REVIEW ARTICLE

STRUCTURE—ACTIVITY RELATIONSHIPS

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The relationship between the chemical structure of a compound and its effect on a biological system is most frequently considered in rather a narrow sense, referring only to synthetic organic compounds or even to a limited class of compounds. Moreover, consideration is often restricted to a limited type of biological response, or to an ill-defined end response such as death of the organism. All naturally occurring compounds in living systems are possessed of biological "activity" of some kind, and even if their function is somewhat static as in structural components, they have at least been synthesised by dynamic biochemical processes. Even the simplest of compounds, water, is biologically active, as it participates not only as a solvent but as one of the most important reactants in the living cell. A full analysis of structure-activity relationships must therefore embrace the whole of classical biochemistry, to which must be added the growing mass of knowledge accumulating as the result of the development of chemotherapy, the fermentation industries, the plant protection industry and the control of organisms attacking manufactured goods. The temptation to narrow the field of enquiry is strong, because by doing so, it is possible to arrive at greater precision in correlating structure and activity. Moreover, it is often said, and with a considerable measure of truth, that fundamental knowledge is so fragmentary that any attempt at broad generalisations is premature. This negative attitude should be resisted, for hypotheses are valuable in crystallising thought and suggesting experiments. There are, in fact, certain valuable basic data, or at least practical working rules, which can act as guides, and a broad enquiry can at least help to define those areas where the gaps in knowledge are greatest and upon which research should be concentrated.

Living organisms, from the point of view of this essay, are considered as dynamic physico-chemical systems. They are dynamic, not necessarily in the sense of movement, but because the living process is one of rapid and orderly chemical change, from the intake of nutrient to the output of waste products. The structure of every molecule must be viewed in relation to its environment, and to its metabolic precursors and successors. Living organisms vary greatly in their biochemical complexity, and to a large extent the study of structure-activity relationships is dependent on the possibility of biochemical dismemberment of the organism, so that the working of the component parts may be examined in isolation. The metabolic processes of micro-organisms, for example, are susceptible to experimental study not only by correlation of the effect of nutrients and foreign compounds on the metabolism, but also by the isolation and

study of individual enzymes and their substrates. Again, it is possible by isolating a particular organ from an animal, for example, thyroid gland, liver, or nerve, to study the effect of chemical compounds on the functioning of the organ in question. Natural associations of enzymes may be studied in isolation from the whole organism by the use of particles such as mitochondria or chloroplasts. This kind of approach has been of immense value, and it will continue to provide basic data of crucial importance. Nevertheless, it has its limitations, and erroneous conclusions may be made because the behaviour of a biochemical system in isolation may not be the same as when it is fully integrated with the interdependent system of the whole organism. Questions such as cell permeability and the inductive formation of intracellular enzymes may have a decisive influence in the natural condition, but may be largely ignored in some of the classical types of biochemical experiment. In the higher organisms, the controlling influence of the nervous and endocrine systems, each with its own complex biochemical characteristics, plays an important part in determining the response of the whole organism to a particular chemical compound. The ability to express psychosomatic medicine in chemical terms is still far distant, though courageous speculations are beginning to appear in certain directions. A special word of caution is needed when "biological activity" means death. A bactericide kills the organism but structure can hardly be correlated with this kind of activity except in restricted series of chemical compounds. It may be legitimate, for example, to correlate structure and bactericidal activity amongst phenols, quaternary ammonium compounds, sulphonamides, or penicillins. It is useless to search for a significant common structural feature in the four classes of compounds named, because they have none. What matters is the biochemical mechanism of the process which results in death of the bacteria.

In a complex living organism, there are many factors which determine whether a foreign compound can exhibit a particular type of biological activity. First and foremost, the compound must be able to reach, in adequate concentration, the site at which the desired biochemical action can result. This may be a particular organ or a parasite. In its transport to the site of action, the compound must overcome a number of barriers. These barriers are partly physical and partly chemical. Physical factors, all affected by the chemical constitution, include quantitative aspects of solubility in aqueous and lipoid phases, membrane permeability, and capacity for adsorption on macromolecular species in solid or solution form. All these affect the rates of absorption and excretion of the compound in question, and hence the concentration at any particular place in the organism. The principal chemical factor determining access to the site of biochemical action is the reactivity of the compound. If the compound is highly reactive, it is likely to be wasted by chemical interaction of a non-specific nature with various chemical components of the organism, even with water itself. If it contains groupings which are susceptible to enzymatic attack, it may undergo chemical change. This change is generally known as detoxification because it frequently

results in the formation of a derivative, for example, glycuronide or other conjugate, which is rapidly excreted, and which has lost the characteristic biological activity of the parent compound. Metabolic change of this kind can, however, actually increase the biological activity, and examples will be quoted later. The second necessary property of a compound which is expected to be biologically active in a complex organism hardly requires statement. It is the intrinsic ability to participate, preferably in a specific way, in the functioning of the particular biochemical system concerned. Thus in the biological activity of a foreign compound, structure-activity relationships are governed by a large number of competing factors. Certain structural features will favour intrinsic activity, and certain features will favour the access of the compound to the desired site. Only when these features are favourable in both respects will activity result. Any one structural feature may thus limit the overall effect.

