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Drug Latentiation

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To suggest that the discovery of a new drug is a result of chance observation is probably as misleading as to accept the other extreme view which attributes an entirely fundamental and rational basis to drug design.

A chance observation may lead to a prototype molecule but the conversion of this into a clinically acceptable drug is usually the result of a team effort by medicinal chemists, pharmacologists, biochemists and pharmacists.

The biological effect of a drug is determined by many factors, all of which are influenced by the chemical structure of the compound. The biological activity of a compound in the animal body is not only a function of its effect at a particular site but also of its ability to reach and be removed from the locus of action. Therefore, a knowledge of the physicochemical properties of the compound which influence its absorption, distribution in tissues, its enzymic biotransformation and its excretion after enzymic detoxification are essential for a complete understanding of the speed and duration of action, the relative toxicity and the possible routes of administration. In any approach to drug design this total spectrum of biological activity must be considered, since the various parts will undoubtedly influence both the quantitative and qualitative pharmacological effect of the drug.

The problem of presenting a potentially useful drug in a stable, non-irritant, relatively non-toxic, easily administrable clinically acceptable form with the desired activity is not one which should be left solely to the ingenuity of the pharmaceutical formulator. In a co-operative effort, the medicinal chemist at the synthetic stage can often help to reduce certain objectional pharmaceutical properties, e.g. the preparation of less soluble salts of penicillin

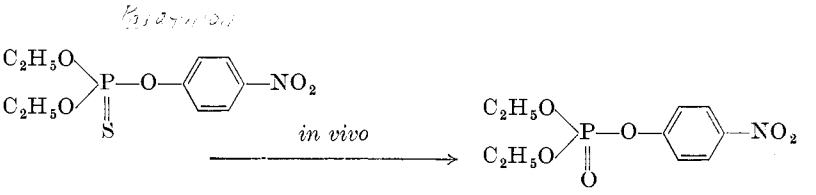
helped in the formulation of reasonably stable aqueous suspensions for oral or parenteral administration, while the synthesis of various esters of steroids provided oil-soluble material suitable for slow release.

Recent developments in drug research indicate that such an approach will become increasingly important in the design of drugs, and the medicinal chemist will become increasingly concerned in what might be termed *drug latentiation*.

Drug latentiation is defined as the chemical modification of a biologically active compound to form a new compound, which upon *in vivo* enzymatic attack will liberate the parent compound. The chemical alterations of the parent compound are such that the change in physicochemical properties will affect the absorption, distribution and enzymatic metabolism. Allied with this concept is *structural formulation*, by which is meant the modification of a biologically active compound at a point not essential for binding to an active site in the biological receptor, so that although the desired biological effect is retained the resultant changes in physicochemical properties cause alteration in the absorption, distribution or metabolism of the drug; the parent compound, however, is not liberated in the body. In contrast, *pharmaceutical formulation* may be defined as the techniques involved in the presentation of a pharmacologically active compound in a stable, non-irritant, relatively non-toxic, easily administered form by modification of the physical properties of the compound, by physical combination with some inert material or, in some cases, by adjustment of the physical properties of the medium in which the compound is administered: changes in the chemical structure of the compound are not involved.

As Brodie and Hogben[1] have pointed out, there are two facets to the problem of the biochemical and physical aspects of drug action—the influence of drugs on the body and the influence of the body on drugs. The pharmacological effect of a compound may be due to the intact molecule or a metabolic derivative formed during the transportation to, or at the locus of action. The metabolic product may be as active or more active than the intact molecule, or may exert a pharmacological effect not apparent in the intact molecule. Brodie *et al.*[1] quote a number of interesting examples, i.e. ephedrine, amidopyrine, methampheta-

mine and methylphenobarbitone all yield active metabolites which exert the same sort of pharmacological activity as the parent compound. Ephedrine is so rapidly dealkylated to the nor-compound in dog that its action may be considered to be due to the dealkylated product.[2] In man, phenacetin is so rapidly dealkylated to the active *N*-acetyl-*p*-aminophenol that it is difficult to determine plasma levels of the parent compound.[3]



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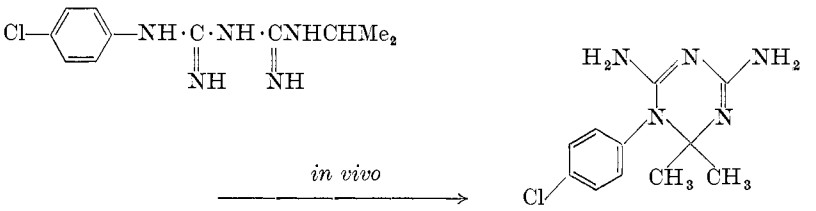


(III) (IV)

The unmasking of a pharmacological response not apparent in the parent compound is often associated with the formation of a new functional grouping, e.g. prontosil (without *in vitro* activity) is metabolized *in vivo* to produce sulphanilamide.[4] The insecticide parathion (I) is relatively inactive *per se* but *in vivo* is converted to a powerful anti-cholinesterase inhibitor (II),[5] while the anti-malarial proguanil (III), without *in vitro* activity on certain malarial parasites, is transformed *in vivo* to an active cyclic derivative (IV).[6]

A recent example of metabolic breakdown to give a more active compound is the muscle-relaxant 2-amino-5-chlorobenzoxazole (Flexin) (V) which has been shown to be metabolized to the active compound (VI).

(V) (VI)

(For further details of some of the above and other examples see Brodie and Hogben.)[1]

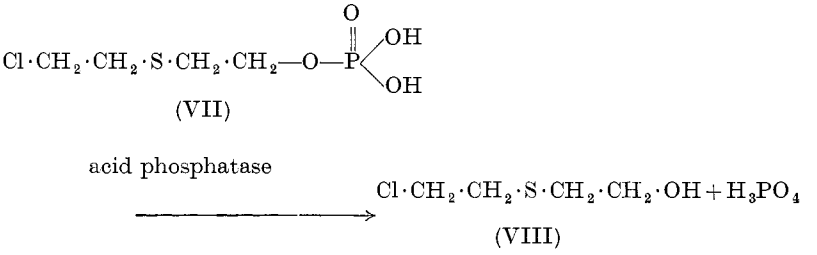
This *in vivo* formation of an active compound from an inactive one immediately suggests a possible approach to drug design by drug latentiation. Provided the interconversions can be localized in a particular target cell or tissue the possibility of: (a) direct exclusive attack in the target cell with the active compound (particularly useful in the case of compounds with systemic toxicity), and (b) the differentiation between the various possible effects of a compound become feasible. Since enzymes are responsible for the interconversion of inactive to active compounds, a completely logical use of this approach depends upon a prior knowledge of the enzyme composition of the target cell and the utilization of this particular enzyme system present in the target area, but absent or only present to a slight extent in other organs or body fluids. Unfortunately, the development of comparative biochemistry of various tissues and cells has lagged behind the ingenuity of the synthetic chemist, so that although in many cases the gun could be loaded with suitable ammunition we lack information concerning the target. Despite these obvious limitations, some recently introduced therapeutically active agents may be considered as examples of drug latentiation.

