Correlation between Structure and Function of Certain Selective Toxins[1]

E. Campaigne, Indiana University

Although the term "selective toxin" has only recently come into use, the concept embodied in this phrase is an old one, originally presented by Ehrlich. Ehrlich assumed that organisms possessed a whole series of different "chemoreceptors", and that it was only necessary to discover among these one specific "chemoreceptor" in the parasite which had no analog in the organs of the host, to have the possibility of a chemical poison which would attack the parasite but not the host. He used this concept to explain the existence of antibodies, the naturally-occurring "magic bullets", and presented the same idea in the old "lock and key" theory. The "lock" is the specific chemoreceptor which was fitted by the "key", which might be drug or antibody.

These same concepts have been used repeatedly in the development of modern theory of drug action. For example, such terms as "specific enzyme inhibitor" and "antimetabolite" are merely efforts to locate more exactly Ehrlich's "chemoreceptor". We can broadly define a "selective toxin", then, as a substance which preferentially inhibits the growth of or destroys one type of organisms in the presence of other organisms or tissues.

Dr. Koffler, in planning this symposium, has posed the question: "If the postulates of comparative biochemistry are true, and all living things utilize essentially the same reactions in their processes, how is selective antibiosis possible?" The answer to this question lies in the word "essentially". This recognizes the idea that various organisms may use similar, but not necessarily identical, mechanisms in their metabolism. The problem posed for the chemotherapist is to discover and differentiate between the minor differences in the metabolism of invading organisms and host. Indeed the entire development of chemotherapy has depended on man's ability to make finer and finer differentiations in the metabolism of various organisms. The earliest clearly defined example of such differentiation probably occur in the 18th century, when the juice of the male fern was used to treat hook-worm disease. The clearly visible excretion of the worms in the feces showed the effective differentiation between host and parasite. The next significant development was Ehrlich's use of dyes and arsenicals on protozoan infections, between 1900 and 1910. The sulfa drugs, developed between 1932 and 1936, provided a tool for distinguishing bacteria from host tissues. It has been much more difficult to discriminate between viruses and host tissues, but some success has been experienced recently with certain

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antibiotics, including the synthetic chloromycetin. The supreme challenge lies in cancer tissues, which is actually host tissue gone wild, but certainly the differences between the metabolism of the cancer cells and the normal cells will be detectable by the proper chemical tool.

The nature of Ehrlich's "chemoreceptor" has intrigued many research workers, and led to a number of theories. One of the most productive has been that elaborated by Woods and Fildes (12) based on Fildes' observations on the relationship between growth factors and essential metabolites (3). According to Fildes, "essential metabolites" are substances formed in each stage of a synthesis necessary for growth. A "growth factor" is an essential metabolite which cannot be synthesized by the organism. An antibacterial substance functions by interfering with essential metabolites.

This interference may occur in at least two ways, either by blocking a reactive group, or by competitive inhibition. In simple terms, competitive inhibition is the displacement of an essential metabolite from a reaction by an "inhibitor". The laws of mass action apply, and relatively large amounts of drug (inhibitor) are required and this high concentration must be maintained by continued high dosage. A simple analogy exists in the "common ion" effect. One liter of 0.1 N acetic acid contains approximately 0.001 equivalents of H^+. If an equivalent amount (0.1 mole) of acetate ion is added to this solution, the $[H^+]$ is decreased one hundred fold, to 10^{-5} equivalents; to decrease the $[H^+]$ further requires much more acetate ion, however. A ten-fold excess (1.0 moles) is required to reduce the $[H^+]$ to 10^{-6}, and one hundred fold, (10 moles) to reduce it to 10^{-7}. If one imagines that the hydrogen ion is an essential metabolite, required in a minimum concentration of 10^{-6}, then more than 10 times as much acetate ion must be added as an inhibitor.

A number of examples are available for this kind of action. The classic one is that of p-aminobenzoic acid and sulfanilamide. Strauss, Lowell, and Finland (10) demonstrated that the ratio of the concentration of P. A. B. required to cause reversal of bacteriostatic action of sulfanilamide, sulfapyridine or sulfathiazole, to the concentration of the sulfa drug was constant, thus proving the law of mass action applied. Similar relationships have been proven in many other cases of bacteriostatic compounds, and have been suggested in many more.