Activity Through Physical Effects

There are certain compounds (or even elements) which have little or no chemical reactivity but which nevertheless can exhibit biological activity, perhaps by a rather non-specific damping down of vital biochemical reactions in the living organism. Such activity is usually reversed on removal of the drug and is often described by a broad use of the term narcosis. Narcotics, in this sense, owe their activity to physical effects and there is a close correlation between biological activity and certain particular physical properties. In so far as these physical properties can be correlated with structure, the structure can be correlated with biological activity. It is found, however, that elements and compounds of a widely different chemical nature exhibit the relevant physical property in like degree; correlation of structure and activity in these cases would therefore probably require resolution at the atomic level.

There have been various theories or empirical rules for correlating activity and physical properties. There is the Overton-Mayer concept whereby narcotic activity was related to the lipid: water partition ratio of the compound, and the Traube hypothesis relating narcotic activity to the effect on the surface tension of water. Each of these has served its usefulness, and they were for a time considered as rival theories. Considerable clarification resulted from an analysis of the situation made by Ferguson in 1939[1]. He recognised that in homologous series of compounds, the variation in toxic concentrations exhibited the same type of relationship as variation in physical properties. Thus, straight lines were obtained by plotting the number of carbon atoms on the chain of normal primary alcohols against the logarithm of either the toxic concentration against *B. typhosus* or the solubility, vapour pressure, surface-tension lowering capacity or partition coefficient betwen water and cottonseed oil. Ferguson emphasised that the concentration required to produce a given biological activity was usually expressed as the concentration in the surrounding medium, but that what is really required is to express the concentration at the site of action, the "biophase". Assuming an

equilibrium state between the various phases in the organism, the chemical potential ("activity" in the thermodynamic sense) will be the same in all phases. All that is necessary, therefore, is to calculate the thermodynamic "activity" in the surrounding medium, which can be done from measurements of vapour pressure or, approximately, from solubilities. In this way, it was shown that ascent of an homologous series revealed a gradual increase of the "activity" towards unity, at which point there was a cut-off, higher members having reduced biological potency. This, of course, is a familiar experimental finding, well exemplified by the fact that the insecticidal activity of normal thiocyanates reaches a maximum at about C_{10} and the bacteriostatic action of *p*-alkylphenols at C_5.

Physical toxicity of the kind described may be exhibited by elements and compounds of widely differing structures. Even though the external concentrations required to produce a particular biological response may vary greatly, the chemical potential lies within a fairly narrow range which is a characteristic of the biological system in question. Illustrations have been provided by Ferguson[1] and by Gavaudin[2]. One particularly striking example compares the mitotic activity on plant cells of nitrous oxide, nitrogen, argon and propane[3].

Effect of Physical Properties on Chemical Toxicity

From the phenomenon of physical toxicity, as outlined above, it follows that the biological activity of a compound which acts chemically will be greatly influenced by its physical properties. This is a familiar experience in chemotherapeutic research, where substituent groups in any particular chemical type can influence both the intrinsic activity and the ability to penetrate to the site of action. The homologous series effect is not confined to purely physical toxicity, but has frequently shown up in compounds where the intrinsic activity is undoubtedly associated with the occurrence of a chemical reaction *in vivo*. The same consideration applies for example to the effect of halogen and alkyl substituents in an aromatic or heterocyclic ring. Many examples have been quoted by the author[4].

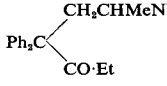
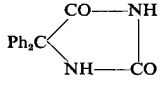
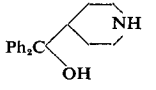
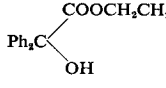
Apart from the effects of structure on such physical properties as solubility and volatility, one of the most important of physical properties is that of molecular shape. Most biochemical reactions in the living organism are mediated by the intervention of macromolecular catalysts, the enzymes. All enzymes so far discovered are proteins, and their characteristic specificity is undoubtedly the consequence of an ordered three dimensional arrangement of the component parts, permitting the access of the natural substrates (and certain structurally related molecules) to the site where they are activated. The shape of the substrate molecule, which may be relatively small, is thus of vital importance. One has only to mention the differences in biological activity between stereoisomers to illustrate this.

A different kind of example of the effect of substituents on the shape of the molecule is provided by certain substituted *p*-aminobenzoic acids. Substitution of a halogen atom at the position *ortho* to the carboxylic group provides derivatives can can function biologically as analogues of

p-aminobenzoic acid, but there is a progressive lowering of the potency as the size of the halogen atom is increased from fluorine through chlorine to bromine. Here, one may envisage the largest halogen atom as providing the greatest impediment to the approach of the molecule to the enzyme "surface". Similar reasoning could explain the inactivation of synthetic auxins of the phenoxyacetic acid class by the introduction of two *ortho* substituents. In other words, steric hindrance is as significant in biochemical structure-activity relationships as it is in preparative organic

TABLE I

SOME DRUGS AFFECTING THE NERVOUS SYSTEM

Formula	Type of activity
$Ph_2CH{\cdot}OCH_2CH_2NMe_2$	Antihistamine
	Analgesic
	Anticonvulsant
$(Cl{\cdot}C_6H_4)_2\,CH{\cdot}CCl_3$	Insecticide
	Ataraxic
	Anti-acetylcholine

chemistry. Other examples have been provided by the work of Landsteiner[5] and Pauling[6] on the serological reactions of artificial antigens prepared by attaching foreign molecules, notably substituted benzene derivatives, to proteins. The immune sera to such artificial antigens exhibit cross-reactions with proteins derived from determinant groups of related shape.

