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FOURTH SMISSMAN AWARD ADDRESS

The Long Search for Valid Structure–Action Relationships in Drugs†

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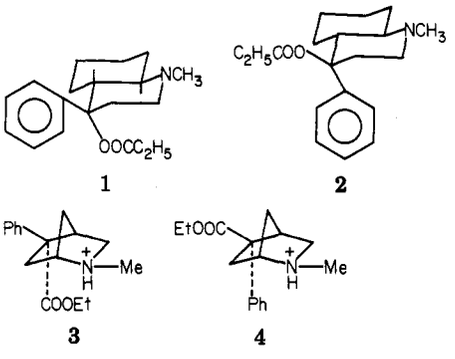
In 1869, Crum Brown discovered the first structure–activity link by showing that alkaloids, even convulsive ones, were converted by N-methylation to muscle *relaxants* resembling curarine (itself a quaternary amine). This led to an attempt to link every type of drug action to its own cluster of atoms. This quest was jolted when Loewi (1926) found that a quaternary amine (acetylcholine) was the principal *activator* of muscle! Suddenly it was seen that a chemical group could be either an agonist or antagonist, depending on its molecular setting. That the agonists were smaller molecules suggested operation of a steric factor. Moreover, Cushny (1926) had focused attention on optical enantiomers: usually only one member of each pair had biological activity, although both were identical in all other properties. The stage was now set for physical properties to play the leading role in relating structure to activity. People recalled the demonstration by Overton and Meyer (1900) that the depressant action of a drug was linked to its lipophilicity. Unhappily, further physical correlations were slow to appear. My colleagues and I, who had been studying (from 1941) the antimicrobial action of aminoacridines and hydroxyquinolines, established quantitatively the role of ionization and chelation (two electronic influences) in the action of drugs. Today most people would agree that the most important properties in determining the action of drugs are not some particular nucleus or substituent but a trio of physical properties: lipophilicity, electron distribution, and shape. Although these properties govern the *activity* of drugs, their *selectivity* is due, as I have long maintained, to another trio of properties, namely, comparative distribution (not necessarily lipophilic), comparative biochemistry, and comparative cytology.

Let me begin by expressing my pleasure in being selected for the Smissman Award. It is heartwarming to realize that all countries, even ones as remote as Australia, receive attention from the selection committee.

Edward Smissman, whom we honor today, died at the early age of 48 while his scientific powers were at their very height. Born in Illinois, in 1925, he found his first academic position at the University of Illinois College of Pharmacy. He remained always in the Middle West and reached his final position in 1960 as Chairman of Medicinal Chemistry at the University of Kansas, where the most characteristic and original of his research projects were conceived.

In these, he excelled in syntheses that led to separable pairs of conformational isomers. The aim of this work was to illuminate the nature of receptors, of which cholinergic, adrenergic, and analgesic receptors particularly served him as goals. In his lectures, which were always lit by a rare enthusiasm, he showed himself to be a skilled expositor of stereospecificity. He used this special knowledge sometimes to devise inhibitors for a particular enzyme and sometimes to map out the steric parameters within which the structure of a drug could be varied, but his long-term goal was always to foreshadow the design of better drugs.

As an example of this work, let me cite his preparation in 1966 of the two stereoisomers of an analgesic, a substituted decahydroquinoline.[1] No difference could be found in the analgesic potency of these two isomers (1 and 2). This result indicated that the conformational requirements for analgesic action may not be as strict as had originally been thought. Two years later, Portoghese and colleagues found that one of a pair of stable conformers (3 and 4), designed as rigid conformers of pethidine, had four times the analgesic potency of the other. Analysis of



1 2

3 4

the brain showed that the active isomer penetrated more rapidly than the other. Moreover, the two conformers differed greatly in partition coefficients and in ionization constants, and they did so in directions conducive to this accumulation by the brain.[2] Thus, members of a pair of conformers can have very different physical properties, and any difference in activity may depend very directly on these differences.

These examples serve to remind us that, in the last 2 decades, stereochemistry, distribution, and electronic disposition have become the key factors in relating the structure of a drug to its action. How was this position achieved? The answer must be that it was achieved only with great difficulty and over a long period.