Drug Latentiation to Give Drug Localization

Stilboestrol shows marked cytostatic activity[8,9] in addition to its well known oestrogenic effect. The use of stilboestrol diphosphate (Honovan) in the palliative treatment of prostatic carcinoma[10–16] emphasizes that by suitable latentiation it is possible to specifically enhance the cytostatic activity. This approach was based on the proposal of Druckery and Raabe[17] that use be made of the high acid phosphatase activity in the cancerous tissue

of the prostate compared with other tissues such as bone, kidney and intestines. Brandes and Bourne[18] demonstrated the *in vitro* dephosphorylation of stilboestrol diphosphate by phosphatase of the prostate, as well as by rat tissues other than prostate. The water-soluble, oestrogenically inert[15] diphosphate is thus preferentially dephosphorylated in the carcinomatous prostatic tissue with the release of the cytostatic stilboestrol[19] (the cystostatic effect of stilboestrol is 300 times greater than that of the diphosphate[17]). An interesting development of this work is the possibility of increasing the therapeutic value of oestrogen phosphates in treating prostatic carcinoma by incorporating ^{32}P in the molecule.[18]

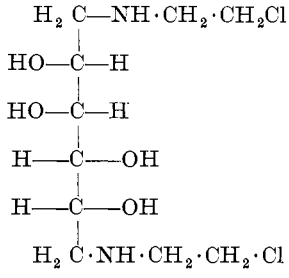
Seligmann *et al.*[20] hoped that the high acid phosphatase activity of the prostatic carcinoma could be utilized to give localized hydrolytic cleavage of a normally well tolerated mustard phosphate (VII) to the unesterified and necrotizing mustard (VIII); unfortunately the latter proved to be too toxic.



(VII) (VIII)

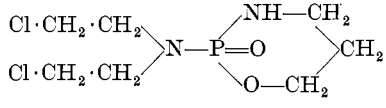
The problem of transport becomes important in the case of compounds which are known to have a definite beneficial therapeutic effect in diseased cells but show high systemic toxicity. Nitrogen mustards have marked anti-cancer activity but have a low therapeutic index since the cytotoxicity which is the basis of their therapeutic effect extends to normal cells, particularly those of the bone marrow. That this systemic toxicity can be overcome by suitable formulation is shown by the use of nitrogen mustard derivatives such as 1,6-*bis*-(β-chloroethylamino)-1,6-desoxy-D-mannitol dihydrochloride (Mannomustine, Degranol) (IX) as an antineoplastic and cytostatic agent.[21–26] The mannomustine dihydrochloride (LD50 in rats 100 mg/kg) is considerably

less toxic than nitrogen mustard (LD50 in rats 1·6 mg/kg), the therapeutic index being about five times as favourable. Although specific evidence concerning the mechanism of action of mannomustine is lacking it is possible that the activity is associated with the release of the nitrogen mustard in the target cells.



(IX)

In an attempt to produce new cytostatic drugs with maximum therapeutic effectiveness and minimum toxicity, Brock and his co-workers[27-31] prepared the cyclic propanol amido ester of *bis*-(β-chloroethyl)-phosphamide (Endoxan, B518) (X) and demonstrated its therapeutic effectiveness in graft tumours of the rat. The minimum lethal dose of B518 in rats was found to be 160 mg/kg[27] (*cf.* 50 mg/kg for the *N*-oxide mustard and 1·5 mg/kg for nitrogen mustard). In therapeutic trials on fully developed



(X)

tumours of the rat, e.g. the solid Yoshida sarcoma, Walker–256–carcinoma, and Jensen sarcoma a definite cure was obtained with a single intravenous injection of B518, the minimum effective dose amounting only to 2–4 per cent of the minimum lethal dose.[27] *In vitro* tests in which B518 was incubated with tumour cells at 37°C showed complete inertness of the compound up to a concentration of 10^{-3} g/ml, the cells then being grafted on to healthy young rats and their taking capacity checked.[27] Brock[29] con-

cluded that B518 was an inactive transport form of the drug which under the conditions prevailing in the organism gave rise to the active compound possibly by enzymic cleavage of the ring.

The converse of such an approach to drug design is of interest and is represented by the investigations of Boyland[31] on 3-hydroxyanthranilic acid, which causes cancer of the bladder in rats by being released from its glucuronide by urinary β-glucuronidase. Boyland[31] has suggested that by the administration of a specific inhibitor of β-glucuronidase, e.g. glucosaccharo-1⟶4-lactone, it may be possible to prevent the release of the carcinogen from its glucuronide transport form.

The problems of concentrating the release of an active drug moiety in a target cell by utilizing a particular enzyme is closely allied to those confronting the histochemist in enzyme histochemistry. In general, those concerned with drug design have not fully appreciated the analogous nature of the problems.

Staining methods for many enzymes have been devised,[32] but with few exceptions these have been restricted to enzymes of low specificity for which suitable chromogenic substances could be prepared. Holt,[33,34] in an attempt to assess the potentialities of such methods, adopted theoretical and experimental approaches and analysed a general histochemical staining process as follows:

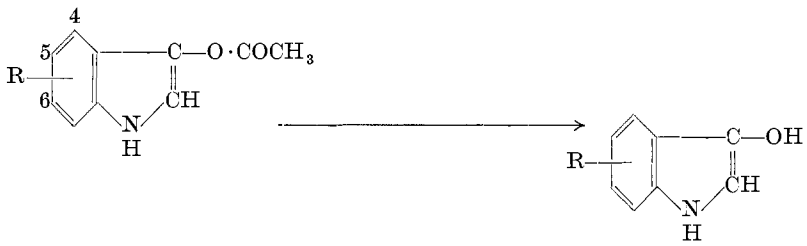
$$\text{Substrate} \xrightarrow[\text{(Zero order)}]{\text{Enzymic Reaction}} \text{Stain Precursor} \xrightarrow[\text{(First order)}]{\text{Capture Reaction}} \text{Visualizing Substance}$$

which is not too dissimilar to a possible concept of drug action:

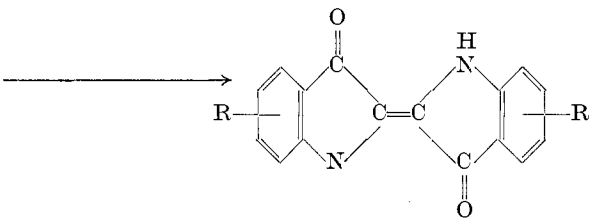
$$\text{Transport Form of Drug} \xrightarrow[\text{Reaction}]{\text{Enzymic}} \text{Active Drug Moiety} \xrightarrow[\text{at Receptor}]{\text{'Capture Reaction'}} \text{Biological Response}$$

Holt considered that only under zero order conditions will the rate of breakdown of substrate be entirely dependent on levels of enzymic activity at all points. In the capture reaction, first order conditions were considered essential to ensure that the visualizing substance is formed in amounts proportional to the concentration of diffusing substance at any point, since only in this way could the final distribution of visualizing substance quantitatively reflect the distribution of enzyme. It was shown that, under such

conditions in systems in which the visualizing substance is completely non-diffusible, the degree of localization depends upon three major factors: (a) the diffusion constant of the primary product of enzyme action, (b) the size of the site and (c) the velocity constant of the capture reaction. It has also been established that the binding of the diffusing substance and final products to tissue components was important if quantitative localization was to be achieved without a very rapid and possibly unattainable



(XI) (XII)



(XIII)

R = Halogen

rate in the capture reaction. The particular reaction investigated was the esterase hydrolysis of indoxyl acetates (XI) to indoxyl (XII) followed by oxidation to an indigoid dye (XIII).

