RELATIONS BETWEEN MOLECULAR STRUCTURE AND BIOLOGICAL ACTIVITY: STAGES IN THE EVOLUTION OF CURRENT CONCEPTS

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The discussion of what are at present called "Structure-Action Relationships" is fundamental to the teaching of both pharmacology and medicinal chemistry. Such discussions assist also in the rational evolution of new drugs, either from first principles or, more often, by the perfection of chance discoveries. The subject, so frequently treated in this *Review,* will be considered here from a somewhat historical aspect: how the really significant concepts began, progressed, and are continuing, also what the words "structure," and "action" implied at different times in the last hundred years.

THE FIRST CORRELATIONS

The year 1869 was a turning point. Before then, structure had been correlated with action only in simple salts. Long before Arrhenius introduced his theory of electrolytic dissociation (namely: salts dissolved in water are dissociated into oppositely charged ions) it was understood that the biological activity of a salt was due to its basic *or* its acidic component, and not to the whole salt as such. Thus the poisonous entity in lead acetate and lead nitrate was known to be the lead moiety and not the acetate or nitrate part. It was similarly known that the toxicity of sodium, potassium, and calcium arsenites resided only in the arsenite portion of these salts.

However in 1869, Crum Brown & Fraser (1) made a major discovery. They showed that several alkaloids, when quaternized, lost their characteristic pharmocological properties (many of them spasmogenic or convulsant) and acquired the muscle-relaxing powers of curarine, whose site of action at the junction between nerve and muscle had been neatly located, a few years earlier, by Claude Bernard (2). Strychnine, bruceine, thebaine, codeine, morphine, nicotine, atropine, and coniine were quaternized into curarimimetic substances, by reaction with methyl iodide but only coniine was endowed with *two* quaternary amino-groups.

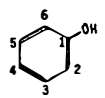
"CONSTITUTION" VERSUS "STRUCTURE"

The Scottish authors wrote: "There can be no reasonable doubt that a relation exists between the physiological action of a substance and its chem-

ical composition and constitution, understanding by the latter term the mutual relations of the atoms in the substance" (1). They went on to say that "constitution" meant more than "structure" (the arrangement of the atoms in a molecule), although they could suggest only one constitutional property, namely the energy required to break the bonds between pairs of atoms.

These discoverers of the first structure-action relationship among organic substances titled their paper "On the Connection between Chemical Constitution and Physiological Action." In this lies a paradox because, although they preferred "constitution" to "structure" yet, writing more than half a century before the discovery of the electronic theory of valency, they could see little more "constitution" in a substance than its structure. Contemporary writers, on the other hand, habitually write "structure" when they mean "constitution" because a rich harvest of chemical and physical properties can be gleaned from a chemical formula.

As examples of "reading out" constitutions when confronted by structures, phenol (I) and pyridine (II) will serve, but most broadly trained chemists can deal similarly with structures much more complex than these. The formula of phenol (I) shows it to be a weak acid of pK_a about 10; moreover if it were a substituted phenol, the rise or fall in acidic properties could be read out at once by subtracting the Hammett σ constant for that substituent [memorized, or taken from a table (3)] from the pK_a of phenol. Further, inspection of formula (I) shows a high density of electrons at positions 2, 4, and 6, and hence the molecule can be readily attacked at these positions by electrophilic reagents. In addition, the hydroxy-group is available for esterification and ether-formation. The ease with which all these reactions can take place in a substituted phenol is governed by the sign and magnitude of Hammett σ constants for the substituents, electron-attracting groups disfavoring and electron-releasing groups aiding the reactions. Nucleophilic attack and reactions of addition are clearly shown by formula (I) to be unlikely, reduction to be difficult, and oxidation to lead to quinones and hence to self-condensation. Sufficiently powerful, electron-attracting substituents (-CN, $-NO_2$, $-CONH_2$) will favor nucleophilic attack, addition reactions, and reduction; but will disfavor oxidation. The distribution of a phenol between an aqueous and a lipoid solvent can be approximately calculated by use of the π-constants for each substituent, as derived by Hansch and his school (4).



Phenol

I

Pyridine

II

Similarly the formula of pyridine (II) reveals at once that it is a weak base, with a pK_a about 5, and the basic strength of substituted pyridines can quickly be calculated (5). Further, the formula shows that there is a large deficiency of electrons in the 2, 4, and 6-positions, which are therefore liable to nucleophilic attack; only the nitrogen atom is open to addition reactions; electrophilic reactions are extremely unlikely; reduction, although not easy, is possible and oxidation unlikely. The rules, allowing these categories of information to be read out of the formulae of various kinds of heterocycles, are easy to memorize and have been set out (6) in convenient form for this purpose.

Thus when we speak of "structure-action relationships," we really mean "constitution-action relationships" because the discoveries of this century have trained our minds to read out many kinds of chemical and physical properties (many more, in fact, than the above sampling) by mere inspection of the printed structure. There are some kinds of drug action where the structure itself plays an outstanding part, namely in the use of metabolite analogs. In many other kinds of action, a physical property is its main source, and this property could be provided by many kinds of structure. It seems appropriate to present, as the first example, a biological action that is practically independent of structure, namely metabolic depression (narcosis).