The first correlation between structure and biological activity was announced in 1869, when Alexander Crum Brown and Thomas Frazer in Scotland showed that a great many alkaloids, even convulsive ones, were converted to muscle relaxants when their tertiary nitrogen atom was

† Presented on August 26, 1981, at the 1981 Annual Meeting of the American Chemical Society, New York, NY.

(1) E. E. Smissman and M. Steinman, *J. Med. Chem.*, **9**, 455 (1966).
(2) P. S. Portoghese, A. Mikhail, and H. Kupferberg, *J. Med. Chem.*, 11, 219 (1968).

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quaternized by methylation.[3] In fact, this simple chemical change had converted strychnine, bruceine, codeine, morphine, thebaine, nicotine, atropine, and coniine into substances with the biological property of the alkaloid tubocurarine, itself a quaternary amine.

We must not underestimate the stimulating effect of this discovery on pharmacologists and medicinal chemists. Like rain in the desert, a simple connection had at last been found between a constitution and a biological property. This correlation started the search for other chemical groups or nuclei (ring systems) to which a unique pharmacological action might be assigned. However, even as late as 1910, all that could be added was that organic arsenicals (but only some) could cure syphilis.[4]

A more fundamental approach was begun by John Langley who, in 1878, put forward the concept of drug receptors while working in Cambridge (England) on the mutually antagonistic effects of atropine and pilocarpine on the salivary flow of cats. Langley wrote: "There is some substance or substances, in the nerve endings or gland cells, with which both atropine and pilocarpine are capable of forming compounds. On this assumption, then, the atropine or pilocarpine compounds are formed according to some law, of which their relative mass and chemical affinity for the substance are factors".[5]

Langley's idea was greatly clarified by Paul Ehrlich, in Germany, at the turn of the century. It was he who coined the name *receptor* and defined it as a chemical group, normally active in the cell's metabolism, which, by combining with the drug, triggers the observed response. He showed that the receptors for arsenical drugs in trypanosomes were mercapto groups and that the (reversible) formation of As–S bonds brought death to the parasite.[6]

Several advances took place in the late 1920's. For some time A. J. Clark (London) had been showing that the action of drugs on receptors quantitatively followed the Law of Mass Action; i.e., the combinations were reversible and obeyed the law that Guldberg and Waage had worked out from ordinary, unbiological chemicals in 1864. Because no receptor had ever been isolated, Clark was obliged to work with, at best, single cells, but the quantitative and repeatable nature of his work, which used a variety of drugs and many tissues, created a much wider acceptance of receptor theory.[7]

Further evidence for the existance of drug receptors was provided by substances that form pairs of optically active isomers, as do atropine, morphine, and adrenaline. The two forms of each of these bases, namely, the dextro- and levorotatory isomers, differ strikingly in biological potency. Because the two members of such pairs have otherwise identical chemical and physical properties and differ only in that their molecules are bult as mirror images of one another, it became evident that the *shape* of a drug molecule can be crucial for its action and that a part of the molecule was obliged to fit a structure complementary to it (Cushny, 1926)[8] Other factors seen at that time to favor the idea of receptors were (a) the low concentration at which many drugs act (several of them even at 10^{-9} M), suggesting that a complementary structure must exist in the cell to rescue the drug from such great dilution, and (b) the high biological specificity of drugs.

In 1926, the world of pharmacology was shaken when Otto Loewi (Vienna) discovered the constitution of the first neurotransmitter.[9] It was acetylcholine, and the shock arose from the following paradox. Although a quaternary amine, acetylcholine was no muscle relaxant (like tubocurarine and all of Crum Brown's artifacts) but was actually nature's number 1 muscle activator! This discovery should have put an end to any more advocacy of the dogma "one chemical group gives one biological action", but this school of thought proved to be conservative.

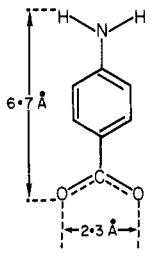
That a given chemical group could produce either an agonist or an antagonist, depending on its chemical setting, was explained by H. R. Ing (London) as follows: acetylcholine and tubocurarine act on the same receptor, but the smaller molecule exactly fits the site and activates it, whereas the larger molecule simply lies on top of the receptor and blocks it.[10] We now know of many series where the lower members are agonists, but the homologues of higher molecular weight are antagonists.

