RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY[1]

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The readers of these *Annual Reviews* have been presented with several articles on relations of chemical structure to biological activity in the last few years. Among them were the superb and comprehensive review by Bloom & Laubach (1) and the interesting synopsis by Fastier (2). Neither the biochemically based nor the heuristic approach to explanations of such relationships has advanced startlingly in the last two years, although many problems have been focused more sharply. The present survey is therefore an expansion and reconsideration of some of the same topics from a medicinal-chemical point of view.

A number of analyses of structure-activity relations have appeared elsewhere. Some of them concern broad principles and are based on careful physicochemical measurements (2–7). Other reviews of the same subject restrict themselves to one series of structurally related compounds (8) or of compounds exerting similar biological activities (9, 10). Almost all of the authors of these reviews, and many others before them, have expressed the hope that a clear understanding of bioreceptors would go a long way in eliminating the high percentage of failures in drug design. These failures are due to our empirical approach to medicinal science. Even in situations in which relations of compounds of apparently identical biological activity, measured in the same test system, are supported by similar relations of one or two physical properties, the question remains whether these particular physical properties have anything to do with the biological performance of the compounds. Yet nobody can deny that it would be of great value to be able to describe exactly what goes on in the interaction of a drug with a reactive cellular constituent. It appears appropriate, therefore, to comment briefly on the present state of our knowledge in this area.

Theories and explanations prove their worth only if they permit predictions and extrapolations. The medicinal scientist is confronted with the unabating need for new drugs for unsolved therapeutic problems; their discovery, evaluation, and useful development compete for his attention to fundamental principles of drug action. There is no point in dwelling on methods of chance discovery of therapeutic agents by random screening or by the accidental observation of a biological effect, although our knowledge of structure-activity relationships is enriched by

[1] The survey of the literature pertaining to this review was concluded in September 1964.

the results of such work. As much as we are forced into random discovery by the urgency of crash programs, we do not learn much from these activities about the mode of drug action. Neither does the slightly more sophisticated search for new drugs in plants, recommended by primitive therapeutic folklore, contribute to fundamental medicinal knowledge. Only two methods are of intellectual appeal in medicinal science. One is a deeper understanding of biochemical reactions in a given specific response situation. The other one is intelligent molecular modification of an existing lead structure, based on significant physicochemical properties and guided by biological findings. In both cases, scientific restraint is necessary, since attempts to transpose the limited success in one series to another series of responses or chemical structures have failed too often.

Even though biologists and chemists try to see eye to eye on fundamental questions affecting the understanding of biology and of chemistry, most of them have acquired through their specialized training a viewpoint, a vocabulary, and a subconscious approach to such problems as emphasize the significance of their own research area, and are apt to play down the requirements at the other end of the scale. Thus, the reviews of relations of chemical structure to biological activity often reflect, if written by a chemist, a naive disregard of biological experience. If written by a pharmacologist, they frequently raise chemical speculations whose meaning becomes obscure as molecular weights increase above perhaps 10,000, let alone if these speculations concern physiological or psychological states. Fastier (*2*) remarked that "pharmacologic activity is not something which can be described precisely in chemical terms. The responses with which the pharmacologist is ordinarily concerned take such forms as muscle tone, inhibition of bacterial growth, or relief of pain . . ." Nobody can argue with this observation, especially with the word "precisely." Moreover, biological function depends on the structural organization and integrity of catalytic systems in a tissue organ, not just on reactions of isolated enzymes. But in view of the rapid progress of experimental chemical evidence at the border of biology and chemistry, the above statement should perhaps be amplified by the words "as yet." Descriptions of pharmacologic phenomena cannot yet be made in precise chemical terms, but the gap is beginning to close for the structurally simplest chemical substrates and drugs which elicit a distinct biological response. The chemist, reassured by the enormous advances in mechanistic and kinetic comprehension during the last 30 years, is more liable to foresee a unified and exact chemical explanation of biological phenomena in the near future.

The pharmacologist, by contrast, has witnessed a centrifugal diversification of details of biological phenomena. As more histological and chemical differences between cells and tissues and their reactions have

become known, a more discreet explanation of each phenomenon becomes necessary. Everybody recognizes, of course, that the fundamental metabolism of all cells follows a very few primordial pathways, but evolutionary changes in tissue design have raised the demands for metabolic variations to such a degree that an overall chemical unification of most biological responses appears unlikely.

BIOCHEMICAL APPROACHES TO STRUCTURE-ACTIVITY PROBLEMS

The experimental supports for theories of drug action lie in the search for descriptions of biochemical receptors and reactions catalyzed by biomacromolecules, and kinetic studies of agonistic and antagonistic processes between two or several chemicals in enzymatic or biological systems. A third type of study concerns biotransformations, but these reflect only the premature, timely, or belated removal of a chemical from the scene of its biological action, or in a few cases, the conversion of a chemical to a more active metabolite. For an excellent review of drug metabolism, the reader is referred to an article by Gillette (11; cf., 12).

It may be true that the organic chemist is going overboard on incomplete evidence in the field of receptor hypotheses, but it should be remembered that extrapolation of known to hypothetical data has become very rewarding in theoretical organic chemistry. The application of electronic and orbital theories, of reaction rate measurements, and of spectral interpretations of structures gives considerable credence to postulates of even very complex structural predictions. This assurance decreases with increasing molecular weight and becomes quite unreliable in the range of "biological" macromolecules. However, at least a few points in the tertiary structure of some proteins and enzymes have been clarified (13) and this accelerating line of research holds promise of an early three-dimensional description of a few of these substances. X-ray diffraction data have already defined helical and randomly coiled portions of large molecules, and have shown that remote sections of a long molecule may approach each other sufficiently to permit interaction of some of their functional groups with one low-molecular chemical that fits into the space between these overhanging sections. Some of the factors which bend such sections in a characteristic way so that two functional groups may "grip" a substrate molecule have become recognized: they are, among others, deformation of the protein chains by metal chelation and hydration.

Bioreceptors

Even though nobody has yet been able to study details of the structure of chemoreceptors, the following considerations make it likely that receptors are macromolecules, mostly of protein-like properties, with a specific capacity

to interact at least with natural substrates at their active site.

(*a*) A wide variety of foreign molecules (drugs, etc.) can be bound at apparently several molecular locations by receptors. This suggests a conformational adaptability typical of proteins.

(*b*) Receptors behave stereospecifically in many (and as we may well learn, in all) of their reactions. This is reminiscent of enzymes which, of course, are proteins.

(*c*) The well-studied receptor of the structurally simple substrate acetylcholine shares its specificity for this metabolite with the enzyme (a protein) acetylcholinesterase. This strengthens the suggestion that the cholinergic receptor is an enzyme-like protein. This is supported further by the observation that labeled ACh antagonists (currarine, muscarine) are fixed at the cholinergic end plates (14). Belleau (15) has suggested that differences in the rates of reaction of acetylcholinesterase and the muscarinic receptor with organophosphorus inhibitors can be accounted for by regarding the muscarinic receptor as acetylated acetylcholinesterase. The normal reactivity of the esteratic site would be masked in the acetylated receptor, but the receptor would retain the ability to form complexes, as it actually is known to do.