At the turn of the century, Overton & Meyer (7) independently advocated the lipoid hypothesis of narcosis: chemically inactive substances of widely different structures have depressant properties, especially on cells that are particularly rich in lipids, such as nerve cells; the higher the partition coefficient of the agent (between a lipid and water) the greater the depressant action. If after the words "partition coefficient" we may be allowed to insert "up to the point where hydrophilic properties are almost lost," this statement is as true today as when it was written. It was challenged in vain by the surface-tension hypothesis of Traube (8), and the ice-structure of Pauling (9) that was put in question by Miller et al (10). Ferguson (11) reduced the Overton-Meyer rule to terms of thermodynamic activities, which for aqueous solutions are roughly equal to the relative saturation of equiactive solutions. Table 1 exemplifies how substances with molecules of different sizes and structures, that cause narcosis of tadpoles at very different concentrations, are nevertheless functioning within as little as a twofold span of thermodynamic activity (12). In the last two decades, it has been shown that chemically inert gases such as xenon (and even nitrogen under pressure) behave as typical general anesthetics in man. The biophase where they act is still not quite clear, although mitochondrial membranes of neurones are indicated. But it is clear that a large variety of substances, of the most diverse structure, can cause one particular physiological effect by virtue of a physical property, lipophilicity, which causes them to accumulate in the central nervous system where they interrupt communication (reversi-

TABLE 1. SUPPRESSION OF MOTILITY OF TADPOLES[a]

Substance	Minimal depressant concentration, moles/litre	Minimal depressant activity, $\times 10^2$
Ethanol	0.41	2.8
Butanol	0.02	2.1
Octanol	0.0001	2.8
Acetone	0.28	3.0
Chloroform	0.001	1.9
Ether	0.03	4.0
	400-fold	2-fold

[a] From Brink & Posternak (12)

bly) simply by being foreign matter. This is almost the only kind of biological activity in which structure simply does not matter.

At the beginning of this century, the Overton-Meyer rule was deeply disturbing, because in the preceding 30 years no important correlation between structure and activity had come to light. In short, the Crum Brown & Fraser example stood alone, and its force was attenuated by the finding that quaternary amines of lower molecular weight had only slight curarizing action, and that tetramethylammonium salts could actually antagonize this effect. However help was about to arrive from two sources, (*a*) the upwelling of the concept of a "receptor," and (*b*) a vague feeling that the action of drugs on cells had something in common with the inhibition of enzymes which were, at that time, said to be "poisoned" by their inhibitors. These two influences will now be discussed more fully.

RECEPTORS, EARLY CONCEPTS

The concept that cells contained "receptors" for drugs was first advocated in 1878 by Langley (13). After studying the opposed actions of atropine and pilocarpine on the flow of saliva in the cat, he wrote:

> We may, I think, without much rashness, assume that 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.

However, the word "receptor" was introduced later by Ehrlich, early in this century. From his experiences in the new science of chemotherapy, he saw receptors as chemical groups that gave a biological response by uniting with chemically complementary groups of natural or foreign molecules. Hence he defined (14) a receptor as: "That combining group of the protoplasmic molecule to which a foreign group, when introduced, attaches itself." Ehrlich maintained, as we still do, that the mercapto-group (-SH) is

the arsenic receptor in trypanosomes, and that the blockade of this metabolically essential group, by arsenic, causes the death of these organisms. Although this reaction, between drug and receptor, involved covalent bonds, Ehrlich was aware that other bonds, more easily reversible than these, were concerned in the action of most drugs because he wrote (15): "If alkaloids, aromatic amines, antipyretics or aniline dyes be introduced into the animal body, it is a very easy matter, by means of water, alcohol, or acetone, to remove all these substances quickly and easily from the tissues." Thanks to Pauling's codification of the various types of chemical bonds (16) (first put forward by him in 1939) the contemporary biological worker knows well their variety and characteristics. Drugs that act by forming covalent bonds are still relatively uncommon but include such important compounds as penicillin, and organic phosphates.

Early confirmation for the existence of drug receptors was provided by substances that form pairs of optically-active stereoisomers. Numerous drugs, including morphine, atropine, and epinephrine, have dextro- and levo-rotatory isomers which differ strikingly in potency. Because the members of such pairs have identical chemical and physical properties, and differ only in that their molecules are mirror images of one another, it is evident that the *shape* of a drug molecule can be crucial for its action and that a part of such a molecule has to fit a structure complementary to it. This evidence was convincingly summarized (17) by Cushny in 1926.

The receptor concept was received with considerable skepticism, but it became more firmly established by the quantitative work of A. J. Clark which was begun about 1920 [see (18) for a summary]. Clark was the first to show that drug-receptor combinations obeyed the law of mass action (as foreseen by Langley). He showed that a great deal of the most accurate quantitative data on drug action could be interpreted as the result of the formation of a bond (not necessarily covalent) between a drug and a receptor specific for this drug. He further saw that, for agonists (as opposed to antagonists) the situation was more complex but could be analyzed further as follows: "The action of acetylcholine depends on at least two separable factors, firstly the fixation of the drug by certain receptors, and secondly the power to produce its action after fixation" (18). Further experimental work by Ariëns led to similar concepts, namely "affinity" and "intrinsic activity" respectively (19). This idea of intrinsic activity was soon supplemented by Stephenson's concept of "efficacy," namely the different proportions of a receptor that must be occupied by various drugs to elicit the maximal response (20).

These concepts, from Langley onwards, have laid the foundations on which contemporary ideas about receptors rest. More will be said about these later.

Analogies with Enzymes

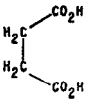
Even in the last century, it was seen that the reaction of a receptor with a drug resembled the reaction of enzymes with their substrates, coenzymes,

and antagonists. The great specificity of structure (even stereospecificity) required for a drug to show activity, had well-known parallels in enzyme work. Moreover the specificity of enzymes can be split into two independently exerted components: (*a*) different substrates are bound with different affinities, and (*b*) different substrates are chemically changed at different rates. But here any analogy between a drug and a substrate breaks down, because the most careful investigation of the action of drugs on isolated cells and tissues has never produced any evidence of chemical change in the drug. The analogy was therefore diverted to comparisons with enzyme inhibitors (for chemotherapeutic agents and other antagonists) and with coenzymes (for agonists).

The notion that many drugs act as enzyme antagonists was swept into prominence in 1940 by the work of Woods on the antibacterial action of sulfonamides (21), but it had much earlier (though rather vague) origins.

