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Review Article

Aspects of drug action: a comparison with intramolecular processes occurring in pharmaceutical and biochemical systems*

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THIS article discusses intramolecular processes involving nucleophilic reaction, nucleophilic catalysis, general acid catalysis and general base catalysis. These intramolecular processes either alone or in certain combinations can account for the instability of pharmaceuticals such as aspirin, atropine and acetylcholine in aqueous solution, the increase in glycogenolysis effected by adrenaline and the mechanism of action of enzymes (e.g. esterases) and hormones (e.g. oestradiol, vasopressin). As a natural extension of these concepts, the current drug-receptor site theory has been re-appraised and the view advanced that the activity of certain classes of drugs may be attributed to the *active* participation of a functional group at a biological surface.

It is pertinent to differentiate between two types of processes in which reactions occur either *with* or *without* catalysis.

Chemical reaction

Chemical reactions entail the formation of new bonds and the scission of old ones and proceed by substitution (eqn *1*, page 530), addition (eqn *2*), or elimination (eqn *3*).

A nucleophilic reaction constitutes attack by a nucleophile (e.g. OH^-, CO_2^-, $\equiv\ddot{N}$, $-\ddot{O}-$) at an electron deficient centre (eqn *1*) whilst an electrophilic reaction describes the attack of an electrophile (e.g. NO_2^+) at an electron rich centre (eqn *4*, page 530).

Chemical reactions between molecules and ions are termed intermolecular (eqn *1–3*) whereas intramolecular reactions occur between adjacent groups within the same molecule (eqn *5*).

NUCLEOPHILIC REACTION

The tendency of a nucleophile to form a bond with an electrophilic centre, as determined either by examination of the products of a reaction or by kinetic measurements, is known as the nucleophilic activity of that species. The nucleophilic activity of a species is dependent on a number of factors which include: (i) solvation (Miller & Parker, 1961; Parker,

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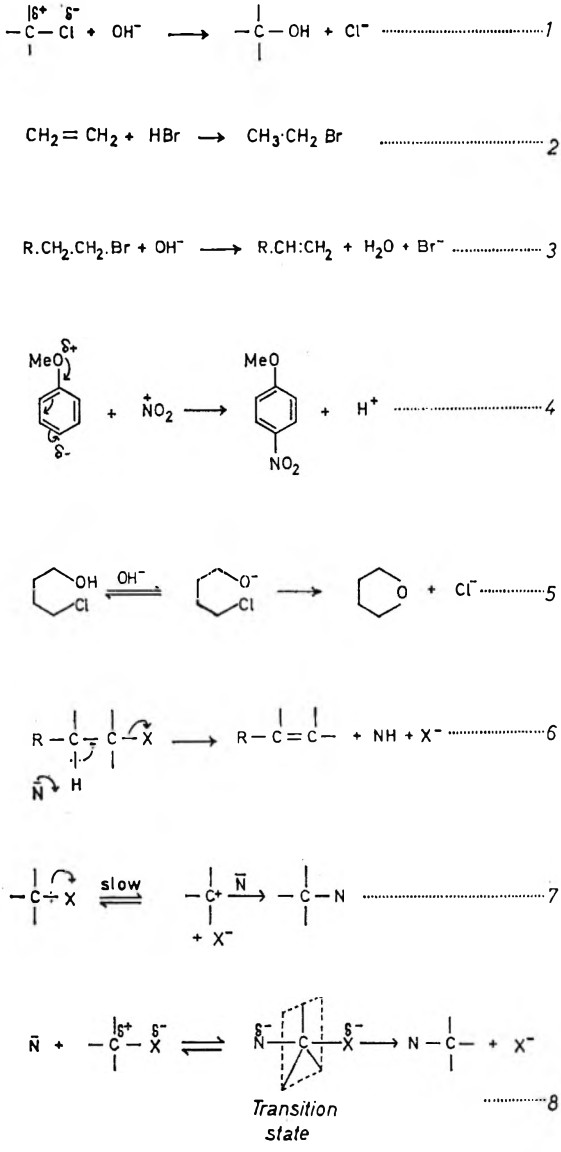
* The first part of a review on this topic. The second part including the references will be published in the next issue of this Journal.

1962; Miller, 1962); (ii) polarisability (Jencks & Carriuolo, 1960; Parker 1961a); (iii) carbon-basicity (Bunnett, Hauser & Nahabedian, 1961; Parker, 1961b); (iv) nature of the electrophilic centre (Hudson & Green, 1962); (v) partitioning of the intermediate in attack at unsaturated carbon



(Bruice, Bruno & Chou, 1963). The relative contribution of each of these factors is dependent on both the nucleophile and the electrophilic centre concerned and only recently has a limited attempt been made to relate

the observed nucleophilic activity of a species with the theoretically calculated nucleophilic activity (Miller, 1963).

Reaction by a nucleophile at a saturated *carbon* centre leading to substitution or replacement (eqn *1*) competes with an elimination reaction which is characterised by attack on *hydrogen* (eqn *6*, page 530). The actual course taken by the reaction depends upon structural features in the reactant molecule as well as environmental factors. Substitution reactions may be classified on mechanistic grounds into two main categories, S_N1 and S_N2, although other minor categories are known (S_Ni, S_N2').

Reaction by an S_N1 (substitution, nucleophilic, first order) mechanism involves preliminary ionisation of the electrophilic reactant in a slow kinetic step which is followed by rapid combination of the carbonium ion formed with the nucleophile (eqn *7*, page 530). The rate of formation of the products of the reaction is dependent on the rate of ionisation of the electrophilic reactant since this is the slowest step in the reaction sequence. The reaction is said to follow first-order kinetics since the rate at any time is dependent only on the concentration of the electrophile and is independent of the concentration of nucleophile (see page 534). Displacement reactions occurring at an optically active centre by an S_N1 mechanism are characterised by extensive racemisation of the products formed. The almost planar carbonium ion formed is attacked by the nucleophile from either side, but due to partial shielding of one face by the leaving group, X^-, rearside attack by the nucleophile is favoured, leading to some retention of optical activity.

S_N2 (substitution, nucleophilic, second-order) reactions involve simultaneous bond making and breaking without a preliminary ionisation step and are considered to proceed through the transition state shown (eqn *8*, page 530). The rate of the reaction is dependent upon the concentration of both reactants and the kinetics are described as second order (see page 534). Substitution reactions occurring at an optically active centre by an S_N2 mechanism are accompanied by an inversion of configuration in the products of the reaction. This behaviour constitutes the well-known Walden Inversion and is due to attack by the nucleophile on the rear-side of the molecule away from the leaving group.

Intramolecular reactions of the S_N2 type usually occur much more rapidly than their analogous intermolecular reactions. A nucleophile located in the same molecule in close proximity to the carbon centre at which substitution occurs will spend more of its time in a position favourable for attack than the nucleophile in a corresponding intermolecular reaction. The influence of one group on a reaction occurring between an external reagent and another site in the same molecule, is described by the general term, "neighbouring group participation" (see Capon, 1964 for review). Neighbouring group participation may lead to either stable cyclic products as a result of intramolecular reaction, (eqn *5*, page 530), or unstable products resulting from bonding to the reaction centre. In the latter instance, the steric course of the overall intermolecular reaction (eqn *9*, page 533) can change.

Catalysis

The rate of a reaction may sometimes be increased by a process of catalysis where the catalytic species, although participating in the reaction, does not appear in the products or become modified chemically by the reaction.

Catalysis of an intermolecular reaction, when effected by a catalyst which is a separate entity from either of the reactants is known as intermolecular catalysis (eqn *10*). A catalytic function which constitutes part of one of the reactants will bring about intramolecular catalysis (eqn *11*).

$$A + B + \text{catalyst} \rightarrow AB + \text{catalyst} \quad .. \quad .. \quad .. \quad (10)$$

$$\begin{array}{ccc} C & & C \\ | & & | \\ A + B & \rightarrow & A{-}B \end{array} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

C=catalytic function

INTRAMOLECULAR CATALYSIS

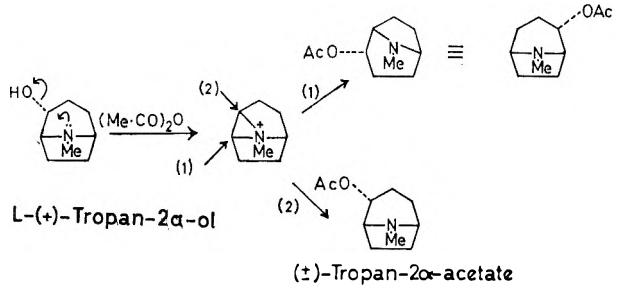
When neighbouring group participation stabilises a transition state or produces a reactive intermediate (eqn *9*) it increases the rate of an intermolecular reaction. This constitues intramolecular catalysis in which the neighbouring group is said to provide anchimeric assistance to the reaction.

On mechanistic grounds catalytic processes whether inter- or intramolecular can be divided into three types: specific and general acid, specific and general base and nucleophilic catalysis.

Specific acid—general acid catalysis. Catalysis of a reaction by hydrogen ion is termed specific acid catalysis and a well known example is the acid catalysed hydrolysis of esters. The mechanism of this reaction is shown in equation *12* page 533 (Ingold, 1953). A general acid catalyst is an electrophilic species other than hydrogen ion which can catalyse a reaction in a similar manner to hydrogen ion. Examples of general acid catalysts are $\overset{+}{N}\equiv$ ions and the hydroxyl function ($-\overset{\delta-}{O}-\overset{\delta+}{H}$) of carboxylic acids and alcohols.

The catalytic influence of the hydrogen ion in the hydrolysis of esters is probably due to the alteration in the electron distribution in the protonated form (I, eqn *12*), so that the carbonyl-carbon atom becomes relatively more electrophilic and so more reactive to water. A general acid catalyst could exert its action in a similar manner.

Alternative explanations for the mechanistic function of a general acid catalyst which are equally acceptable are, (i) the change in the electronic distribution within the ester brought about by bonding increases the reactivity of the ester by stabilising the transition state or intermediate formed in the reaction (eqn *13*, page 533), (ii) a negatively charged leaving group has its charge distributed over more atoms and so has a greater tendency to leave the transition state or intermediate to form products in the reaction (eqn *14*, page 533). Equations *13* and *14* depict examples of intramolecular general acid catalysis.



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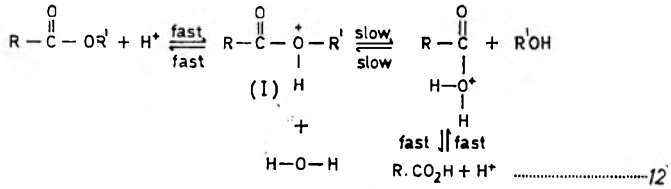

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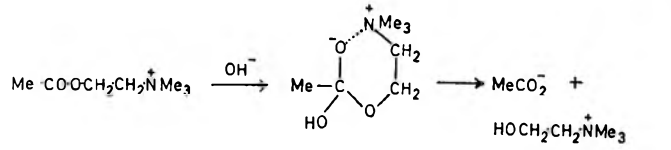

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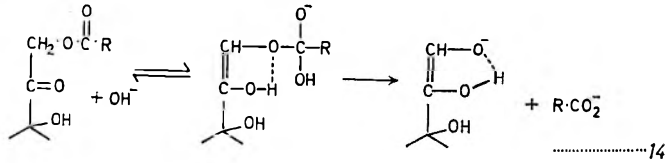

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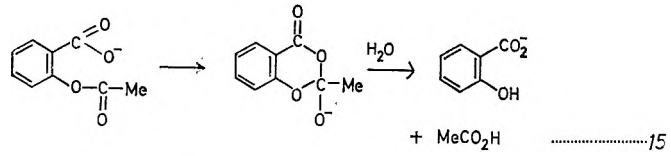

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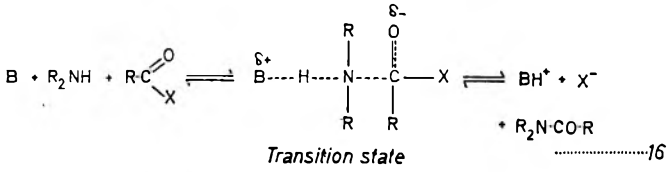

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Nucleophilic catalysis. A nucleophilic catalyst is a nucleophile which enters into either intermolecular or intramolecular reaction with an electrophilic *carbon* centre (cf. base catalysis). The reactive intermediate thus formed then undergoes *intermolecular* reaction with another reactant to give the final products of the reaction and regeneration of the nucleophile as seen in the example of intramolecular catalysis in equation *15* page 533. The nucleophile performs its role as a catalyst by increasing the overall rate of the intermolecular reaction and is not itself consumed in the reaction.

Specific base—general base catalysis. Base catalysis is effected by nucleophiles. The specific base catalyst is the hydroxyl ion whereas general base catalysts include $R.COO^-$, $\equiv\ddot{N}$. Base catalysis of a reaction can be mechanistically distinguished from nucleophilic catalysis in that the catalytic species attacks *hydrogen.* However, a molecule such as imidazole behaves as either a nucleophilic or general base catalyst depending upon the system in which it is exerting its influence.