Suggestions concerning the events at the receptor, which must lead to a stimulus, have been made by Belleau (16). It is agreed that a drug or metabolite (S) must reach a receptor or enzyme (E) and form a complex at a rate k_1; the reaction is reversible, since this is only a process of adsorption, and the rate k_2 determines the dissociation of the complex into the original components E and S. The biologically still inert complex ES can then also decompo
molecular complex, and at the rate k_3 can furnish a reaction product (P) accompanied by recovery of E which is then ready for recycling.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [ES] \xrightarrow{k_3} E + P$$

This simple kinetic equation was introduced into enzyme chemistry by Michaelis, and adapted to interactions between drugs and receptors by Clark (17, 18) and by Gaddum (19). As pharmacology began to approach biochemistry, more quantitative interpretations were proposed, notably by Ariens (20) and Stephenson (21), and then by Paton (22). During a discussion of difficulties arising from these divergent hypotheses, Ariens (3, 20) first suggested that intrinsic activity (or efficacy) is analogous to the reaction velocity constant k_3, i.e., the rate at which the final product P is formed, and presumably, the biological effect becomes manifest. However, structural variations of S, leading from agonists via partial agonists to antagonists, greatly affect k_3, while Ariens' hypothesis calls for insensitivity of k_3 in the case of pure agonists. It is more likely that the transition of an initially inactive complex (ES) to an activated state is more

closely related to the production of a stimulus, than is the rate of decomposition of ES to E + P. Paton (*22*) proposed that activation of receptors would be proportional to the total number of collisions per unit of initial time, that is, the rate of complex formation would determine the response. High stimulant activity should reflect not only a high k_1, but also the reverse, a high k_2. Thus, the quality of drug action could be accounted for in terms of rates of dissociation (k_2) from the receptors, agonists having a high k_2, partial agonists an intermediate value, and antagonists having a low k_2. Antagonists will "stick" to the receptor, and be reinforced for this purpose by various molecular surface forces. This could mean that a receptor would lose its biocatalytic properties if the agonist would remain adsorbed on it. If the receptor is to regain potential catalytic properties, the substrate would have to leave it rapidly (high value for k_2). This is contrary to experience, and so is the effect of structural variation on complex formation and complex persistence. A good agonist has structural features which facilitate complex formation. For example, the CH_3 portion of the acetyl group of acetylcholine must promote complex formation, since formylcholine which lacks this CH_3 is much less stimulating. Acetylcholine should then (according to Paton) have a higher k_2, since its k_1 is structure-dependent. On the other hand, butyrylcholine, with two more CH_2 groups than acetylcholine, also has a lower potency towards acetylcholinesterase *in vitro*. Here, the lowering of k_2 would have to be attributed to an increased force of binding. It is unlikely that both deletion and addition of CH_2 groups would have the same effect on k_2, leaving the acetyl group of acetylcholine at an unjustifiable apex of the curve.

Such discrepancies are not likely to be resolved as long as one assumes that all chemicals with qualitatively similar actions are adsorbed at the same site of the receptor-enzyme. Although it has been intimated for some time that different chemicals may be attracted to different sites in the same macromolecule,[2] these thoughts have been clarified by Koshland (*23*) in his ideas about the perturbation of the shape of macromolecules by substrates, hormones, and inhibitors.

While it is generally assumed that the active site of an enzyme (and most likely, a receptor) is biochemically specific for a given reaction, it should be remembered that many enzymic reactions depend on adequate concentrations of widely encountered cofactors (NAD^+, NADH, ATP, etc.). In the case of a low concentration of such a cofactor, there will be competition for cofactor molecules by various enzymes which depend on it. The dominant reaction, which may overtake all others, will be determined by the rate limit imposed by the cofactor.

It is to be expected that a drug will shift these competing reaction

[2] The receptor theory of Paul Ehrlich, *Angew. Chem.*, **23**, 2 (1910), advocated such a distinction between "side-chains" to which a nutrient could attach itself, and others which were specific for cell poisons.

rates by its antagonistic effect on the most sensitive of these processes. The emergence of a previously subdued reaction as the dominant one in a multireceptor system would go a long way to explain profound changes in ultimate physiological effects. Similar ideas have been proposed for intracellular reactions (24).

Selectivity of Drug Action

Absolute selectivity of drug action (Ehrlich's magic bullet) has never been achieved, and near-absolute selectivity only in very few cases. The cause of this failure becomes apparent if one considers the very nature of chemical reactions: they seldom go quantitatively in one direction only, even in very simple systems. Only in the case of some ionic reactions, insoluble products are formed whose precipitation shifts the equilibrium rapidly to one side. For organic compounds, much careful manipulation of conditions is needed to raise yields to above 95 or 97 per cent. Most complex organic compounds can and do react by ionic as well as radical mechanisms, and the overpowering interest of organic chemists in chromatographic separation methods and structural spectral analysis bespeaks the frequency with which complicated mixtures of reaction products are obtained. They also indicate the difficulties of separation, purification, and identification of products from such mixtures, even in the relatively simple artificial systems of the organic chemical laboratory.

These difficulties are multiplied by several magnitudes in reactions *in vivo,* and biochemically, even *in vitro,* unless homogeneous enzymes (rarely available) are used with very simple substrates. The drug may be placed anatomically near its expected site of performance, but never right at that site without some intervening barrier. Thus, it has to pass by sites of loss, i.e., chemical reactions which cause premature metabolic alteration and membranes which retain it. If the approach of the drug to its receptor requires active transport mechanisms, the drug molecule may be too inert to enter into such reactions. The concentration of drug molecules arriving at the site of action is, at best, only a small fraction of the administered dose. Altogether, a successful accumulation of a drug concentration adequate to perform its task at its receptor is subject to considerable chance.

Molecular Perturbation Theories

The conformations of both enzymes (25) and noncatalytic proteins (26) are disturbed by interaction with substrates or foreign molecules. The specificity for a given substrate makes it likely that chemical reactions between receptor-enzyme and substrate occur at a narrowly defined section of the receptor molecule, usually called the active site (27). This appears to be a three-dimensional region formed by functional groups and side-chains of protein constituents, and shaped by the tertiary structure

of the protein to admit readily the substrate molecule. The snug fit of the latter permits maximum anchoring by weak, short-range forces, and the receptor protein is probably drawn about the substrate molecule by such forces and thereby deformed (perturbed).

Koshland (23) has illustrated this effect as follows. "The substrate is like the spider at the center of the web. It can change the conformation near the center and start a perturbation which will cause a change in a far distant corner of the web. It follows, of course, that a change in a far distant corner of the web may be felt by the spider at the center."

It is unlikely that the usually much less specific inhibitors are adsorbed at the active site; they probably attach themselves less selectively at some structural segment of the receptor-enzyme which complements their particular structure. Such a segment need not have any relation to the active site, but as it is occupied by a foreign molecule, it will be deformed and perturb the remainder of the receptor protein, including the portions making up the active site. The substrate molecule probably needs at least two functional groups of the receptor in juxtaposition for a rapidly catalyzed transformation. After a deformation, it may find these groups at the catalytic site so spread out that they can no longer interact with the substrate simultaneously. It should be noted that the inhibitors may be attracted to sections of the receptor protein without the need for high steric complementariness, and therefore short-range forces need not be the prime consideration for the stability of the complexes formed. Still, they will help to promote adsorption, and in the case of long-lasting, or near-irreversible inhibitors, firm complexation by auxiliary short-range forces must come into play. For less potent inhibitors, hydrophobic groups in the inhibitor molecule, attracted by nonpolar sections of the receptor protein, will suffice for complex formation. Graphic representations of the effect of inhibitors on molecular perturbations and of the effect of bulky groups on inhibitory action have been given by Fastier (2) and Koshland (23).

Perturbations of macromolecules by such small hydrophobic groups as methyl have been held responsible for alterations of the conformation of nucleic acids. For example, the structure of polyribothymidylic acid (with several 5-CH_3 groups) is more highly ordered, and more extensively hydrogen bonded to polyadenylic acid, than is polyuridylic acid (without 5-CH_3) (28). If small alkyls within the structure of a macromolecule can bring about such conformational changes with far-reaching consequences, similar groups on molecules intruding into the macromolecular structure may be expected to produce analogous effects.