This notion of metabolite antagonism was foreshadowed (22) by the work of Ringer (1883) who found that the sodium (cations) in a solution of sodium chloride could not maintain the beat of an isolated heart unless balanced by calcium and potassium. By 1910, it was known, more relevantly, that many enzymes could be blocked by substances whose molecular structure resembled those of their substrates. Thus amylase, whose substrate is starch, is strongly inhibited by dextrin, maltose, and glucose all of which have a configuration similar to the unit of which starch is a polymer. Later simpler examples were found, e.g. malonic acid (III) which competitively inactivates the enzyme succinic dehydrogenase by displacing the normal substrate, succinic acid (IV), from the enzyme (23).

III



IV

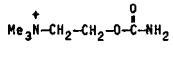
As early as 1910, a parallel was found in physiology, as follows: the toxic action of carbon monoxide (C-O) was recognized as resulting from the displacement of a similarly shaped molecule, oxygen (O-O), from combination with hemoglobin (24).

No close connection between these discoveries and the action of drugs was suspected until it was shown (25) that physostigmine (eserine) caused the heart to contract, not by direct action, but by blocking the local enzyme, acetylcholinesterase, thus allowing environmental acetycholine to act on the heart. Here at last was exact knowledge of the nature of a receptor, for this receptor was quite plainly an enzyme. (However this enzyme is not a receptor for acetylcholine, but a "site of loss." The receptors for acetylcholine are still little known chemically.)

The Enzyme as One Kind of Receptor

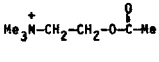
It was soon found (26) that the portion of the physostigmine molecule that inhibited esterases was the methylcarbamoyloxy-group (V). When, in 1932, it was shown that carbachol (VI), which had a carbamoyloxy-group, exhibited very much the same biological effects as acetylcholine, it was correctly supposed that physostigmine blocked acetylcholinesterase by causing that enzyme to adsorb it instead of its normal substrate, acetylcholine (VII). However this relationship was a little too complex to be widely appreciated.

V



Carbachol (cation)

VI



Acetylcholine (cation)

VII

By 1934 it was recognized that acetylcholine is the natural substance that transmits the nervous impulse across the synapse between nerve and muscle (27). Ing (28) quickly saw that tubocurarine must block neuromuscular transmission by competing with acetylcholine for the latter's receptors which tubocurarine could not operate. Because both substances were quaternary amines, Ing went on to interpret the somewhat weaker curariform action of innumerable quarternary ammonium, phosfonium, arsonium, stibonium, and sulfonium salts as arising from their competing similarly with acetylcholine.

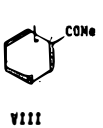
These interpretations shed a new light on the "one-group one-action" hypothesis that had dominated the thoughts of many workers in this field, especially in the drug industry (29–32). This hypothesis had been born with the Crum Brown & Fraser correlation (1) and was nurtured by Ehrlich's successes in the cure of trypanosomiasis and syphilis with the aromatic arsenicals, and by the wide range of barbiturates (and other ureides) that had shown hypnotic action. It was now clear that drugs with a given group could have two quite opposite actions, one action if they could mimic a natural metabolite, but the exact opposite action if they interfered with the working of this metabolite. Both actions spring from close similarity of one part of the drug to a corresponding part of the metabolite: which of the two actions was elicited was seen to depend on very small differences in the rest of the molecule (for example, see tetramethylammonium salts, above).

About this time, the first antivitamin was discovered by a happy acci-

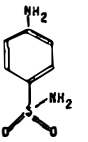
dent. Woolley (33) tested analogs of nicotinic acid, particularly 3-acetylpyridine (VIII). In the belief that these analogs would have, at least qualitatively, the biological action of the vitamin, they were fed to dogs suffering from a nicotinic acid deficiency which the analogs (to everyone's surprise) made much worse. A clear picture of what was happening emerged in 1940 when Woods demonstrated the reversal of the antibacterial action of sulfanilamide (IX) by *p*-aminobenzoic acid (X) and pointed out that this reversal depended on the structural similarity of these two substances (21).

It is worthwhile listing here what these two substances have in common: (*a*) each has a primary amino-group attached directly to a benzene ring, (*b*) situated *para* to this group (i.e. not *ortho,* not *meta*) is a weakly acidic group (pK_a 4.8 for *p*-AB[1]) that is not essential for the action, but it cannot be replaced by a stronger acidic group that would interfere with distribution, (*c*) lengthwise the molecules measure between 6.7 and 6.9 Å. The amino-group attached to sulfur is inserted at a sharp angle to the rest of the roughly planar molecule; it provides the opportunity for subsitution by various groups (usually heterocyclic) that can favorably modify electron distribution in the rest of the molecule without interfering sterically with the location on the receptor-enzyme.

The selectivity of the antibacterial sulfonamides, so favorable to man, depends on the fact that although *p*-aminobenzoic acid, which is part of the molecule of folic acid (XI), is an essential requirement for many microorganisms, it is not utilized by mammals, which, on the contrary, cannot synthesize their own folic acid but must absorb it from the diet [very few bacteria can absorb preformed folic acid (34)]. These constitute two most fortunate differences between bacterial and mammalian biochemistry. The

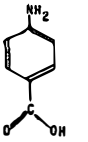


VIII



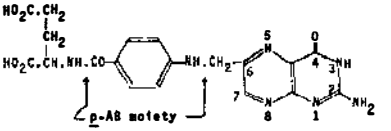
Sulphanilamide

IX



p-Aminobenzoic acid

X



Folic Acid

(pteroylglutamic acid)

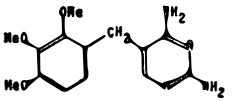
XI

[1] *p*-AB = *p*-aminobenzoic acid.

Woods correlation took drug designers by storm: many thousands of analogs of vitamins and other natural metabolites were made and tested, usually without any useful result because they proved to be just as toxic to the host as to the parasite. It was only slowly realized that it is quite unusual and rare to find large metabolic differences between bacteria and man, and that unless these are first sought at the level of comparative biochemistry, the time of a whole army of chemists can be wasted in the production of metabolite analogs of no possible use in clinical medicine.

