bromo analogue of the same family of blocking agents is a competitive inhibitor of adrenergic stimulation has theoretical implications most compatible with the previously proposed characterization of the anionic site of the adrenergic receptor as a phosphate grouping [Belleau (233)].

As a further step in characterizing the adrenergic receptor, the same research group approached the question of whether norepinephrine triggers an excitatory response in the manner of a true enzyme substrate undergoing chemical transformation, or whether the response merely involves nonbonded interactions between the catecholamine and its receptor site [Belleau *et al.* (234)]. Although evidence at hand strongly favored the latter alternative, it seemed desirable to eliminate the possibility that oxidative deamination of

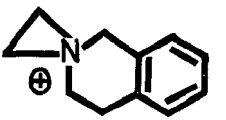


Fig. 8. Spiro ethyleneiminium ion.

the transmitter substance by monoamine oxidase was a functional part of the sequence of events leading to an excitatory response. To do this, an ingenious experiment was devised which involved measuring the adrenergic excitatory responses elicited by tyramine, tryptamine, and norepinephrine, and comparing these responses with those caused by the analogous *α-bis*-deuterated amine molecules.

This experiment represents the first application of the kinetic isotope effect to a pharmacological problem. The effect is based on the fact that isotope differences are not reflected in the physicochemical properties of the molecules containing them, except when a covalent bond to that isotope is made or broken during the rate-determining step of a sequence of reactions. Under those circumstances, bonds formed by the several isotopes of an element will be made or broken at different rates reflecting the difference in zero-point energies of the several isotopes. The deuterated adrenergic amines cited above would be expected to show a prolonged duration of action compared to their natural hydrogen counterparts if the physiological disposition of these amines involved enzymatic rupture of the relatively stronger C–D bond as a rate-determining step. The pharmacological results observed were a pronounced intensification of the blood pressure effects and nictitating membrane contractions caused by *α,α-bis*-deuterotyramine and *α,α-bis*-deuterotryptamine, but no corresponding effect was observed in the norepinephrine series. From these observations the authors arrived at the following important conclusions: (*a*) Monoamine oxidase must be intimately associated with adrenergic effector cells and must be the limiting factor in the action of tyramine and tryptamine. (*b*) Norepinephrine is not a substrate of monoamine oxidase at the adrenergic effector cell. Oxidative deamination is, therefor, not part of the sequence of events leading to a response. (*c*) The role of monoamine oxidase in adrenergic mechanisms can be postulated to be a protective device for the rapid disposition of circulating or endogenous nontransmitter amines.

In view of the results obtained, it became important to establish whether the isotope effect observed had been stereospecific. This necessitated the synthesis of both optical isomers of α-(mono)deuterotyramine, which were prepared by enzymatic decarboxylation of the pair of correspondingly labelled tyrosines [Belleau & Burba (235)]. In this corollary experiment, it was demonstrated that the physiologically important transformations effected by aromatic amino acid decarboxylase proceed by a mechanism involving complete retention of configuration about the carbon atom bearing the amino group. Experiments with the specifically deuterated tyramines revealed a completely stereospecific isotope effect in both the nictitating membrane preparation and studies with enzyme preparations from rat liver and brain *in vitro*. This suggests a marked mechanistic similarity between the monoamine oxidase operating at each of these distinct sites of action. This stereospecific isotope effect also implies that three-point bonding interactions of phenethylamine-like substrates with monoamine oxidase involve the amino

group, one α-hydrogen and either the other α-hydrogen or the β-methylene group. This visualization is consistent with the well-known lack of optical or substrate specificity of monoamine oxidase [Belleau *et al.* (236)].

Not only does the sameness of pharmacological response at adrenergic receptors for α,α-bisdeutero-norepinephrine and norepinephrine preclude any intervention by monoamine oxidase, it also minimizes the possibility that the α-methylene hydrogen atoms of norepinephrine are involved in binding to the receptor site. Other studies have shown that when this type of binding is involved, the deuterated member of a given pair of compounds will form a notably looser Michaelis complex with its receptor protein [Belleau (233)].

These experimental results, then, cast doubt on the likelihood that the energy for a stimulatory response at the adrenergic effector derives from an enzymatic transformation of the transmitter substance. At the same time, support is provided for the attractive alternative conclusion that the excitatory response derives from a simple electrostatic interaction between the receptor and the catecholamine. The process visualized is closely analogous to the proposed mechanism for myosin contraction and involves charge neutralization of a phosphate anion through ion-pair formation with the ammonium head of the protonated adrenergic amine [Belleau (50)]. The possibility that this phosphate grouping on the catalytic surface is part of an adenosine triphosphate-like moiety is emphasized, in light of the knowledge that adenosine triphosphate is involved in the storage of catecholamines and is intimately associated with the process of muscle contraction.