In the late 1920's drug scientists began to visualize an agonistic drug as relating to a receptor in much the way that a coenzyme is related to an enzyme. Similarly, an antagonistic drug and an enzyme antagonist were seen to have much in common.

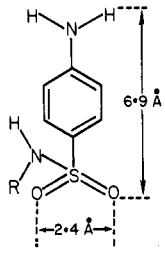
It was soon shown that some receptors actually were on enzymes. Edgar Stedman (Scotland) discovered in 1929 that the alkaloid physostigmine, which has an acetylcholine action, does not act directly on the receptor but blocks the enzyme (acetylcholinesterase) which has the task of destroying acetylcholine (ACh) after it has acted on the receptor. This blocking of the enzyme allows natural ACh to accumulate, so that the patient gets a continuing dose of his own neurotransmitter.[11] Since that time, many receptors have been found to be active sites on enzymes. However, the receptor for ACh is not on an enzyme but on a different kind of protein, one that regulates the passage of sodium and potassium ions in and out of the muscle cell without effecting any chemical change. This permease (as such a protein is called) was isolated in 1969 by J.-P. Changeux (Paris) from the electric organ of a fish.[12] When purified, it can be seen as rows of rosettes under the electron microscope. The permeases of other neurotransmitters have since been isolated but not in such large quantity. All appear to be glycoproteins, highly phosphorylated. However, as will be seen in what follows, not all receptors are situated on proteins.

Meanwhile, let us return to Stedman's location of a drug receptor on an enzyme. It seems curious that no extension of this phenomenon from pharmacodynamics to chemotherapy occurred until 1940, when Donald Woods (London) demonstrated the reversal, by *p*-aminobenzoic acid (PAB), of the antibacterial action of sulfanilamide.[13] He attributed this effect to a general steric and electronic similarity of the two substances **5** and **6**), and these two kinds of relationships were later quantified by Paul Bell and Richard Roblin.[14] Very small changes in these physical properties were shown to alter the action of the drug, for worse if the properties became too divergent but for the better if they converged. For example, the introduction of a π-deficient heterocyclic ring into the sulfonamide group increases its ionization as an acid, a change

(3) A. Crum Brown and T. R. Fraser, *Trans. Roy. Soc. Edinburgh*, 25, 151 (1869).
(4) P. Ehrlich and S. Hata, "Die Experimentelle Chemotherapie der Spirillosen", Springer, Berlin, 1910.
(5) J. N. Langley, *J. Physiol. (London)*, 1, 339 (1878).
(6) P. Ehrlich, *Ber. Deutsch. Chem. Ges.*, 42, 17 (1909).
(7) A. J. Clark, "The Mode of Action of Drugs on Cells", Edward Arnold, London (1933); *J. Physiol. (London)*, 61, 530 (1926).
(8) A. R. Cushny, "Biological Relations of Optically Isomeric Substances", Ballière, Tindall, and Cox, London, 1926.
(9) O. Loewi and E. Navratil, *Pflugers Arch. Ges. Physiol. Menschen Tiere*, 214, 689 (1926).
(10) H. R. Ing, *Physiol. Rev.*, 16, 527 (1936).
(11) E. Stedman *Am. J. Physiol.*, 90, 528 (1929).
(12) J.-P. Changeux, *Proc. Nobel Symp.* 11, 235 (1969).
(13) D. D. Woods, *Br. J. Exp. Pathol.*, 21, 74 (1940).
(14) P. Bell and R. O. Roblin, *J. Am. Chem. Soc.*, 64, 2905 (1942).



5 (*p*-aminobenzoic acid)



6 (sulfanilamide), R = H

that increases its resemblance to PAB and, hence, makes it a more potent antibacterial.