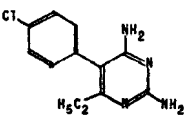
The receptor for sulfonamides is an enzyme, dihydrofolate synthetase. This has been isolated (35) and was found to catalyse the condensation of *p*-aminobenzoic acid with 2-amino-4-oxo-6-pyrophosphoryloxmethyl-7,8-dihydropteridine, an important step in the biosynthesis of folic acid.

Folic acid plays a leading role in the biosynthesis of purines and pyrimidines in all forms of life. The active coenzymes are tetrahydropteridines produced by the enzyme 7,8-dihydrofolate hydrogenase. This enzyme in bacteria and protozoa is very susceptible to inhibition by 2,4-diaminopyrimidines such as trimethoprim (XII), whereas the corresponding enzyme in man is over 10,000 times less sensitive (36). This is exemplified in Table 2. The corresponding drug for the malarial parasite is pyrimethamine (XIII), which is widely used to prevent malaria.



Trimethoprim

XII



Pyrimethamine

XIII

TABLE 2. EFFECT OF TRIMETHOPRIM (XII) ON ISOLATED DIHYDROFOLATE HYDROGENASE

Concentration ($\times 10^8$ M) causing 50% inhibition

Source: Mammalian liver		Bacteria	
Man	30,000	*E. coli*	0.5
Rat	26,000	*S. aureus*	1.5
Rabbit	37,000	*P. vulgaris*	0.4

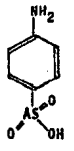
From (36)

These 2,4-diaminopyrimidines occupy a receptor on dihydrofolic hydrogenase normally reserved for the 2-aminopyrimidine ring of 7,8-dihydrofolic acid. It has been most fortunate, because unusual, that the analogous enzymes in different species should show such different susceptibilities to these drugs. 2,4-Diaminopteridines, although very efficient inhibitors of the

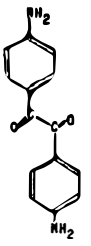
enzyme, do not differentiate in this way. Hence the clue to selectivity lies in the alkyl- and aryl- groups which are shown here on the left hand side of formulae (XII) and (XIII). It is possible that these groups make the molecule more selective because of their steric effect. As Fastier wrote in these *Reviews* (37): "Some structural features of a good drug are important, not so much because they aid interaction with particular receptors, as because they preclude interaction with numerous other receptors."

The sulfonamide antibacterials, which, soon after their discovery in the late 1930's, seemed to reinforce the "one-group one-action" hypothesis, ended by dealing it a mortal blow. It soon became evident that the presence of a sulfonamide group would not introduce antibacterial properties unless the other conditions (given above) for fitting the enzyme-receptor were observed. Moreover many nonsulfonamides were found to compete strongly with *p*-AB for a place on the receptor. Typical examples were *p*-aminobenzenearsonic acid (XIV), 4,4′-diaminodiphenylsulfone, and 4,4′-diaminodiphenylglyoxal (XV) (diaminobenzil). The necessary feature (as for sulfanilamide) is an electro-negatively charged group separated from a primary amino group by the same distances as in *p*-AB [see Section 6.3 of (38)]. On the other hand, many sulfonamide drugs have been evolved which, although lacking antibacterial properties, are excellent carbonic anhydrase inhibitors (and hence diuretics), or antidiabetics. There is some evidence that the diuretic effect follows from the masking of zinc (by the sulfonamide group) in the enzyme carbonic anhydrase, but special steric, distributional, and electronic (39) features must be built into the supporting nucleus to produce a useful drug.

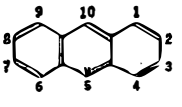
Thus the period of the Second World War was a turning point in the study of structure-action relationships. Mere "paper resemblances" between two formulae began to lose favor as a guide to biological action. Less often were groups (or nuclei) assumed to be the direct source of some pharmacological effect, and more attention was given to the physical properties which these groups (and nuclei) introduced and maintained. The chief physical properties studied were (*a*) electron distribution (e.g. ionization), which could facilitate or forbid the combination of a drug with its receptor, and



XIV



XV



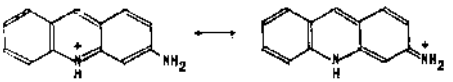
Acridine

XVI

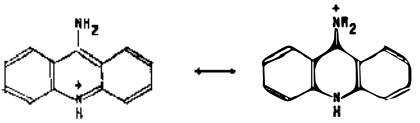
(*b*) steric properties which governed access to the correct receptor and a good fit upon arrival there. Both of these factors came to the fore in studies of the aminoacridines which will now be described.

The Aminoacridine Story

A discovery made in 1941, that the antibacterial activity of aminoacridines was proportional to the fraction ionized as cation (40), constituted the first step in uncovering the physical basis for their biological properties. Acridine itself (XVI) is only a weak base, with a pK_a of 5.3 in water at 37°, and hence only 1 percent is ionized at pH 7. However two of the five monoaminoacridines are strong bases, and this strength arises from a resonance in their cations that is not possible in the other isomers (41). The resonances that confer these strong basic properties on 3- and 9-aminoacridine are shown in formulae (XVII) and (XVIII) respectively.



XVII



XVIII

The positive correlation between ionization and bacteriostasis is demonstrated in Table 3 with *Staphylococcus pyogenes*. It has also been shown with a large variety of other bacterial species including aerobes and anaerobes, Gram-positive and Gram-negative types (42). The first five substances in Table 3 (all of them mono- or di-aminoacridines) are feebly ionized under the conditions of the test, and they proved to be feebly antibacterial. The next five substances, isomers of the five above them, are highly ionized, and they were found to be highly antibacterial.