Hydrophobic (or lipophilic) character implies repulsion or displacement of water molecules, while pre-existing bonds between nonpolar chains are disrupted only a little. In the case of the well-studied cholinergic receptor and of acetylcholinesterase, the quaternary ammonium ions of the common substrates and of many analogues may be considered hydro-

phobic (15). They could pry loose water molecules from the macromolecular lattice, and these water molecules would then be available for the transport of Na^+ and K^+ across cell membranes (16). Moreover, expulsion of water molecules by a methyl group from the active site would create an anhydrous environment for nucleophilic attack of the ester carbonyl of acetylcholine. Nucleophilicity is often increased considerably in anhydrous media (29), and the high rate of acetylation of acetylcholinesterase by the quaternary substrate as compared with N,N-dimethylaminoethyl acetate could be explained on this basis. There is no reason to assume that similar perturbations should not take place in other receptor-proteins as well. Care should be taken not to base any conclusion of this type on experiments with drugs for which dose-response curves are not available (3, 30).

The most apparent benefit the perturbation hypothesis offers is an appreciation of random structures as receptor inhibitors. Although the structures of such drugs may be totally unrelated to those of known substrates, they may now be regarded as changing the conformation of receptor proteins and thereby inhibiting the proper formation of the catalytic site.

Effects on Drug Design

The suggestion that inhibitors may be adsorbed at receptor locations which are not related to the active site should be of interest to the molecular design of drugs. It is generally accepted that drugs inhibit or modify biocatalysts. If drug molecules can achieve their mission by perturbing the receptor protein without direct contact with the active site, the need for structural similarity to the substrate to be displaced is no longer required. Unfortunately, as long as nothing is known about the structure of the peptide section of the receptor protein which attracts the inhibitor molecules, no direct practical effect on the design of drug structures will be realized.

One way of discovering such drug structures, however, is to study carefully the biotransformation of a substrate. It is not uncommon that a terminal metabolite, chemically quite removed from its progenitor, can inhibit the first degradative step of the original metabolite by a feedback mechanism that has been called allosteric inhibition or end-product inhibition (8, 31). It is of interest that binding of the terminal metabolite by the feedback mechanism need not involve the active site of the first catabolic enzyme that is inhibited. This first enzyme may lose its sensitivity to the inhibitory action of the terminal metabolite without losing its catalytic activity for the original substrate. As pointed out above, the inhibitor brings about a reversible change in the structure of the enzyme (here called allosteric transition) which changes the properties of the active site.

The net effect of this change is the same as inhibition at the active

site by an inhibitor which structurally resembles the original substrate. However, new inhibitors (nonclassical antimetabolites) may now be designed by taking off from the structure of the finite metabolite instead of that of the original substrate. Thus, a structural lead may be obtained by identifying all those metabolic products of the substrate, which could (or do) act as feedback inhibitors.

This identification may be accomplished by using an inhibitor of one step of a biochemical sequence. The product of the preceding step—perhaps otherwise undiscovered—may now be isolated. In a typical example, L-azaserine has been helpful in elucidating the roles of 5-phosphoribosyl pyrophosphate during the enzymatic formation of glycinamide ribotide intermediates in the biosynthesis of inosinic acid (32).

Such biochemical explanations expedited by the use of drug structures have been the most fruitful application of the antimetabolite approach to drug synthesis. Even a weakly active antimetabolite, if logically designed, may be a valuable tool in gathering information about the metabolism or biosynthesis of the compound with which it is supposed to compete. Work with such antimetabolites has shown that except in such rare cases as the sulfonamides and *p*-aminobenzoic acid, most antagonists do not simply compete with the metabolites whose structure they mimic most closely. Thus, 5-fluorouracil is not an antagonist of uracil *in vivo* but seems to exert its antileukemic activity by being converted to the deoxyribonucleotide, 5-fluorodeoxyuridine phosphate, which now interferes with the biosynthesis of thymidylic acid (33). Similarly, 6-azauracil is converted to its ribonucleotide which then blocks orotidylic acid decarboxylase (34), and 6-mercaptopurine also acts at the ribonucleotide level (35).

Reversible Antimetabolites

The above examples emphasize that direct structural modification of a metabolite will lead only to reversible antimetabolites at best. Such compounds may be useful to undermine biochemical reactions of pathogenic bacteria and some other organisms whose metabolism and synthesis of cellular membranes have little analogy to those of the host. If necessary, the concentration of the antimetabolite can be raised sufficiently for therapeutic purposes without affecting the host, for whom the corresponding metabolite is of no significance. In infections with parasitic animal cells, and especially in neoplastic and functional diseases, such reversible antimetabolites are of limited use. The advantage of overcoming any toxic effects of an antimetabolite by administration of the corresponding and available metabolite is overbalanced in such cases by the small difference in rate at which the metabolite is depleted in the pathogenic (or tumor cell), as compared with the rate of depletion in the host (or communal) cell. This means that the therapeutic index of structural analogues of metabolites is usually quite low.

The ratio of metabolite to antimetabolite at which inhibition occurs

depends on the relative stability of their complexes with the receptor and the rate of the subsequent reaction of these complexes. Here predictable structural changes may be made to affect these factors. For example, the 4-amino analogues of PGA which are used in certain leukemias are much more firmly bound to folic reductase than is the normal substrate of this enzyme (PGA) (36). This has been explained by a firmer attachment of the 4-amino group of the antagonists to an acidic site of the enzyme, as compared with a weaker bond of the acidic site to 4-OH in PGA (35).

One might classify such compounds as less reversible antimetabolites. Some of them are distinguished by a long duration of action. Examples may be found in the long-lasting inhibitors of monoamine oxidase; after an initial lag period caused by the slow depletion of amine substrates, these inhibitors are hard to remove from the blocked enzyme, but they can be removed ultimately. Since they are usually amines, hydrazines, or hydrazides, they may conceivably react with a coenzyme of monoamine oxidase (MAO), in some cases pyridoxal phosphate, and form Schiff's bases which are known to hydrolyze slowly at different pH.

The acidity or basicity of the functional groups of the enzyme at which binding occurs, may be studied by measuring dissociation constants of enzyme complexes with analogues in which the electrophilic character of only one group of the substrate molecule has been altered. Considerable knowledge about such enzymic functions can be gained by measuring dissociation constants over a wide pH range. By the same token, one can leave the character of the functional groups of the substrate unaltered, but modify the size of the molecule or the relative distance between the groups. This furnishes some information about the shapes of the binding sites. Both methods have been used to define, as well as possible, receptor sites on acetylcholinesterase (37).

Irreversible Inhibitors

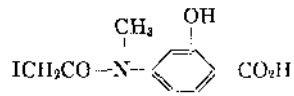
As a compound is bound to an enzyme-receptor by bonds of increasing strength, its ability to become dislodged will diminish. Examples of progressively firm binding have been cited above for 4-amino analogues of PGA (polar and hydrogen bonds), and for MAO inhibitors (probably C = N bonds). Maximum bond strength will be achieved in covalent and certain semipolar bonds (C–O, C–N, C–S). Inhibitors which can attack a macromolecule and combine with it by way of such bonds will deform the macromolecule permanently, and thereby inhibit its catalytic activities irreversibly.

The functions in the inhibitor molecules which produce strong bonds to the receptor sites are groups readily capable of carbonium ion formation. Among them are $>N(CH_2CH_2Cl)$, $-OSO_3H$, and $-CHN_2$ (biological alkylating agents). They need not be structurally related to the metabo-

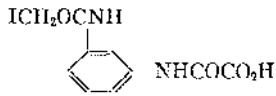
lite, or to the macromolecule (or its constituent parts) to be attacked, although some structural similarity to the metabolite may confer desirable pharmacological transport properties on them. It is assumed that the alkylating agents irreversibly deform a receptor by bonding at positions other than those which attract the metabolite.

Such reactive compounds are often referred to as exoalkylating agents, a term used especially by Baker (38). If the alkylating group is part of a molecule which also resembles a metabolite, they are spoken of as endoalkylating agents. Examples are L-azaserine [$HOOCCH(NH_2)CH_2OCOCHN_2$] and 6-diazo-5-oxo-L-norleucine (DON) [$HOOCCH(NH_2)CH_2CH_2COCHN_2$] in which the amino acid portion of the molecule, which fits the catalytic site, aids the alkylating group to combine with a nearby high electron density function of the receptor.

