CHEMICAL ASPECTS OF SELECTIVE TOXICITY*

By Prof. ADRIEN ALBERT
Department of Medical Chemistry, Australian National University, Canberra

SELECTIVE toxicity may be defined as the injuring of one kind of cell without injuring some other kind of cell with which the first is in intimate contact[1]. Thus, in agriculture, it is concerned with ridding crops and seeds of noxious insects and fungi, without causing harm to the higher plants. In veterinary and human medicine it is well exemplified by chemotherapy, which safely rids the host of internal parasites. A more delicate form of selective toxicity is met in pharmacodynamics. Here both types of cell are in the one species, and the task of selective toxicity becomes more difficult. This difficulty is intensified by the necessity of exerting a graded response, and of allowing the cells to recover in the course of time. Thus a patient with insomnia can be put to sleep by a drug which acts on the central nervous system, but the nerve cells which have been poisoned (and poisoned to a predetermined degree) must recover in time for the patient to go to his work next morning.

That selectivity is possible seems to rest on two main principles: (*a*) different kinds of cells exhibit very different kinds of distribution phenomena, and (*b*) the anabolic processes of different cells differ widely (catabolic processes appear rather similar). Whereas detailed knowledge of anabolic differences awaits further work in the field of comparative biochemistry, much is already known about differences in distribution.

When a substance is administered, it has to penetrate at least one semi-permeable membrane before it enters into circulation. Sometimes the substance, as administered, is only a 'pro-drug' which has to be broken down to give the true drug. Examples of this kind are phenacetin, chloral hydrate, pentavalent arsenicals and two of the antimalarials: pamaquin and proguanil. But this seems to be a rare phenomenon, and it appears that most substances act in the form in which they are given.

The circulating drug (and by 'drug' is meant any biologically active substance) then takes part in five other phenomena, to various degrees depending on its nature and on the nature of the cell[2]. It can be stored, excreted, or chemically destroyed; it can penetrate another semi-permeable membrane, or combine with its receptor. Storage can be useful, as with the antimalarial mepacrine, which is stored in capillaries from which it is released (under mass action conditions) to maintain a therapeutic level in the bloodstream. Examples are also known where storage is wasteful or the drug is entirely segregated on 'sites of loss'[3]. Chemical destruction (detoxication), usually by primitive (not highly specific) enzymes in microsomes[4], takes place by one of seven or eight standard chemical reactions which increase partition coefficients in favour of water so that the penetration of further membranes is diminished. Excretion is usually by the kidney or bile duct in mammals, and these are so constituted that the chemical changes brought about by detoxication greatly increase the excretability of the drug[5].

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If, as seems usually to be necessary, the drug has to penetrate yet other semi-permeable membranes before its meets the receptor, the chances of selective toxicity are heightened. Four different types of membranes are distinguished[6,2]: (*a*) where diffusion is regulated by pore-size, (*b*) where a lipoidal layer causes substances to diffuse at a rate proportional to their lipoid/water partition coefficient and excludes the majority of ions, (*c*) where a lipoid anion (for example, oleate) assists the passage of cations, and (*d*) where specific nutrients, particularly anions, are transported (often enzymatically), utilizing energy obtained from the simultaneous oxidation of (for example) glucose. Mixed types of membrane have also been found.

Bearing in mind the various types of membrane made available by different kinds of cells, and the various rates at which a drug, imprisoned within any pair of these membranes, will be subjected to the processes of storage, destruction and excretion, it is easy to see how specificity can be promoted merely by small changes in the rate or equilibrium constants governing any of these processes. To be added to these factors, which in sum govern the rate at which the drug will reach the receptor, is the affinity which the drug has for the receptor.

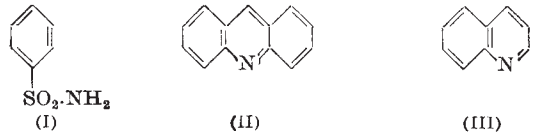
Receptors, according to Ehrlich[7], are chemical groups containing, perhaps, less than a dozen atoms, which normally perform one of the metabolic duties of the cell: when blocked by the drug they can no longer function and thus the pharmacological effect occurs. At the present time workers tend to equate 'receptor' with the active group of an enzyme.

The combination between drug and receptor seems usually to occur by secondary valencies, namely, hydrogen bonds, ionic bonds and multiple van der Waals bonds. Covalent bonds must be rare because such bonds are seldom easily broken at 20°–37° C., whereas most drug-receptor effects can be terminated by washing with saline. Ehrlich was aware of this distinction when he wrote[8]: "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". This was in 1898, before knowledge of bond-strengths, -types and -distances became as exact as it is to-day[9].

Nevertheless, a few examples are known of the formation of covalent bonds with receptors, mainly by those drugs which can acylate or alkylate (penicillin, the nitrogen-mustards and the phosphorus anti-cholinesterases), but also by a few substances which become chemically incorporated into vital parts of the cell (for example, *p*-aminosalicylic acid into folic acid, and azaguanine into nucleic acid).