Many substituted acridines and aminoacridines were then synthesized and tested, and it was always found that the substituents exerted no direct effect on the antibacterial action, but modified it through their effect on the ionization, as predictable from the sign and magnitude of the Hammett sigma constant of each substituent (see above). Thus Table 3 shows several amino-methyl- and amino-chloro-acridines that are poorly ionized and hence poorly antibacterial, as well as an equal number of isomers of these compounds that are highly ionized and hence highly antibacterial. This correlation between ionization and bacteriostatic activity was found to apply also to the bactericidal action of these substances (42). This work led to the introduction of 9-aminoacridine into the clinic for the local treatment of

TABLE 3. DEPENDENCE OF BACTERIOSTASIS ON IONIZATION

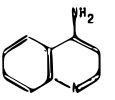
-acridine	Minimal Bacteriostatic Concentration (*Strept. Pyog.*) 1 in	Percentage Ionized as Cations (pH 7.3, 37°C)
4-Amino-	5,000	<1
2-Amino-	10,000	2
1-Amino-	10,000	2
4,5-Diamino-	<5,000	<1
2,7-Diamino-	20,000	3
3-Amino-	80,000	73
9-Amino-	160,000	100
3,9-Diamino-	160,000	100
3,7-Diamino-	160,000	76
3,6-Diamino-	160,000	99
2-Amino-9-methyl-	20,000	3
1-Amino-4-methyl-	20,000	1
4-Amino-5-methyl-	<5,000	<1
2-Amino-6-chloro-	<5,000	<1
3-Amino-9-chloro-	<5,000	11
3-Amino-6-chloro-	40,000	33
9-Amino-2-methyl-	160,000	100
9-Amino-3-methyl-	160,000	100
9-Amino-4-methyl-	320,000	100
9-Amino-2-chloro-	160,000	96
9-Amino-3-chloro-	160,000	94
9-Amino-4-chloro-	160,000	86

From (42)

deep, infected wounds (43), and this substance is now included in the pharmacopoeias of several countries.

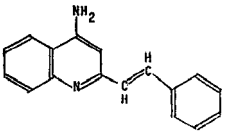
The antibacterial action of aminoacridines takes place at high dilution, even in the presence of serum proteins, and without harm to mammalian tissues. Because no other cations known at that time (1945) had this combination of properties, my colleagues and I decided to alter the molecule of 9-aminoacridine in a bold but systematic way (43). Our aim was to learn more about the physical basis of "aminoacridine-type" bacteriostasis. The stepwise deletion of rings gave 4-aminopyridine [see (II)] and 4-aminoquinoline (XIX), but these lacked antibacterial activity although they were as highly ionized as 9-aminoacridine. It seemed to us that the only physical difference between 4-aminoquinoline and 9-aminoacridine was the loss of about 10.6 sq.Å of flat area (the acridines, quinolines, and pyridines are en-

tirely planar molecules). By inserting a styrl-group into 4-aminoquinoline, to give *trans*-4-amino-2-styrylquinoline (XX), a molecule was created with 49.9 Å of flat area: this substance had most of the antibacterial activity of 9-aminoacridine (38.5 sq.Å). The latter was probably the optimal area because the addition of another benzene ring to 9-aminoacridine, which gave the corresponding aminobenzacridine (XXI) (48.9 sq.Å), did not increase the antibacterial action. From these experiments, we concluded that 28 sq.Å of flat surface was too little to allow typical aminoacridine bacteriostasis, but 39 sq.Å was enough. Investigation of other isomers and derivatives supported this conclusion (43).



4-Aminoquinoline

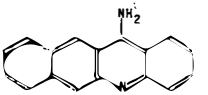
XIX



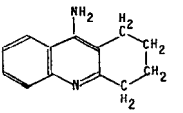
4-Amino-2-styrylquinoline

XX

To test this hypothesis further, flatness was taken away from part of the 9-aminoacridine molecule by hydrogenation of one ring to give 9-amino-1,2,3,4-tetrahydroacridine (XXII). In this formula, the right-hand ring has become three-dimensionally bulky (i.e. space-filling) by the loss of conjugated double bonds that always ensure flatness. Thus, the flat area was decreased to 27.9 sq.Å. As expected, this substance lacked antibacterial properties,[2] although completely ionized at the pH of the test (pH 7.3).

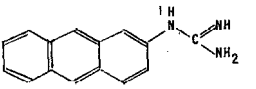


XXI



9-Amino-1,2,3,4-tetrahydroacridine (tacrine)

XXII



2-Anthrylguanidine

XXIII

The approximate areas quoted above were those of the minimal rectan-

[2] However 9-amino-1,2,3-4-tetrahydroacridine is a clinically successful analeptic. Its lack of affinity for nucleic acids allows it to reach the necessary receptor in high concentration.

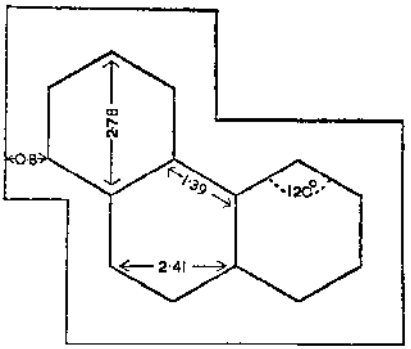


FIGURE 1. Minimal rectangular envelope (drawn at the van der Waals distance of 0.8 Å from carbon and nitrogen atoms) used as an approximate measure of the area of the planar position of a molecule. From (43)

gular envelopes into which the molecule (plus 0.8 Å for the van der Waals distance of closest approach) could be fitted. As an example, phenanthrene is shown in Figure 1, which also serves as a model for aminophenanthridines that obey the same rules as aminoacridines.

Further exploration showed that aminoacridine-type bacteriostasis could be obtained with nonheterocyclic cations, provided that at least 39 sq.Å of flat surface was present (43). A typical example is 2-anthrylguanidine (XXIII) where the anthracene nucleus provides the flat area, and the guanidino-group ensures complete ionization as cation.