Taking into consideration the hypothesis that in the metabolic function of neoplastic cells lactic dehydrogenase (LDH) plays an important role (39), the observation was of interest that phenoxyacetic acid can inhibit LDH under certain conditions (40). The failure of phenoxyacetate and some of its derivatives to inhibit the growth of experimental animal tumors could be blamed on the high concentration of pyruvate *in vivo* with which these enzyme inhibitors had to compete. Baker suggested that the poor performance of phenoxyacetic acids might be improved by introducing biological alkylating groups into the molecule which would convert the compounds to less reversible inhibitors. After exploratory studies of the optimal "carrier" of such alkylating groups, compounds related to oxanilic, phenylpyruvic, and salicylic acid were equipped with halogenoacetylamino groups, especially with the iodoacetylamino (ICH_2CONH–) group (41). Some of these alkylating agents indeed inhibited LDH much more potently than their carrier molecules alone, and they were also inhibitory for glutamic dehydrogenase (GDH). Several of the derivatives were selective in their action on one of these two enzymes while others exhibited overlapping activities; thus, N-methyl-4-iodoacetamidosalicylic acid inhibited LDH irreversibly but not GDH, while *m*-iodoacetamidooxanilic acid irreversibly interfered with GDH but not with LDH (42).



N-methyl-4-iodoacetamidosalicylic acid

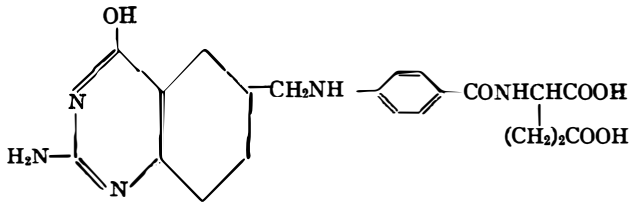


m-iodoacetamidooxanilic acid

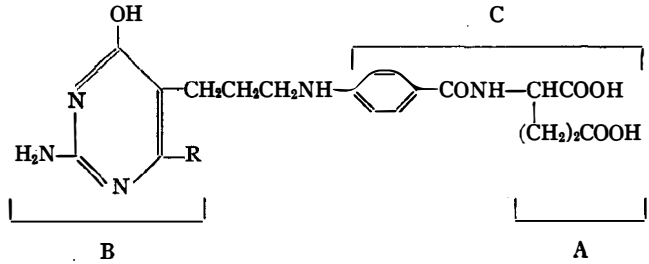
In a similar vein, Baker and his co-workers have extended the idea of developing irreversible inhibitors of tetrahydrofolic acid (THF) which is formed through intracellular reduction of folic acid by the enzyme folic reductase. The cofactor form of folic acid is believed to par-

ticipate in at least 14 known enzymatic reactions for acceptance and transfer of one-carbon fragments (43, 44, 45).

Knowing that the nitrogen atoms in positions 5 and 10 of tetrahydrofolic acid are involved in one-carbon transfer reactions, the nitrogen atom in positions 5 and 8 were first replaced with a methylene group (46). Such a compound, namely 5,8-dideaza-5,6,7,8-tetrahydrofolic acid, exhibited a 50 per cent inhibition of the growth of *Streptococcus faecalis* on



a Flynn folic acid medium containing folic acid. Further studies with aminopterin analogues (47) revealed that the amino group in position 4 was of greater importance for antifolic activity than the changes around the nitrogen atoms in positions 5 and 10. In view of the trend toward structurally less stringently similar analogues, a number of bioisosteres with the general formula were prepared.

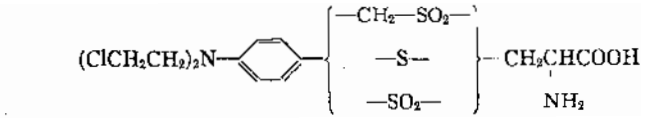


1.

The 6-phenyl analogue of Formula 1, (R = C_6H_5), [N-1-(2-amino-4-hydroxy-6-phenyl-5-pyrimidyl)-3-propyl]-*p*-aminobenzoylglutamic acid, was found to inhibit folic reductase and bind the enzyme far better than the substrate folic acid (48).

Studies on compounds related to Formula 1 with changes in the *p*-aminobenzoyl-L-glutamate fragment revealed that (*a*) binding by carboxy-L-glutamate moieties (A) was minimal but necessary, and (*b*) both the pyrimidyl (B) and benzoyl-L-glutamate (C) moieties joined together were necessary for binding to folic reductase (49). In those instances where simultaneous changes at the 4- and 6-positions of the pyrimidine ring were made, the effects were not additive (50). Thus, there are two areas which are not in contact with the enzyme, i.e., the area at the 6-position of the pyrimidine moiety, and part of the area of the carboxy-L-glutamate section.

The interesting biological properties of phenylalanine mustards have stimulated a search for related compounds with enhanced activity. Examples of such compounds are the nitrogen mustards of S-phenyl- and S-benzylcysteine (51). One of these analogues, 3-{*p*-[bis(2-chloroethyl)-amino]-benzylsulfonyl}-L-alanine (E), caused complete inhibition of implanted carcinoma 256 in rats at a single dose of 50 mg/kg and though



active at higher doses than L-*p*-phenylalanine mustard, it had a therapeutic index comparable to that of the latter. Analogue F was active at higher dose levels than analogue E, whereas G, having a strongly electron-withdrawing group in the *para* position to the mustard group, was ineffective in causing inhibition.

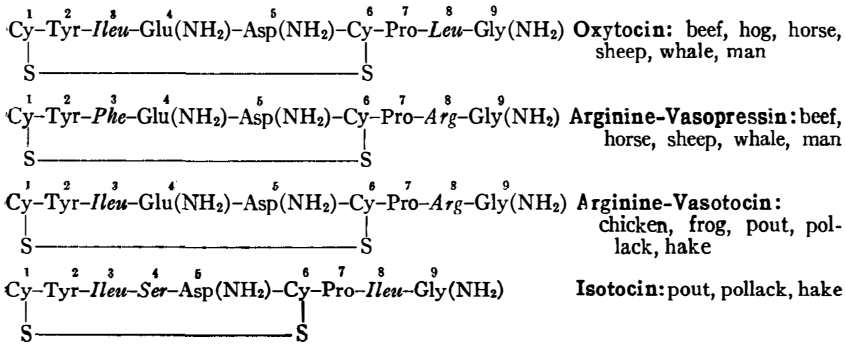
Differences in the biological activities of two analogues such as F and G may have interesting biological implications (52). For example, if the normal tissue contained an oxidative enzyme that was lacking in the tumor tissue, analogue F might be biotransformed in the normal tissue by oxidation to G, while retaining its effect on the tumor tissue.

MOLECULAR MODIFICATION

The origins of molecular modification for the study of relationships between chemical structure and biological activity may be found in naturally occurring biologically active compounds of animal and botanical origin. In the course of genetic steering of metabolic processes, structurally related compounds are often elaborated in the very same animal or plant organ, or in different species.

Some of the polypeptide hormones of the posterior lobe of the pituitary gland may illustrate this observation. The analogue with the ring of oxytocin and the side-chain of arginine-vasopressin ("arginine-vasotocin") (53), first synthesized by du Vigneaud and his group, was found later to be present in pituitaries from frogs (54) and chicken (55; cf., 56, 57, 58). The analogue of oxytocin with serine in place of the 4-glutamine residue and isoleucine in place of the 8-leucine ("isotocin") has also been found, after its synthesis, in several nonmammalian species (59). An oxytocin structure with isoleucine instead of leucine in the 8-position ("mesotocin") was discovered in a species of bony fish. These nonmammalian hormones were probably contrived by nature early in the evolutionary scheme.