Physiological action is highly dependent upon the finest details of chemical structure. If these details are varied ever so slightly, the degree of action is usually radically changed. For example, both the C-methyl-groups in thiamine are essential for its vitamin action. In synthetic drugs, a similar dependence on minute detail is observed. Thus in benzenesulphonamide (I), an amino-group can be inserted in

three different positions : in two of these it gives rise to an inactive substance, but in the other it gives the highly antibacterial substance sulphanilamide[10]. Again, in acridine (II), an amino-group can be inserted in five different positions : in three of these it gives aminoacidines which are almost inert, but in either of the other two it gives a powerful antibacterial[11]. Once again, in quinoline (III), a hydroxy-group can be inserted in seven different positions : in six of these, completely inert substances are created, but in the remaining position a strong antibacterial and antifungal substance is produced.



(I) (II) (III)

In each of these three examples, it is known why the active isomers are active, and why they alone are the active ones. The activity of the sulphonamides depends on the *para*-isomer being a metabolite analogue for *p*-aminobenzoic acid, to which it shows a close structural resemblance[10]. The activity of the acridines depends upon something quite different : only in the 2- and 5- positions can an amino-group acquire enough resonance stabilization in the cation to produce a highly ionized substance (the antibacterial action of the acridines depends on a high degree of ionization, see below). The activity of the quinolines depends on yet another principle : only in the 8-position can a hydroxy-group collaborate with the ring-nitrogen atom to bind metal cations by that pincers-like process known as chelation, and the antibacterial action of 8-hydroxyquinoline depends upon chelation[12,13].

Since the discovery of the mode of action of sulphonamides, metabolite analogues have received closer investigation and have led to the discovery of many substances which block specific enzymes and hence are of great value to the biochemist. Antivitamins, of similar nature, have contributed usefully to nutritional studies[14]. But in human medicine the most successful antimetabolites are, so far, all connected with pteroylglutamic acid (folic acid). This acid, a vitamin or vitamin-precursor, consists of a pteridine nucleus to which are linked *p*-aminobenzoic acid and glutamic acid. Sulphonamides exert their antibacterial action by preventing the incorporation of *p*-aminobenzoic acid into this substance, whereas *p*-aminosalicylic acid actually becomes incorporated in its place. Some antileukæmic agents of clinical value have been made by substituting amino for hydroxyl in the pteridine ring, and a much used antimalarial (pyrimethamine or 'Daraprim') was evolved as a metabolite analogue of the pteridine portion of folic acid[15]. A new type of synergism has been devised, known as sequential blocking, where metabolite analogues of two stages of the folic acid cycle are used together.

The great success of these various anti-folic acid drugs depends on a singular set of coincidences : man does not require *p*-aminobenzoic acid but must absorb folic acid from his diet, and pathogens require *p*-aminobenzoic acid to form their own folic acid, but cannot absorb it if supplied (it is possible, of course, that their folic acid is slightly different from ours). In searching for useful analogues of other metabolites, these facts have to be borne in mind.

The majority of biologically active substances are capable of ionizing to different degrees at different *p*H values ; also the extent of their ionization can be controlled by small alterations in the chemical structure. This variability in ionization is important because an ion and its corresponding neutral molecule undergo different chemical reactions, penetrate membranes differently, and become adsorbed on different types of substance[16]. Because the combination between ions and receptors must be reversible (from the very nature of the bond) the degree of ionization under the conditions of the experiment is all-important for effecting combination. This does not mean, however, that complete ionization is always an advantage, because foreign ions do not readily penetrate membranes ; also, a few examples are known where a pharmacological effect seems to be brought about by the neutral molecule[1,2].

Only two factors affect the degree of ionization of a substance : the ionization constant, and the *p*H at which the experiment is conducted. Given these, the percentage ionization can at once be read from a table[1].

The acridine antibacterials are the most studied example of positive correlation between ionization and biological activity[11]. It was shown, using 107 variously substituted acridines, that at least 50 per cent of the substance must be present as cation to give a significant bacteriostatic effect. This work produced the antibacterial substance, aminacrine, which is now included in the British Pharmacopœia.

Metal-binding substances are of two types, those like 8-hydroxyquinoline (oxine) which bind by chelation (see above), and those like dimethyldithiocarbamic acid which bind without the aid of chelation (these are usually sulphur-derivatives). Biologically, both types act similarly, provided the stability constants are high enough. Stability constants, which are derived from mass-action equations, form a reliable indication of the avidity with which a metal is held by a particular binding agent. Contrary to general belief, the various binding agents show very little differences in their preference for the different metals.