One of the best known examples of the deliberate use of the concept of molecular shape in synthesising biologically active molecules is the oestrogenic compound, stilboestrol. Here, the characteristic biological activity of the natural oestrogens was achieved by making a molecule in which two hydroxyl groups were separated by a structure giving the same spatial characteristics as in the natural steroids. Reproduction in a similar way of the biological activity of important natural products has been achieved in several other well-known cases. Examples are to be found amongst vitamins, in synthetic morphine-like analgesics and in the substituted phenyl- and phenoxyacetic acid auxins, which behave like indolylacetic acid. More recently, it has become apparent that a diphenylmethyl substituent, attached to various functional groups, confers properties which favour activity in the nervous system. The reason for

this is not clear, but it may well be a question of access to the site of action. Examples are given in Table I.

Activity Through Chemical Reactions

The biological activity of many of the normal chemical constituents of a living organism is due to their ability to enter into a chemical reaction. They may, in addition, use forces of a physical nature. This is particularly, and perhaps predominantly, the case with macromolecules, where interaction is due to a multiplicity of points of loose attachment in stereochemical juxtaposition. Thus the binding of an apoenzyme to its substrate may depend on the forces of electrostatic attraction, hydrogen bonds or van der Waals forces. The same is probably true of antigen-antibody interactions. Many of the simpler chemical reagents (as distinct from catalysts) of the living organism, however, are the smaller molecules, the metabolites and the coenzymes. These compounds enter into well-defined chemical reactions, and the same is true of many biologically active foreign compounds. One of the simplest ways in which a foreign compound may affect a living organism is by entering into an irreversible chemical reaction with a vital metabolite. A good example is the reaction of vital thiol compounds (enzymes or smaller molecules) with heavy metals such as copper or mercury, or with a reagent like iodoacetic acid. Biological activity in such cases is usually non-specific, and manifested as a general toxic effect. The foreign compound is highly reactive, and with highly reactive compounds the possibilities of finding biological activity of a specific kind are remote. Many biologically active compounds have the character of acid anhydrides. Highly reactive anhydrides will enter into non-specific reactions with the first chemical constituents of the organism which they encounter, even with water. They therefore have no chance to exhibit specific effects. With a lower degree of reactivity (but not too low) they may resist attack by water and certain other agents, and so be able to penetrate to a point in the organism where there is an appropriate natural chemical with which they may react. Examples of such anhydride-like compounds are A.T.P., acetylcoenzyme A, penicillin, and the organophosphorus insecticides. Chemical reactivity, therefore, must be delicately poised.

When considering structure-activity relationships in compounds foreign to an organism, it is necessary to discuss the phenomenon of competition between metabolites. Briefly stated, this amounts to the simple conception that the place of a normal metabolite in a biochemical sequence may be taken by a foreign compound of related structure and electrochemical properties, with disruption to varying extents of the dynamic function. The metabolite competition hypothesis has resulted in considerable theoretical advances during the last two decades. The empirical discovery of antibacterial agents has led to intensified research on the biochemistry of micro-organisms, particularly since the discovery of the metabolic importance of *p*-aminobenzoic acid in 1940. Even if it is accepted that in this field theoretical explanation has hitherto generally followed after the empirical discovery of activity, the hypothesis has a

predictive value which will become more important as basic biochemical knowledge increases. Although Quastel and Wooldridge had clearly demonstrated competitive antagonism in an enzyme system as far back as 1928[8], the antibacterial action of sulphanilamide was not predictable in 1935, because at that date the biochemical significance of *p*-aminobenzoic acid was not known. Competitive antagonists to various essential metabolites and coenzymes have since been successfully synthesised, and there are many examples in the cases of nicotinamide, pantothenic acid, thiamine, biotin and various amino acids, purines and pyrimidines[4].

If, in a biochemical reaction sequence, it is desired to synthesise a competitive antagonist to a particular component, there are certain considerations which can be applied. There should be a structural similarity to the metabolite, so that the antagonist can occupy the site on any enzyme "surface" which is normally occupied by the metabolite. Groups which are responsible for the enzyme-substrate binding must, therefore, be retained, but groups which participate in the reaction sequence may be modified. Thus sulphanilamide resembles *p*-aminobenzoic acid in its shape, in the aminophenyl residue and in the fact that they are acids of comparable strength. The antagonist differs from the metabolite in that the natural carboxylic group, which in the sequence must react with glutamic acid, is replaced by a grouping which can undergo no such reaction. Similar considerations undoubtedly apply to antagonists of thiamine, biotin and riboflavine, but space does not permit of more detailed illustration. A more complex example may be cited in relation to pantothenic acid, illustrated as part of the formula of coenzyme A, below.