Other examples for structural variations among natural hormones are epinephrine and norepinephrine, thyroxine and its lower iodination products, the insulins, glucagons, and adrenocorticotrophic hormones in



various animal species, and the innumerable steroids found in varying amounts, or specifically, in different species. The sex hormones illustrate particularly well the principle of natural molecular modification.

In the plant kingdom, alkaloids and other toxic substances are apparently waste and end products of plant metabolism. Frequently, closely related compounds are formed, or substances which are obvious metabolic precursors of accompanying materials. In this category are the opium alkaloids of the benzylisoquinoline (papaverine) group which could be metabolic precursors of morphine, codeine, and thebaine. Different *Rauwolfia* species produce closely related alkaloids of reserpine-type structures; the various A and K vitamins present series of interesting natural congeners from the same source. In other cases, genetically related plants or organs can produce structurally related compounds of considerable divergence in activity, e.g., cocaine from *Erythroxylon* species, and hyoscyamine and scopolamine from plants of the Solanaceae family.

Examination of the individual members of each of these groups of compounds reveals gradual or profound differences in chemical structure. Each member has its own pharmacological identity. Morphine is ten times more potent as an analgetic than codeine, but the antitussive effect of codeine is relatively much more pronounced. Thus, in a given biological test, and for a given therapeutic purpose, each compound of a group of structural relatives presents certain advantages which must be weighed against the disadvantages of undesired side effects.

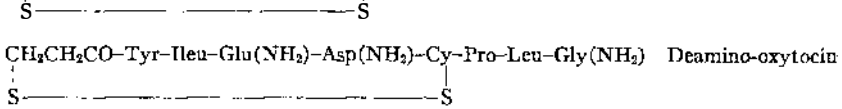
These facts convinced medicinal chemists as early as 1890 that (*a*) nature does not necessarily produce the most valuable compounds for mammalian therapy; and (*b*) that an imaginative chemist may often be able to improve upon naturally occurring drugs by a careful scrutiny and mental "dissection" of the molecule, and by continuing the work of structural modification in the laboratory. What is needed is a prototype structure (a "lead" compound) exhibiting activity in a meaningful biological test. Variation of the lead structure is now seldom done by a random pattern. Most commonly, the principles of bioisosterism are ap-

plied to this activity. Occasionally, the chemical difficulties of placing the right group in the right position may be so great that a compromise with synthetic opportunism has to be chosen. However, this may never serve as an excuse for not carrying out a defendable plan, since any structure can be synthesized with sufficient effort.

The biochemically based paths to choosing a prototype structure have been considered earlier in this review. The following discussions will deal with examples which illustrate the philosophy of molecular modification and the biological ramifications of this approach to drugs. A short survey of the methodology of molecular modification will also be presented.

Analogues of Hormones and Vitamins

Structures as similar as possible to that of a natural hormonal agent give the best promise of attaining similar activity. In their extensive variations of the structures of the oxytocin-vasopressin group, du Vigneaud, Boissonnas, and their associates found that the spectrum of the activities (pressor, avian depressor, oxytocic, antidiuretic, milk-ejecting) could be maintained in spite of small structural alterations, while other changes brought about profound separations of individual activities. For example, replacement of the tyrosine residue at position 2 by phenylalanine (deoxy-oxytocin), that is, deletion of the phenolic OH, left the spectrum of the activities of oxytocin unchanged (60) although quantitatively decreased (61, 62, 63). By contrast, blocking of the OH group of the tyrosine moiety by methylation leads to an antagonist (64, 65); the ether inhibits both the oxytocic activity of oxytocin (66) and the pressor effect of arginine-vasopressin (64).



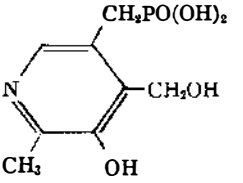
Replacement of the amino group of the half-cystine residue at position 1 by hydrogen (deamino-oxytocin) (66–69) raises the various pharmacological properties, and combines high antidiuretic (5 x oxytocin) with low rat pressor (1/3 oxytocin) activity. This might make deamino-oxytocin a candidate for clinical trial as an antidiuretic in diabetes insipidus in place of lysine-vasopressin in the male and for the nonpregnant and nonlactating female.

Similarly, 1-deamino-4-decarboxamido-oxytocin exhibits extremely low pressor and antidiuretic activities while its milk-ejecting activity is high (70).

It appears that the amino group of oxytocin is involved in the formation of a complex with a protein, neurophysin (71), but is not needed for

the pharmacological properties of the mammalian hormone. Similarly, the carboxamide group of the glutamine residue in position 4 is not essential for high oxytocic activity, but the $CONH_2$ of the asparagine group (position 5) is all-important and that of glycine at position 9 appears to be necessary. These groups can be visualized as being involved in the binding of the hormone to its receptor site (72).

A similar dramatic difference is observed when one atom of oxygen is deleted from pyridoxine-5-phosphoric acid. The 5-phosphoric acid group is believed to be a point of attachment of the coenzyme precursor to the apoenzyme only. By contrast to the coenzyme activity of the 5-phosphoric acid derivative, the analogous 5-deoxypyridoxine-5-phosphonic acid, in



which a C–P bond has been established, is without noticeable activity (73). In the series of steroid hormones, it has been suggested convincingly by Wolff et al. (74) that the steroid is in contact with its receptor surface, perhaps segments of coiled (75) nucleic acids, in two discreet areas: the β-face of rings A, B, and C, and the α-face of ring D. The two principal binding sites are ring A, where a π-bond is formed, and the relatively unhindered 17β-function, which can be attached by nonbonded interactions such as hydrogen or hydrophobic bonds. The remaining areas in contact with the macromolecular receptor are bound by hydrophobic or van der Waals bonds. There is no chemical interaction, not even of 17β-ester groups which could be hydrolyzed to 17β-ols, but instead the steroid induces a conformational change in the receptor. The receptor on the β-face, for example, might be altered to accommodate the angular C-19 methyl group.

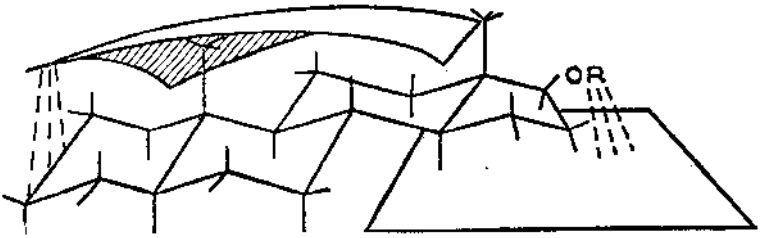


FIG. 1. Simultaneous interaction of the androgen molecule with two spatially separate surfaces at the receptor site (74).

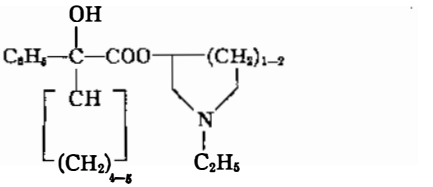
Such ideas might implement earlier systematic modifications of natural steroids, even though these may have been based on rational chemical explanations. For example, substitution by halogens in the 9α-position was correlated with the electronegativity of the substituent (76) and the glycogenic activity of the analogue. Similarly, the observation that 17α-methylprogesterone is three times as active as progesterone led to the testing of other 17α-substituted analogues whose favorable effect might be due to blocking of the biotransformation of the side-chain in position 17.

DRUG DESIGN BY MOLECULAR MODIFICATION

A new experimental compound is usually tested for a limited number of activities only. First of all, there is seldom available more than a few grams of the material, and much of this is used up in a determination of the dose range and initial tests for the activity for which the drug was designed. It is unfortunate that such factors usually prevent a follow-up of other actions, desirable or undesirable, observed during the initial screening. If the early tests hold out promise that suitable structural alterations may increase potency to a needed level, or serve to decrease side actions, molecular modification will usually be begun without delay, using the principles of bioisosterism.