It was clearly shown that the position of the substituent in the aromatic ring had a profound effect on the physicochemical properties of the derived indigoid dye. All substrates with halogen substituents in the 7 position gave dyes of high lipoid solubility, which decreased in those substituted in the 4 position, while 5 or 6 substituted substrates gave dyes of minimal lipoid solubility. That the insoluble dyes were highly associated by hydrogen bonding between the imino and carbonyl groups of

adjacent molecules was established by consideration of the relationship between molecular structure of the dyes, their solubilities, melting points and infrared absorption. This was supported by the x-ray diffraction studies of von Eller on indigo.[35] Fig. 1, which is a scaled drawing based on x-ray diffraction data, shows the relationship between two associated molecules; van der

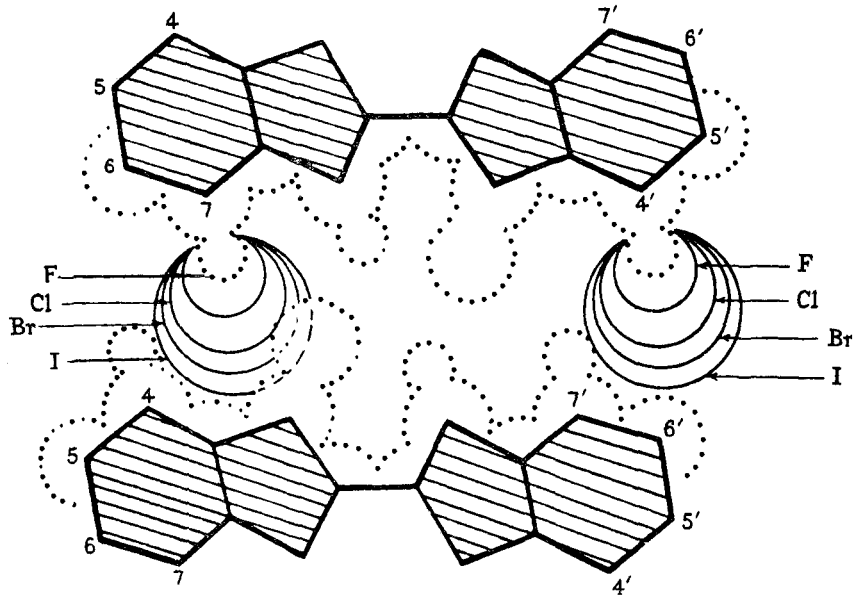


Fig. 1. Scaled drawing based on x-ray diffraction data showing the relationship between associating indigo molecules. (Van der Waals' envelopes of adjacent parts of the molecules and the corresponding radii of fluorine, chlorine, bromine and iodine are shown)

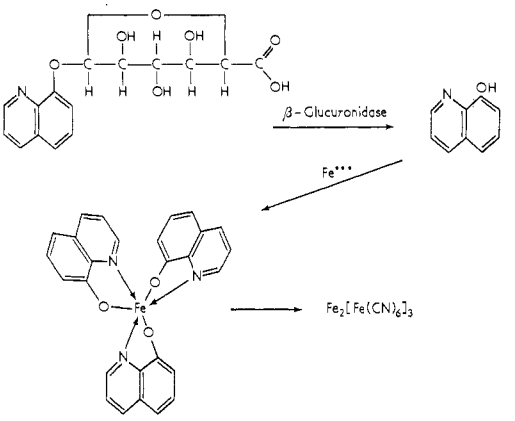
Waals' envelopes of adjacent parts of the molecules and the corresponding radii of fluorine, chlorine, bromine and iodine are also shown.[34]

It is clear that substituents in the 5 and 6 positions could not constitute a steric factor preventing intermolecular binding of the indigo molecules. On the other hand, a substituent in the 7 positions would be completely within the confines of the intermolecular space and would therefore interfere with intermolecular association. The prevention of association of the molecules

results in greater solubility of the dye. In the 4 positions steric hindrance due to a substituent would be less pronounced but would increase with increasing atomic size of the substituent. As Holt pointed out,[34] the importance of this concept is that factors controlling the associations of dye molecules are probably also those responsible for the binding of the dye to protein. Holt[34] decided to prepare a highly substantive dye of low solubility. On the basis of the known facility with which 4,4-dichloro-indigo binds to protein[36] and the solubilizing effect of substituents in the 5 position, 5-bromo-4-chloro indoxyl acetate was prepared and this gave a dye 5,5-dibromo-4,4-dichloro indigo which fulfilled these expectations.

From the medicinal chemist's viewpoint these investigations emphasize the optimum conditions for transporting a drug to the target area, show clearly the importance of steric factors in the 'fit' of the drug molecule and the receptor site, and emphasize the importance in drug transport of the bonding of drugs with protein. Jarowski[37] has commented that in a series of analogues in the tetracycline group of compounds a direct correlation existed between the extent of oral absorption and the degree of binding to an aqueous dispersion of hog mucin, and suggests that one could generalize that the more tightly a given drug is bound to plasma proteins the longer will be the duration of blood levels when compared with an analogue or homologue in a similar series.

Some recent investigations by Fishman and Baker[38] on the cellular localization of β-glucuronidase leads one to speculate on the possibility of the latentiation of chelating agents. The importance of trace metals in enzyme systems is well known, and the use of chelating agents as selectively toxic substances to remove essential trace metals required for abnormal enzyme reactions due to a diseased condition, without impeding the normal enzyme sequence, has always appeared attractive.[39] This might be accomplished, (a) if it were possible to transport the chelating agent direct to the target cell, or (b) if a selective chelating agent, which would chelate metals essential only in the abnormal enzyme system, could be developed. For the cellular localization of β-glucuronidase, an enzyme associated with the action of gonadol hormones and neoplastic growth, Fishman *et al.*[38] used the following reaction.



The release of a chelating agent such as 8-hydroxyquinoline in the target area appears to offer possibilities worthy of consideration. However, such an approach to drug design depends upon a prior knowledge of the enzyme make-up of the target area.

Drug Latentiation to Affect General Transport

The development of a dextran–iron complex (Imferon)[40–43] for the parenteral administration of iron as a haematinic illustrates how the latentiation of iron in a suitable transport form can be used to overcome problems of absorption, toxicity and stability.