The exact function of a general base catalyst is not clearly defined on mechanistic grounds (see Bender, 1960, for review) but it will be convenient here to consider that the base functions as a catalyst by removing a proton from the transition state of a reaction which leads to the formation of products in a shorter time (eqn *16*, page 533).

DETECTION AND MEASUREMENT OF CATALYSIS

Catalytic processes may be detected directly or indirectly by a study of the rate of the reaction in which they are participating and it seems relevant to examine some of the basic kinetic concepts of reaction rates before considering examples of intramolecular catalysis occurring in pharmaceutical and biological systems.

The rate of a reaction which obeys first-order kinetics (see page 531) is dependent only on the concentration of one type of molecule (A) and may be expressed as

$$\text{rate } (v) = k[A],$$

where k is the reaction constant for the reaction. In a second-order reaction where two types of molecules (A) and (B) are reacting, the rate is dependent on the concentration of both (A) and (B) and,

$$v = k'[A]\,[B]$$

It follows that if the concentration of either molecule is kept constant throughout the reaction then the rate becomes,

$$v = k''[A]; \qquad v = k'''[B],$$

and this reaction obeys first order kinetics for either (A) or (B). A reaction showing kinetics of this type is known as a pseudo-first order reaction. A well-known example is the specific acid catalysed inversion of sucrose (eqn *17*).

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_{12}O_6 \qquad .. \quad (17)$$

where the hydrogen ion concentration is unaffected and the water concentration is not measurably affected during the reaction. The rate is given by,

$$v = k_{H^+} [H_3O^+] [\text{Sucrose}] = k [\text{Sucrose}]$$

where k_{H^+} is the catalytic constant for hydrogen ion. In a similar manner the specific base catalytic constant for a specific base catalysed reaction can be determined from the equation,

$$v = k_{OH^-} [OH^-] [A]$$

In the general case where the rate of a reaction can be affected by specific acid or base catalysis or alternatively by "spontaneous" reaction with water, then the rate becomes,

$$v = k_0[A] + k_{H^+} [H_3O^+] [A] + k_{OH^-} [OH^-] [A],$$

where k_0 is the rate constant for the uncatalysed reaction. The overall (observed) rate constant, k, can be related to the individual catalytic constants as follows,

$$k = k_0 + k_{H^+} [H_3O^+] + k_{OH^-} [OH^-]$$

The rate-pH profile for a reaction gives considerable information about the type(s) of catalysis occurring in the reaction. The profiles obtained for combinations of acid and base catalysis and "spontaneous" reaction are shown in Fig. 1. Dependence of rate on pH in acid and

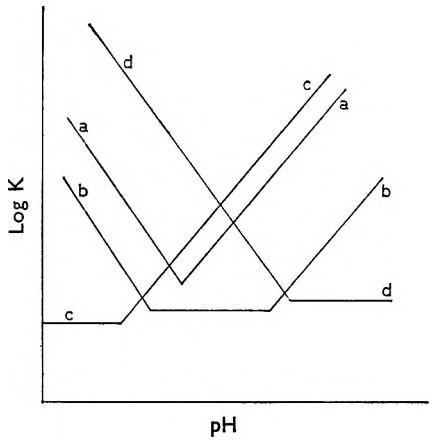


FIG. 1. Rate - pH profiles for combinations of acid and base catalysis and "spontaneous" reaction.

alkaline regions indicates combined acid and base catalysis respectively (curve a) but independence of rate on pH in the intermediate region shows a "spontaneous" (uncatalysed) reaction (curve b). Curves c and d show base and acid catalysis respectively.

The overall rate constant, k, for a reaction may include terms due to general acid and general base catalysis as well as the other terms mentioned and is then expressed by the equation,

$$k = k_0 + k_{H^+} [H_3O^+] + k_{OH^-} [OH^-] + k_{HA} [HA] + k_{A^-} [A^-]$$

where A^- is a general base and HA is a general acid.

Base catalysis is readily determined from a consideration of the rate-pH profile for a reaction as previously described. Intermolecular general base catalysis cannot be separated from specific base catalysis by this method but is readily estimated by measuring the rate of a reaction at a constant pH using different concentrations of the general base at constant ionic strength. Intermolecular general acid catalysis may be differentiated from specific acid catalysis in a similar manner.

Intramolecular catalysis by a catalytic function is more difficult to detect since the concentration of the catalyst is invariable as it is part of one of the reactants in the intermolecular reaction. However, catalysis can be detected provided the catalytic function is ionisable within the normal working pH range and the ionised and unionised forms have widely different catalytic activities. For example, intramolecular nucleophilic catalysis of an intermolecular reaction by a carboxylate anion in the intermediate pH range (over $\pm$ 2 pH units of the pK_a of the corresponding acid) gives an overall rate constant, k, for the reaction, assuming absence of general acid and general base catalysis,

$$k = k_{OH^-}[OH^-] + k_{H^+}[H^+] + k_o[R.COO^-]$$

which expands to,

$$k = k_{OH^-}(k_w/[H^+]) + k_{H^+}[H^+] + k_o/(1 + [H^+])//K_a \quad ..(18)$$

where K_a is the dissociation constant for R·COOH (Garrett, 1962a). Agreement between the values calculated for k_o from equation *18* over the whole intermediate pH range would indicate nucleophilic catalysis by $R{\cdot}COO^-$.

The effect on a reaction of an intramolecular catalytic function which does not ionise over the normal pH range (e.g. a hydroxyl group) cannot be observed directly and its catalytic role can only be recognised by comparison of the catalysed reaction rate with that of a similar reaction in which the catalytic function is absent. Here, the assumption that polar and steric effects on the reaction centre are similar in both systems is only really valid if the catalytic function and the reaction centre are separated by a long carbon chain.

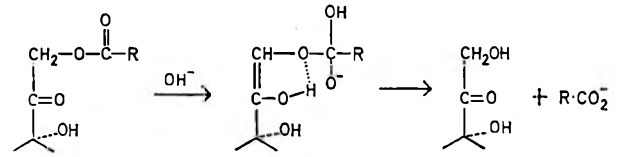
Intramolecular catalysis in pharmaceutical systems

GENERAL ACID CATALYSIS

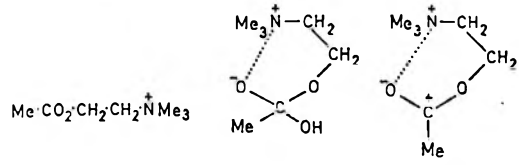
The spontaneous hydrolysis of hydrocortisone hemisuccinate may be attributed either to nucleophilic catalysis (see later) or general acid catalysis by the hydroxyl group of the enol form of the C-20 carbonyl function in the undissociated acid (eqn *19*, page 537) (Garrett, 1962a). The alkaline hydrolysis of hydrocortisone hemisuccinate above pH8 definitely involves general acid catalysis of the ionised form since this is hydrolysed 36 times faster than ethyl hemisuccinate itself (eqn *19*) (Garrett, 1962b).

The esters of alkylated ethanolamines have a faster rate of hydrolysis in neutral or alkaline aqueous solution than would be expected from the

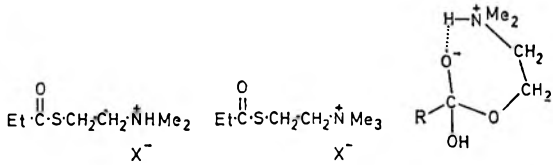
attachment of a quaternary nitrogen atom to a β-carbon atom. Acetylcholine (2), below, for example, is readily hydrolysed in alkaline solution and this is attributed to general acid catalysis by the positively charged nitrogen atom which can either stabilise the intermediate by distributing the charge on the oxygen anion (3) or increase the electrophilic nature of the carbon reaction centre (4) (Davis & Ross, 1950). On the other hand,



Hydrocortisone hemisuccinate ($R=[CH_2]_2\cdot CO_2^-$) Intermediate 19



(2) (3) (4)



(5) (6) (7)

Fellman & Fujita (1963) have attributed the rapid rate of reaction of acetylcholine to the inductive influence of the quaternary nitrogen rather than to the cyclic conformation (4). This followed from a correlation of the infrared stretching frequency of the ester carbonyl group in acetylcholine and a number of its homologues with the rate of reaction at this centre with hydroxylamine. Hansen (1962), has reported that the protonated tertiary amine salt (5) is hydrolysed 240 times faster than the corresponding quaternary ammonium salt, propionylthiocholine (6). In general, the more pronounced effect of a protonated nitrogen atom on hydrolysis compared with a quaternary nitrogen may be attributed to more efficient stabilisation of the transition state by a labile proton, e.g. (7) (Bender, 1960). This effect has also been noted in a study of the relative rates of hydrolysis of acetylcholine, ethyl acetate and diethylaminoethyl acetate hydrochloride in the pH region 5·5 – 8·4 (Zaslowsky & Fisher, 1963), and acetylcholine, ethyl acetate and dimethylaminoethyl acetate in alkaline solution (Davis & Ross, 1950) (see Table 1). The 110-fold difference between the rates of hydrolysis of the two dialkylamino-compounds in alkaline solution and in the pH range 5·8–8·4 can be attributed to general

acid catalysis by a protonated nitrogen at the lower pH and to absence of catalysis by a neutral nitrogen atom at the higher pH value. At the lower pH value, the protonated nitrogen of diethylaminoethyl acetate is twenty-times more effective as a general acid catalyst than the quaternary nitrogen atom of acetylcholine.

TABLE 1. RELATIVE BIMOLECULAR RATE CONSTANTS FOR HYDROLYSIS (1 mol.$^{-1}$sec^{-1})

	pH 5·5–8·4*	Alkaline pH†
Acetylcholine	28·2	141
Diethylaminoethyl acetate HCl	516	—
Dimethylaminoethyl acetate	—	4·4
Ethyl acetate	1·1	1·0

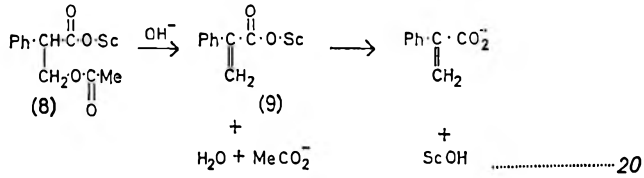
* Zaslowsky & Fisher (1963).
† Davis & Ross (1950).

TABLE 2. BIMOLECULAR RATE CONSTANTS FOR ALKALINE HYDROLYSIS (1 mol.$^{-1}$sec.$^{-1}$) AT 30°

Compound	k_1	k_2	k
Tropine phenylacetate	$1·8 \times 10^{-3}$	0·13	—
Nor-atropine	$8·8 \times 10^{-3}$	0·42	—
Atropine	$9·8 \times 10^{-3}$	0·25	—
Homatropine	$5·2 \times 10^{-2}$	2·05	—
Atropine methyl bromide	—	—	0·54
Homatropine methyl bromide	—	—	2·34

Atropine salts are stable in aqueous solution at low pH, even at autoclave temperatures, whereas at higher pH values hydrolysis occurs to give tropine and tropic acid and the hydrolysis is enhanced by increased hydroxyl ion concentration. Zvirblis, Socholitsky & Kondritzer (1956) have studied the hydrolysis of atropine in alkaline solution and this has been followed by a more comprehensive study by Patel & Lemberger (1958, 1959, 1963) involving atropine, homatropine, nor-atropine and a number of other tropine esters. These workers found that in strongly alkaline solution (pH 12), the bimolecular rate constant (k_1) obtained for the reaction related to hydrolysis of the free base, whereas when the hydrolysis was conducted below pH 8·5 the rate constant (k_2) obtained related to hydrolysis of the protonated form. Patel & Lemberger's results are summarised in Table 2. Examination of the Table shows that k_2 for the protonated forms of atropine and homatropine is about 30-fold greater than k_1 for the free bases. The increased rate constant for hydrolysis due to a charged nitrogen atom has been interpreted (Garrett, 1957b) in terms of a field effect which results in an increased concentration of hydroxyl ion in the neighbourhood of the ester molecule. We prefer to consider that the quaternary nitrogen atom functions as a general acid catalyst and stabilises the transition state in a manner analogous to that described for acetylcholine. The unexpected similarity in rate constants for the protonated tropine esters and their quaternary salts may be attributed to prevention of proton transfer as a consequence of the greater distance between the quaternary nitrogen atom and the reaction centre when compared with previously cited cases.