Since the process of charge neutralization through ion-pair formation requires a very close approach of interacting positive and negative charges, the steric effects introduced by alkyl substitution on the nitrogen atom of norepinephrine should be reflected in profound modification of biological activity. This offers a convenient experimental basis for further structural analysis of the adrenergic stimulatory response. A useful correlation of catecholamine activity at α- and β-receptor sites has recently been presented along these lines [Ariëns (237)].

As a means of gaining knowledge about the beta-adrenergic receptor site, a family of tropolones biologically isosteric with the catechol system was shown to inhibit O-methyltransferase non-competitively [Belleau (233)]. The chemical nature of these inhibitory substrates clearly implicates chelation as the primary mechanism of substrate binding to O-methyltransferase. Since these tropolones have also proven able to decrease markedly the sensitivity of β-receptors to isopropylarterenol and to block the metabolic effects of norepinephrine in the cat, the possibility arises that these several receptors are mechanistically interrelated. Belleau tentatively concludes from these tropolone studies that Michaelis complex formation with O-methyltransferase and binding to β- and metabolic receptors belong to the same category of mechanisms. Certainly, at the very least, the availability of these novel enzyme inhibitors opens a new and promising approach to the biochemical pharmacology of the adrenergic receptor.

As an interim summary of the experimental evidence currently available, the unitarian concept depicted graphically in Figure 9 has been put forth. It is proposed that α- and β-receptors are really part of the same structural entity and that the type of response elicited at the adrenergic receptor will be determined by which of these sites is attacked first by the agonist. The known actions of norepinephrine, epinephrine, and isopropylarterenol are quite compatible with this visualization of the receptor as long as one recognizes the implicit assumption that molecules capable of forming an ion-pair at the α-site will produce an excitation response even while simultaneously chelating with the β-site of the receptor [Belleau (233)].

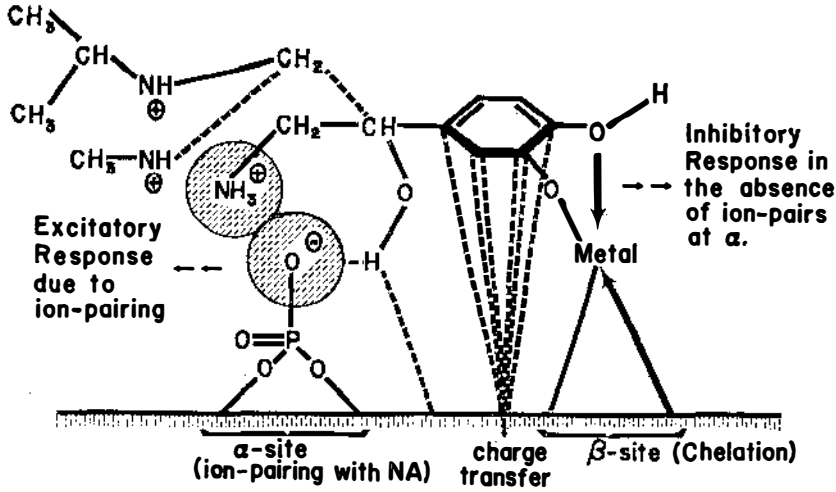


Fig. 9. Adrenergic receptor (Belleau).

Although limitations of space prohibit any extensive discussion of other important attempts at receptor site characterization which have appeared in the recent literature, several of these studies should be mentioned for the sake of completeness. These include the extensive series of publications by Waser (25) and Friess (23) on cholinergic receptors, the detailed depiction of acetylcholinesterase-catalyzed hydrolysis by Krupka & Laidler (238), the structural analysis of nerve cell membranes by Mullins (239), characterization of the receptors for neurohumoral amines in storage granules as reviewed by Born (240), experiments on the serotonin receptor by Woolley (241), the mapping of the analgesic receptor by Beckett (242), and a study of the histamine receptor by Rocha e Silva (243). Also of pertinence to analyses of drug-receptor interactions is the provocative theory of drug action which assumes that excitation by a stimulant drug is proportional to the rate of drug-receptor combination, rather than to the proportion of receptors occupied by the drug [Paton (244)].

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