The first essential for safe administration is stability of the iron preparation in the blood since ionic iron is highly toxic. The iron must be administered in a suitable transport form in order that the ferrous and ferric ions are not released in the muscle, in the lymphatics, or in the bloodstream. It is essential, however, that when the iron complex has reached the intracellular environment of the reticulo–endothelial cells the latent iron ions are released and become incorporated into storable and utilizable forms such as ferritin. That this is achieved with Imferon is shown in Fig. 2 which represents diagrammatically the sequence of changes undergone by the iron–dextran complex after its injection into muscle, and indeed Imferon is well absorbed, has a low toxicity and is a satisfactory haematinic.[41]

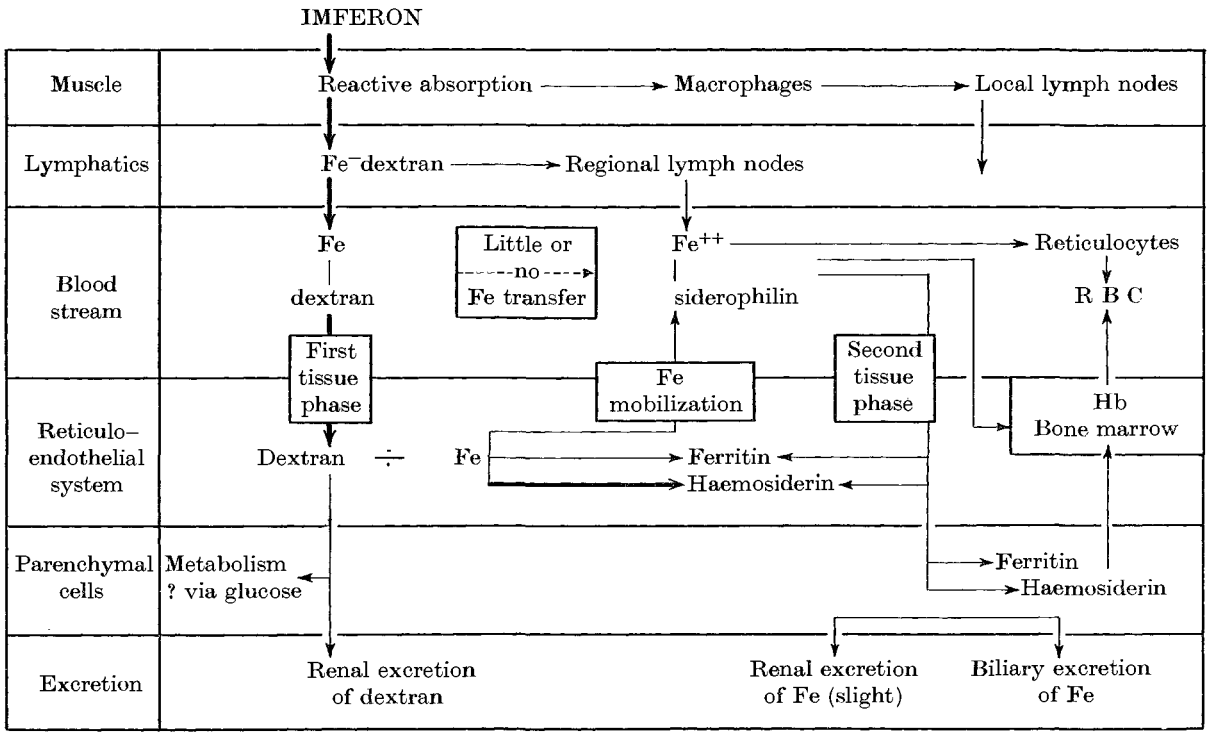
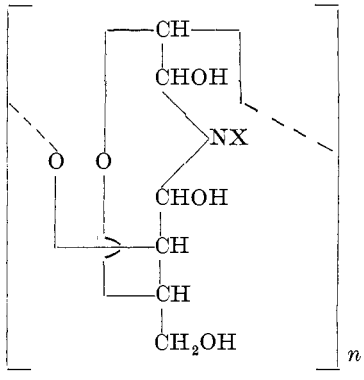


Fig. 2. Diagrammatic representation of the sequence of changes undergone by the iron–dextran complex after its injection into muscle

In formulating transport forms of drugs, the requirement that the complex is capable of being preferentially attacked by a specific enzyme in the target area with the release of the active moiety is vital. It is, however, also important that the physicochemical properties of the transport form are such that actual penetration to the target area is possible.

In this respect, a new concept of the physiological role of vitamin B_{12} by Heathcote and Mooney[44] is of interest. It was considered that inadequate absorption of vitamin B_{12} is due not to the absence of an intrinsic factor but to inadequate proteolysis of the



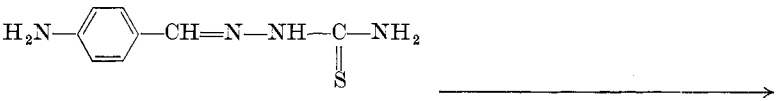
X = —NH·CO·C_5H_4N and
—C_6H_4·CH=N·NH·CS·NH_2
on alternate glucose residues.

(XIV)

naturally occurring protein-bound Vitamin B_{12} present in food, as a consequence of which the vitamin–protein complex is not broken down to a form which can be absorbed. This hypothesis was tested clinically by treating a group of patients with pernicious anaemia using a dialysable vitamin B_{12} peptide complex of relatively low molecular weight, isolated from a fermentation of streptomyces; this complex was shown to be the most effective oral preparation yet available.

The introduction of hinconstarch (XIV) and related compounds by Barry and his co-workers[45–50] suggests that complex formation of a drug might be used to overcome the acquired resistance of an organism to a particular drug. Hinconstarch is a mixed polymer obtained by condensing periodate-oxidized starch through its

potential aldehyde group with an equimolar mixture of isoniazid and *p*-aminobenzylthiosemicarbazone.[46, 49] It is active in human pulmonary tuberculosis[51] and effective in experimental tuberculosis of mice and guinea-pigs whether the infecting bacilli are susceptible or resistant to isoniazid.[45, 52] Barry and Edward[53, 54] isolated urinary metabolites of hinconstarch, namely *iso*nicotinic acid, *iso*nicotinylglycine, monoacetyl-isoniazid (similar metabolites are obtained from uncombined isoniazid[55]) and 5-*p*-acetamidophenyl-2-amino-1,3,4-thiadiazole (XVI), the latter derived from the thiosemicarbazone (XV) by oxidative cyclization and acetylation. These findings appeared to provide direct chemical



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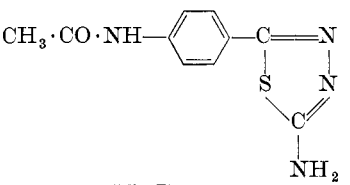


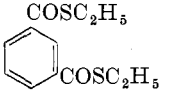
(XVI)

evidence that both active principles of the drug are absorbed.[53] Barry and Edward[53] suggest, however, that the processes of digestion do not simply liberate isoniazid and ABT from the hinconstarch. This conclusion was based on the following observations: (a) combined thiosemicarbazone is less toxic than uncombined thiosemicarbazone; (b) thiostarch, the polymer obtained when oxystarch is condensed with thiocarbazide, exerts a suppressive effect on experimental tuberculosis, whereas thiosemicarbazide itself has no such effect; (c) polymers derived from oxystarch break down under mild conditions to yield derivatives of glyoxal and erythrose, suggesting that linkages formed during condensation are not most readily cleaved. The activity of the complex against isoniazid-resistant organisms may be due to different metabolic pathways being involved for the combined and uncombined agent.