We postulated that this need for a large, flat surface in antibacterial cations meant that they had to fit an equally flat receptor; moreover the need for cationic ionization suggested strongly that this receptor was anionic. What could it be? In 1940, Strugger (44), while investigating the phenomenon of vital staining, showed that aminoacridines accumulated in the nucleic acids of living cells (DNA fluoresces green and RNA orange). The cells of higher organisms are unharmed by this staining, and continue to reproduce. But bacteria, which have a less differentiated structure, are vulnerable to low concentrations of aminoacridines. In bacteria, the DNA is not in a nucleus but attached to the cytoplasmic membrane; hence it can accumulate more of an aminoacridine than does the DNA of a higher organism.

It seemed to us, then, that the nucleic acids were the target for antibacterial action. This hypothesis was strengthened in 1953 when Watson & Crick published their twin-helix model for DNA (45). This model showed the now well-known ladder-like assembly of purine-pyrimidine pairs, each presenting about 50 sq. Å of flat surface, and held in place by two continuous sugar-phosphate spiral strands. This, we felt sure, was the receptor for aminoacridines.

In 1961, Lerman proposed that aminoacridine molecules interacted with

DNA by intercalation, namely by lying
ecule of the acridine on any one base pair, and not every pair so occupied, at saturation). To make the necessary space between the pairs, which are normally tightly packed, the spiral molecule of DNA had to untwist (46). This hypothesis was based on Lerman's observations that aminoacridines (*a*) increased the viscosity of DNA, hence these cations must have stiffened the rod-like molecules of this nucleic acid, and (*b*) decreased the sedimentation of DNA, showing that they decreased mass per unit length. The concept of stiffened rods was confirmed by small-angle X-ray scattering (47), flow dichroism, polarization of fluorescent light (48), and inhibition of diazotization of 3,6-diaminoacridine (proflavine) when this was adsorbed on DNA (49).

More direct evidence was provided by Cairns who showed, by autoradiography of ^{3}H-labelled DNA (in phage), that proflavine caused a good deal of lengthening of the DNA molecule (51). Valuable confirmation came also from the discovery (52) that proflavine increased the "melting temperature" of DNA, by about 20°, and that most of this acridine was released suddenly when the DNA complex melted (i.e. changed from a twin strand to two single strands). From this increase in the temperature of thermal denaturation, free energy calculations were made on the basis of various models. The results (53) strongly supported the intercalation model, and were incompatible with an edgewise attachment of the acridine. Many other aminoacridines and aminobenzacridines, examined in the same way, reinforced this conclusion (54).

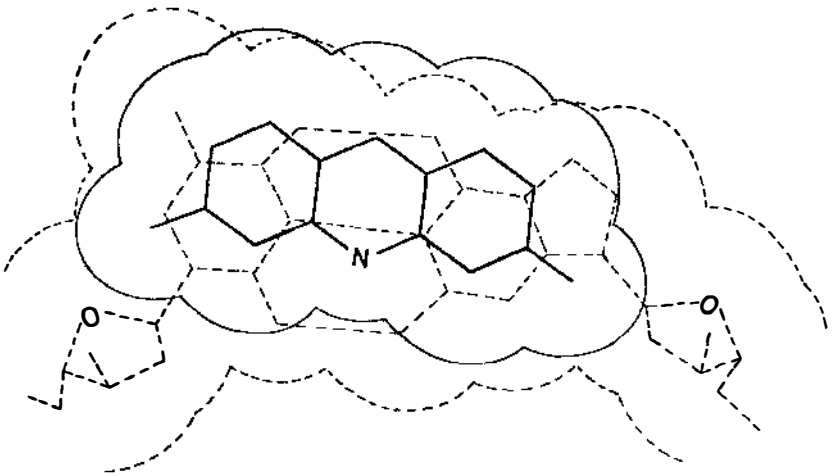


FIGURE 2. Intercalation of 3,6-diaminoacridine (proflavine) into DNA; section through the complex, showing one proflavine molecule lying on one base pair and forming ionic bonds with phosphate anions. From (50)

Because only those acridines that were highly basic caused marked physical changes in DNA, Lerman postulated an ionic (salt-like) link between the aminoacridine cations and phosphate anions in the backbone of the helix. He noted that this link, which otherwise could only be ephemeral, was maintained by a large number of van der Waals bonds between each aminoacridine molecule and the purine-pyrimidine pair on which it lay (50). This conception is reproduced in Figure 2, and the total effect (i.e. on a strand of DNA)is shown diagrammatically in Figure 3. To increase the distance between base pairs to 6.72 Å (to make room for an aminoacridine molecule) requires only 12° of untwisting (55).

It appears that the injurious effect of an aminoacridine on microorganisms is caused by its blocking the "starter." This starter is a DNA molecule required by enzymes that synthesize DNA and RNA, and also repair

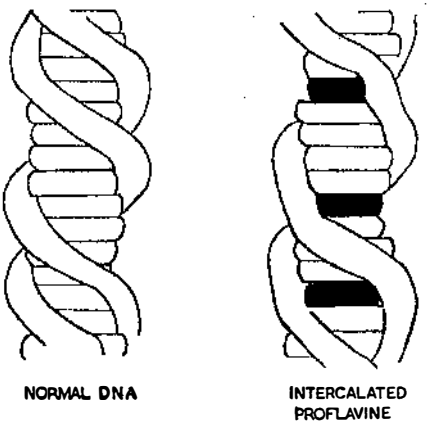
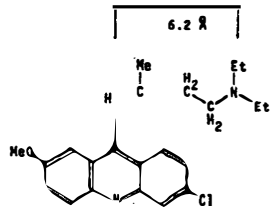


FIGURE 3. Sketches representing the secondary structure of normal DNA (left) and DNA containing intercalated proflavine molecules (right). The helix is drawn as viewed from a remote point, so that the base-pairs and the intercalated proflavine appear in edgewise projection, and the phosphate deoxyribose backbone appears as a smooth coil. From (50).

injured DNA. Thus, in vitro, proflavine (30 μM) inhibits DNA polymerase by 85 percent and RNA polymerase by 30 percent (56), and the action can be shown to be exerted mainly on the starter by plotting the reciprocal of the velocity against the reciprocal of the DNA starter concentration.