This correlation was confirmed in 1962 when Gene Brown (Massachusetts) isolated the enzyme dihydrofolate synthetase which makes an important coenzyme, dihydrofolic acid, of which PAB forms the central part. This enzyme has a receptor site for PAB which the sulfonamide antibacterials fit very well. The enzyme comes to equilibrium, quite reversibly, with either the natural substrate, the inhibitor, or both, depending on the concentrations present. When the sulfonamide is in excess, the enzyme is blocked and no dihydrofolic acid is made.[15]

This enzyme can also be antagonized by PAB analogues that are free of sulfur, such as 4,4′-diaminobenzil, which is much more antibacterial than sulfanilamide.[16] Here, as before, the physical properties (rather than the presence of a certain chemical group) created the required biological activity. Moreover, many successful diuretic and antidiabetic drugs which contain a sulfonamide group have been found, with the result that the "one chemical group gives one biological action" school lost most of its support.

Extension to insecticides of the idea that receptors could occur on enzymes was made soon after the end of the Second World War, when organic phosphates began to be used for their inhibition of acetylcholinesterase in insects.

I would now like to say something about the work which my colleagues and I did for the Australian Army during the Second World War. At that time, the aminoacridines, because of their high selectivity, were often used to irrigate deep, infected wounds. In short, they killed bacteria without harm to either leucocytes or abraded tissues, and the wounds rapidly healed. This work proved to be interesting in two ways, because it established, quantitatively for the first time, the importance of ionization in the action of a drug; also, it led the way to recognition that some drug receptors were not on proteins but, as here, on nucleic acids.

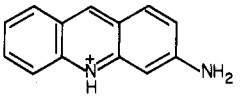
At first, it seems puzzling that of the five possible aminoacridines, two were highly antibacterial, whereas three had little activity.[17] At that time, almost nothing was known about the ionization of heterocyclic bases, so we began to determine the pK_a values of a great many examples, and later we published the main rules that connect structure with basic strength.[18] Fortunately, the correlation that we needed for our acridine work was found early. It turned out that the 3-amino- and 9-aminoacridines had a resonance in their cation that was lacking in the neutral species, and this made them very strong bases (e.g., 7 ↔ 8). The other three aminoacridines could not, for reasons of valence, acquire this resonance and, hence, were very little stronger than acridine itself, a weak base (see Table I). We called this the 4-aminopyridinium

Table I. Increased Bacteriostasis of Aminoacridines through Ionization

substituted acridine	% ionized as cation under conditions of test (pH 7.3; 37 °C)	minimal bacteriostatic concn (*Streptococcus pyogenes*)
1-amino	2	1 in 10 000
2-amino	2	1 in 10 000
4-amino	<1	1 in 5 000
3-amino	75	1 in 80 000
9-amino	100	1 in 160 000
2,7-diamino	3	1 in 20 000
4,5-diamino	<1	1 in <5 000
3,6-diamino	99	1 in 160 000
3,7-diamino	76	1 in 160 000
3,9-diamino	100	1 in 160 000

Table II. Failure of Electron-Attracting and Electron-Releasing Substituents to Modify Bacteriostatic Action of Aminoacridines, Apart from Any Direct Modification of Ionization

substituted acridine	% ionized as cation under conditions of test (pH 7.3; 37 °C)	minimal bacteriostatic concn (*Streptococcus pyogenes*)
4-amino-5-methyl	<1	1 in 5 000
1-amino-4-methyl	1	1 in 20 000
2-amino-9-methyl	3	1 in 20 000
9-amino-2-methyl	100	1 in 160 000
9-amino-3-methyl	100	1 in 160 000
9-amino-4-methyl	100	1 in 320 000
2-amino-6-chloro	<1	1 in <5 000
3-amino-9-chloro	11	1 in <5 000
3-amino-6-chloro	33	1 in 40 000
9-amino-3-chloro	94	1 in 160 000
9-amino-2-chloro	96	1 in 160 000
9-amino-4-chloro	86	1 in 160 000



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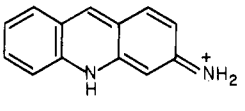

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type of base strengthening because we had demonstrated it first in 4-aminopyridine.