While discussing the problems of drug transport, reference must be made to a striking method of directing the attack of antibiotics to the lymphatic system. Málek *et al.*[56] have shown that antibiotic distribution could be markedly altered by merely preparing a macromolecular salt. Based on the fact that macromolecules and colloidal particles have a particular affinity for the lymphatic system, Málek *et al.*[56] combined streptomycin, neomycin, viomycin and streptothrycin with high molecular weight compounds such as polyacrylic acids, sulphonic or phosphorylated polysaccharides and polyuronic derivatives. The parenteral administration of these unique compounds produced low blood levels of the antibiotic which were maintained for a much longer period of time, while lymph levels were high. Streptomycin sulphate, for example, gave low lymph levels and high blood levels while the reverse was the case with streptomycin polyacrylate. Such an alteration in distribution caused the streptomycin to be more sustained in its sojourn in the body, the lymphatic circulation being much slower. These macromolecular compounds had the additional advantage of being less toxic. Although the classical chemist would consider the dissociation of salts to be rapid, and as a consequence of little value in modifying drug potency and distribution, the work of Málek *et al.* indicates that factors other than simple salt dissociation may be involved.

Drug Latentiation as an Adjunct to Pharmaceutical Formulation

Medicinal chemists are already contributing to work with the object of overcoming or minimizing certain objectionable pharmaceutical properties encountered during the development of drugs.



(XVII)

A classical example is the development by I.C.I. of Etisul (diethyl dithiol*iso*phthalate) (XVII), an anti-tubercular and anti-leprosy compound[57–63] which illustrates most of the objects of drug latentiation. In 1951, while investigating a claim that a mixture of sodium 2-mercaptobenzthiazole-5-sulphonate and

sodium *S*-ethyl thiosulphate prevented the development of tuberculosis in infected guinea-pigs, sodium *S*-ethyl thiosulphate was found to be active. This could be decomposed *in vitro* and presumably *in vivo* to give ethyl mercaptan, and the latter when tested was found to be active in mice. Ethyl mercaptan is an evil smelling, low boiling, inflammable liquid and the initial problem was to prepare an odourless, non-toxic derivative which *in vivo* would release ethyl mercaptan. A large number of thioesters were prepared and of these diethyl dithiol*iso*phthalate (XVII), a bland almost odourless oil, which in experimental tuberculosis is more effective parenterally than orally, was selected. Subcutaneous administration is particularly effective as there is depot formation. After absorption the drug is hydrolysed, presumably by esterases, to give ethyl mercaptan which is considered to be the active agent.

An observation that the drug was quickly absorbed from the skin and after a short while the smell of the ethyl mercaptan could be detected in the breath led to the formulation of Etisul as a cream, a novel method of systemic treatment with an anti-bacterial substance. The drug was tested in leprosy since it was considered that tuberculosis in the mouse was representative of all diseases caused by mycobacteria: tuberculosis, leprosy and Johne's disease of cattle. Recent clinical trials[62] have shown that, as an anti-leprosy drug, Etisul is very effective and rapid in its action.

Drugs having an obnoxious taste which cannot be masked by blending with flavouring agents often present the pharmacist with a problem during the formulation of oral preparations. Chloramphenicol (Chloromycetin) has a bitter taste and many of its tasteless derivatives show far less anti-bacterial activity. Glazko *et al.*[64, 65] prepared a number of esters and of these selected the palmitate, a tasteless compound with no appreciable anti-microbial activity, which, however, was readily hydrolysed by esterases of the small intestine, to give active chloramphenicol. The intact esters are poorly absorbed and indirect evidence suggests that the rate of absorption of the chloramphenicol depends upon the rate of enzymatic attack. The rate of hydrolysis of the ester appeared to depend upon its state of physical subdivision, the chemical nature of the ester grouping and the wett-

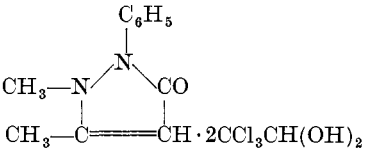
ability of the compound. Pauletta[66] found significant rate differences in the enzymic hydrolysis of such closely related esters as the stearate, palmitate and oleate. Increased water solubility of the esters might be expected to increase the rate of hydrolysis but Glazko *et al.*[65] found that some of the more water-soluble esters are hydrolysed more slowly than water-insoluble esters such as the palmitate, provided the latter had a sufficiently large surface area exposed to enzymic attack.

The formulation of a satisfactory parenteral preparation of chloramphenicol is made difficult by its limited solubility in water.[67] The intramuscular administration of microcrystalline suspensions of this antibiotic, although clinically successful in some cases,[68] results in slow absorption which is unsatisfactory when high initial blood levels are required immediately after dosage. Concentrated solutions of chloramphenicol in various organic solvents,[69] although yielding high blood levels after intramuscular injection, can give rise to some irritation at the site of injection. In an attempt to prepare a water-soluble compound which could be given intramuscularly with a minimum of side-reactions and which would be absorbed rapidly to produce bacteriostatic blood levels of chloramphenicol, Glazko *et al.*[67] investigated the mono- and disuccinic esters in the form of their sodium salts. The pattern of absorption, metabolism and excretion of the succinic acid esters appears to be similar in rat, dog and man, the disuccinate being hydrolysed to the monosuccinate which in turn is hydrolysed to chloramphenicol. The monoester appears to be the drug of choice[70, 71] since only one hydrolytic step is necessary and in man produces high blood levels whether given orally or parenterally.

Chloral hydrate has long been recognized as one of the best and safest hypnotics. Its unpleasant odour and objectionable taste and the gastric and intestinal irritation which it sometimes causes are disadvantageous. These disadvantages have been overcome by complexing two molecules of chloral hydrate with one molecule of phenazone to give dichloralphenazone (Welldorm) (XVIII).[72,74] Dichloralphenazone possesses all the hypnotic and sedative properties of chloral hydrate (clinical trials have shown that the order of effectiveness in producing rapid onset of sleep was: sodium amylobarbitone > dichloralphenazone > chloral

hydrate[72]), and is less toxic and free from the unpleasant odour and taste of chloral.[74]

Rice and McColl[74] found that the hypnotic dose of (XVIII) was slightly larger than that expected on the basis of its molecular content of chloral hydrate. It was suggested that this may be



(XVIII)

due to the fact that the phenazone component of dichloralphenazone is released before the conversion of chloral hydrate to its metabolite, trichlorethanol.

Some Basic Concepts of Drug Latentiation

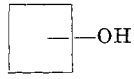
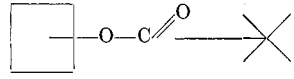
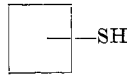
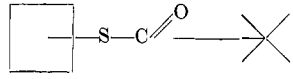
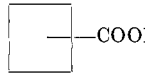
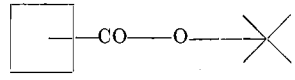
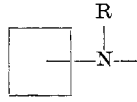
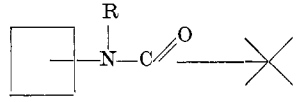
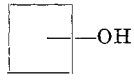
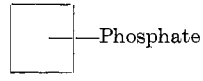
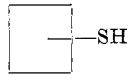
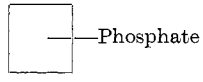
The above quoted examples illustrate successful applications of drug latentiation. Despite the lack of information about the comparative biochemistry of cells and tissues, it is important to consider some of the basic principles. Reduced to its simplest terms, drug latentiation involves the conversion of an active drug into an inactive transport form which upon enzymic attack releases the active drug. A consideration, therefore, of those factors influencing the hydrolytic enzymic attack on the transport form of the drug is important. Obviously many of these factors also apply to the design of a drug itself, but in the present context we are primarily interested in the attachment of a carrier group to the active drug to alter its physicochemical properties and then the subsequent enzymic attack to liberate the active drug moiety.