Garrett (1957b) has studied the alkaline hydrolysis of scopolamine, acetylscopolamine (8) and their corresponding quaternary salts and has shown that acetylscopolamine and its salts undergo a concomitant elimination and hydrolysis reaction to give initially aposcopolamine (9) which is subsequently hydrolysed to the alcohol and dehydrotropic acid



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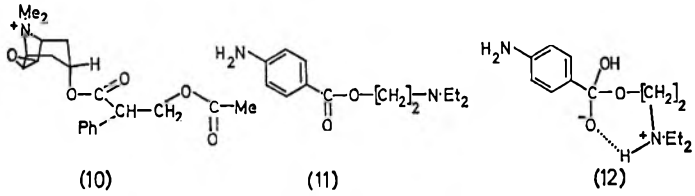

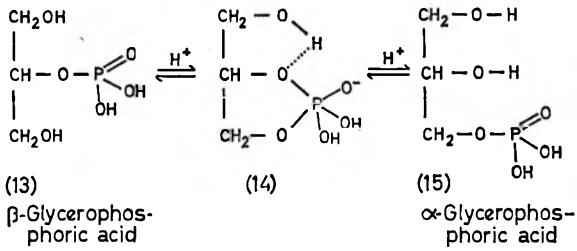

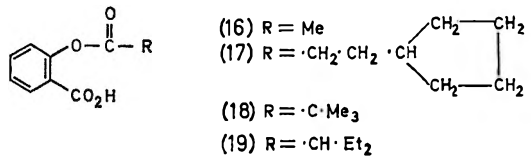

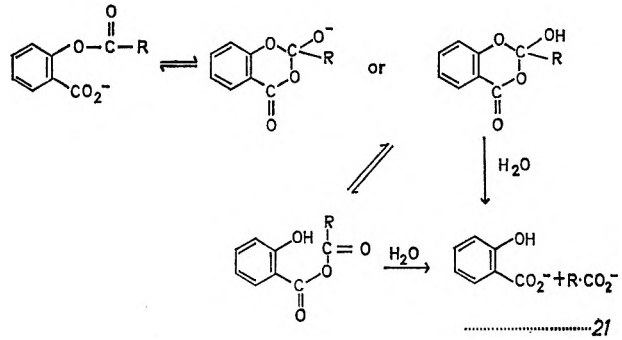

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in accordance with equation *20* (page 539). The rate constant for alkaline elimination of the acetyl linkage in the acetylscopolamine methyl bromide was five times greater than that for the corresponding base but the rate constant for the second ester linkage was 500 times greater for aposcopolamine methyl bromide than for aposcopolamine base. Consideration of a model of acetylscopolamine methyl bromide (10) shows that the differences noted may be accounted for on the basis of the proximity of the quaternary nitrogen atom to the reaction sites concerned (Garrett, 1957b).

Hydrolysis of procaine (11) readily occurs in alkaline solution and Higuchi, Havinga & Busse (1950) have studied the hydrolysis of this compound at above pH 9 where the compound exists mainly as the free base, and at below pH 9 where the monoprotonated form is present. The bimolecular rate constant is 300-fold greater for the protonated form than for the free base. We consider that this result may be cited as another instance of general acid catalysis by a protonated tertiary amine as depicted in (12).

Bailly (1938, 1939) found that β-glycerophosphoric acid (13) is isomerised by acid to α-glycerophosphoric acid (15) without liberation of phosphoric acid and the α-form predominates in the equilibrium mixture obtained. These findings were later confirmed by Verkade, Stoppelenburg & Cohen (1940), and it was shown that this conversion involves an intramolecular rearrangement since the β-acid on treatment with acid in the presence of radioactive sodium phosphate gave the α-form which did not contain radioactive phosphorus (Chargaff, 1942). We consider that these facts may be explained in terms of general acid catalysis by a hydroxyl group in the cyclic *ortho*-ester (14) suggested (cf. Verkade & others, 1940; Baer & Kates, 1948) as an intermediate in this intramolecular reaction. The common intermediate (14) for these interconversions has the secondary hydroxyl oxygen anion stabilised as a leaving group by hydrogen bonding to the primary alcohol group. Consequently, preferential bond fission in the intermediate will occur to give the most stable leaving group and formation of the α-acid will be favoured.

NUCLEOPHILIC CATALYSIS

A well established example of nucleophilic catalysis by a carboxylate anion is the "spontaneous" hydrolysis of acetylsalicylic acid and other esters of salicylic acid in aqueous solution over the pH range 4–8 (Garrett, 1957a) (Fig. 2). The rate of hydrolysis of the esters (16)–(19), is dependent upon the pH of the solution over the pH ranges 1–4, and 8–14, where catalysis by hydrogen and hydroxyl ions respectively occurs. However, in the intermediate pH range (4–8) the rate of hydrolysis is independent of pH and considerably faster than expected by extrapolation of the specific hydrogen and hydroxyl ion curves. This enhanced rate is attributed to nucleophilic catalysis by the carboxylate anion with the formation of a reactive anhydride-type of intermediate which readily decomposes in accord with equation *21* (page 539) to give the products of the reaction directly or indirectly through the anhydride (Chanley, Grindler

& Sabotka, 1952; Garrett, 1957a). Evidence for the existence of the intermediary anhydride comes from two sources. "Aspirin" when hydrolysed in water (i.e. nucleophile) enriched with $H_2{}^{18}O$ at pH6 gives a mixture of salicylic and acetic acids. The salicylic acid produced contains only 6% of the ^{18}O available (Bender, Chloupek & Neveu, 1958) which is in accord with the production of an anhydride intermediate during the hydrolysis, since acetylbenzoyl anhydride is partitioned in an analogous manner in its reaction with the nucleophile hydroxylamine (Wieland & Stimming, 1953). Direct evidence for the existence of an anhydride intermediate during spontaneous hydrolysis of the mono-*p*-methoxyphenyl ester of exo-3,6-endoxo- Δ^4-tetrahydrophthalic acid (20) (page 543) has been provided by Bruice & Pandit (1960). This compound gave *p*-methoxyphenol and an intermediate which presumably was the anhydride (21), since it was hydrolysed at the same rate as the anhydride to give the dicarboxylic acid (22).

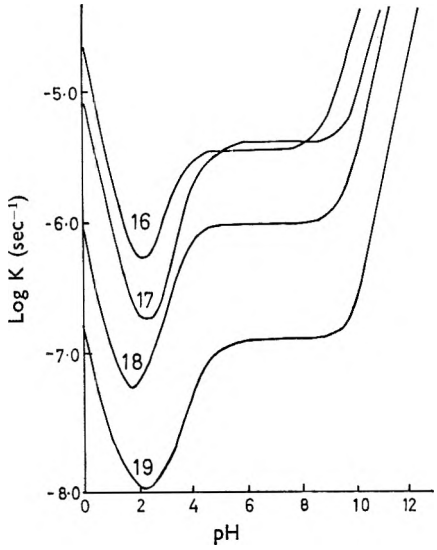


FIG. 2. Apparent first order rate constants for esters of salicylic acid. (16 = acetyl–; 17 = cyclopentylpropionyl–; 18 = trimethylacetyl–; 19 = diethylacetyl). (cf. page 539.)

Phillips (1953) has examined the course of the reaction between succinylcholine (23), a potent neuromuscular blocking agent, and aqueous potassium hydroxide in equimolar proportions, in an attempt to prepare the half-ester (24) for biological evaluation. The products from this reaction were succinic acid (corresponding to 30-35% di-ester) and unchanged di-ester (35-40%) (23); the required half-ester (24) was not isolated. A satisfactory explanation of these results is that the half-ester (24) is hydrolysed as fast as or faster than the di-ester (23). This is contrary to expectation since hydroxyl-ion attack on (24) would be

repulsed by the field of the negatively-charged carboxylate ion (Ringshaw & Smith, 1964). The enhanced rate of hydrolysis of the half-ester was attributed to intramolecular nucleophilic catalysis by the carboxylate ion which presumably proceeds through the anhydride (eqn *22*, page 543).

Hydrocortisone hemisuccinate (25) exhibits spontaneous hydrolysis in aqueous ethanol in the intermediate pH region (Garrett, 1962a) (Fig. 3). The enhanced rate of hydrolysis noted in the intermediate pH region may

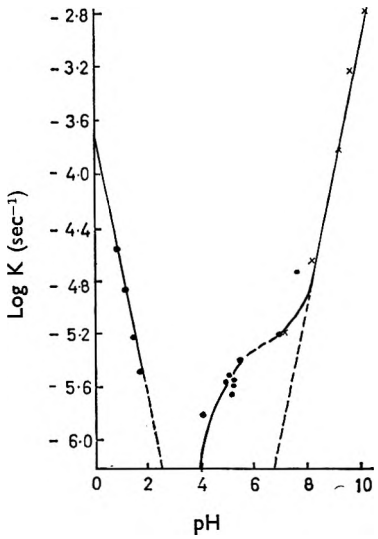


FIG. 3. Rate constants for the hydrolysis of hydrocortisone hemisuccinate at 70°, in 30% ethanol. ((●) = Rate constant as determined by colorimetric assay; (×) = rate constant as determined by constant pH titrations).

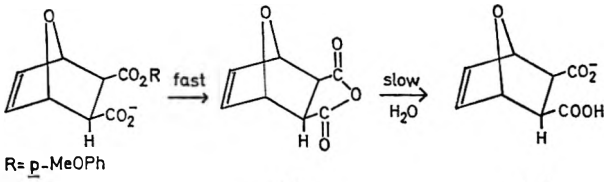
be attributed to either nucleophilic catalysis by carboxylate ion in a manner analogous to that noted previously with aspirin and similar esters (e.g. eqn *23*, page 543) or alternatively to a kinetically indistinguishable process involving general acid catalysis by the enolised C-20 carbonyl group in the unionised ester (see page 536).

Bifunctional catalysis

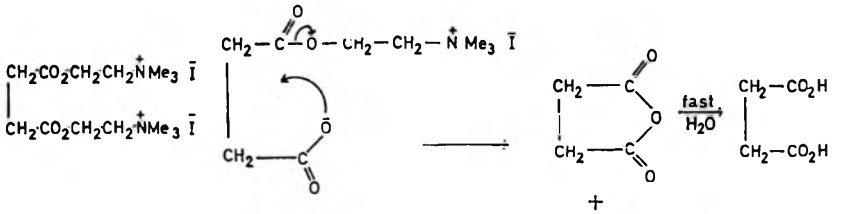
The three catalytic processes so far discussed have been illustrated with examples where only one type of catalysis is involved. This section deals with simple systems where two different but complementary catalytic processes are occurring. The simultaneous occurrence of two such processes each of which is capable of increasing the rate of a reaction leads to a rate of reaction far in excess of expectation.

GENERAL ACID-NUCLEOPHILIC CATALYSIS

The hemisuccinate ester of salicylic acid (26) (page 543) is rapidly hydrolysed in aqueous solution and the rate-pH profile (Fig. 4) exhibits a

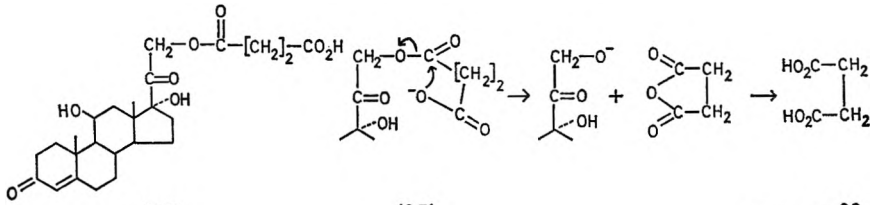


(20) (21) (22)



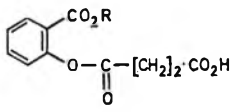
(23) (24)

.....22



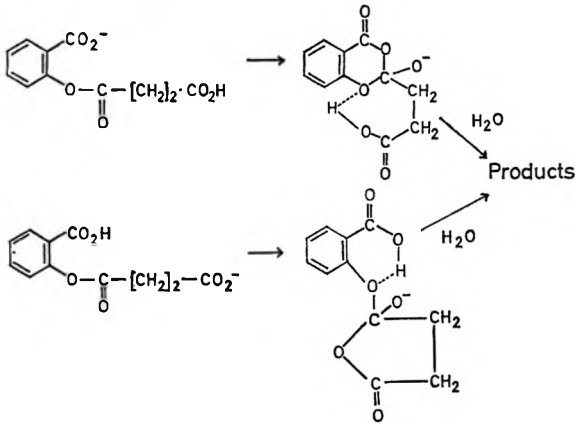
(25) (25)

.....23



(26) R=H

(27) R=Me



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maximum at pH 4 (Morawetz & Oreskes, 1958). The methyl ester of this compound (27) by contrast shows "spontaneous" hydrolysis in the intermediate pH region in a manner similar to that noted for acetylsalicylic acid (16). It is considered that bi-functional intramolecular catalysis occurs during the hydrolysis of (26) at pH values around 4 where only one of the carboxyl groups is ionised. In this region the ionised carboxylate ion acts as a nucleophilic catalyst and the unionised carboxyl group functions as a nucleophilic catalyst and the unionised carboxyl group functions as a general acid catalyst. The course of the reaction is shown in equation *24* (page 543). Nucleophilic catalysis (cf. acetylsalicylic acid) alone occurs in the intermediate pH region where both carboxyl groups are ionised. The bifunctional catalysis occurring in the hemisuccinate of salicylic acid considerably enhances the rate of hydrolysis at pH 4, so that it is hydrolysed 66 times faster than its methyl ester and 24,000 times faster than acetylsalicylic acid at this pH.

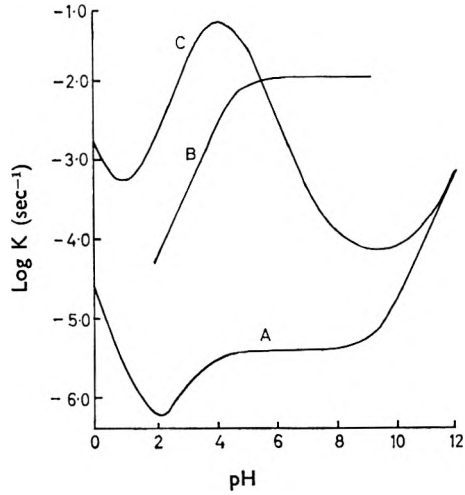


FIG. 4. Rate constants for hydrolysis of esters of salicylic acid, at 25° A = Acetylsalicylic acid; B = methyl succinylsalicylate; C = succinylsalicylic acid.