The second interference with an essential metabolite is by blocking, or "masking" of prosthetic groups. Drugs which act in this way have the following characteristics:

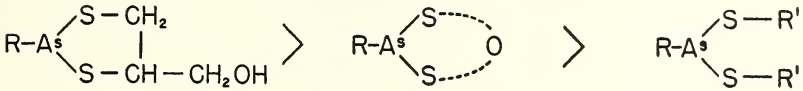
1. They will follow an adsorption curve type of action.
2. They are usually effective at very low dosage.
3. They have a persistent action.

To extend our analogy of simple ions, this type of action may be compared to removal of an ion. Thus if our metabolite was hydrogen ion, in the 0.1 N acetic acid, then addition of 0.1 equivalent of hydroxide ion reduces the $[H^+]$ to 10^{-7}. The base was the blocking drug, active

in low concentration, and not easily reversible. Clearcut examples of this type of action are not readily available, since the action is harder to study. However, "Hetrazan", N-methyl-N'-dimethyl-carbamidopiperazine, which is effective against filariasis, is active at relatively low dosage (about 300 mg./day), while Mapharsen is effective against syphilis at dosages of 40 mg./week.

The problem of the mechanism of drug action is more complicated than consideration of these two simple types of action would indicate. Since only a few of the possible "essential metabolite" steps are clearly understood, whereas thousands probably exist, there is a great possibility that chemotherapeutic agents may act on more than one site. This can be illustrated by some observations on arsenic poisoning, arsenicals, and British Anti-Lewisite.

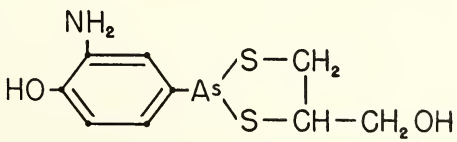
The suggestion by Voegtlin, Dyer, and Leonard (11) that arsenicals exerted their toxic effects through combination with sulfhydryl groups was brilliantly parleyed by the British investigators into the development of B.A.L. (7). During the course of these investigations it was shown that although a large excess of monothiol would reverse the toxicity of arsenoxide, dithiols were much more active. This is attributed to the greater stability of the ring compounds.



B.A.L. complex thiol enzyme complex monothiol complex

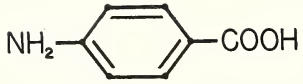
Since B.A.L. was quite effective in preventing, and even reversing, pathological damage by Lewisite, it was not surprising that "in vitro" tests showed that B.A.L. reversed the toxic effect of arsenoxides on trypanosomes, and it seemed apparent that the mechanism of action of the arsenicals was to interfere with sulfhydryl groups in the trypanosomes.

However, recently Friedheim and Vogel (4) reported that the compound formed by the combination of Mapharsen and B.A.L. (I):

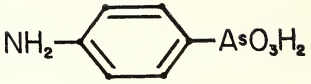


I

is quite active against both trypanosomes and spirochetes, although it is much less toxic than the parent Mapharsen. This indicates an entirely different site of toxic action in host and parasite. This is also indicated in the work of Sandground and Hamilton (8) who considered that the aromatic arsenicals (Atoxyl) might act as anti-PAB compounds, like the sulfa drugs.



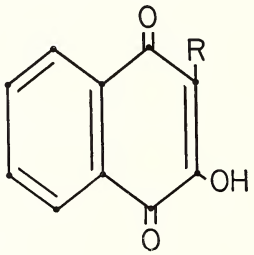
PAB



Atoxyl

During the course of experiments designed to test this hypothesis, it was discovered that administration of PAB on or before injection of Carbarsone protected rats against several toxic doses of the arsenical. No protection of the animals against trivalent arsenic or the arseno-compounds, and more remarkable, no inhibition of the trypanocidal activity of Carbarsone by PAB was observed!

Solvent effects and permeability are also important in considering the mechanisms of action of selective toxins. In several instances optimum actvity has been found to correlate quite closely with a certain distribution coefficient between water and fat-solvent. For example, Fieser, et al (2) found such a correlation in the antimalarial activity of a series of alkylhydroxynaphthoquinones (II).