In Table I a number of examples of hypothetical active drugs and possible transport forms of these are diagrammatically represented (X represents the main portion of the carrier molecule). The nature of the carrier group X will obviously influence not only the absorption and distribution of the inactive transport form but also the rate of release of the active drug moiety by enzymic attack.

Rate of Release of the Active Drug

The rate of enzymic release of the active drug from the transport form will depend on the electronic, the steric and the configurational characteristics of the carrier group X. That such factors do play an important role in *in vitro* and possibly in *in vivo*

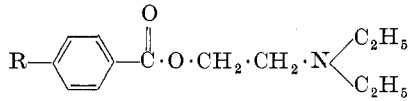
Table I. Diagrammatic representation of some hypothetical active drugs and some possible transport forms.

	Active drug	Inactive transport form
(1)		
(2)		
(3)		
(4)		
(5)		
(6)		

enzymic reactions is established by the investigations of Levine and Clark,[75] Glick [76-78] and Fu and his co-workers.[79]

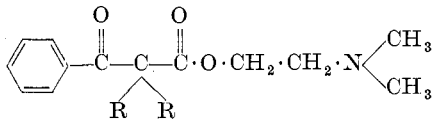
In case (1) (Table I), if we suppose that the active drug is an alcohol and is formulated as an ester, the rate of release of the active alcohol will depend upon the nature of X. That a change in the alectronic character of a group can influence enzymic hydrolysis is clearly shown by the investigations of Levine and

Clark[75] who found that (XIX) is hydrolysed *in vitro* by human serum much more rapidly than (XX). The introduction of a steric factor at a point near the location of attachment of the carrier group X would be expected to influence the rate of hydrolysis, the rate decreasing with increasing steric hindrance. Levine and Clark[70] demonstrated the operation of such a steric factor by



(XIX) R=F 3,000*
(XX) R=NH_2 500

substituting in the α carbon of the acid moiety of an ester, the rate of hydrolysis to yield the amino alcohol decreasing rapidly as the steric hindrance was increased (*cf.* XXI–XXIV). Similar considerations to the above might reasonably be expected to apply to



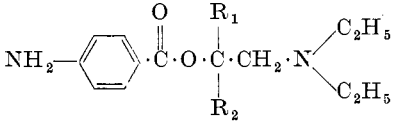
	R	R	
(XXI)	H	H	500
(XXII)	CH_3	CH_3	35
(XXIII)	CH_2	CH_2	25
(XXIV)	CH_2	CH_2	0

the rate of release of the mercaptan from its transport form (Table 1, case (2)).

Case (3) represents a biologically active acid formulated as an ester. The rate of release of the active acid can be controlled in much the same way as the active alcohol in case (1). The introduction of steric hindrance by substituting on the α carbon of the alcohol would be expected to decrease the rate of enzymic attack and the release of the acid fraction. The *in vivo* hydrolysis by

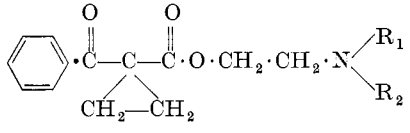
* Figures associated with formulae (XIX)–(XXIX) are those of Levine *et al.*[70] and represent the rate of hydrolysis in human serum—μg. split/ml. serum/h.

human serum of compound (XXV) was much more rapid than compounds (XXVI and XXVII).[70] Similar results were obtained from the hydrolysis of choline derivatives. If the carrier group X were an amino alcohol, the rate of release of the active acid could be controlled by variation of the nature of the basic group, e.g. in compounds of the type (XXVII) the rate of *in vitro* hydrolysis decreased as the size of the basic group increased[75] (*cf.*



	R_1	R_2	
(XXV)	H	H	500
(XXVI)	H	CH_3	15
(XXVII)	CH_3	CH_3	0

XXVIII–XXX). On the other hand, Glick[80] found that whereas dibutylethanolamine benzoate was not hydrolysed by horse serum, the diethyl derivative was hydrolysed, the rate of hydrolysis of the latter, however, being greater than that of the dimethyl derivative. Where the carrier group X is an amino alcohol the rate of hydrolysis will probably depend on the length of the



		R_1	R_2	
(XXVIII)		CH_3	CH_3	59
(XIX)		C_2H_5	C_2H_5	32
(XXX)	$CH(CH_3)_2$	$CH(CH_3)_2$		29

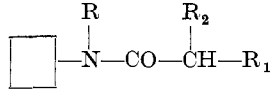
alcohol chain. In general, esters of two carbon chain alcohols are more rapidly hydrolysed.

The stereochemical specificity of many enzymes is well known (for examples see Beckett[81]) and, therefore, the configuration of the carrier group X may well have a profound effect on the rate of the hydrolytic enzymic attack. The introduction of asymmetry in X (Fig. 3) will give rise to two enantiomorphs of the transport form of the drug which will probably differ in their rates of hydrolysis. Asymmetry can be obtained by introducing the group Z. If Z is a small, chemically inert grouping such as methyl, profound changes in the physicochemical properties of the drug transport

form may be avoided. The configuration of the carrier group X may, however, in some cases assume an even more important role if at least one of the substituent group (Fig. 3, Y or Z) were such that in the correct orientation an additional point of attachment with the enzyme surface was formed.

Fig. 3

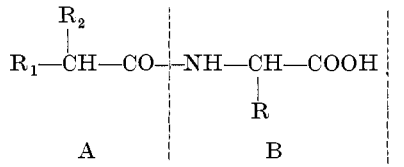
In case (4) (Table I), which illustrates the formulation of a supposedly pharmacologically active amino compound, the rate of release of the active drug moiety will once again depend upon the electronic, the steric and the configurational characteristics of the acyl carrier Group X. Introduction of asymmetry into X (e.g. XXXI) will give rise to two enantiomorphs, the rates of hydrolysis of which might be expected to differ. Changes in the electronic character of the acyl group would also affect the rate of hydrolysis, as would the introduction of steric factors, particularly near the point of attachment of the carrier group. In a compound such as (XXXI) changes in the nature of the groups R_1 and R_2 would



(XXXI)

affect the rate of hydrolysis, the extent depending upon the balance of electronic and steric factors. If $R_1 = H$ and R_2 was an electronegative group, the rate of hydrolysis would probably increase; however, as the bulk of R_1 increased, the steric factor might become predominant with a resultant decrease in the rate of hydrolysis. Such generalization appears not unreasonable in the light of the investigations of Fu and his co-workers[79] on the hydrolysis by renal acylase I of *N*-acylated-L-amino acids with optically active acyl groups (XXXII). Fu *et al.*[79] found that the susceptibility of an acylated amino acid to enzymic attack was influenced by the configuration of B (XXXII), the nature of the

carbon chain R (XXXII) and the nature of A (XXXII). The influence of A, the acyl group, was expressible in terms of its electronic, steric and configurational characteristics (see Table II).