Some metal-binding agents are used in medicine as antidotes to poisoning because they withdraw toxic metals and metalloids. Examples of this kind are dimercaprol (arsenic, antimony and gold), and ethylenediamine tetracetic acid (lead). Others act by forming toxic complexes with such metals of variable valency as are present in the medium. Such a substance is oxine, which does not injure bacteria in the absence of iron[13]. Toxic complexes of this kind appear to initiate oxidative chain reactions, and death occurs very rapidly. Thus, the chelation of a metal of variable valency does not necessarily decrease its biological action, but often increases it. Measurements of oxidation-reduction potentials now in hand should give a better guide to the choice of inactivators.

Several much-used drugs, such as the salicylates, aureomycin, and isoniazide, bind metals at least as strongly as do the amino-acids, and hence could well act by chelation. Some convincing evidence concerning isoniazide has already been obtained. Chelating agents are widely used in crop-protection, for example dimethyldithiocarbamic acid and its derivates, the mode of action of which resembles that of oxine[17].

Toxicity can be brought about in other ways than through covalent bonding, metabolite analogues, ionization and metal-binding. The rupture of cell-membranes, as affected by phenols, quaternary

lipophiles and polymixin, has been reviewed recently[18] and shows some selective effects.

The class of substances known as 'depressants' is unique in that the toxic effect is dependent on chemical structure only to the limited degree necessary to make the substance lipophilic enough to reach the central nervous system. Thus the inert gas, xenon, has been successfully used as a general anæsthetic in man[19]. The lack of a dipole moment indicates that this gas acts in the bulk phase rather than by adsorption at a surface. Thus it seems to be merely a diluent separating biochemical components which should react together. The mode of action of all volatile anæsthetics is almost certainly the same as that of xenon, because they all act at those concentrations which produce a standard thermodynamic activity. This was first demonstrated by Ferguson[20], and the anæsthetic activity of xenon was predicted from Ferguson's principle[21], and verified.

This presentation of the phenomena of selective toxicity has necessarily been from the chemical angle. It is put forward in the hope of supplying biologists with a framework which may help to classify results and to suggest new, and often more decisive, experiments.

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FORMATION OF A HELICAL STEROID COMPLEX

By Drs. ALEXANDER RICH* and DAVID M. BLOW*

Section on Physical Chemistry, National Institute of Mental Health, Bethesda, Maryland

THE purpose of this communication is to describe the formation of a helical complex of macromolecular dimensions from the bile acid steroid, sodium deoxycholate.

The reaction was first observed when an aqueous solution of sodium deoxycholate was buffered with glycylglycine. Within a few minutes of making up a solution containing 0·1 *M* sodium deoxycholate and 0·1 *M* glycylglycine, unusual changes occur in the physical properties of the solution. There is a large increase of viscosity, and the solution can be drawn out into very long glassy fibres which are stable and dry into clear brittle rods. Soon the solution can almost be lifted clear of its container as a cohesive jelly-like aggregate. It has a mucous-like stickiness when handled. If the solution is allowed to stand, it will eventually form a clear gel which has considerable rigidity.

The complex can be formed most simply by adding acid to an aqueous solution of sodium deoxycholate. The solution becomes very viscous when the *p*H falls below about 6·8 (about 0·2 equivalent of acid). Addition of much larger amounts of acid causes a precipitate of the insoluble deoxycholic acid to form, but there is a range of *p*H and concentration in which the solution is clear and viscous. High ionic strength evidently assists the complex to remain in solution, and solutions of less than 0·01 *M* are always turbid in this *p*H range unless salt is added. The formation of the complex is immediately reversed on raising the *p*H.

By working with these dilute solutions, the phenomenon can be studied in a viscometer. In an aqueous solution containing 0·01 *M* sodium deoxycholate, 0·01 *M* glycylglycine and 0·04 *M* sodium chloride, a maximum viscosity is reached in about 15 min. The macromolecular dimensions of the complex can be demonstrated in the analytical ultracentrifuge, which shows a rapidly migrating peak with a sedimentation constant of 12–15 S. The solutions which have been described as clear always exhibit the Tyndall effect to some extent.

We should explain that we have had some difficulty in finding an adequate terminology for describing these phenomena. The above observations are characteristic of the formation of an elongated polymer. There is no reason to believe, however, that any covalent bonds are formed in this system, and so we have preferred to use the word 'complex'. Although the particles of the complex are of macromolecular size, we have avoided calling them macromolecules.

X-Ray Diffraction Studies

To study the internal structure of the complex, X-ray diffraction studies were carried out on air-dried fibres, some of which contained glycylglycine in addition to the steroid. The fibres are usually glassy clear and brittle, and have weak negative birefringence ($-0{\cdot}002$). The diffraction patterns are unusually detailed. Two characteristic types of diffraction pattern are reproduced in Figs. 1 and 2. Fig. 1 was obtained from a fibre with a glycylglycine : deoxycholate mole ratio of 0·5 : 1. It can be seen that the more intense layer lines are clearly resolved into a series of spots. Heavier exposures show that the weaker lines are also made up of a series of closely spaced diffraction spots. The equatorial spacings correspond to a hexagonal lattice with $a = 39{\cdot}2$ A. The first layer line spacing is 49·2 A.

* Now at the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.