We went on to show, using 102 different acridines and 22 species of bacteria, that the antibacterial action of aminoacridines increased with the proportion that was ionized at the pH and temperature of the bacteriostatic test. The nature of any nonamino substituent, whether electron attracting or electron releasing, made absolutely no difference so long as it permitted at least 50% cationic ionization under these conditions.[19] Some examples are offered in Table II.

As a result of this work, we were able to replace the army's favored deep-yellow wound irrigant, proflavine (3,6-diaminoacridine), by the more selective and nonstaining aminacrine (9-aminoacridine).

Next, we made several stepwise alterations to this molecule, to discover the parameters of its efficacy. What we found was that any nucleus would do as well as acridine, so long as the candidate was (a) basic enough to be ionized at least 50% at the pH of our test and (b) had no less than 38 Å^2 of flat area. 4-Aminopyridine and 4-aminoquinoline, although ionized enough, had too little flat area, but when

(15) G. M. Brown, *J. Biol. Chem.*, **237**, 536 (1962).
(16) R. Kuhn, *Angew. Chem.*, **53**, 1 (1940).
(17) A. Albert, S. D. Rubbo, and R. J. Goldacre, *Nature (London)*, **147**, 332 (1941).
(18) A. Albert, R. J. Goldacre, and J. N. Phillips, *J. Chem. Soc.*, 2240 (1948).
(19) A. Albert, S. D. Rubbo, R. J. Goldacre, M. Davey, and J. Stone, *Br. J. Exp. Pathol.*, **26**, 160 (1945).

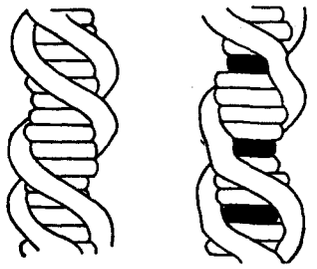
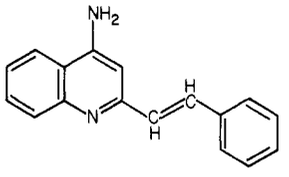


Figure 1. Sketches representing the structure of normal DNA (left) and of DNA with intercalated proflavine molecules (right). The base pairs and the intercalated aminoacridine appear in edgewise projection, whereas the phosphate deoxyribose backbone appears as a coil.[22b]

supplied with more of that property by inserting a coplanar substituent, as in 4-amino-2-styrylquinoline (**9**), the an-



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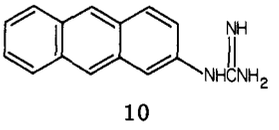

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tibacterial activity returned. Moreover, the order of the rings comprising the acridine molecule was found relatively unimportant, for many highly antibacterial aminobenzoquinolines and phenanthridines were soon brought to light. Not surprisingly, our standard, 9-aminoacridine, was deprived of its activity by hydrogenating one of the outer rings, an operation that deleted flatness from one-third of the molecule.

Finally, we boldly left the heterocycles behind and began to make basic anthracenes, such as 2-guanidinoanthracene (**10**). This has enough of both requirements: ionization and flatness. Moreover, it had the typical aminoacridine-like bacteriostatic properties, namely, activity against a wide range of Gram-positive and Gram-negative organisms at high dilution, in the presence of serum, without harm to phagocytes.[20] Here, just as with the antibacterial sulfonamides, the required biological action depends on the correct steric and electronic properties and not on the presence of a particular nucleus or substituent. Needless to say, such a conclusion was far less acceptable at that time than it is today.

That aminoacridines were accumulated only by the nucleic acids of the living cell became known through their use in vital staining.[21] The reason for a need for molecular flatness was explained in 1961 when L. Lerman (Colorado) showed that aminoacridine molecules became intercalated into DNA by stacking between the layers of base pairs, to which they clung by van der Waals forces supplemented by stronger ionic bonds to the phosphate ions of the DNA backbone (see Figure 1). The resultant increase of 20 °C in the T_M ("melting temperature") showed that intercalation had interfered with separation of the strands and, hence, with normal functioning of the DNA.[22]

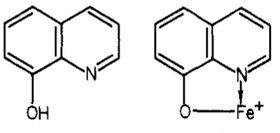
In the next year, J. Hurwitz and his colleagues in New York demonstrated that aminoacridines injure bacteria by blocking the DNA starter required by the polymerases that synthesize bacterial DNA and RNA.[23]

These studies of structure–action relationships in the aminoacridines established that nucleic acids can be receptors. In fact, the drug–receptor interaction was observable here in unusual detail, much of it at the level of molecular biology. It is now recognized that nucleic acids are receptors for all of the steroid hormones and many other kinds of drugs, including the nitrogen mustards used in treating cancer, as well as for many plant growth factors and several insect hormones.