Only after considerable interest in an experimental drug has been created will large enough quantities become available for the evaluation of related or fringe activities. In addition, more biologists will then be motivated to undertake such additional work. It is at this point that molecular modifications may be started in new directions to raise previously negligible, but potentially valuable, side actions to a more prominent level. This will be based on the experience that with enough effort it is frequently possible to find structures which separate one or two activities from other pharmacological properties. Such molecular modifications broaden the scope of the usefulness of the lead compound beyond the limits visualized in early tests.

Pharmacodynamic examples.—Illustrations for such structure-activity variations may be found in many fields. Atropine and scopolamine have been varied to bring to the fore inherent antiulcer, antidiarrheal, antiparkinsonism, mydriatic, and a variety of CNS effects. In some of these series, compounds with a fairly high specific activity have been evolved on this basis. For example, certain psychotomimetic esters were first pre-



pared as standard anticholinergic agents, and their predominantly central effects were elaborated only during early clinical tests (77, 78). The majority of present-day local anesthetics trace their ancestry to cocaine; the molecular dissection leading to synthetic local anesthetics and other anticholinergic agents has been reviewed (79).

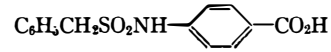
These large and varied series of anticholinergic drugs, and similar work on antihistaminics, have illustrated principles of exchanging and replacing certain nonfunctional moieties such as ring systems or sections of carbon chains by similar segments. If similar activity is hoped for, a minimum of branching of alkyl chains, especially in the vicinity of a metabolizable functional group, appears indicated. On the other hand, considerable branching promotes antagonistic or protective properties, especially if the larger branchings are aromatic, or contain other π-electron-rich moieties.

The aromatic groups have been regarded differently by various investigators. Pfeiffer (80) called them an umbrella structure; he envisaged the compounds as attaching themselves loosely, by means of their protonated ammonium group, to an anionic site ordinarily anchoring the basic amino group of the natural metabolite. The function of the aromatic rings would be to block sterically the approach of further metabolite molecules which might loosen the inhibitor from the receptor. The role of π-electron binding by the aromatic rings as reinforcement for the stability of the antagonist at the receptor was taken into account.

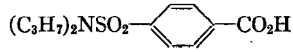
A somewhat different view is held by van Rossum (4; cf., 81). He points out that metabolite-like potency decreases as heavier substituents are introduced into the molecule until finally antagonistic properties are observed. The affinity of such antagonists can be increased by introducing large, planar ring systems, and this may mean that these rings become bonded to regions of the peptide chain of the receptor which do not have to be involved in the active site. Translated into the meaning of molecular perturbation hypotheses, the π-electron-rich planar rings may deform the receptor so that the catalytic site can no longer be formed around the protonated amine function.

Sulfonamides.—The lead provided by the bacteriostatic activity of sulfanilamide ($H_2NC_6H_4SO_2NH_2$) has had far-reaching consequences both in the therapy of infectious and functional disorders. By applying the time-honored method of inverting functional groups in the molecule, bis-(4-aminophenyl)sulfone, $(H_2NC_6H_4)_2SO_2$, was devised. This compound has remained a valuable drug in the therapy of leprosy. Certain N-alkylsulfonamide derivatives of *p*-aminobenzoic acid (such as carinamide) and later N,N-dipropyl-*p*-carboxybenzenesulfonamide (probenecid) proved to cause retention of penicillin in the plasma by blocking renal clearance. When it was discovered that probenecid also interfered with

the renal tubular reabsorption of uric acid, probenecid became useful in the treatment of gout.

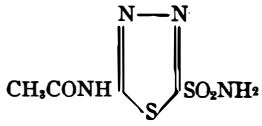


carinamide

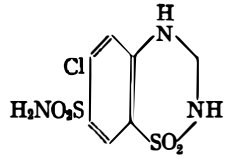


probenecid

The next therapeutic advance arose from observation of another side effect of the anti-infectious sulfonamides, again concerned with renal clearance problems. Patients treated with these drugs lost sodium ions, and a study of the cause of this phenomenon showed that sulfanilamide derivatives inhibit the enzyme carbonic anhydrase which catalyzes the exchange between CO_2 and sodium bicarbonate. Since water is needed to dissolve inorganic salts, an inhibitor of the salt-retaining enzyme could be a model for a diuretic. Indeed, introduction of the sulfonamide group into various heterocyclic rings led to the potent saluretic acetazolamide, and cyclization of one of the sulfonamide groups of an aromatic "disulfanilamide" derivative led further to the thiazide diuretics. During the de-



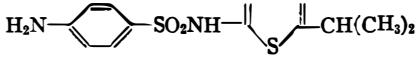
acetazolamide



dihydrochlorothiazide

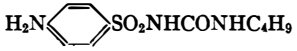
velopment of chlorothiazide, attention was drawn to the antihypertensive effect of the saluretic "rice diet" in hypertensive patients, and the antihypertensive activity of chlorothiazide was predicted correctly (82).

Another advance based on the SO_2NH group came from the observation of an additional side effect of the bacteriostatic sulfonamides. Many of these drugs produce a modicum of hypoglycemia, but this effect may become pronounced in some cases, for example, in 5-isopropyl-2-sulfa-

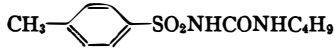


IPTD

nilamido-1,3,4-thiadiazole (IPTD) (82). During simple changes in the structure of this compound the hypoglycemic activity was lost, for example, in the 5-methyl and 5-ethyl homologues. The $-SO_2NHC(-S-) = N-$ grouping present in these 1,3,4-thiadazole derivatives led to the synthesis



carbutamide



tolbutamide

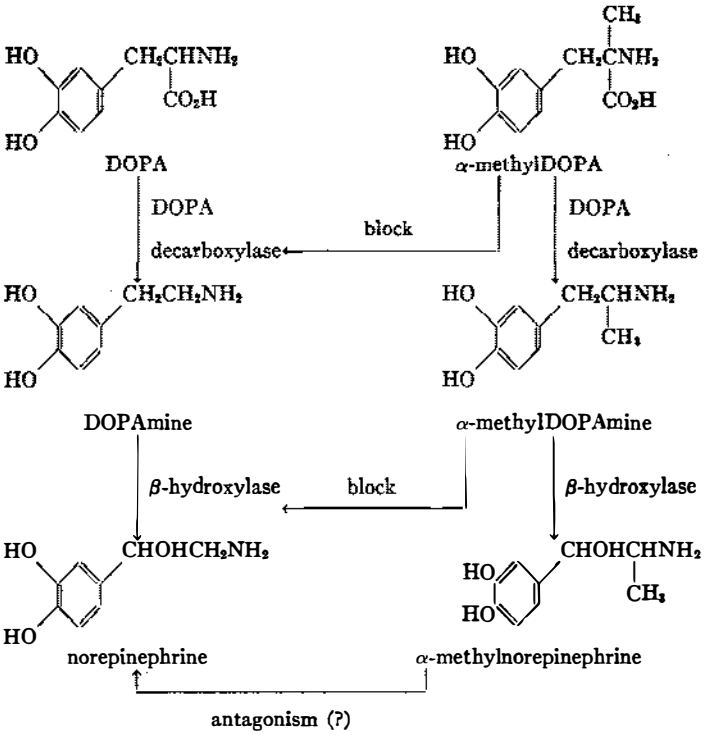
and testing of noncyclic sulfonylureas $-SO_2NHCONHR$; one of the *p*-amino analogues (carbutamide) was too toxic but its methyl isostere tolbutamide is used as an oral hypoglycemic agent.