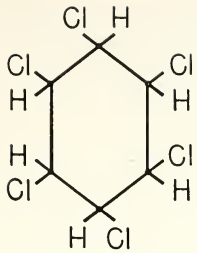


II

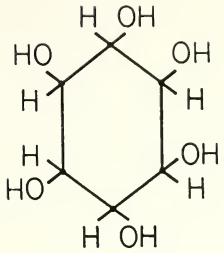
Blowfly larvae are unaffected by immersion for one hour in kerosene, or in absolute ethanol, but a mixture of kerosene and alcohol is toxic in a matter of seconds. This has been attributed to altered permeability of the cuticle (5).

The manner in which selective toxins combine with or displace essential metabolites is not always clear, but great emphasis must be placed on similarities in molecular structure. Bell and Roblin (1) were able to show the close relationship between the para-aminobenzoate ion and sulfanilamide. The activity of fluoroacetic acid as a rat-poison (1080) is at least partially due to its interference with the acetic acid oxidase system. Since chloroacetic acid does not interfere in this system, it has been assumed that the fluoro group may replace hydrogen in the acetic acid-enzyme complex, but the chloro-group is too large to permit formation of the complex.

Similarly the unusually high activity of the gamma isomer of hexa-chlorocyclohexane in the inhibition of yeast growth has been shown to be due to its similarity to inositol in stereochemical configuration (6).

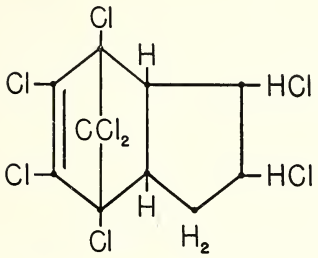


"Gammexane"

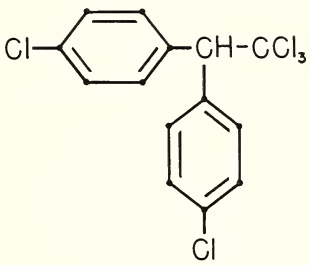


inositol

This similarity must permit blocking of some essential inositol reaction in yeast. On the other hand, no such relationship is apparent in its activity as an insecticide, and "gammexane" is grouped with other chlorinated hydrocarbons, such as chlordan (III) and DDT (IV), which are effective insecticides.



III



IV

The high activity of DDT against various insects has led to many studies on related molecules, and the mode of action, but no likely theory has as yet been developed. Some compounds tested against lice are reported in Table I, after Sexton (9).

It is apparent from these data that certain structural requirements are present for activity against lice. A functional group must be present on one of the benezene rings. This may be halogen or methoxy, but hydroxy inactivates the molecule. Only one substituted benzene ring is required, but the trichloromethyl group is essential, and is apparently not hydrolyzed "in vivo", since the coresponding acid is inactive. The gradual decrease in activity with increase in group size in the alkyl-

TABLE I. Toxicity to Lice of Certain DDT Analogs

$(p\text{-}ClC_6H_4)_2CHCCl_3$	+++	$(p\text{-}C_2H_5OC_6H_4)_2CHCCl_3$	++
$(C_6H_5)_2CHCCl_3$	+	$(p\text{-}C_3H_7OC_6H_4)_2CHCCl_3$	+
$(p\text{-}FC_6H_4)_2CHCCl_3$	+++	$(p\text{-}C_6H_5CH_2OC_6H_4)_2CHCCl_3$	0
$(p\text{-}CH_3C_6H_4)_2CHCCl_3$	++	$p\text{-}ClC_6H_4CHOHCCl_3$	+++
$(p\text{-}C_2H_5C_6H_4)_2CHCCl_3$	+	$p\text{-}ClC_6H_4CHClCCl_3$	+++
$(p\text{-}HOC_6H_4)_2CHCCl_3$	0	$(p\text{-}ClC_6H_4)_2CH\text{-}CHCl_2$	+
$(p\text{-}CH_3OC_6H_4)_2CHCCl_3$	+++	$(p\text{-}ClC_6H_4)_2CHCOOH$	0

and alkoxy-substituted compounds is significant, particularly in view of the loss of activity with the polar hydroxyl substituent which is comparable in size to the fluoro group. This indicates that the polarity as well as size is important in any "lock and key" theory which can be developed for the DDT series of compounds.

In summary, we may say that compounds are selective toxins when their molecular structure and chemical properties are such as to permit them to react with specific "chemoreceptors", which may be enzymes, to inactive the receptor either by displacing an "essential meabolite", or preventing formation of a necessary active complex. Only when the nature of the "chemoreceptor" is known can quantitative experiments be made which will confirm or deny the proposed mechanism of action.

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