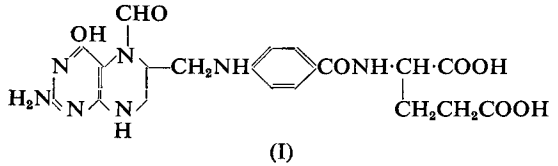
H— ⟵Pantothenic Acid⟶ —OH

Nucleotide— —O·CH_2CMe_2CHOH·CONH·C_2H_4·CO·NH·C_2H_4SH

H— ⟵Pantoic acid ⟶OH

Antagonists to pantothenic acid result by substitution its COOH group with SO_3H, SH, –CO⟨⟩Me, $-SO_2$⟨⟩Me or $-CONHNH_2$, all of which groupings are incapable of reacting with mercaptoethylamine. Replacement of the terminal OH group responsible for combining with the nucleotide by hydrogen produces an antagonist under defined experimental conditions. An antagonist of pantoic acid was also produced by replacing the terminal OH by H. The simultaneous replacement in pantothenic acid of the terminal OH by H and of the COOH by SO_2NH_2 gave a compound which was biologically inert[9,10]. This may well be because of the inability of the synthetic compound either to bind to an enzyme or to react with either of the adjacent compounds in the biochemical sequence. In other words, its structure is such that it is prevented from any type of participation in the specific chemical reactions concerned.

Another interesting class of metabolite antagonists has been derived from folic acid. This is depicted in the catalytically functional form of folinic acid (I), the *N*-formyl derivative of tetrahydro-pteroylglutamic acid.

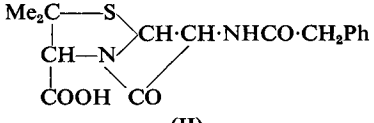


(I)

In this molecule, the *N*-formyl group is believed to provide the carbon atom which mediates various important "one-carbon" biosyntheses. Examples are the formation of purine rings by cyclic formylation of an open-chain intermediate, conversion of glycine to serine, uracil to thymine and homocysteine to methionine. Although exact details of the biochemical reactions are not yet known, it has been observed that various derivatives will function as folic acid antagonists, and their activity is now readily understood from their structures. One is pteroylaspartic acid, which obviously upsets the function of the glutamic acid moiety of the natural compound[11]. In aminopterin, the pyrimidine OH is replaced by NH_2, thus providing an opportunity for "fixation" of the formyl group by ring closure[12]. In methopterin, the linking $-CH_2NH-$ group is *N*-methylated. There is evidence, at least from the natural occurrence of 10-formylpteroic acid (rhizopterin), that this N atom is concerned in the formation or function of folinic acid, to which purpose methylation would be inimical. In contrast to the case of pantothenic acid, simultaneous alteration at two vital points in the molecule has also produced antagonists. These are A-methopterin, which contains both the amino group of aminopterin and the methyl group of methopterin[12], and the (4) amino analogue of pteroylaspartic acid[14]. Obviously in spite of the double change of structure, both these compounds retain an affinity for the vital enzyme or enzymes concerned.

In the examples mentioned above, the competitive metabolites result in a major disruption of the total metabolism, resulting often in the death of the cell. The overall effect of introducing competitive metabolites, however, need not be so drastic. If *E. coli* is grown in a medium containing *p*-fluorophenylalanine, this synthetic amino acid is incorporated into proteins in the places normally occupied by the structurally related amino acids, phenylalanine and tyrosine. Some, but not all, of the resulting proteins retain their characteristic biological activity[15]. In a similar way, structural analogues of the normal purine and pyrimidine bases can become incorporated into nucleic acids. In fermentation of *Penicillium chrysogenum*, a variety of penicillins can be produced, and in the normal manufacture of penicillin G, it is the practice to add phenylacetic acid or a suitable derivative to the medium in order to provide the mould with an excess of one of the normal precursors. This results in the predominant production of penicillin G, with its phenacyl side chain (II). If phenoxyacetic acid or various substituted derivatives of this or of phenylacetic acid are employed "unnatural" penicillins are obtained, such as the important penicillin V (phenoxymethylpenicillin). In the same way, numerous biologically active analogues of vitamin B_{12} have been produced experimentally, by addition to the culture medium of structural analogues of the

natural base, 5:6-dimethylbenziminazole. Examples of analogues which are effective in this way are benziminazole itself, and some halogen- or even nitro-substituted derivatives, 4-chlorobenztriazole, adenine and some substituted derivatives[16]. At a relatively simple chemical level the production of halogen-containing mould metabolites can be influenced by the quantity and nature of the inorganic halide in the culture solution. Examples are griseofulvin (III) and the tetracyclines, for example, aureomycin (IV).