GENERAL ACID-GENERAL BASE CATALYSIS

A study (Kupchan, Eriksen & Shen, 1963) of the methanolysis of derivatives of the alkaloid cevadine from *Ceveratrum* species has shown that reaction occurs at the C-16 position to give methyl acetate and the C-16 alcohol. This reaction is aided by general acid catalysis by an adjacent hydroxyl, and by general base catalysis by the heterocyclic nitrogen atom in the manner shown in (31) (page 546). General acid catalysis by the adjacent hydroxyl group increases the rate of solvolysis of (30) by 1000-fold over (28) where the hydroxyl group is absent. Similarly, the effect of the general base catalysis is to make the reactivity of (30) 25 times greater than (29) where the basicity of the nitrogen is removed by formylation.

"Intramolecular" reactions in biochemical systems

"INTRAMOLECULAR" REACTION

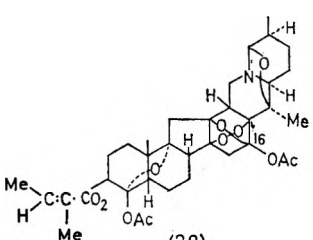
In the reaction between a nucleophilic and electrophilic centre in a biological system, one of the functions constitutes part of an enzyme at whose surface the inhibitor or substrate molecule, bearing the second function, is held by the relevant forces. This enables the second function to become orientated in a definite spatial arrangement favourable for reaction with the first function. Although such reactions are strictly intermolecular when compared with reactions in non-biological systems, we have described them as "intramolecular" throughout this article. We consider that a reaction between functions arranged in the manner described will derive the same benefit from their close proximity as has been noted for intramolecular reactions where they are part of the same molecule.

REACTIVATION OF CHOLINESTERASE INHIBITED WITH ORGANOPHOSPHORUS COMPOUNDS

The enzyme cholinesterase, which is responsible in the body for the hydrolysis of acetylcholine produced during humoral transmissions at the nerve endings, is readily inhibited by organophosphorus compounds such as tetra-ethylpyrophosphate (TEPP) (32) (page 546), di-isopropylphosphonofluoridate (dyflos, DFP) (33) and isopropyl methylphosphonofluoridate (Sarin) (34). Although inhibition of cholinesterase by dyflos has proved useful in the control of myasthenia gravis, exposure to nerve gases such as Sarin may result in death from the accumulation of acetylcholine in the tissues. Many organophosphorus compounds such as TEPP, Parathion and E600, are in constant use as agricultural insecticides and the possibility of poisoning by misadventure is very real (Conley, 1957; Namba & Hiraki, 1958).

The organophosphorus compounds irreversibly inhibit cholinesterase at the active site of the enzyme in accord with equation *25* (page 546) since the phosphorylated enzyme is only slowly hydrolysed to regenerate the enzyme (see Aldridge, 1956, for review). A search for antidotes to organophosphorus poisoning has led to the discovery of a number of compounds (Childs, Davies, Green & Rutland, 1955; Wilson, 1955; Hobbiger, O'Sullivan & Sadler, 1958), which are capable of displacing the phosphorus moiety from the phosphorylated enzyme and afford some protection against organophosphorus poisoning in animal experiments (Hobbiger & others, 1958; Hobbiger & Sadler, 1958). The most effective reagents for reactivation of the inhibited cholinesterase are hydroxamic acids (R·CO·NHOH) (Childs & others, 1955; Wilson, 1955) and oximes (Davies & Green, 1955; Hobbiger & others, 1958; Hobbiger & Sadler, 1958). These compounds presumably react as the anion, $R{\cdot}CO{\cdot}NH{\cdot}O^-$ or $R{\cdot}CH{:}N{\cdot}O^-$, since reaction between these compounds and the inhibitors themselves proceeds in this manner (Hackley, Plapinger, Stolberg & Wagner-Jauregg, 1955; Green & Saville, 1956).

The most potent reactivators complex with the polypeptide chain adjacent to the inhibited active site and then by an "intramolecular" reaction undergo nucleophilic attack on the phosphorus atom. A positively charged reactivator such as pyridine-2-aldoxime methiodide



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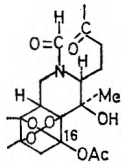

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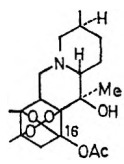

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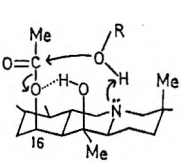
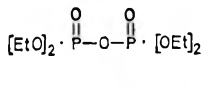


(31)

Relative rates of solvolysis of C_{16}-acetate

(28)	(29)	(30)
1	40	1000



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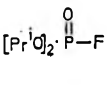

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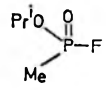

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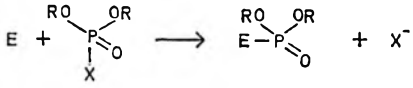
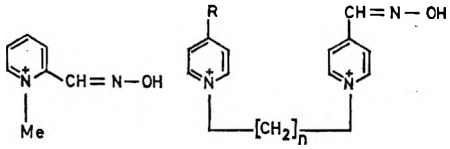


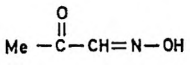
(DFP; X=F, R=Pr^i)

25



(35)

(36) R=CH:N·OH; n=2-5
(37) R=H; n=3-5



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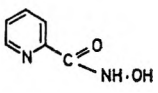

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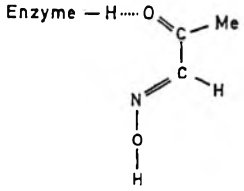

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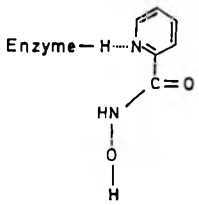


(41)

(P-2-AM) (35) in common with the natural substrate for the uninhibited enzyme, complexes at the anionic site (which is probably a carboxylate anion) where it is firmly held by electrostatic attraction in the correct spacial configuration for attack by the oxime anion on the phosphorus atom (Green & Smith, 1958a). More potent reactivators than P-2-AM are known (36) (37) (Hobbiger & others, 1958; Hobbiger & Sadler, 1958) and these are probably held firmly at the inhibited enzyme surface in an analogous manner.

The most efficient uncharged reactivators are mono-isonitroso acetone (38) and picolinhydroxamic acid (39) (Childs & others, 1955). These compounds are considered to be held at the inhibited enzyme surface by hydrogen bond formation between the surface and either the carbonyl oxygen of the reactivator (40) or the heterocyclic nitrogen atom (41) (Green & Smith, 1958b).

The oximes and hydroxamic acids used as reactivators exhibit nucleophilic activity which is far greater than expected from a consideration of their basicities. The enhanced nucleophilic activity of these compounds is attributed to the presence of the α-nitrogen atom (Bruice & others, 1963; Jencks & Carriuolo, 1960; Green, Sainsbury, Saville & Stansfield, 1958).

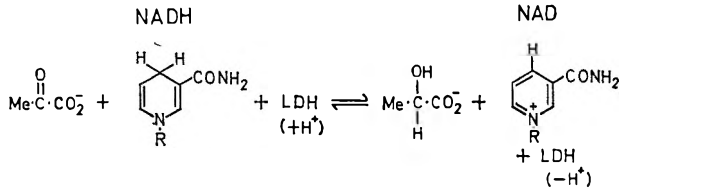
ACTIVE-SITE DIRECTED IRREVERSIBLE INHIBITION

Recent work on the biochemical differences between normal and neoplastic tissues has shown that neoplastic tissues have a lowered oxygen uptake and a higher lactate formation than normal tissues (Warburg, 1956a,b; Weinhouse, 1956). The oxidative processes of oxygen-deficient neoplastic tissues are catered for by reduction of pyruvate to lactate by the enzyme lactic dehydrogenase (LDH) with its co-enzyme, reduced nicotinamide-adenine dinucleotide (NADH) (Baker, Lee, Skinner, Martinez & Tong, 1960) (eqn *26*, page 548). Recent work on anti-cancer agents has been concerned with the design of specific inhibitors of LDH which although inactivating LDH in normal and tumour cells will only affect the oxidative metabolism of tumour cells since this function of LDH is probably not necessary in normal cell tissues (Baker & others, 1960; Baker, Lee, Tong & Ross, 1961; Baker & Alumaula, 1963; Baker & Patel, 1963; Baker, Patel & Alumaula, 1963; see Baker, 1964 for review). Baker and co-workers have designed inhibitors of LDH which complex at the active site in the manner normal for reversible inhibitors and then react with a functional group present in the polypeptide chain of the enzyme adjacent to the active-site to form a covalent bond. This results in irreversible inhibition of the enzyme since access of normal substrate to the active-site is prevented. The "intramolecular" reaction involves a nucleophile on the polypeptide chain and an electrophilic centre such as $-CH_2 \cdot I$ or $-HN \cdot CO \cdot OR$ in the inhibitor.

A closely related enzyme to LDH is glutamic dehydrogenase (GDH) and the behaviour of potential inhibitors to the two enzymes has been studied since GDH represents an enzyme system necessary to the host. It is considered that this approach will provide information about the specificity

of an inhibitor to closely related enzyme systems necessary for the well-being of host and tumour cell.

Enzymes carrying out similar functions may have similar functional groups present at the active site, but it seems highly probable that the polypeptide chain adjacent to each site will have a different amino-acid sequence and consequently a different arrangement of functional groups. This concept is the basis for an explanation of the specificity shown by certain inhibitors towards GDH and LDH.



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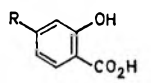


(42) R=NH·CO·CH$_2$I
(43) R=NH·CO·CH:CH·CO$_2$H
(44) R=NH·CO·O·Ph

The irreversible inhibitors studied may be arranged into three classes: iodoacetamides, substituted maleamides and phenylurethanes. 4-(Iodoacetamido)salicylic acid (42) is an inhibitor of GDH and LDH (Baker & others 1961) whereas 4-maleamyl salicylic acid (43) although a potent reversible inhibitor to both enzymes inhibits LDH irreversibly and selectively (Baker & Alumaula, 1963). 5-(Carbophenoxyamino)salicylic acid (44) irreversibly inhibits GDH but not LDH (Baker & Patel, 1963).

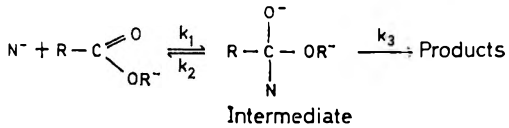
NATURE OF THE NUCLEOPHILIC GROUP

The selectivity of these classes of inhibitors is connected with the nature of the nucleophilic group adjacent to the active site. The polypeptide chain of the enzyme protein contains numerous free amino-, thiol and hydroxyl groups, each present as a third group in a constituent amino-acid moiety of the peptide chain. Baker & Patel (1963) consider that whereas compounds containing the iodo-acetamido function (42) react with a wide range of such functions present in a limited number of amino-acid residues, the phenylurethanes (44) are by contrast more selective in their choice of nucleophile and only react with a primary amine function. 4-Maleamylsalicylic acid (43) is capable of reacting with thiol, amino- and hydroxyl groups since compounds containing these functions react with maleamic acids by the Michael reaction in the order of increasing reactivity, hydroxyl $<$ amino $<$ thiol (Baker & Alumaula, 1963).

THEORETICAL CONSIDERATIONS

The factors affecting the activity of a nucleophile at saturated and unsaturated carbon centres are not identical and this probably accounts

for the selective reaction of the unsaturated carbon centre present in the phenylurethanes with a primary amino-group, whereas the electrophilic iodoacetamido centre is non-selective in its choice of nucleophile. The important difference between reaction at saturated and unsaturated carbon centres lies in the structure of the transition state for these reactions.



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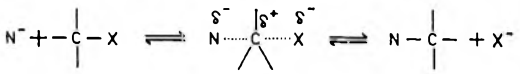

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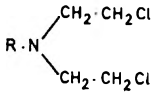

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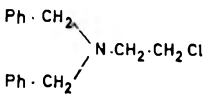

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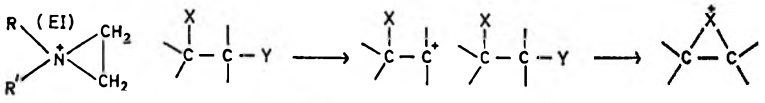

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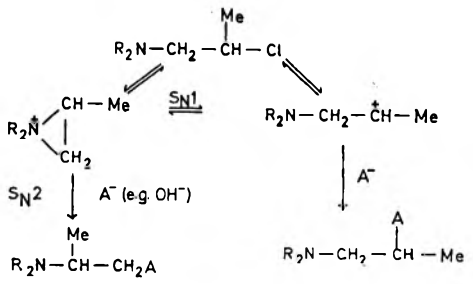


IV

Reaction between a nucleophile and an ester is considered to proceed through a transition state to give an intermediate of similar structure (Hudson & Green, 1962) (eqn *27*, above). The energy term for the dissociation energy of the C–N bond in the intermediate contributes to the

activation energy or energy barrier to formation of the intermediate and this factor can be reflected in the nucleophilic activity of the nucleophile (Hudson & Green, 1962). The dissociation energy term is unimportant when reaction at saturated carbon is considered since incomplete bond formation occurs in the transition state due to the repulsive forces between the entering and leaving groups (eqn *28*, page 549).