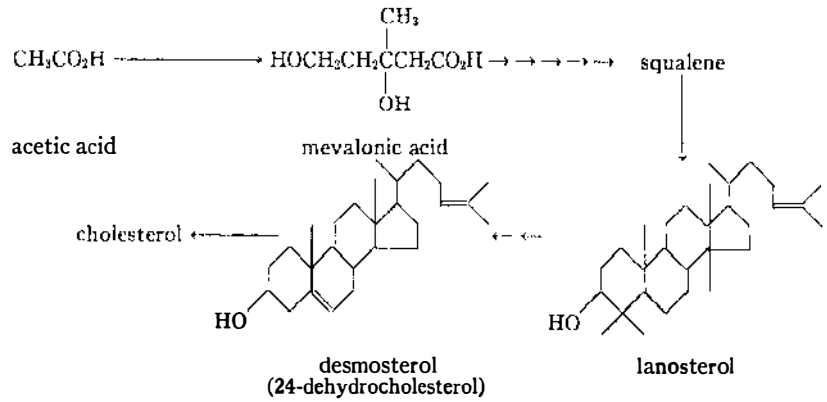
This sequence of events demonstrates the value of molecular modification not only in improving an existing lead but also in opening up new therapeutic areas. The condition for this is an open mind and keen pharmacological observation. Although these qualities may occasionally call the attention of an investigator to minor activities while the compound is still in its early testing stages, it is more common that the wide use and study of a drug after its introduction into therapy lead to the examination of side effects.

The Precursor Approach

Some recent cases have shown that biochemical reasoning can be used successfully for the practical design of new drugs. They demonstrate both the usefulness of biochemical rationale, and some of the limitations imposed upon it by our present state of relative ignorance.

α-MethylDOPA.—This homologue of DOPA was synthesized by Pfister et al. (83) as an agent that might depress the decarboxylation of DOPA to DOPAmine. Both DOPAmine and norepinephrine, formed from the latter biosynthetically by β-hydroxylation, and epinephrine, formed





entific grounds; some others are based more on experience than on strictly defendable data. Although a more thorough understanding of biochemical reactions should precede a strictly scientific program of structure-activity variations, the decisive details of macromolecular reactions, especially in histologically fixed organization, are not likely to be available for maybe some decades. In the meantime, intuitive and empirical methods of varying the structures of lead compounds cannot be abandoned. In this dilemma, the thoughtful medicinal chemist bases his plans in drug synthesis essentially on a gradual variation of electronic and steric effects which have been summarized in the concept of bioisosterism (87, 88). Because the majority of modern pharmacologists are preoccupied with other facets of medicinal science, a brief survey of bioisosteric rules will be given below.

Briefly, if one wants to design a new compound whose spectrum of potency and activities approaches very closely that of a known prototype compound, the best promise of success will lie in a minimum of structural deviation. In other words, both the distribution of electric charges and the three-dimensional shape of the prototype molecule should be changed as little as possible. These requirements put in jeopardy the smallest of all structural changes, that is, the construction of conformational and other stereoisomers in which only hydrogen atoms or small functional groups have been moved to opposite sides of the molecule, and the puckering of flexible chains or rings may have been changed accordingly. In so many cases the drug receptors have such high steric requirements that this simplest of all molecular manipulations cannot be applied without considerable or total loss of biological activity.

These effects were disregarded in the classical applications of isosterism (89) to biological problems which considered principally atoms, ions, or molecules in which there is identity or near identity of the peripheral

layers of electrons. Thus, such similar groups were called pseudoatoms (CN, OCN, SCN, etc.: pseudohalogens; C = O, pseudo-N = N^+, etc.); compounds containing such groups often have overlapping immunochemical properties. Later, ring equivalents were added to the list; the CH = CH group in benzene or pyridine was equated to the sulfur atom in thiophene or thiazole, respectively. A molecular orbital treatment of these compounds (87) provides a picture of real electronic similarity between a sulfur atom and a combination of two aromatic carbon atoms.

Such accurate comparisons are not feasible if, for example, furan (cyclic oxygen), pyrrole (cyclic aromatic NH), and similar systems are compared to benzene or thiophene. However, the attachment of the same pharmacophore group ($-CH_2CH_2NH_2$; $-CH_2CH(CH_3)NH_2$; $-CH = CHCO_2H$; $-CO_2R$; NHAc; etc.) leads to compounds with at least overall similar behavior in the same pharmacological test system. There may be considerable variations in side effects, toxicity, and their quantitative performance, but evidence of the same main measurable type of activity can be expected. This is not always true for aliphatic analogues, and less true for analogues with the same side-chain attached to saturated ring systems of the same general size. It must be assumed that the combination of a certain side-chain and a quasi-aromatic ring system furnishes compounds with overall similar biological properties. And yet, these ring systems are not strictly isosteric in the classical sense of the term. They are nonclassical isosteres or, better, bioisosteres.

To be sure, the term bioisosterism represents a dilution of chemically and physically descriptive comparison. However, bioisosterism is descriptive of analogous chemical structures which elicit analogous effects in the same test system. It serves as a useful means of comparing chemical structures in medicinal chemistry, and of planning more concisely the next move in a program of molecular modifications. It derives support from the fact that so very few compounds have one clear-cut biological activity without side effects. If one would only prepare compounds which fulfill the requirements of near-complete spatial and electronic likeness, one could expect to find nearly the same distribution of main and side effects in the resulting structural analogues. Bioisosteric structural variations carry the promise of similarity of activity, but because the structures are no longer strictly comparable, they should produce a separation or quantitative redistribution of their multiple activities. In almost every case of developing a useful drug from an interesting lead, a number of simple variations of the prototype molecule must be made to find the therapeutically most acceptable structure. A complicated molecule containing a small alkyl ether or ester group will barely be isosteric with a homologue with a much larger ether or ester function. But the large groups may serve to point up the preponderance of the most desirable therapeutic feature of the compound and decrease bothersome side effects.

Such homologous ethers or esters may then be termed bioisosteric because they retain the functional characteristics of the lower homologue together with its principal biological properties.

The most useful aspects of bioisosterism lie in the direction it can give to molecular modification. Suppose ethyl *p*-aminobenzoate, *p*-$H_2NC_6H_4COOC_2H_5$, has been found active in a given test. Literally hundreds of structural variations come to mind which could be applied to this molecule with a fair hope of retaining or segregating the test activity. It would be nice if all these variations could be prepared and tested, but the time and manpower needed, the cost, and the ennui of repetitive chemical and biological operations will usually be prohibitive. Therefore a limited choice will have to be made of analogues to be prepared and tested. This should encompass representative examples of molecular alterations, spaced in such a way that the activities of other variants can be extrapolated with a reasonable degree of certainty. This choice should be made essentially on chemical grounds, and should therefore lend itself to a better predictability than if thoughts about biological reactions were involved. The structures to be prepared should differ from the prototype in decisive variations of polar properties and solubilities in representative solvents, and in steric and electronic effects. They should permit a clear prediction about similar reactivities and physical properties in analogues which lie between themselves and the prototype. In other words, they should represent parameters of structural changes.

In the example of ethyl *p*-aminobenzoate, the following variations could be proposed along these lines. The compound is an aromatic amine, and an ester. The acid, *p*-$H_2NC_6H_4COOH$, to which the ester is related, should be tested for the same activity, and so should one or two other esters, e.g., one higher alkyl ester. If such an ester with a much larger alkyl group, i.e., a higher solubility in ether, chloroform, and other lipid solvents, shows an increased activity, then the preparation and testing of a few additional higher alkyl esters with perhaps branched or cyclic alkyl groups would be justified. If activity had diminished in the higher alkyl ester, as compared with the original ethyl ester, further study of higher alkyl esters would be inadvisable at this stage of the investigation.

Since the reactivity of ester groups compares with that of amide groups, the corresponding amide, *p*-$H_2NC_6H_4CONH_2$, may be studied. Again, if potency or selectivity of action takes an interesting turn other amides with secondary or tertiary amide groups may then be chosen. If the easily accessible primary amide behaves unsatisfactorily in the test, this line of variation should not be pursued further in this initial search for information.