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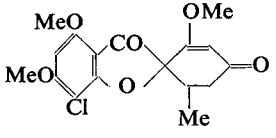

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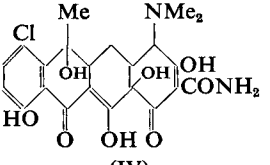
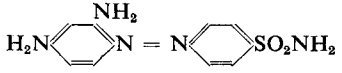


(IV)

Drug Metabolism

When a foreign compound enters a living organism, there are two effects to be considered, the response of the organism and the chemical effect of the organism on the drug. The second of these is of great practical importance in chemotherapy, where one of the problems is to ensure an adequate concentration of the biologically active compound at the site of action. Drugs can undergo metabolic alteration to varying degrees. This results from interaction with the normal biochemical reagents of the cells, though there are special circumstances, discussed later, where the drug can alter the cell metabolism in a manner inimicable to its own molecular integrity. Such metabolic changes are commonly referred to as detoxification processes, because they frequently result in the excretion of the foreign compound. The term is unfortunate, however, because the chemical changes undergone by a drug may on occasion result in the production of a derivative which is more toxic to the organism (or a parasite) than the drug itself. Well known examples are the greatly increased trypanocidal activity of pentavalent arsenicals on reduction to the trivalent state in the animal body, the reduction of the azo dye, Prontosil Rubrum (V), to sulphanilamide, and the increased antimalarial activity of proguanil (VI) through its metabolic oxidation to a dihydrotriazine (VII)[17]. Both these examples relate to parasites, but there are also cases where parasites are not involved. The carcinogenic action of certain aromatic amines is probably indirect, the true active agents being oxidation products. The auxin-like activity of substituted ω-phenoxyaliphatic acids $ArO(CH_2)_nCOOH$, where n is an odd number, arises through their degradation by β-oxidation to the lowest homologue ($n = 1$) which is active in its own right[18]. The same applies to the

toxicity of ω-fluoro-aliphatic acids containing an even number of carbon atoms[19]. Similarly, the activity of esters of synthetic auxins probably follows their hydrolysis, though this has never been proved experimentally. The toxicity of methanol to various types of organism may well be due as much to its oxidation to formaldehyde as to the inherent toxicity of the alcohol.



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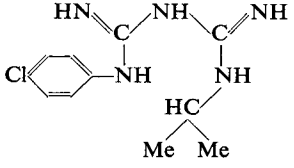

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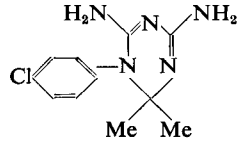


(VII)

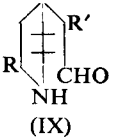
The commonly occurring metabolic reactions whereby a foreign compound may be changed chemically are (*a*) oxidation and reduction, (*b*) hydrolysis and condensation, (*c*) alkylation and dealkylation. Oxidation of aliphatic chains has already received brief mention in the previous paragraph, and it offers an explanation of a structure-activity relationship which is occasionally encountered, namely an alternation in potency between odd and even numbered members of an homologous series. Aliphatic or aralkyl alcohols and amines may be oxidised by animals through aldehydes to carboxylic acids. Hydroaromatic or reduced heterocyclic rings may be aromatised by oxidation. The hydroxylation of aromatic compounds to phenols (and their subsequent conjugation with solubilising groups) is a familiar "detoxification" process in animals exemplified in the case of aniline and the polycyclic carcinogenic hydrocarbons. Reductions in the animal body have already been mentioned in the case of pentavalent arsenic and a particular azo compound. They also occur with nitrocompounds. Other azo compounds which are split in the animal body are certain carcinogenic azo dyes. In yeast and green plants, aliphatic aldehydes, for example, chloral, are reduced to alcohols.

Condensation reactions in normal biochemical processes are represented in the biogenesis of many important macromolecules such as proteins, polysaccharides and nucleic acids, and in processes of esterification such as fat formation. Many of these reactions can be looked upon as acylation reactions, and acylation of foreign substances is frequently encountered. In the animal body, amines are frequently acetylated, as for example, in the case of aromatic amines such as the sulphonamide drugs and aniline itself. The actual process of acetylation is mediated through the intervention of acetyl-coenzyme A, which functions as a mixed anhydride. Foreign carboxylic acids will often combine in the body with amino acids, as in the well known excretion of benzoic in

the form of hippuric acid. In birds, ornithine takes the place of glycine. Phenylacetic acid may be eliminated by some mammals in the form of a conjugate with glutamine. In dogs, some benzoic acid is excreted after condensation with glucuronic acid. Phenols frequently form sulphuric esters ("ethereal sulphates") but the choice of the conjugating molecule is influenced by substituents in the benzene ring[20]. Condensation of alcohols and phenols with sugars, the formation of glycosides, is more frequently encountered in green plants, and the nature of the conjugating sugar varies both with the nature of the alcohol or phenol and with the plant species[21]. Hydrolyses of foreign compounds are encountered in the breakdown of barbiturates by fission of peptide links, and in the hydrolysis of simple penicillin esters (themselves not bactericidal) in certain animal species, though not in man, to the bactericidal free acid penicillin.