Table III. Necessity for a Metal Cotoxicant for the Bactericidal Action of Oxine, as Shown by Incubating in Distilled Water at 20 °C and Plating Out after 1 h

oxine, 1/M	ferrous sulfate, 1/M	growth (*Staphylococcus aureus*)
nil	nil	prolific
100 000	nil	prolific
nil	100 000	prolific
100 000	100 000	undetectable

Meanwhile another project had been set up by the Australian Army, and once again some new general principles emerged. We had been asked to report on the mode of action of oxine (8-hydroxyquinoline) (**11**), which is

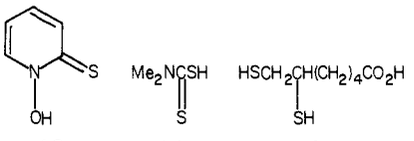


11 **12**

strongly fungicidal as well as antibacterial. We began by making the six isomeric hydroxyquinolines. We soon found that none of these chelated metals and none was antimicrobial. We formed the opinion that oxine was acting by chelation, a mode of action not accepted for any chemotherapeutic drug at that time. A typical chelated product (with ferrous iron) is shown as **12**. In this 1:1 complex, the iron is unsaturated for oxine because, given more oxine, it can form the 2:1 complex. To confirm our conclusion, we blocked the chelating properties of oxine by methylating, in turn, the nitrogen and the oxygen atom. The two products, as expected, were neither chelating nor antimicrobial.

However, we did not leap to the conclusion that oxine was removing a metal from the bacterium, as the antidotes dimercaprol and EDTA do from poisoned patients. In fact, oxine proved to be quite nontoxic for cells provided that certain metals were excluded, even in traces, namely, iron for bacteria and copper for fungi. Clearly oxine does not function without a metal cotoxicant, whose presence may be only accidental. This surprising conclusion was soon fortified by incubating *Staphylococcus aureus* in distilled water with oxine, with iron, and then with both chemicals. Subsequent plating out on nutritive media showed that only bacteria that were exposed to *both* oxine and iron were killed (see Table III).[24]

In soon became evident that drugs of quite different chemical structure, such as pyrithione (**13**), which is used



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in dermatology of the scalp, and sodium dimethyldithiocarbamate (**14**), a common agricultural fungicide, had the

(20) A. Albert, *Med. J. Aust.*, 1, 245 (1944); A. Albert, S. D. Rubbo, and M. I. Burvill, *Br. J. Exp. Pathol.*, **30**, 159 (1949).
(21) S. Strugger, *Jena. Z. Med. Naturwiss.*, **73**, 97 (1940).
(22) L. Lerman (a) *J. Mol. Biol.*, **3**, 18 (1961); (b) *J. Cell. Comp. Physiol.*, **64**(Suppl 1), 1 (1964); J. Chambron, M. Daune, and C. Sadron, *Biochim. Biophys. Acta*, **123**, 306 (1966).
(23) J. Hurwitz, J. Furth, M. Malamy, and M. Alexander, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 1222 (1962).

typical oxine mode of action. This action can be defined as the rapid killing of bacteria and fungi at high dilution, requiring the presence of either iron or copper, and preventable by a trace of cobalt (but no other metal).[25]

What was happening at the molecular level was revealed in 1959 when Sijpesteijn and Janssen (Holland) showed that these metal complexes catalyzed the destruction of lipoic acid (15), the coenzyme of pyruvic oxidase.[26] This chain reaction of consecutive oxidations, accompanied by reduction of the metal ion, is uniquely blocked by cobalt, as in nonbiological situations. Clearly, lipoic acid has to be recognized as the receptor for this drug, but at the time it caused surprise to find one of such low molecular weight.