Next one may question the significance of the primary aromatic amino group. By means of simple chemical one-step transformations, NH_2 can be converted to $N(CH_3)_2$ or chloro substituents. Each of them

can serve as a point of departure if the first variation is of interest. Several different tertiary-amino esters could be tested if the dimethylamino compound is active; in addition, the activity of the secondary methylamino derivative can be extrapolated from the data on the NH_2 and $N(CH_3)_2$ compounds. If the *p*-chloro ester, *p*-$ClC_6H_4COOC_2H_5$, should be active, other halogeno and the cyano analogues could be prepared easily. Such activity would also discredit the need for the amino group, and this could be corroborated further by testing the *p*-hydroxy ester, or just ethyl benzoate.

The position of the substituents in the aromatic ring should be examined, and either the *ortho* or the *meta* isomers of ethyl *p*-aminobenzoate should be tested. If it is suspected that the amino group is biotransformed too rapidly to permit a satisfactory duration of action of the prototype compound, one could block one or both *ortho* positions with readily introducible chlorine to inhibit metabolic attack upon the amino group. If this would be shown to be successful biologically, other *ortho*-substituted analogues might be tested.

However, if it is felt that a nuclear substituent provides not only bulk, but also inductive electronic effects, a second derivative carrying a small substituent of opposite electronic character in the same position should be prepared and tested. Common electron-donating substituents for this purpose include CH_3, OH, OCH_3, electron-withdrawing substituents CF_3, CN, NO_2, and others.

One could also go further afield by studying one amino ester of another aromatic or aromatic-heterocyclic system. This would point up the significance of the benzene rings (see below).

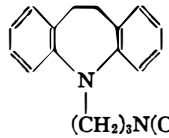
To summarize the special case of a small-alkyl *p*-aminobenzoate, five or at most seven compounds in this series should circumscribe the structural parameters of activity in one pharmacological test. They should serve as a basis for further molecular modification and may be regarded as new lead compounds. The effort to prepare and test these informative analogues is minimal compared with older random methods of structural variation.

For complicated multifunctional molecules, more possibilities for even the most significant structural alterations will exist. In such cases, the first choice of variations may well be based on personal preferences or synthetic expediency, as long as other equally significant variations are not disregarded in further experiments. An additional proven modification is employed often in such complex structures; rings may be replaced by chains of atoms, and vice versa. A series of recent examples of this thinking may be found in the work by Baker et al. (48) who have replaced the pyrazine moiety of the pteridine portion of PGA by an open chain.

In many pharmacodynamic agents (pressor, various blocking agents) aromatic rings with their high π-electron density are needed to produce a

desirable degree of potency. The function of these aromatic rings is believed to be a reinforcement of ionic linkages of polar functional groups, by van der Waals forces which anchor the drug molecule at a flat receptor surface. A benzene ring can furnish 2 to 3 kcal/mole of supporting attachment, whereas nonaromatic rings will be of much less value as a source of short-range bonds. The number of ring atoms and the area of the rings may be expected to have an effect on such auxiliary forces. Studies by Burger and his associates (90) have shown that pressor activity of analogues of phenylethanolamine decreases progressively as the aromatic ring is replaced by more saturated and by smaller rings (phenyl > 1-cyclohexenyl > cyclohexyl ≅ 1-cyclopentenyl > cyclopentyl). In accord with these observations, 2-cyclobutylethylamine (300 mg/kg by mouth, in rats) failed to exert any overt activity, and the branched homologue representing an amphetamine type did not increase motor activity in mice (91). On the other hand, the small saturated cyclobutyl group can replace 1-cyclohexenyl effectively in hypnotic barbiturates (91).

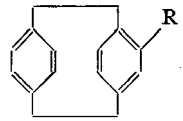
Aromatic through-resonance is not required, however, to produce certain pharmacodynamic and psychopharmacological activities. This can be seen in systems in which two aromatic rings are twisted out of a common plane, for example, in the antidepressant and motor-activating drugs imipramine and desipramine (92).



Imipramine: R = CH_3

Desipramine: R = H

In derivatives of [2.2]paracyclophane substituted with functional side-chains which are associated with pressor (R = $CHOHCH_2NR_2$) and anticholinergic-antihistaminic [R = $CH(CH_3)OCH_2CH_2N(C_2H_5)_2$] activities, these effects were retained qualitatively (93). The penicillin derivative of



this bent ring system was qualitatively indistinguishable from benzylpenicillin in its behavior toward sensitive and resistant pathogens (93).

Statistical Methods

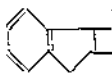
In a series of analogues containing a common carbon skeleton but different substituents at the same or different positions, a qualitatively similar response may sometimes be observed, varying in order of magnitude.

Assuming that the effect of various groups in a structurally related series is additive, Free & Wilson (94) have presented a mathematical model which attempts to summarize the contributions of such group variations to the prediction of certain biological activities. It is based on statistical calculations but does not compensate for steric and physical requirements of the compounds, or for the cumulative effect of several substituents. As it is only a preliminary model, future modifications may take these factors into account. The prerequisite for such calculations is a precise biological measurement, and therefore data on *in vitro* microbiological inhibition lend themselves best for these methods. *In vivo*, LD_{50} values may be used as a basis for calculations.

In a typical example, the known LD_{50} values of some analgetic 1-acylamino-2-phenylindanes permit the calculation of the contributions of changes in the substituents R^1 and R^2 on the LD_{50}, from the figures in Table I.

TABLE I

LD_{50} VALUES OF SEVERAL 1-ACYLAMINO-2-PHENYLINDANES



$NHCOCHR^1R^2$ where $R^1 = H$, $R^2 = N(CH_3)_2$; $R^1 = H$, $R^2 = N(C_2H_5)_2$; $R^1 = CH_3$, $R^2 = N(CH_3)_2$; $R^1 = CH_3$, $R^2 = N(C_2H_5)_2$.

R^1	LD_{50}		Average LD_{50}
	$R^2 = N(CH_3)_2$	$R^2 = N(C_2H_5)_2$	
H	2.13	1.28	1.705
CH_3	1.64	0.85	1.245
	Average 1.885	Average 1.065	Total average 1.475

There are four compounds possible; by subtracting the average value (1.065) for the compounds with $N(C_2H_5)_2$, from the total average (1.475), the contribution of the diethylamino group becomes -0.41. Similarly, for the dimethylamino compounds (average, 1.885), the contribution of the $N(CH_3)_2$ group may be expressed as $+0.41$. Here the symmetry of these contribution values is built into the solution, because if a substituent x contributes a value m to the average value of two compounds, a substituent y will contribute a value of $-m$ to the average.

Assuming that there is such an "additivity" in a series of analogues, the mathematical model may be written by the formula

Response = Average + Effect of R^1 substituent + Effect of R^2 substituent.

This formula may be applied to the example in question as follows:

$LD_{50} = K + a[H] + a[CH_3] + b[N(CH_3)_2] + b[N(C_2H_5)_2]$, where K is the overall average and the values in the brackets refer to the contributions by the respective substituents R^1 and R^2.

By placing the known LD_{50} values of the four compounds in the above equation, we obtain four equations with five unknowns. However, it follows from the earlier assumption of additivity that the contribution by $a[H]$ equals $-a[CH_3]$.

When this restriction has been imposed, four equations and three unknowns are left. This can be solved readily by least squares (95).

Other mathematical methods suggested recently include a simplification over earlier methods (96–99) of predicting the carcinogenicity of polycyclic aromatic molecules by application of the Dewar localization energy approximation (100). This is one of the simplest molecular orbital approximations. Calculations of the *ortho* and the *para* localization energies of the *K* and *L* regions of various carcinogens correlate well with the observed values of carcinogenic activity.

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