More recent research by Baker & others (1963) has been concerned with the design of inhibitors which are more strongly bound in the complex and contain a more reactive electrophilic centre than the existing irreversible inhibitors. The reactive bromomethyl ketone substituent group has been incorporated into salicylic acid. This approach is unlikely to lead to an increase in specificity when a more reactive but closely related electrophilic centre is introduced into the basic structure of the known inhibitors. Thus, in a reaction between a series of closely related nucleophiles (e.g. pyridines) and a closely related series of electrophiles (e.g. *p*-nitrophenyl acetate, acetic anhydride, 2,4-dinitrophenyl acetate), the more reactive system shows the lowest selectivity towards the nucleophile (Bender, 1960).

ALKYLATING AGENTS

Certain drugs are capable of effecting the alkylation of nucleophilic groups associated with biologically important receptors. Such alkylating agents are exemplified in cancer chemotherapy by the nitrogen mustards (45) (page 549) and in adrenergic blockade by dibenamine (46). The biological importance of such 2-haloalkylamines is related to their ability to form reactive ethyleniminium (EI) ions I (page 549), under physiological conditions (Golumbic, Fruton & Bergmann, 1946; Bartlett, Ross & Swain Gardner, 1949). The entropy changes involved in the formation of such small rings are generally less than those associated with bimolecular displacements. Furthermore, the three-membered EI ion, being a strained ring, will react readily with a number of nucleophilic groups (Streitweiser, 1956).

In nucleophilic displacement reactions of compounds bearing a neighbouring group X on a β-carbon atom, the rate determining step has been shown (Winstein, Grunwald, Buckles & Hanson, 1948; Winstein & Grunwald, 1948; see Streitweiser, 1956, for review) to be either the formation of a carbonium ion II, or an internal nucleophilic displacement by the neighbouring group III. Ross (1958, 1962a) suggests that the probability of reaction with relevant nucleophilic sites varies with the alkylating agent, which can follow a monomolecular (S_N1), a bimolecular (S_N2) or a mixed type of reaction mechanism. Thus, in aromatic nitrogen mustards the nitrogen atom is not sufficiently basic for stable EI ion formation so that they effectively react by the S_N1 mechanism.

The possible courses of reaction open to a typical 2-haloalkylamine may consequently be summarised as in IV. The polarity of the solvent and the concentration of nucleophilic centres (A^-) will also determine the reaction mechanism. The distribution of the positive charge on the EI ion confers partial carbonium ion character on the ring carbons.

Consequently, neutral hydrolysis leads to substitution at the most substituted carbon atom since the intermediate secondary carbonium ion is energetically more stable than the alternative primary carbonium ion. However, attack by OH^- is favoured at the primary position since here the electron density is lower (Schatz & Clapp, 1955).

NATURE OF THE NUCLEOPHILIC CENTRE

Whether or not a particular biological nucleophilic group can react with a carbonium ion at physiological pH will depend on the dissociation constant of that group. A study of pK_a values (Ross, 1958) has revealed that in proteins the reactive groups will be carboxyl, thiol, imidazole and terminal-amino, whilst in nucleic acids reaction could occur with primary and secondary phosphoryl and aromatic amino-groups, e.g. guanine, adenine. Under physiological conditions, however, alkylation is most likely to occur at phosphoryl and aromatic amino-groups of nucleic acids (Stacey, Cobb, Cousens & Alexander, 1958; Ross, 1962b). The alkylation of guanine moieties in deoxyribonucleic acid could effect changes due to quaternization, elimination of the alkylated moieties and fission of the polymer chain (Lawley & Wallick, 1957; Lawley, 1957; Lawley & Brookes, 1963). The greater effect of difunctional alkylating agents as tumour inhibitors led Goldacre, Loveless & Ross (1949) to put forward their "cross-linking hypothesis" which envisaged such a molecule reacting at two distinct points leading to a greater coiling of the DNA structure.

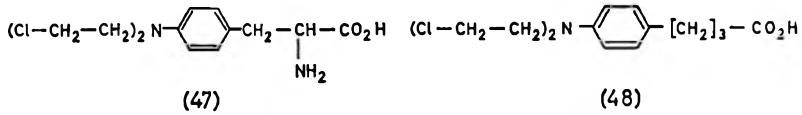
"INTRAMOLECULAR" ALKYLATION

A discussion of theories relevant to the activity of nitrogen mustards will serve to support our contention that alkylation proceeds with the impetus of an "intramolecular" reaction following an initial alignment of the drug to a receptor surface.

Bergel (1958) has suggested that nitrogen mustards consist of a carrier group and an alkylating group and that differences in effects and side-effects on tumours might be related to differences in the carrier group. Later, this hypothesis was extended into a rationale for the design of specific irreversible enzyme inhibitors (Gram, Mosher & Baker, 1959) in which the alkylating group is attached to a carrier which is a metabolite or metabolite analogue. Thus, phenylalanine mustard (47) (page 552) (Bergel, Burnop & Stock, 1955) and chlorambucil (48) (Everett, Roberts & Ross, 1953) are thought to exert their anti-tumour effect by initially fitting the site normally occupied by L-phenylalanine during protein synthesis. Since both (47) and (48) are effective it is assumed that in the protein synthesizing system they become attached to an enzymatic site only by their carboxyl groups. Once these mustards have occupied the site for phenylalanine their alkylating groups can then combine irreversibly with an adjacent nucleophilic group, thus blocking some enzymic conversion of L-phenylalanine, possibly to L-tyrosine and 3-(3,4-dihydroxyphenyl)-L-alanine. It is suggested that in those systems where (47) possesses

anti-tumour activity whilst (48) is inactive, then (47) fits the enzyme site by attachment through its amino-group and carboxyl group.

The search for specific irreversible inhibitors has led to selective cytotoxic nitrogen mustard derivatives of such substances as DL-tryptophane, indole-3-carboxylic acid (De Graw & Goodman, 1962a, b; 1964), serine and threonine (Bergel & Wade, 1959), phenoxyalkanoic acids (Skinner,



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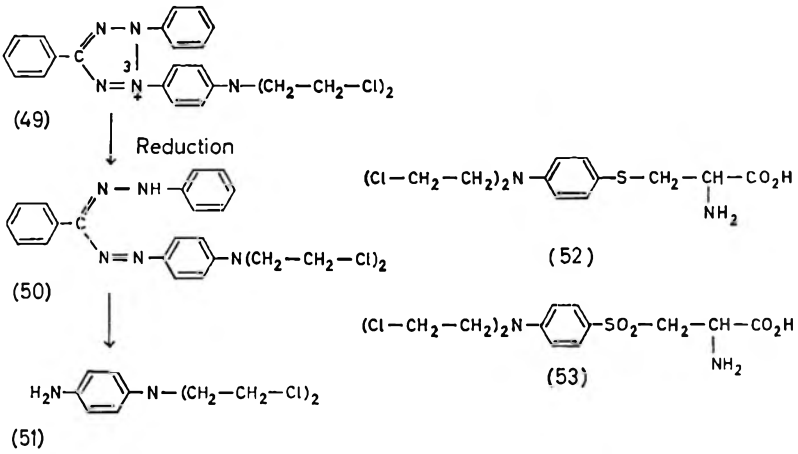

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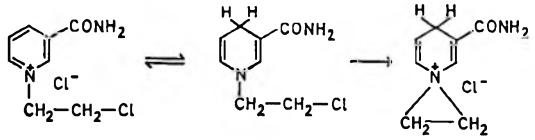
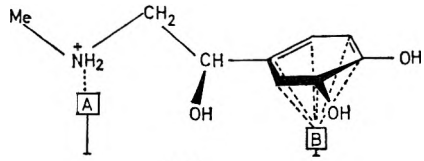


(54) (55) (56)

Martinez & Baker, 1961), 6-mercaptopurine (Levin, Sagiura & Brown, 1964) and uracil (Lyttle & Petering, 1958). Acceptance of Baker's rationale suggests that "two-armed mustards" could be replaced by "one-armed mustards" provided that the alkylating group is attached to the right substrate. The supposition that such a compound might be as good as or possibly better than the "two-armed" derivative as an anti-tumour agent, led to the preparation of monochloroethyl derivatives of uracil (Benitez, Ross, Goodman & Baker, 1960) and 6-amino-6-deoxy-D-glucose (Reist, Spencer & Baker, 1960).

The synthesis of chemotherapeutic compounds based on differences in the distribution of enzymes between normal and cancer cells constitutes a

promising area of investigation. Tsou & Su (1963) exploited the different dehydrogenase activity of normal and cancer tissue. Thus, tetrazolium



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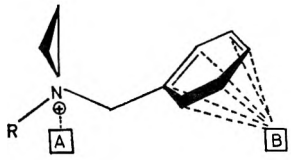

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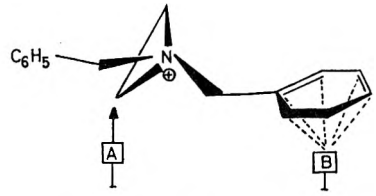

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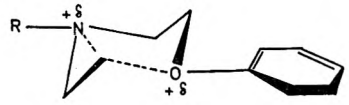

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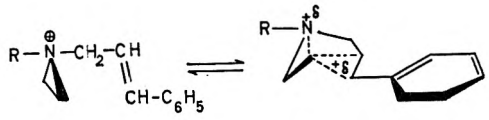

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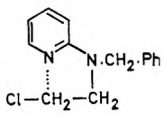

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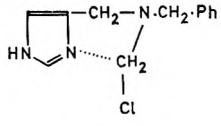

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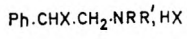


(65)

salts (49) (page 552) bearing a nitrogen mustard have a low rate of EI ion formation and lower toxicity since the tetrazolium group exerts a strong electron-withdrawing effect on the aromatic amine. However, the high

succinic dehydrogenase activity found in uterine and other types of cancer reduces this group to the formazan (50) in which the electromeric effect of the N-3 atom imparts an increased electron density to the mustard nitrogen thereby increasing the basicity of the amine and toxicity of the compound. Reduction beyond the formazan stage yields an even more potent nitrogen mustard (51). Iwamoto, Acton, Ross, Skinner, Baker & Goodman (1963) suggest that normal tissues can oxidize the cytotoxic derivative (52) to the inactive form (53) in which the strongly electron withdrawing sulphone group decreases the alkylating activity. In a new class of latently cytotoxic monofunctional agents (Friedman, Pollak & Khedouri, 1963) exemplified by (54), the pyridinium form is unable to effect alkylation. Host bearing tumours, however, can reduce (54) to the dihydro form (55) which is then converted to the reactive EI ion (56).

The dichloracetyl group may also react specifically at some receptor sites. Thus, the importance attached to this group in chloramphenicol (Feitelsen, Gunner, Moualim, Petrow, Stephenson & Underhill, 1951) has led to the preparation of dichloracetyl derivatives of DL-serine (Levi, Blondal & Lozinski, 1960), inositol, methyl anthranilate and dienoestrol (Sweeny, Salmon, Fenster, Bekersky & Canter, 1964) all of which possess anti-tumour activity.

ADRENERGIC BLOCKING AGENTS

The mode of action of these compounds is also relevant to our studies in "intramolecular" alkylation. Considerable evidence exists (Chapman & James, 1953, 1954; Nickerson, 1957; Graham, 1957) to indicate that dibenamine (46) (page 549) and its related compounds owe their activity to corresponding ethyleniminium (EI) ions which are easily formed under physiological conditions. Belleau (1958, 1959a,b; 1960) has written an analysis of the structural isosterism relating the dibenamine EI ion to adrenergic β-phenylethylamines. The non-competitive block produced by dibenamine is thought to be due to a chemical interaction with the same receptor sites which normally bind the adrenergic hormone. All adrenergic blocking agents are believed to adhere to a "phenylethylamine pattern" which requires the electrophilic group capable of interacting with an anionic site to be situated approximately 3 interatomic distances away from the aromatic ring. This allows the ammonium ion of the agonist amine (57) (page 553) to be equated with the partial carbonium ion of the antagonist, in so far as interaction with a nucleophilic group at the receptor is concerned. (It has previously been indicated that the ring carbon atoms of the EI ion possess carbonium ion character.) Thus, it is postulated that the EI ion is initially attracted electrostatically to the anionic site through its quaternary nitrogen (58) and this is followed by a rearrangement enabling the isosteric electrophilic carbon to approach the anionic site close enough (59) to allow alkylation (or esterification) of the latter. The rearrangement of the EI ion to the "phenylethylamine pattern" is assisted by van der Waals' forces operating between receptor site B and the aromatic nucleus. This mechanism is in line with Nickerson's (1957)

observation that in the establishment of an adrenergic block, an initial competitive phase is followed by a non-competitive one.