(XXXII)

L-Acylated amino acids were more susceptible than the corresponding D-acylated acids. In (XXXII) if $R_1 = H$, and R_2 was changed from H to Cl, the rate of hydrolysis increased, but when $R_1 = CH_3$ a similar change in R_2 gave a decreased rate. In the

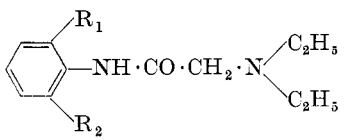
Table II. Hydrolysis of acylated L-amino-acids with optically active acyl groups by renal acylase I.

Terminal amino acid residue	Rate of hydrolysis (micromoles hydrolysed) per h					
	α-chloropropionyl derivative XXXII A, $R_1 = CH_3$, $R_2 = Cl$			Alanyl derivative XXXII A, $R_1 = CH_3$, $R_2 = NH_2$		
	L-form	D-form	L : D	L-form	D-form	L : D
Glycine (XXXII B, R=H)	23	13	1·8	240	0·5	480
L-Alanine (XXXII B, $R = CH_3$)	87	19	4·6	1,200	3	400
L-Butyrine (XXXII B, $R = C_2H_5$)	290	28	10·4	5,650	12	470
L-Norvaline (XXXII B, $R = nC_3H_7$)	1,240	106	11·7	9,500	28	340

first pair, the electronic effect must predominate upon introducing Cl, while in the second pair the steric factors must outweigh the electronic factor upon changing from H to Cl. In L- and D-chloropropionyl alanine ($R = CH_3$, $R_1 = CH_3$, $R_2 = Cl$) the steric and electronic factors are similar and the differences in the rates

of hydrolysis must be due to configuration. When $R_2 = NH_2$, configuration played an increasingly important role, and it was assumed that the NH_2 group in the L-configuration formed an additional point of attachment to the enzyme surface and this results in increased hydrolysis. Incorrectly orientated as in the D-configuration of the acyl group, it did not constitute a point of attachment.

That such an approach to drug design is of more than academic interest is illustrated by the activities of some local anaesthetics. The favourable anaesthetic activity of xylocaine (XXXIII,



(XXXIII) $R_1 = R_2 = CH_3$

(XXXIV) $R_1 = H, R_2 = CH_3$

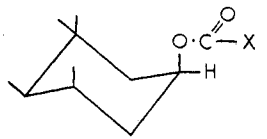
(XXXV) $R_1 = R_2 = H$

$R_1 = R_2 = CH_3$) and especially its prolonged duration of action can be attributed to the presence of two *ortho* methyl groups.[82] This effect has been attributed to an inhibition of resonance between the nitrogen and the aromatic nucleus and to increased lipophilic character,[85] but Sekera *et al.*[82] concluded that increase in activity observed with progressive substitution of the *ortho* positions was associated with increased stability of the amide linkage. They were of the opinion that the xylocaine type compound was most likely to be attacked *in vivo* at the anilide bond by peptidases, a view supported by the investigations of Ther[85] on the xylocaine analogue histacaine, but in direct opposition to McMahon *et al.*,[86] who found no evidence for *in vivo* hydrolysis of the amide linkage in dogs. However, Sekera *et al.*[82] were able to show a distinct parallelism between local anaesthetic activity and resistance to acid hydrolysis with increased *ortho* methyl substitution (compounds XXXIII–XXXV).

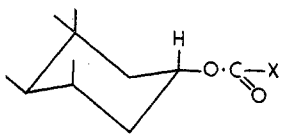
Cases (5) and (6) represent examples in which the active product has been formulated in such a way as to utilize the attack of a specific type of enzyme, e.g. the phosphatase enzyme which may be relatively abundant in the target cell or tissue. Honovan (stilboestrol diphosphate) is an example of this particular type of drug latentiation (see above).

In certain cases, for example a pharmacologically active cyclic

alcohol formulated as an ester, the enzymic release of the active alcohol might well be affected by the conformation of the ester carrier group. The ester grouping could exist in an axial (XXXVI) or equatorial conformation (XXXVII). In a compound in which the ester grouping was held in the axial conformation, e.g. by a large group attached to the ring, it should be more subject to steric hindrance by the erect hydrogens or substituents on the

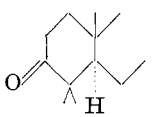


(XXXVI)



(XXXVII)

β carbon atoms than would be the case with an equatorial conformation. Consequently, by analogy with chemical hydrolysis, one would expect the equatorial acyloxy grouping to be hydrolysed more readily than the axial acyloxy grouping. The conformational effects possibly influencing the reaction between the transport form of the drug and an enzyme need not necessarily be associated with that portion of the drug molecule complementary to the enzyme. It is known that, in semi-rigid ring systems,



(XXXVIII)

long-range conformational effects may be relayed through the system, e.g. variations in the rate of reactions of some triterpenoid-3-ketones with a common group (XXXVIII) were shown to be dependent upon group arrangement in more distant parts of the molecule.[87] Such effects may also operate in the enzymic hydrolysis of the transport form of a drug.

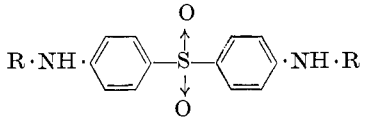
Influence of the Carrier Group on Absorption and Penetration

While it is not the intention to consider in detail the formidable problem of the absorption and penetration of drugs in the animal

body, an excellent account of which has been given by Brodie *et al.*,[1] it is perhaps pertinent in the present context to consider how these factors will be influenced by the nature of the carrier moiety X in the transport form of the drug. In general, the absorption and penetration of a drug will depend upon its lipoid solubility and also, in the case of electrolytes, on the pK_a values, unless enzyme transport is involved, e.g. permeases. With a systemically active drug, oral administration involves absorption from the gastro-intestinal tract, transport by the blood stream, and passage across various barriers to the locus of action. While such barriers must deal with lipoid soluble compounds, lipoid insoluble compounds and inorganic ions, the general lipoid character of the membranes facilitates the transport of lipoid soluble materials, e.g. succinyl-sulphathiazole is poorly absorbed from the gut because even its uncharged form is too lipoid insoluble for rapid absorption.[1] The ease with which a substance penetrates the blood–brain barrier will obviously effect its penetration into the central nervous system. Although the mechanism of penetration of this barrier is not fully understood, it is known that water-soluble substances like sucrose penetrate the central nervous system very slowly, while lipoid-soluble substances penetrate rapidly. In this context, the anaesthetic Viadril[88,90] (21-hydroxy-pregnane-3,20-dione sodium succinate), a water-soluble ester is of interest. This would be unlikely to cross the blood–brain barrier but rapid hydrolysis by the non-specific esterase of serum allows a concentration of the drug to be quickly achieved.[89]

In designing a drug with central nervous system activity it might, therefore, be advisable to try to enhance its penetration by increasing the lipoid character of the carrier group. Such an approach might perhaps be used in the case of central nervous system acting drugs which had unwanted side-effects due perhaps to their partial water-solubility characteristics. In some cases the insolubility in water of a drug may present a problem in formulating an easily administrable form. This might perhaps be overcome by including a basic grouping in the carrier portion of the transport form, thus allowing the formation of a more water soluble salt. Alternatively, increased water solubility may be achieved by using a carbohydrate carrier grouping. In this connection, the investigation of Foye *et al.*,[91] who compared the acute toxicities

and anti-bacterial activities of various sugar derivatives of *bis*-(4-aminophenyl)-sulphones (XXXIX) with those of the α amino acid derivatives, is of interest. It was shown that the α amino acid



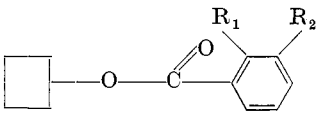
(XXXIX)

substituents were less effective in reducing the toxicity of the sulphone in mice than the sugar derivatives, but that there was a comparable retention of anti-tubercular activity with both types of compounds.