Alkylation processes are more frequently considered in relation to the mode of drug action than to so-called detoxification processes, though the ubiquity of transmethylation reactions involving choline and methionine suggests that methylation of foreign compounds ought to be of frequent occurrence. A few examples are quoted by Williams[24], notably the conversion of pyridine to methylpyridium hydroxide which has been known for seventy years, and the similar quaternisation of various pyridine derivatives, including quinoline. This process, apparently, has some species selectivity, and does not occur in rabbits. The conjugation of many halogeno compounds, for example, benzyl chloride and halogen-substituted benzene compounds, with *N*-acetylcysteine amounts to an alkylation of the thiol group rather than a condensation. Dealkylation of, for example, ethers or tertiary amines is perhaps properly regarded as a special case of oxidation. The alkyl group may come off in the form of aldehyde, as in the case of the removal of the first methyl group from the carcinogen, *p*-dimethylaminoazobenzene[22]. In the dealkylation of the side-chain tertiary amino group in chloroquine[23], a suggested intermediate is the *N*-oxide. One of the metabolic changes of cinchona alkaloids in man is hydroxylation in the quinuclidine ring, and the author has suggested[4] that this may be a special case of oxidative dealkylation on the lines of VIII–X.

(VIII)



(IX)

(X)

Drug Resistance

The biological activity of many compounds is affected in a quantitative way of continued exposure to sub-active concentrations of the compound. This is of great practical importance in the chemotherapy of bacterial infections and in the control of insect pests. Under these conditions, the bacterial or insect population may acquire a resistance to the action of much higher concentrations of the toxicant. This overall statistical

result on a multiplying population is probably due to a combination of two types of phenomena, one genetic and one physiological. In the present context it is only profitable to discuss the latter, in which the toxicant induces biochemical changes which favour the survival or continued function of the cells in question. The possible physiological basis for drug resistance has recently been discussed in general terms by Davis[25], who questions the general validity of two hypotheses which at one time found considerable favour. One was that the blocking of a biochemical pathway by a drug forced the organism to develop an alternative route. The other was that the organism elaborated increased quantities of the metabolite which was antagonised by the drug. The increased synthesis of *p*-aminobenzoic acid under sulphonamide bacteriostasis had early been advanced as a possible cause of sulphonamide resistance, but Davis considers this to be of dubious quantitative significance. A doubled level of thiol compounds in arsenic-resistant ticks has recently been reported[26], but unless the level of a particularly significant thiol compound was masked in the modest overall thiol increase reported, this also is a questionable explanation of the acquisition of arsenic-resistance.

A profitable field of enquiry has been opened up in recent years by the growing knowledge of enzyme adaptation and of bacterial permeases. It is only possible here to give an extremely brief and over-simplified account of these phenomena. The reader is referred to several recent review articles for more extensive, and to some extent speculative accounts[15,27–30]. The bare facts are that the production of normal enzymes within a cell can be stimulated to excess by the presence of their substrates and certain structural analogues thereof, and that the penetration of certain polar compounds into cells is mediated by enzyme-like permeases in the cell wall. Permeases, like intracellular enzymes, are capable of being induced or inhibited. Adaptive enzyme formation has mostly been studied in relation to the formation of penicillinase by *B. cereus*, induced by minute amounts of penicillin, and the induced formation of β-galactosidase. The increased quantities of penicillinase in *B. cereus* cause the organism to be resistant because the penicillin is destroyed by the enzyme. The induction is specific to compounds (penicillins, cephalosporins) of closely related structure[31]. Adaptive enzyme (and permease) formation occurs in the absence of cell division, but is dependent on the active synthesis of protein, which in turn is linked to the synthesis of nucleic acid. It is possible for a particular foreign compound to affect adaptive formation of either a permease or an intracellular enzyme, but not necessarily both. "Induced" *E. coli* metabolises various structurally related galactosides, but exchange of the glycosidic oxygen for sulphur affords compounds which, though they can penetrate the cell wall, are not attacked by galactosidase. When glactosidase is induced in *E. coli* in a medium containing *p*-fluorophenylalanine, the normal amount of the enzyme is formed, but no additional permease.

The theoretical relevance of all this to the problem of drug resistance is obvious, but examples of its application to drugs are as yet very few in

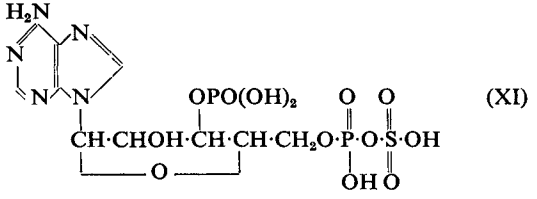
number. Penicillin resistance has already been mentioned. An interesting case has been provided by certain carcinogenic hydrocarbons. Administration of methylcholanthrene or of 3:4-benzpyrene to rats induces the formation in the liver of increased quantities of enzymes which hydroxylate the hydrocarbon and which demethylate carcinogenic methylated amino-azo compounds. The induction is prevented by ethionine, which is countered by methionine[32]. Insects rendered resistant to the insecticide, DDT, contain increased amounts of an enzyme "DDT-dehydrochlorinase" which converts it to an inactive compound. Some structural analogues of DDT which exhibit a synergistic effect with DDT against resistant insects appear to do so by inhibiting the enzyme[33]. The key position of nucleic acid in the phenomenon of drug resistance is emphasised in a recent brief report. If DNA, extracted from *Dip. pneumoniae* cells rendered resistant by exposure to A-methopterin, is added to normal susceptible cultures, these cultures acquire resistance to the drug[34]. It has been observed that azaguanine will inhibit the formation of adaptive enzymes in *Staph. aureus*. Guanine reverses this, not by preventing incorporation of azaguanine into RNA, but by allowing an increased production of RNA[35].