The efficacy of aminoacridines against protozoa can be greatly increased by furnishing the molecule with a basic side chain, although this does not increase the antibacterial properties. A typical example is the powerful antimalarial, mepacrine ('Atebrin', quinacrine). Mepacrine is accumulated by the organism from very dilute solutions because, in addition to intercalating, this drug (XXIV) forms an extra ionic link between the far end of the

basic side-chain and a slightly remote phosphate anion (57). At first it was thought that each of the two ionic links (that from the side-chain and that from the nucleus) involved a different strand, but it is likely that mepacrine binds two (consecutive) phosphate anions on the *same* strand where they are only 7 Å apart (58). This concept followed from the discovery that ethylenediamine, which is too short to cross even the minor groove in DNA, can stabilize the helix. The pK_a of the basic group at the far end of the chain of mepacrine is 10.48, whereas that at the near end of the chain (in resonance with the nitrogen atom of the nucleus, as explained above) is 7.92. Hence, although both ends of the chain are ionized at pH 7, any shortening of the distance between the two nitrogen atoms must greatly decrease the lower pK_a (by Coulomb's Law) and, in fact, it does cause nuclear ionization to decline and finally disappear. Consonant with this, the antimalarial action of mepacrine analogs declines and disappears as the chain is shortened.



XXIV

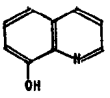
This account of structure-action relationships in acridines has shown that nucleic acids can be receptors. In fact, the drug-receptor interaction has been observable here in unusual detail. The need for studying physical properties, both ionization and steric effects, is well illustrated. The reason for the high selectivity of mepacrine, however, is not yet understood.

Oxine, and the Chelation of Metal Cations

Several chelating agents are regularly used in hospitals as antidotes for poisoning by metals. These antidotes circulate in the blood-stream without greatly depleting the body's essential metals, a tribute to the strength with which these cations are bound, e.g., by the enzymes that they activate. 8-hydroxyquinoline (oxine) (XXV), a powerful antibacterial and antifungal agent, although a strong chelating agent, does not act by depleting cell reserves of metals. Because it was the first antimicrobial chelating agent to undergo structure-action investigation, thus providing a model for other types, a brief history will be given of the steps by which its mode of action came to be understood.

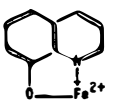
Typical of the way of thinking in the early 1930's, Hata (59) suggested that oxine owes its antibacterial properties to a combination of those of quin-

oline and phenol in the one molecule. Yet neither quinoline nor phenol is at all antibacterial at a dilution of 1:5000, whereas oxine is active at 2 parts per million. That the biological properties of two substances could be combined by introducing their individual groups into a single molecule strikes us to-day as absurd, because the favorable electronic distribution of each of the constituent molecules (here, phenol and quinoline) must, more often than not, be incompatible and hence ruined by condensation into a single molecule. Oxine, moreover, has six nonantibacterial isomers (namely 2-, 3-, 4-, 5-, 6-, and 7-hydroxyquinoline) (60).



Oxine

XXV



The 1:1 – ferric complex of oxine

XXVI

The outstanding chelating properties of oxine are due to the juxtaposition of the oxygen and nitrogen atoms which permits the tight binding of heavy metal cations in a 5-membered ring, as shown in (XXVI). The remaining positive charge(s) on the metal can be progressively removed by binding with further molecules of oxine, and the complexes become progressively more liposoluble. Once we had established, by structural variations, that the antimicrobial action of oxine was closely linked to its chelating properties (60), my colleagues and I posed this question: Does oxine act by removing metals essential to bacterial welfare, or does it cause traces of metals in the medium to become more toxic to the bacteria? The latter proved to be the case (61), as first indicated by the appearance of "concentration quenching," exemplified as follows. Staphylococci were killed in an hour by 0.00001M oxine but were unharmed by 0.0007M oxine; in fact even a saturated (i.e. 0.005M) solution did not kill them. Streptococci behaved similarly (61).

The meaning of this concentration quenching became evident when it was found to occur only in media containing traces of heavy metals (such as bacteriological media commonly have). The viability of staphylococci for at least 24 hours in distilled water permitted the decisive experiments shown in Table 4 to be made.

It can be seen from Table 4 that oxine (0.00001M) is biologically inert, but it becomes bactericidal in the presence of a similar quantity of iron, although the iron alone is not toxic. Clearly the toxic agent is not oxine, but an oxine-iron complex. When broth replaced water, no added iron was necessary because it was present in the medium. When the concentration of oxine was increased to 0.0013M, the bactericidal action disappeared because of concentration quenching, as described above. This last result showed that the toxic action was caused by the 1:1 complex (XXVI), or the 2:1 com-

TABLE 4. THE INNOCUOUSNESS OF OXINE IN THE ABSENCE OF IRON (BACTERICIDAL TEST)

Staph. aureus: pH 6–7 (20°)

Oxine conc. (M)	$FeSO_4$, or $Fe_2(SO_4)_3$ conc. (M)	Growth after 1 hour	
		Glass-distilled water	Untreated meat broth
nil	nil	+ + +	+ + +
0.00001	nil	+ + +	—
nil	0.00001	+ + +	+ + +
0.00001	0.00001	—	—
0.08	nil		+ + +
nil	0.08		+ + +
0.08	0.08		—

+++ means innumerable colonies, —means no growth
From (61)

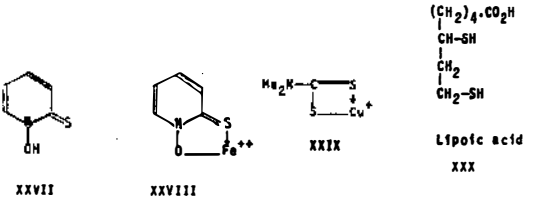
plex, but not by the 3:1-complex which must be the only form present when oxine is in excess. Therefore we added iron in sufficient amount (0.0013M) to equal that of the oxine, and thus restore the 1:1 complex. As expected, this combination proved just as highly bactericidal as the earlier ones (see Table 4). It is noteworthy that 0.0013M iron was not toxic on its own, but the oxine made it so.