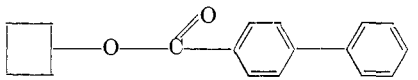
The Planarity of Drug Transport Molecules

In a complex biological system, it is probable that few molecules of the active compound will reach the site of action at which the biological response is produced. Some may interact at sites of secondary importance, while others may be indiscriminately adsorbed in a harmless way or may cause toxic effects in the system. These sites at which loss of drug occurs with respect to its primary action have been termed by Veldstra,[92] 'sites of loss', and it has been shown that such losses may be stereoselective. The loss of drug due to general indiscriminate adsorption may be influenced by the planarity of the transport drug molecule. In general, it seems reasonable to suppose that the more planar the molecule the more readily would it be adsorbed. There is no lack of evidence to show that differences in planarity can affect the adsorption of compounds on non-biological surfaces. Co-planarity factors play an important part in the chromatographic adsorbabilities of conjugated isomeric biaryls and arylalkenes on alumina;[93] the *trans* isomers (co-planar) of stilbene and 4,4-dimethoxystilbene are more strongly adsorbed than the corresponding *cis* isomers (non-planar),[94] in 1,4-diphenylbutadienes, the order of adsorbability is *trans-trans*(co-planar) > *trans-cis* > *cis-cis* (non-planar).[95]

It is, therefore, of interest to speculate on the possibility of altering the planarity or non-planarity of a drug transport molecule. In a compound such as (XL), where $R_1 = R_2 = H$, substitution of R_1 by a relatively large group may perhaps influence the



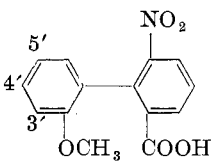
(XL)



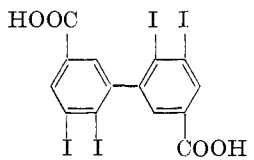
(XLI)

rotation about the carbon carbonyl bond. Such an effect might be enhanced by a 'buttressing effect' due to substitution in the R_2 position.

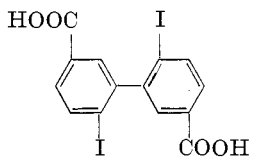
The introduction of asymmetry due to restricted rotation in



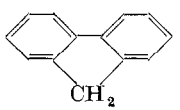
(XLII)



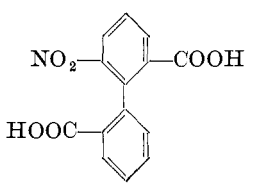
(XLIII)



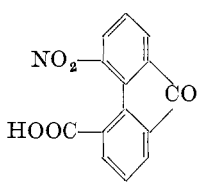
(XLIV)



(XLV)



(XLVI)
Non-planar

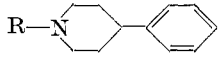


(XLVII)
Co-planar

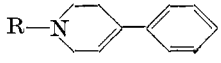
diphenyl type compounds (XLI) by substituting with groups of suitable size in the sterically sensitive *ortho* positions is well known.

Groups other than those in the *ortho* positions can have an effect on the planarity of the molecule. Adams *et al.*[96] compared the optical stability of a series of acids of the type (XLII) and found that substitution in the 3′ position had a profound effect on

the optical stability. A striking example of this 'buttressing effect' was recorded by Reiger and Westheimer[97] who found that (XLIII) was about 30,000 times more optically stable than (XLIV). Co-planarity can be obtained in diphenyl compounds by joining the two phenyl rings through the *ortho* positions (*cf.* XLV and XLVII). Planarity could be introduced into a molecule of the type (XLVIII) by creating an unsaturated linkage in the heterocyclic ring. In (XLVIII) there will be free rotation about the two rings, but in (XLIX) the introduction of unsaturation will give a planar compound to allow conjugation.

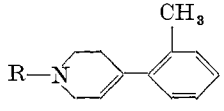


Non-planar
(XLVIII)



Planar
(XLIX)

The introduction of a CH_3 substituent in the *ortho* position of the phenyl ring (L) might at first sight appear a relatively minor change without a profound effect on the planarity of the molecule. The ultraviolet absorption, however, shows clearly that such a substituent does in fact completely alter the planarity of the molecule, e.g. compound (XLIX) shows λ at 243 (typical styrene-type absorption) but compound (L) shows reduced absorption and

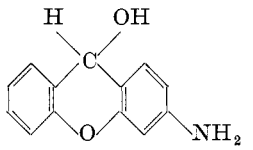


(L)

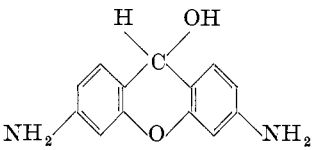
no such peak.[98] The appreciation of such a change is important when one is considering the fit of a drug at an enzyme surface, particularly in the case of a three-dimensional attachment, where the flatness of the phenyl ring in relation to other points of attachment may be an important feature (*cf.* analgesic receptor site proposed by Beckett and his co-workers[99, 100]). Such a minor substituent has the effect of converting a predominantly planar basic molecule into a non-planar one, a change which may profoundly affect its pharmacological activity.

Planarity in a compound may be due to resonance interactions,

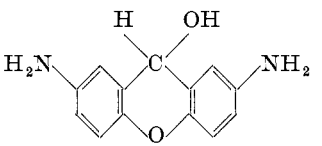
and Hunter[101] has suggested that it is tempting to consider the anti-tubercular activity of some xanthydrols (LI–LIV) in terms



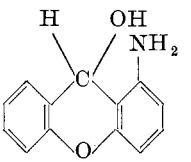
(LI)



(LII)

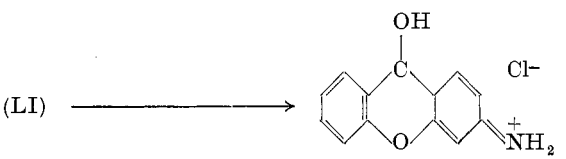


(LIII)



(LIV)

of such planarity. Compounds (L) and (LI), the xanthylium salts of which should be planar, e.g.



were found to have anti-tubercular activity. Compound (LIII) which was inactive would not necessarily give a planar salt except insofar as an oxonium salt may be formed. The inactive compound (LIV), although coloured, and possibly planar, does not appear to offer as much possibility of resonance interaction as compound (LI). It should be remembered that not only is the planarity affected by resonance interactions of this type, but also the polarity of the compound.

It must be emphasized that some of the methods of altering the planarity or non-planarity as a facet of drug design cannot be considered in isolation, since the modification introduced into the molecule will, of necessity, alter the physicochemical properties and thus effect perhaps the absorption of the compound, or in some cases the rate of hydrolysis.

Our knowledge of the detailed action of drugs is meagre and any approach to a concept of drug design must of necessity be speculative. Speculation is, however, the stimulus to directed experimentation and resultant increased understanding, which in time leads to more rational hypotheses. If this article helps to guide interest into profitable speculative channels, it has served its purpose.

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