Biological Action of Specific Structures

We have seen how, on general grounds, activity may be the result of a purely physical process or it may result from chemical reactivity within the cell, as regulated by the structure of the molecule. The concept of toxophoric groupings, by analogy with chromophoric groups, has only limited validity. Whereas all compounds containing chromophores are coloured, a particular grouping may confer biological activity only in some restricted compounds or classes of compounds. There are, nevertheless, certain fairly well-defined chemical reactions whereby foreign compounds can interfere in biochemical reactions, and it is of interest to illustrate by a few examples.

Alkylating agents. The alkylating ability of simple alkyl halides or other alkyl esters in preparative organic chemistry is a type of chemical reactivity which can be measured. In the biological activity of these compounds, the mechanism is chemical if they are sufficiently reactive or physical if they are not reactive. This was demonstrated by Ferguson and Pirie[36], who showed that alkyl chlorides above methyl, or bromides above propyl affected grain weevils by a physical process. Methyl chloride and the lower bromides, being more reactive, showed chemical toxicity. In rats, the ethyl ester of methane sulphonic acid will ethylate the thiol group of cysteine[37]. In more complex compounds, an alkylating function has in recent years been recognised as probably underlying the radiometic activity of nitrogen mustards, epoxides and ethylenimines[38]. These compounds react readily with anions, and the suggested site of chemical reaction in their biological activity is the (ionised) phosphoric acid residue in nucleic acid[39]. Alkylation is probably a cell reaction associated with the insecticidal activity of chlorinated hydrocarbons such as γ-benzene hexachloride. In addition to alkylation as a chemical

reaction of biological significance, one must consider the significance of alkyl groups as substituents in molecules which are active through some other chemical or physical process. The nature of the alkyl group will influence biological activity through its effect on physical properties and on reactivity. Classical examples are the alkyl substituted phenolic antiseptics and the alkyl thiocyanate insecticides. The effect of methyl substituents on the carcinogenic activity of certain polycyclic hydrocarbons is probably a chemically activating influence. In other circumstances, a methyl group may, for steric reasons, interfere with chemical reactivity in such a way as to destroy a characteristic biochemical reaction. Examples have already been given in a modified pantothenic acid and in the folic acid antagonist, methopterin. Another case is provided in the destruction of vitamin activity in nicotinamide by insertion of a methyl substituent in the 2-position.



(XI)

Acylating agents. Several important natural products have been recognised through their function as acylating agents. They are mixed anhydrides, typified by adenosine triphosphate, "active sulphate" (XI), "activated" amino acids and *S*-acetylcoenzyme A. Foreign compounds which have an anhydride-like reactivity may be exemplified by penicillin and by the modern organophosphorus insecticides. Although the mode of action of penicillin in terms of organic chemistry is still obscure, it may well be associated with an acylating action. The case of the organophosphorus compounds is more clear. Here, various esterases (choline esterase, chymotrypsin, etc.), are acylated (possibly at the CH_2OH of serine) with formation of a phosphorus-containing enzyme in which biochemical function has been destroyed. This is particularly important for choline esterase, which is intimately concerned with nervous transmission. The reaction is illustrated for di*iso*propyl phosphorofluoridate and an enzyme, EH.

$$F{\cdot}PO(OPr)_2 + EH \qquad E{\cdot}PO(OPr)_2 + HF$$

Thiol reactors. Thiol groups play an important role in natural processes because of their capacity for reversible oxidation to disulphides, their acylation to give so-called high energy bonds, and other characteristic reactions. Thiol groups are essential components of many apoenzymes, and they are of vital functional significance in smaller molecules such as those of coenzyme A and glutathione. It follows that compounds which can react with thiols may be expected, subject to the limitations imposed by their physical properties, to exhibit biological activity. The simplest examples are ions of heavy metals such as copper and mercury, and certain organometallics such as alkylmercuri salts and organic arsenicals.

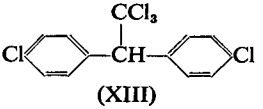
Interaction of arsenicals containing trivalent arsenic with thiols is now the accepted mode of action of these compounds; the dithiol, dimercaptopropanol, acts as an antidote to arsenical poisoning by its preferential (cyclic) attachment to arsenic.

Perhaps the most important purely organic types of compounds which can react with thiols under physiological conditions are those containing the α-β-unsaturated ketonic structure, including open chain compounds and cyclic ones represented by unsaturated lactones and quinones. The cyclic structures are very commonly found in biologically active natural products which exhibit antibacterial, or sometimes herbicidal, activity. Studies of the reactivity of these, and of synthetic analogues, with thiols such as cysteine have been recorded[43,44]. The antibacterial activity of synthetic quinones has also been studied extensively from this viewpoint. Although more than one mode of action is feasible, there is considerable evidence that the activity of quinones, against Gram-negative organisms at least, is associated with their ability to react with thiol groups[45].