In the absence of added heavy metals, oxine enters cells of bacteria (*Staph. aureus*) (62) and fungi without harming them. Yet if a suitable metal is made available at the same time, these organisms are severely injured. Oxine is toxic to bacteria only when one of the following cations is present: Fe^{2+}, Fe^{3+}, or Cu^{2+}; for mycelial fungi and yeasts, only Cu^{2+} will serve.

Derivatives of oxine having a low lipid/water partition coefficient are not antibacterial, but become so when the coefficient is made to rise (by suitable alkyl-substitution of the molecule) until that of oxine is reached (63). Provided this rule is observed, substances with characteristic oxine-like properties can be produced in the cinnoline (2-aza-oxine) and quinazoline (3-aza-oxine) series (63).

Other bactericides have been found that, while having a totally different structure, mimic the action of oxine by being active only in the presence of iron or copper (64). Such a substance is 1-hydroxypyridine-2-thione (XXVII) ('Omadine'), whose chelated forms are exemplified by formula (XXVIII). Similarly other fungicides mimic oxine by being active only in the presence of copper (65). The active chelated form of dimethyldithiocar-

bamic acid, a widely used agricultural fungicide, is shown in formula (XXIX). It has been suggested that this chelate and those of oxine and 1-hydroxy-pyridine-2-thione all act by catalyzing the oxidative destruction of lipoic acid (XXX) (thioctic acid) which is the essential coenzyme for the oxidative decarboxylation of pyruvic acid, and some accumulation of pyruvic acid has been demonstrated (65).



XXVII XXVIII XXIX XXX

In summary, structural requirements for achieving a typical oxine activity are no more rigid than for obtaining a typical aminoacridine activity (see above) provided that the essential physical properties are kept in mind.

Receptors, Current Concepts

We are fortunate to be living at a time when the physical nature of receptors is becoming better understood, because former information was as indirect as what might be obtained from an invisible airfield by landing radar-controlled planes on it. For many inhibitory actions, enzymes are the receptors and three examples were discussed above. Similarly, the hydroxy-group of serine, in the enzyme acetylcholinesterase, has been shown to be the receptor for the organic phosphate insecticides, but here a covalent bond is formed. Penicillin, which is thought to inhibit the enzyme glycopeptide-transpeptidase in the cytoplasmic membrane of bacteria (66), also unites with its receptor by a covalent bond; but this is unusual. Other receptors are coenzymes, e.g. the lethal action of hydrogen cyanide in mammals follows directly from the binding of this poison to the free valence of iron in the porphyrin portion of cytochrome oxidase, thus bringing respiration to a standstill. The receptor for oxine seems to be a coenzyme, whereas that for the aminoacridines is the nucleic acid DNA (see above). This list of common receptor-types is relevant specially for inhibitory agents, but agonists (stimulatory agents) have more complex receptors whose special nature and requirements are becoming better known. Three types will now be mentioned.

(*a*) Many steroid hormones seem to act by de-repressing a length of temporarily inactive DNA so that it can produce its characteristic messenger RNA which in its turn specifies new proteins, especially enzymes. For a

review on such induction of enzymes, see (67). (DNA has been shown to be the primary site of action by the use of actinomycin D as a specific DNA inhibitor).

(*b*) The receptors for the water-soluble hormones, such as those for epinephrine and acetylcholine, are embedded in semi-permeable membranes, which may be a necessary consequence of the rapid, graded response required. Thus the enzyme adenylcyclase, a receptor for epinephrine and some other hormones, is embedded in a membrane and equipped with a supply of adenosine triphosphate as substrate (68). Epinephrine causes this enzyme to convert ATP into cycloadenylic acid (3′,5′-AMP), and thus seems to act as a coenzyme. This acid energizes the enzyme phosphorylase; as a result, liver cells perform glycogenolysis, the heart becomes stimulated, and also other well-known physiological effects of epinephrine are brought about. A general concept of the adrenergic receptors as enzyme-substrate complexes, where ATP is the (exhaustable) substrate, has been convincingly advanced (69).

(*c*) In addition, some workers think that agonists can selectively alter the permeability of membranes, presumably by acting on permeases, which are crystallizable substances that specifically ensure the passage through the membrane of a particular metabolite such as the succinate or isocitrate anion. So far the evidence that some agents act in this way is only fragmentary.

The better understanding of receptors is bearing fruit in practical therapy. The existence of two different kinds of adrenergic receptor (α-and β-) was first suggested in 1948 by Ahlquist (70), and it was soon realized that the inhalation treatment of asthma depended on blocking the β-receptors in the lungs with say, isoprenaline. A more recent subdivision of β-receptors (71) led to the discovery that isoprenaline activates both β_2-receptors in the lung (desirable in therapy) and β_1-receptors in the heart (causing undesirable tachycardia). This differentiation led to selective screening procedures which furnished drugs, e.g. salbutamol, which activate only β_2 receptors (72).

Conclusion

Each early discovery of a structure-activity relationship seemed, to its contemporaries, to be a universal explanation of drug action, and it was not clearly seen that, had this been true, drugs could evoke only one kind of biological effect. Nowadays, more correctly, we accept different explanations for different biological actions, but are still troubled by our nomenclature. Thus when we say "structure," we mean "constitution," namely all the information on physical and chemical properties that is stored in the chemical formula, or that can be discovered by measurement and experimentation. Also when we say "activity" we mean the action on the drug-receptor, but this effect, we know, is often connected to the desired physio-

logical result only through a long chain of other reactions, chemical and biological. It is only with reference to the action at the receptor that the constitution of the drug has any relevance.

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