The well known adrenergic blocking activity of phenoxyethylamines has also been interpreted by Belleau (1958) in terms of the "phenylethylamine pattern."

It was suggested that (60) should lead to a hybrid state (61) as a result of anchimeric interaction between the cationic ring carbons of the EI ion and the vicinal nucleophilic oxygen. The conformation of this hybrid structure enables a cationic carbon to be at a position equivalent to three interatomic distances from the aromatic ring. Evidence for the existence of this hybrid was obtained by the preparation of rigid cyclic analogues in which the distance separating the cationic carbon of the EI ion and the -O- atom was fixed, (Belleau & Cooper, 1963). In this case improved adrenergic blockade is obtained due to the stabilization of a structure analogous to (61) at the receptor site.

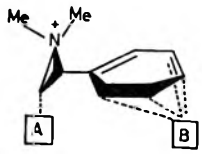
The formation of the required hybrid state (61) is also facilitated by the substitution in the aromatic nucleus of +E or +I groups which increase the nucleophilic character of the ethereal oxygen atom. Thus, a 2-methyl group in the phenyl ring increases adrenergic blockade activity (Ullyot & Kerwin, 1956). The adrenergic blockade activity of cinnamyl derivatives (62) can be explained by the interaction of the π electrons of the double bond with a cationic carbon of the EI ion resulting once more in a "phenylethylamine" alignment at the receptor site (Belleau, 1958).

Compounds (63) and (64) are inactive since they are unable to form EI ions as a result of anchimeric interaction involving the vicinal heterocyclic nitrogen atom. This produces stable five- and six-membered rings respectively.

The *NN*-dialkyl-2-aryl-2-haloethylamines constitute a remarkable class of blocking agents, the most active members being some 10,000-20,000 times more potent than dibenamine as antagonists of adrenaline (Graham & James, 1961). Unlike dibenamine, these contain a secondary or tertiary alkylamino-group and a secondary alkyl halide (65), giving structures closely resembling adrenaline and noradrenaline. The *NN*-dialkyl-2-phenylethyleniminium (EI) ion (66) (page 556) does not appear to fit the adrenaline receptor in the requisite "phenylethylamine pattern." However, Belleau (1958) claims that alkylation of the receptor at the anionic site A enables a better interaction of the benzene ring with the receptor at B (67). Chapman & Triggle (1963) question this interpretation of Belleau's since their examination of the solvolysis of the EI ion in neutral solution revealed an S_N1 mechanism of ring opening V (page 556). Consequently, unless the orientation was changed at the receptor site Belleau's theory would break down when applied to this type of compound. However, when the EI ion is close to the receptor it could be affected by a sufficiently high concentration of nucleophilic species to cause S_N2 ring opening VI.

In a new class of adrenolytic agents (Schipper, Boehme, Graeme, Siegmund & Chinery, 1961) where the chloroethylamine chain is part of a heterocyclic ring, the most effective member was found to be 1-benzyl-2,

5-bis(chloromethyl)pyrrolidine (68). This type of compound readily falls into Belleau's "phenylethylamine pattern." The mechanism of action proposed is based on the transition of a strained EI ion to a strainless piperidine conformation, where the second chloromethyl group could anchimerically assist nucleophilic attack by the active site occurring on the receptor (69).



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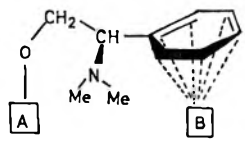

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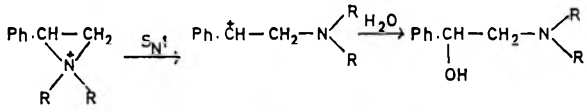

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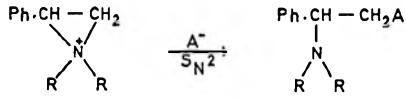

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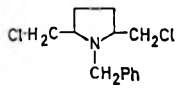

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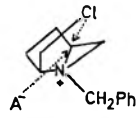

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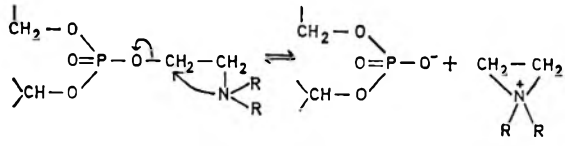

VII

NATURE OF ANIONIC SITE OF ADRENERGIC RECEPTOR.

Belleau (1960) in discussing the relative stabilities of amino-alcohol esters indicates that phosphate esters are much more resistant to hydrolysis than carboxylic esters, and he visualises the phosphate ion as being present in the active site of the receptor. The esterification hypothesis also readily explains the variations in duration of blockade since the hydrolysis necessary to regenerate the receptor may well be anchimerically assisted by the amino-group at physiological pH (see discussion on hydrolysis of acetylcholine). The greater nucleophilic driving force of the *NN*-dimethylamino-group over the *NN*-dibenzylamino-group in this respect explains why the duration of blockade by *NN*-dialkyl-β-halophenyl-ethylamines is much less than that for dibenamine. It has also been reported that certain phosphate esters of β-amino-alcohols undergo slow dealkylation as a result of cyclisation to EI ions (Brown & Osborne, 1957; Durant, Turnbull & Wilson, 1958). Belleau (1960) claims that evidence is accumulating to indicate that this is the pathway for the dealkylation of the phosphate groups of nucleotides and nucleic acids VII.

(To be concluded)

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Research Papers

Viscosity and stability relations of the system ascorbic acid: water: polysorbate 20

J. R. NIXON AND B. P. S. CHAWLA

The viscosity of dispersions of ascorbic acid in solutions of polysorbate 20 has been determined and found to be Newtonian at all concentrations studied. The course of the oxidation in polysorbate 20 appeared to be by the normal chain reaction, but the rate at which it occurred was modified by a number of factors. Incorporation within the micelle appeared to be responsible for an increase in oxidation rate, but the high viscosity of concentrated polysorbate 20 solutions, which would affect the diffusion of oxygen to the reaction site caused a much larger reduction in oxidation rate.

MANY pharmaceuticals have been prepared in recent years in which the medicament has been solubilised by non-ionic surface-active agents. Aqueous preparations of oil-soluble vitamins have been particularly popular and increased stability to oxidation has been claimed.

Previously this department has reported the oxidation of model relatively water insoluble substances in surface-active agents (Nixon, 1958; Mitchell, 1960; Swarbrick, 1963). It is possible to include the water-soluble vitamin ascorbic acid in this type of preparation and we now describe its oxidative behaviour in the model system ascorbic acid: water: polysorbate 20.

Experimental

Ascorbic acid. Assay (iodometric) 99%. M.p. 190–192°. $[\alpha]_D^{20}$ 2% in water + 22°. pH of 2% in water 2·5.

Polysorbate 20 (Tween, Honeywill-Atlas Ltd). This material complied with the manufacturer's specification dated October, 1956.

Copper sulphate. Analar. Used at 1×10^{-4}M $CuSO_4$. $5H_2O$ as a catalyst.

Buffer solution, pH 3·4. Na_2HPO_4/citric acid (McIlvaine, 1921).

Determination of solubility. The solubility of the ascorbic acid was determined by equilibration in glass-stoppered flasks immersed in a water-bath at 25° ± 0·1°. The end-point was taken as the average between an under- and over-saturated dispersion. Because of the viscosity of high concentrations of polysorbate 20 it was necessary to warm the flask to 60° to speed the equilibration period. This did not affect the quantity solubilised. In all instances the excess ascorbic acid separated out as crystalline material.

Determination of viscosity. This was measured using a Ferranti-Shirley cone and plate viscometer fitted with an automatic flow curve recorder. The viscosity was measured at 25° using either a 4 cm (angle

From the School of Pharmacy, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

20′. 26″) or 7 cm (angle 20′. 25″) cone. Flow curves were determined as the shear rate was continuously increased from zero up to 1800 sec^{-1} and then decreased to zero again. The samples were also subjected to recycling.

Measurement of oxygen uptake. The oxidation of the systems was followed by means of a Warburg constant volume respirometer at 25° as previously described (Carless & Nixon, 1957). The oxygen uptake of polysorbate 20 in water was also measured and subtracted from the total uptake as a correction.

Chromatography of oxidised ascorbic acid solutions. The lower layer of a butanol: glacial acetic acid: water system (40: 10: 50) was used to develop the chromatogram. The Whatman No. 1 filter paper was equilibrated for 24 hr with the upper layer of the mixture before development. The spots were made visible with ammoniacal silver nitrate and the chromatograms were also examined under ultraviolet light.

Results

The presence of polysorbate 20 did not cause any large increase in the solubility of ascorbic acid (line AB, Fig. 1) and at high surface-active agent concentrations the solubility fell until in the polysorbate 20 itself only 5% w/w of ascorbic acid was soluble. None of the dispersions

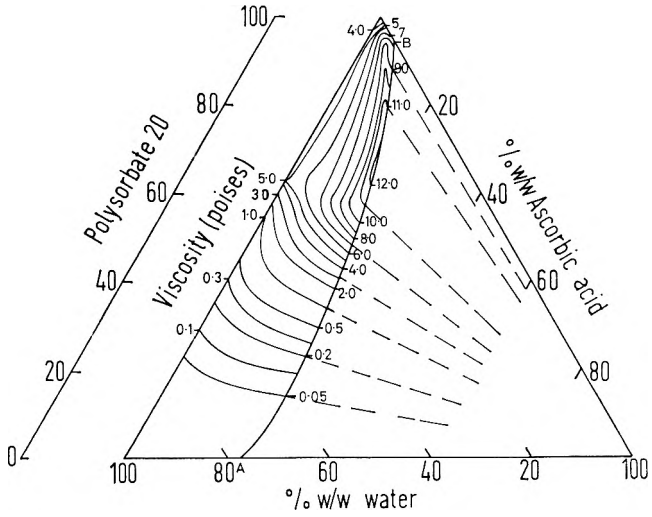

FIG. 1. Viscosity and solubility relationships in the system polysorbate 20/ascorbic acid/water. Line A–B is the solubility curve of ascorbic acid. ——— Viscosity contours of one phase system. - - - - Viscosity contours of equilibrium liquid in contact with excess ascorbic acid.

showed birefringence when examined under polarised light, indicating the absence of liquid crystals.

The viscosity of all the dispersions was Newtonian and did not vary on recycling.

The rather complicated viscosity map, produced by connecting similar viscosities within both the solubilised and solubilised plus excess solid regions, is shown superimposed on the solubility curve in Fig. 1.

In the binary system polysorbate 20: water there was a very slow increase in viscosity to 30% w/w polysorbate 20 after which the increase was extremely rapid and reached a maximum of 5·2 poises at 62·8% w/w polysorbate 20. The viscosity then fell gradually and polysorbate 20 itself had a viscosity of 3·81 poises.

The initial addition of ascorbic acid to any polysorbate 20: water system caused an increase in viscosity. The subsequent behaviour on further addition of ascorbic acid depended on the starting concentration of polysorbate 20. At concentrations up to about 60% w/w polysorbate 20 a point was reached where further addition of ascorbic acid caused little or no change in the viscosity. This occurred after the addition of 10–12% w/w of ascorbic acid. From 60–90% w/w polysorbate 20 the viscosity of the dispersions continued to increase with addition of ascorbic acid until the solubility limit was reached. In the heterogeneous region of Fig. 1, the viscosity of solutions on the same tie line was, as expected, found to be constant. This proved a useful check on solubility data which would otherwise have been difficult to determine. For initial concentrations of polysorbate 20 in excess of 90% w/w a third behaviour pattern was observed. Here the initial increase of ascorbic acid caused a rapid increase in viscosity but on further addition the contours turned back upon themselves and the result was a slight fall of viscosity.

The viscosity of saturated solutions of ascorbic acid in polysorbate 20: water exhibited a similar form to the binary polysorbate 20: water. The peak viscosity was 12·3 poises at 68% w/w polysorbate 20.

The catalysed oxidation of ascorbic acid in water at pH 3·4 and 6·0 was a first order reaction. The rate was approximately twice as fast at the higher pH but both showed a rapid increase in oxidation rate at ascorbic acid concentrations of less than 8% w/w (Table 1).

TABLE 1. OXIDATION OF AQUEOUS ASCORBIC ACID

Concentration of ascorbic acid % w/w	Oxidation rate (ml/kg/hr)	
	pH 3·4	pH 6·0
2·7	7,500	13,600
5·95	3,300	5,900
8·1	1,950	3,400
11·9	1,650	2,900
16·2	1,500	2,800

Catalyst: $CuSO_4.5H_2O$

The induction period of ascorbic acid in polysorbate 20 was much more extended than in water alone, and after the induction period a rise to an approximately steady oxidation rate occurred. The period of declining oxidation rate was also extended.

The oxidation of ascorbic acid in polysorbate 20 is slightly complicated due to the slow uptake of oxygen by the polysorbate 20 itself. This occurs more rapidly at acid pH, and at high polysorbate 20 concentrations

could form a significant proportion of the total oxygen uptake. Fig. 2 shows the uptake of oxygen by polysorbate 20 and it can be seen that although increasing viscosity did initially cause a fall in oxidation rate, at high polysorbate 20 concentrations this had increased again and was now in excess of dispersions with low viscosity. The method of increasing gas: liquid transfer by increasing the shaking rate did not cause any noticeable increase in this oxygen uptake.