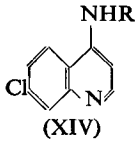
Halogeno compounds. The biological effects of introducing halogen atoms into organic compounds may be divided into physical and chemical effects. This has already been illustrated in the simple case of alkyl halides. The effect of halogen substitution in modifying physical toxicity has recently been made use of in the development of the anaesthetic, halothane (XII)[46]. Other physical effects are exhibited by halogen substitution in aromatic structures. Halogen-substitution in *p*-aminobenzoic acid is an example which has already been quoted. Halogenation of phenols and of quinones raises their bacteriostatic activity; halogenation in the appropriate positions of phenoxyacetic acid raises the herbicidal activity. In many cases, however, it is not possible to apportion the effect of halogen substitution clearly to physical effects or to effects on chemical reactivity. There is overlap, because the introduction of a halogen atom, whilst undoubtedly influencing physical properties, may also effect reactivity. A case in point is the insecticide, DDT (XIII). The biochemical function of the $-CCl_3$ group is probably through its reactivity. Without the two *p*-chlorine atoms the molecule is biologically inactive, but these chlorine atoms may be replaced by methoxyl without loss of activity. It is far from clear whether the *p*-substitution is influencing insecticidal action through an effect on physical properties, or through modifying the reactivity of the $-CH(CCl_3)-$ group, or through both. The same consideration applies to chlorine substitution in quinoline (XIV), acridine (XV) and biguanide antimalarials, in antibiotics such as aureomycin and griseofulvin, and in other chlorinated hydrocarbon insecticides. A clear case of biological activity arising directly from the introduction of a halogen atom into a position where it exhibits chemical reactivity is mustard gas and its nitrogen analogues, β-chloroethylamine derivatives, which are thereby converted into alkylating agents.

$CF_3CHClBr$

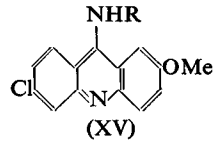
(XII)



(XIII)



(XIV)

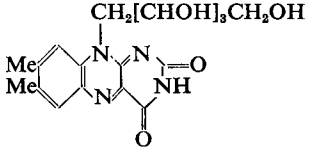


(XV)

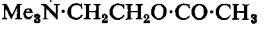
when R = — CHMe $[CH_2]_3$ NEt_2; XIV = chloroquine and XV = mepacrine

The substitution of a halogen atom (non-reactive) for hydrogen or methyl in certain natural metabolites frequently results in the production of competitive antagonists. Thus, fluoroacetic acid is metabolised to fluorocitric acid, an antagonist of citric acid[47]. 6-Fluoronicotinic acid competes with nicotinic acid. Substitution of chlorine atoms for the methyl groups of riboflavine (XVI) produces a riboflavine antagonist. 2-Methylnaphthoquinone has vitamin K activity; 2-chloronaphthoquinone antagonises this.

Combination of two "active" groups in the same molecule. Most workers who have attempted to synthesise biologically active compounds have at some time yielded to the temptation to introduce two "active" groupings into the same molecule. To take a hypothetical example, the untutored might attempt to obtain an improved bactericide by introducing a phenolic group into sulphanilamide, or to combine antimalarial with antibacterial activity by introducing a hydroxy group into the 8-position of the quinoline ring in chloroquine. There is a long history of such attempts, generally unpublished because of the disappointing character of the results. What happens is due to the fact that the antimalarial activity of chloroquine, for example, is intimately associated with the molecular architectural details. These would be greatly affected by the introduction of an 8-hydroxy group, so that although antibacterial activity might emerge in the hypothetical hydroxylated compound, the antimalarial activity would be expected to fall, probably to vanishing point.



(XVI)



(XVII)

Biological activity is, nevertheless, often exhibited by compounds with two or more "active" groups. In such cases the activity is usually of a highly specific kind, in terms of biological effect, and limited to compounds of closely related structure. To take a particular example, acetylcholine (XVII) is hydrolysed by the enzyme cholinesterase. The substrate is attached to the enzyme at two points which have a space relationship determined by the enzyme structure. These two are the anionic site, where negative charge on the enzyme surface attracts the positive

charge on the quaternary nitrogen of acetylcholine; and the esteratic site, where an electron donating atom in the enzyme attacks the carboxylic carbon. Specific affinity for this enzyme is, therefore, confined to compounds which contain two groupings, appropriately spaced and having similar electrochemical properties to the natural substrate. Compounds which do not have this structural relationship may yet attack cholinesterase (for example, the organophosphorus insecticides), but this attack is not specific to cholinesterase. Other esterases or proteolytic enzymes are also attacked by the organophosphorus compounds. This high specificity of action, based upon two- or multi-point attachment of the active molecular to a macromolecular catalyst finds its highest expression in the phenomena of virus multiplication, cell-division and immunity reactions. For accounts of this complicated topic the reader is referred to Landsteiner[5], to Sevag[48], and to various papers by Pauling and his associates published from 1940 onwards[6].

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