We may now sum up all of the foregoing as follows: Drug scientists, after following for too long the seductive clue of 1869, slowly realized that chemical structure in a drug is usually only secondary to the physical properties that the structure can generate and which can be obtained also from distinctly different structures. The relevant physical properties are those that distribute the drug to the receptor and those that bind it when it gets to the receptor. It seems that these properties are three in number: lipophilicity, electron distribution (as evidenced by ionization, chelation, or Hammett σ values), and a steric nature complementary to the receptor. Already in 1900, Overton and Meyer had demonstrated the overriding importance of lipophilicity in the design of general anesthetics and hypnotics. For all other kinds of biological action, we now know that *all three* types of physical properties play a part but in different degree for different drug–receptor combinations.

Corwin Hansch has devised the "multiple regression equation" which, by the use of statistical methods and a computer, helps to predict optimal values for each of these three variables.[27] This approach has given some excellent results and is particularly valuable when time is at a premium. However, the biological situation is more complex than such an equation can accomodate. For example, distribution need not depend on lipophilicity but on the use of facilitated channels that exist for the uptake of natural products, such as sugars, purines, amino acids, and even choline. The steric term in the regression equation presents problems too, because a three-dimensional cavity in a receptor is likely to have *several* relevant dimensions, which cannot be expressed by a single numeral. For these reasons, those who have the time and the interest will continue to examine structure–action relationships in all their fine detail and complexity.

Nor is that the whole of their work! The rules that teach us how to produce *activity* tell us nothing about how to achieve *selectivity*, that very desirable property which enables a drug to act strongly on the designated cells wihout affecting any of the others. How is selectivity to be achieved? In my opinion, and I have been publishing along these lines for a quarter of a century,[28] there are three important approaches for differentiating between the economic and the uneconomic cell, namely, by the use of selective distribution, selective biochemistry, and selective cytological structure. There is no doubt in my mind that attention to these three variables will lead us to more powerful, yet much safer, drugs than have yet been at our command.

(24) A. Albert, M. I. Gibson, and S. D. Rubbo, *Br. J. Exp. Pathol.*, **34**, 119 (1953).
(25) S. D. Rubbo, A. Albert, and M. I. Gibson, *Br. J. Exp. Pathol.*, **31**, 425 (1950).
(26) A. K. Sijpesteijn and M. Janssen, *Antonie van Leeuwenhoek*, **25**, 422 (1959).
(27) The First Smissman Award Address: C. Hansch, *J. Med. Chem.*, **19**, 1 (1976); C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1610 (1964).
(28) A. Albert, "Selective Toxicity", 6th ed., Chapman and Hall, London, and Methuen, New York.

Articles

Synthesis and Pharmacological Evaluation of Conformationally Restricted Phenothiazine Analogues

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The synthesis of 3-(dimethylamino)-2,3-dihydro-4-chloro-1*H*-pyrido[3,2,1-*kl*]phenothiazine, its 10-chloro analogue, and two chloro isomers of 2-[(dimethylamino)methyl]-2,3-dihydro-1*H*-pyrido[3,2,1-*kl*]phenothiazine is described. In these compounds the side chain of chlorpromazine is fixed into a certain position in order to study the dopamine-overlap theory and the role of the substituents in the phenothiazine neuroleptics. The compounds were tested for their influence on dopamine metabolism, while for the second set their ability to displace [^{3}H]spiroperidol from dopamine receptors was assessed. No neuroleptic activity was found from the pharmacological data. The results are discussed on the basis of conformational requirements for dopamine antagonistic activity.

In a previous paper[1] we described our study concerning the role of the 2-substituent in the phenothiazine neuroleptics with flexible side chains. From the measurement of brain concentrations of promazine, chlorpromazine, and triflupromazine and their potential to increase the level of the dopamine metabolite homovanillic acid (HVA), it was evident that the relative potencies based on brain

(1) C. J. Grol, H. Rollema, D. Dijkstra, and B. H. C. Westerink, *J. Med. Chem.*, **23**, 322–324 (1980).

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