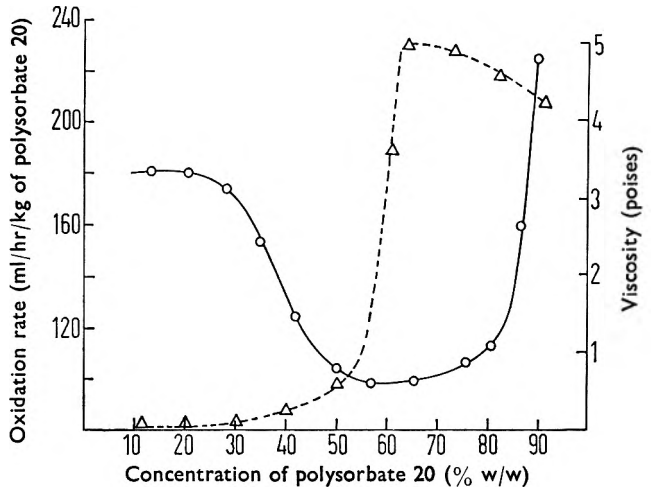

FIG. 2. The oxidation of polysorbate 20. Temperature 25°. Catalyst 1×10^{-4}M $CuSO_4.5H_2O$. pH 3·4.——— Oxidation rate. - - - - Viscosity.

Before studying the oxidation in relation to the viscosity solubilisation diagram (Fig. 1) the effect of catalyst and pH was determined. Saturated solutions in 15 and 30% w/w polysorbate 20 were used. With both polysorbate concentrations the rate of oxidation increased rapidly with

TABLE 2. EFFECT OF COPPER CATALYST ON THE RATE OF OXIDATION OF SATURATED SOLUTIONS OF ASCORBIC ACID

Polysorbate concentration % w/w	Copper sulphate M	Steady oxidation rate ml/kg/hr
15	0	15
	1×10^{-5}	130
	5×10^{-5}	480
	1×10^{-4}	670
	5×10^{-4}	1,100
30	0	15
	1×10^{-5}	220
	5×10^{-5}	580
	1×10^{-4}	750
	5×10^{-4}	1,240

increasing catalyst concentration, although no linearity was found (Table 2). An increase in pH also caused an increased oxidation rate except in the region pH 5·6 to 7·2 where a plateau existed (Fig. 3).

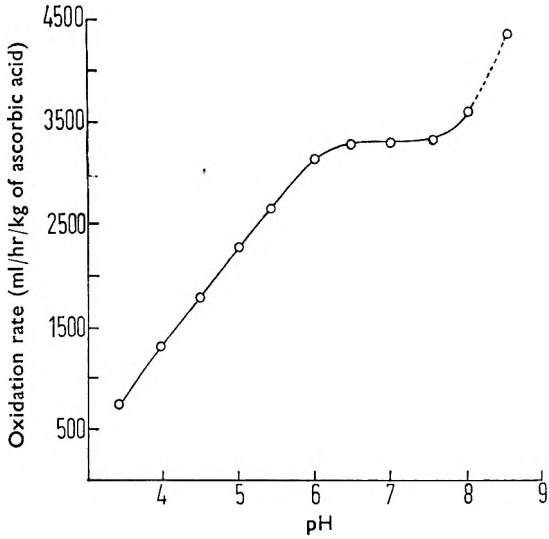

FIG. 3. The effect of pH on the oxidation of ascorbic acid in polysorbate 20. Polysorbate concentration 30% w/w Temperature 25°. Catalyst 1×10^{-4}M $CuSO_4.5H_2O$.

The oxidation of ascorbic acid-saturated dispersions, both catalysed and uncatalysed, was studied at pH 3·4. This pH was adopted to prevent the catalysed oxygen uptake rate becoming too fast to measure. The large increase in the viscosity of concentrated polysorbate 20 dispersions had a negligible effect on the oxidation rate of the uncatalysed reaction. As the concentration of polysorbate increased, the oxidation rate rose slightly, although there was a sharp fall in rate at polysorbate 20 concentrations greater than 90% w/w (Fig. 4).

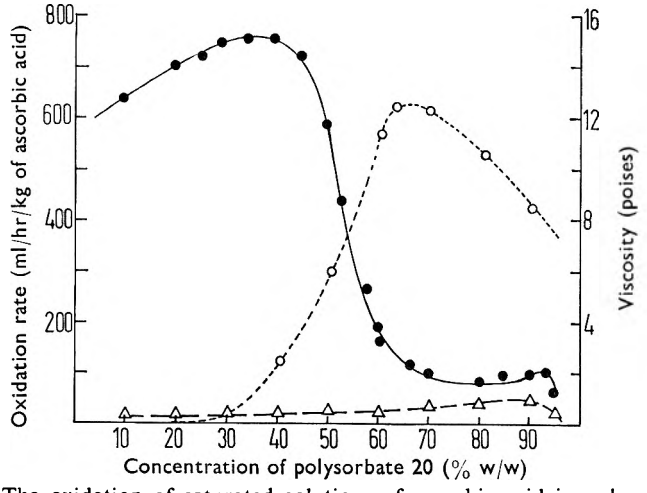

FIG. 4. The oxidation of saturated solutions of ascorbic acid in polysorbate 20. Temperature 25°. pH 3·4. ——— Catalyst 1×10^{-4}M $CuSO_4.5H_2O$. — — Uncatalysed. - - - - Viscosity.

The oxidation of the catalysed systems differed considerably. At lower polysorbate concentrations, where the viscosity remained almost unchanged, the rate of oxidation rose steadily, but once the viscosity started to increase, the oxidation rate fell precipitously and reached a minimum at around 75% w/w polysorbate 20. Thus, even after the maximum viscosity was passed, the oxidation rate showed a further decrease. There was little further change in oxidation rate, although the trend was slightly upward until dispersions in pure polysorbate were reached, when a further sudden fall in oxidation rate occurred. None of these results showed significant variation on increasing the rate of agitation.

The presence of even small traces of polysorbate 20 caused a considerable fall in the oxidation rate of the more saturated solutions of ascorbic acid when compared with the rate of similar aqueous solutions. This suggested that the incorporation of ascorbic acid in the polysorbate, and the consequent depletion of the water pseudophase, was resulting in a measure of protection. At very low polysorbate concentrations the protection may be due to the normally found effect of surface-active agents on the diffusion of oxygen into solutions (Downing, Melbourne & Bruce, 1957). To study this effect, a concentration of 32% w/w polysorbate 20 was used; this being the highest concentration possible before the viscosity commenced its rapid increase, and also because it possessed the highest oxidation rate for a saturated solution (Table 3). Conversely, if the concentration of ascorbic acid was constant and the polysorbate 20 concentration varied, then increased incorporation of the acid in the polysorbate would be expected to confer some degree of protection on the ascorbic acid as is also illustrated in Table 3.

TABLE 3. OXIDATION RATE OF ASCORBIC ACID IN POLYSORBATE 20: EFFECT OF SATURATION LEVEL

Polysorbate 20 32% w/w with ascorbic acid % saturation	Rate of oxidation ml/kg/hr	Ascorbic acid 20% in polysorbate 20 % w/w	Rate of oxidation ml/kg/hr
9·9	12,350	0	1,450
26·2	2,579	10	1,360
57·8	1,124	25	1,100
100·0	763	35	850

Catalyst: $CuSO_4.5H_2O$

Because of the small quantities of material involved and the uncertain interference of the polysorbate 20 with most assays, the quantitative formation of the oxidation products was not followed. However, a number of chromatograms of the oxidising material were made. The polysorbate 20 tended to follow the solvent front, but three spots were observable with Rf values in the ranges (*a*) 0·08–0·09; (*b*) 0·33–0·37; (*c*) 0·53–0·59. The spot (*b*) was faint in all instances. In one system (ascorbic acid 10% w/w, polysorbate 20 30% w/w) a fourth spot was detected under ultraviolet light, Rf value 0·80. This was not identified and was probably due to impurity. The spots *a*, *b* and *c* are considered to correspond respectively to diketogulonic acid, ascorbic acid and dehydroascorbic acid. The slight variation from the Rf values of Mapson &

Partridge (1949) we consider is caused by the polysorbate 20 increasing the hydrophilic property of the material. The large tail of the spots was also due to this cause.

Discussion

Mulley (1961) studied the phase equilibria of systems containing non-ionic surface-active agents and suggested a general form of ternary phase diagram. In the present work solid material separated out once the solubility limit was reached and, therefore, only the left-hand portion of the diagram would apply. No evidence was found of an anisotropic liquid crystalline phase, birefringence and non-Newtonian viscosity being absent. The polysorbate 20 was probably too hydrophilic to allow for its formation. The transfer would appear to be direct from an S_1 to an S_2 type micellar distribution (Windsor, 1954). There was no evidence to show when this commenced, nor the relative proportions of each micellar type at a given concentration. The alternative is that a weak gel structure may exist, but no evidence for this was found since the flow curves were Newtonian and not shear dependent.

The increase in viscosity would appear to be associated with the formation of S_2 type micelles, and the two forms must coexist in dynamic equilibrium over most of the diagram (Fig. 1). At the two extremes, one or other will predominate, the S_1 being the main type at low polysorbate 20 concentrations. Even so, it cannot be assumed that equal quantities of the two micellar entities exist at the highest viscosity although it is probably the antagonism of the two types which causes the viscosity pattern.

If the viscosity contours are examined, it is seen that the addition of ascorbic acid causes very large increases in viscosity in the region where Mulley (1961) predicted the presence of a liquid crystalline phase. As the ascorbic acid would tend to be solubilised towards the outside of the S_1 type micelle rather than in the hydrocarbon interior, the bulk of the micelle would be considerably increased and in consequence the relative density of packing of the micellar pseudophase.

In the S_2 type micelle the ascorbic acid would be towards the centre and the increase in bulk would not be so great, therefore a slight fall in viscosity would result as the S_2 type began to predominate. This coupled with the mutual antagonism of the two micellar types would appear to account for the complex viscosity map.

The mechanism for the oxidation of ascorbic acid in polysorbate 20 dispersions, as shown by the chromatograms, did not appear to be abnormal. However, solubilisation in the polysorbate, the effect of pH and the changes in viscosity did modify the rate at which this oxidation took place. At pH values above 7·5, where the plateau region had been passed and secondary ionisation had commenced, the oxidation rate was too fast to be conveniently studied by the methods available, so that pH 6, corresponding to complete primary ionisation, and pH 3·4 were used. This meant that only one grouping was being attacked and therefore the mechanism of oxidation was simpler.

Unlike previous studies, the part played by solubilisation in preventing oxidation appeared to be small. In previous studies the materials were far less water soluble and therefore at equilibrium the bulk would be in the micelle. In the present instance the solubility in the polysorbate 20 was low when compared with the water solubility and it was in the water where most of the oxidation appeared to take place. Except where the saturation of the ascorbic acid solution was in the region of 10% or less, two effects were apparent. Immediately small amounts of polysorbate were present, the oxidation rate fell. This could not be due to solubilisation and it is suggested that the fall was caused by a reduction in the rate of diffusion of oxygen into the system, similar to the effect of detergents on the aeration of sewage effluents (Downing, Melbourne & Bruce, 1957). Once micelles formed, the close proximity of the solubilised molecules facilitated the continuance of the oxidation chain reaction and until the viscosity began to increase rapidly the rate of oxidation rose. It was in this region of low viscosity that the previous studies were made.

In the region of high viscosity it is probable that the much slower diffusion of oxygen to the site of oxidation is the cause of the very low oxidation rate. However, an increased shaking rate, which in the event of undersaturation with oxygen would normally increase this, produced no effect. Even so, from the shape of the oxygen uptake curve, it appeared probable that this was the explanation. In support of this the uncatalysed oxidation showed no drop over the same region and the oxidation rate even rose slightly as the viscosity increased, showing that here the system contained an optimum amount of oxygen and that gas exchange was fast enough to prevent depletion of the system.

Once the S_2 type micellar system predominated and the viscosity began to fall again, the systems showed little further change in oxidation rate until almost pure polysorbate 20 was reached. In this region the water, where the bulk of the oxidation appeared to take place, was enclosed as a discontinuous pseudophase inside the polysorbate 20 micelle and any ascorbic acid dissolved in it was protected from the oxygen by the polysorbate itself. Once all the water was eliminated, the rate of oxidation in the polysorbate itself showed a further sharp fall which suggested that the presence of water even in small quantities inside the micelles allowed the easier formation of the initiating and propagating free radicles.

Acknowledgement. The authors would like to thank Dr. J. E. Carless for helpful discussions during the preparation of this manuscript.

References

Carless, J. E. & Nixon, J. R. (1957). *J. Pharm. Pharmacol.*, **9**, 963–973.
Downing, A. L., Melbourne, K. V. & Bruce, A. M. (1957). *J. appl. Chem.*, **7**, 590–596.
Mapson, L. W. & Partridge, S. M. (1941). *Nature, Lond.*, **164**, 479–480.
Mitchell, A. G. (1960). Ph.D. thesis, University of London.
Mulley, B. A. & Metcalf, A. D. (1961). Paper presented to 21st International Congress of Pharmaceutical Sciences, Pisa (1961).
Nixon, J. R. (1958). Ph.D. thesis, University of London.
Swarbrick J. (1963). Ph.D. thesis, University of London.
Windsor, P. A. (1954). *Solvent Properties of Amphiphilic Compounds*, p. 7, London: Butterworth.

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An investigation of the pharmacology of macusine B

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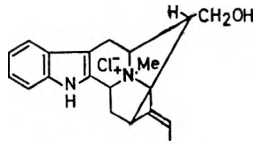
Macusine B, an alkaloid isolated from *Strychnos toxifera*, blocks α-adrenergic receptors and stimulates β-receptors *in vivo* and *in vitro*. It partially blocks the response of the isolated rabbit heart and the cat blood pressure to tyramine. Macusine B is a competitive inhibitor of 5-hydroxytryptamine on the guinea-pig ileum and also blocks the action of this spasmogen on the rat uterus.

THE first recorded observation of the pharmacological potency of the curare obtained from *Strychnos toxifera* was made by Schomburgk (1879) who reported that the arrow poison prepared from this plant by the Macusi tribe of British Guiana was the most potent hitherto observed. Since then over 60 alkaloids have been found in *S. toxifera* (Battersby, Binks, Hodson & Yeowell, 1960). Toxiferine I was one of the first crystalline alkaloids to be isolated from this plant and the potency of the arrow poison was largely due to the presence of this alkaloid. Herring & Marsh (1951) and Paton & Perry (1951) were the first to investigate the powerful neuromuscular blocking action of toxiferine I.

Because of the difficulty involved in the isolation and purification of the alkaloids from *S. toxifera* few of the other alkaloids from this species have undergone pharmacological investigation; Battersby & others (1960) isolated and identified the alkaloids macusine A and B and have since obtained sufficient macusine B for pharmacological investigation. The alkaloid has the structure



It is a white crystalline solid which is readily soluble in water to give a neutral solution.

Experimental

METHODS

Effect on the whole animal. Ten female albino mice (16–20 g) were injected with an approximately LD50 dose (50 mg/kg i.p.) of macusine B and observed for at least 15 min. During this time the mice were periodically tested on a rotating rod and also for their ability to grip a rough surface. The time of onset of any effect of macusine B was noted. For the LD50 determination, female albino mice (16–20 g) were used. After estimating the approximate LD50, four groups of 10 mice were injected intraperitoneally with doses of the alkaloid in a logarithmic series of 0·25, 0·50, 1·0 and 2·0 respectively where 1·0 was the approximate LD50. The LD50 was estimated by the method of Weil (1953).

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Guinea-pig isolated ileum. The ileum was suspended in a 15 ml bath of oxygenated Tyrode solution at 37°. Longitudinal contractions were recorded isotonically. The lever was weighted with 0·5 g and had a $\times 10$ magnification. Submaximal contractions were induced to constant doses of acetylcholine, histamine, barium chloride, nicotine and 5-hydroxytryptamine (5-HT), and when a steady response had been achieved for each agonist macusine B was added to the bath and the effects on the responses to the subsequent doses of the agonist were recorded. The method of Timms (1956) was used to test for the type of inhibition produced by the alkaloid.

Rat isolated colon. The terminal colon was suspended in a 15 ml bath of oxygenated De Jalon solution at 30° as described by Gaddum & Lembeck (1949). The effect of macusine B on the depressor effect of adrenaline on the acetylcholine-stimulated contractions was then observed.

Rat isolated uterus. Virgin rats (90–110 g) were injected subcutaneously with stilboestrol propionate (1 mg/kg) 24 hr before killing. The uteri were suspended in a 15 ml bath of oxygenated De Jalon fluid at 30° as described by Gaddum & Lembeck (1949). The effect of macusine B on the submaximal contractions caused by 5-HT and vasopressin was observed. In other experiments the uterus was stimulated with a standard dose of carbachol and the effect of macusine B on the depressor response of adrenaline on the carbachol-stimulated uterus was observed.

Rat phrenic nerve-diaphragm. This was suspended in a 15 ml bath of oxygenated Tyrode solution at 37° as described by Bülbring (1946). Macusine B was added to the bath and its effect observed on the contractions of the diaphragm that followed the electrical stimulation of the phrenic nerve.

Rat heart rate and electrocardiogram (ECG). Rats (90–110 g) were anaesthetised with pentobarbitone sodium (60 mg/kg; i.p.), after 15 min, the resting ECG was recorded following an intraperitoneal injection of physiological saline (0·1 ml). Macusine B was injected (15 mg/kg, i.p.) and a record made for 30 sec periods immediately after injection and subsequently after periods of 1, 2, 3, 4, 5, 7 and 10 min.

Guinea-pig isolated vas deferens—hypogastric nerve. The tissue was set up in a 50 ml bath of oxygenated Tyrode solution as described by Birmingham & Wilson (1963). The effect of macusine B on the contractions of the vas deferens was observed following stimulation of the pre-ganglionic nerve.

Chick isolated rectum. Approximately 6 cm of rectum from 14–21 day old chicks was suspended in a 15 ml bath of Krebs solution as described by Armitage & Vane (1964). The direct effect of macusine B on the chick rectum was recorded in addition to its effects on the responses of the rectum to adrenaline. The response of the isolated rectum to adrenaline alone was also observed.

Rabbit isolated duodenum. A piece of duodenum was suspended in a 50 ml bath of oxygenated Tyrode solution at 37° and the effect of macusine B on the muscle tone and pendular movement was observed.

Rabbit isolated heart. The rabbit heart was perfused with oxygenated

Ringer solution at 37° by the method of Langendorff. The effect of macusine B on the heart rate and amplitude of contraction was tested at first alone and then following the injection of tyramine and adrenaline into the perfusion fluid.

Test for local anaesthesia. The frog lumbar plexus anaesthesia method of Bülbring & Wajda (1945) was used. The determinations were made using three groups of 5 frogs. The first group received a 0·2% solution of macusine B in 0·65% saline intraperitoneally, the second group was given a 0·2% solution of lignocaine hydrochloride in 0·65% saline and the control group received 0·65% saline alone. The local anaesthetic potencies of macusine B and lignocaine were compared by observing the times taken for the frogs to fail to withdraw their legs from a beaker of dilute hydrochloric acid.

Cat blood pressure. Cats (2–4 kg) were anaesthetised with chloralose (80 mg/kg, i.p.) and the blood pressure was recorded from the carotid artery. The nictitating membrane was attached to a frontal writing level giving a ×15 magnification and the preganglionic fibres of the sympathetic chain were stimulated by means of supramaximal shocks of 10 sec duration at 1 min intervals from a Palmer stimulator.

The effect of macusine B on the normal blood pressure, and its effect on the pressor response to adrenaline, noradrenaline, tyramine, carotid occlusion for 10 sec, and on the depressor response to acetylcholine, vagal stimulation of 5 sec duration and carotid occlusion for 10 sec was observed. All drugs were injected into the femoral vein.

Blood glucose determination. Blood was obtained from mice by making a small incision in the lateral tail vein after first dipping the tail into warm water. Serial samples of blood (0·05–0·1 ml) could readily be obtained in this way. Three groups of 5 mice were used. Group 1 was injected subcutaneously with adrenaline (1 mg/kg); group 2 was injected with the same dose of adrenaline together with macusine B (25 mg/kg, i.p.); group 3 was given the alkaloid (25 mg/kg, i.p.) alone. The control group was injected with physiological saline (0·1 ml, s.c.). The blood glucose was estimated by the glucose oxidase method (Huggett & Nixon, 1956).

Plasma non-esterified fatty acids. Four groups of 4 rats were used. Group 1 was given adrenaline (1 mg/kg, s.c.); group 2 was injected with the same dose of adrenaline together with macusine B (15 mg/kg, i.p.); group 3 was given macusine B (15 mg/kg, i.p.) alone. The control group was injected with physiological saline (0·1 ml, s.c.). All animals were killed by a blow on the head 3 hr after injection; this corresponded approximately to the period of peak activity for adrenaline as assessed in a preliminary experiment. Plasma free fatty acids were then determined by the method of Duncombe (1964).

Results

Effect on whole animal. By 3½ min after the injection of an approximate LD50 dose of macusine B, the mice showed a slight head tremor, followed shortly by ataxia and a reduced grip. The ability of the animals to hold onto a rotating rod was completely lost 5–6 min after injection. Clonic

convulsions occurred after approximately 7½ min and loss of righting reflex, cyanosis and death resulted within 9 min. The mice in the group surviving this dose showed only the first two phases of the seizure. The LD50 on intraperitoneal injection was: 53·7 mg/kg with 95% confidence limits of 42·8–67·2.

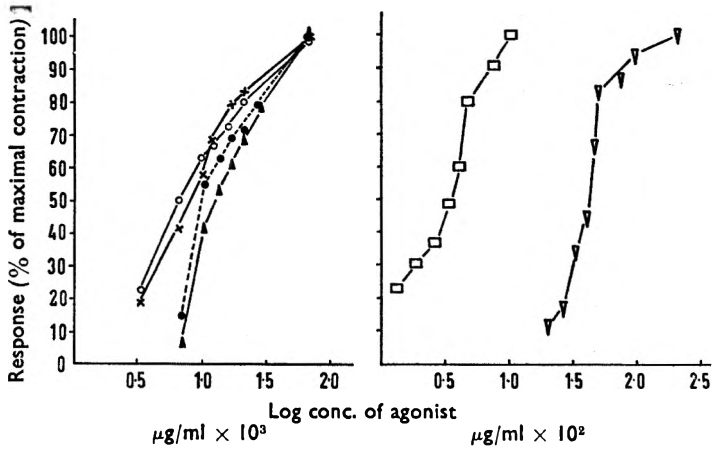

FIG. 1. The effect of macusine B (1·3 μg/ml) on the responses of the guinea-pig isolated ileum to acetylcholine (×, ○), histamine (▲, ●) and 5-hydroxytryptamine (□, ▽). The symbols X, ▲ and □ represent the responses to the agonists; the others represent the responses after equilibration with macusine B. Each curve represents the mean of two experiments.

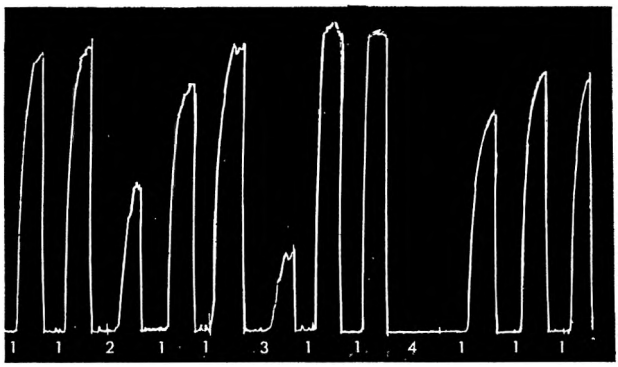

FIG. 2. Effect of macusine B on the responses of the guinea-pig isolated ileum to nicotine. (1) 1·33 μg/ml nicotine hydrogen tartrate, (2) 1·33 μg/ml nicotine + 1·66 μg/ml macusine B, (3) 1·33 μg/ml nicotine + 3·32 μg/ml macusine B, (4) 1·33 μg/ml nicotine + 6·64 μg/ml macusine B.

Guinea-pig isolated ileum. Macusine B had no effect on the contractions caused by the action of acetylcholine, histamine and barium chloride on this preparation. It did inhibit the responses of the ileum to 5-HT, and the parallel displacement of the dose-response curve to the right (Fig. 1) suggests that the alkaloid is competitively inhibiting 5-HT. Macusine B also reversibly inhibited the responses of the ileum to nicotine (Fig. 2).

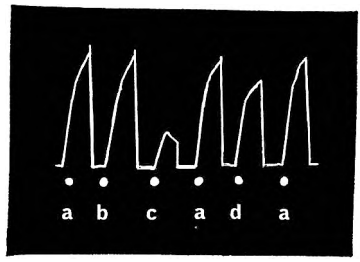

FIG. 3. Effect of macusine B on the rat colon. Colon suspended in oxygenated De Jalon solution at 30°. Response of colon shown to (a), 0·01 μg/ml acetylcholine (as chloride); (b), 0·01 μg/ml acetylcholine + 1·70 μg/ml mascusine B (as hydrochlorine); (c), 0·01 μg/ml acetylcholine + 0·01 μg/ml adrenaline (as hydrogen tartrate); (d), 0·01 μg/ml acetylcholine + 1·70 μg/ml macusine B + 0·01 μg/ml adrenaline.

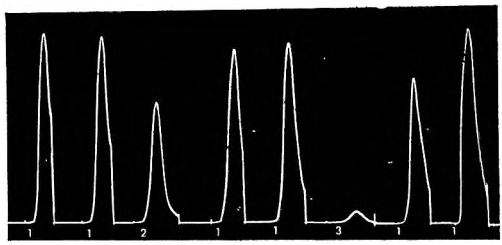

FIG. 4. Effect of macusine B on the responses of the rat isolated uterus to 5-HT. (1) 0·033 μg/ml 5-HT creatine sulphate, (2) 0·033 μg/ml 5-HT + 1·66 μg/ml macusine B, (3) 0·033 μg/ml 5-HT + 6·64 μg/ml macusine B.

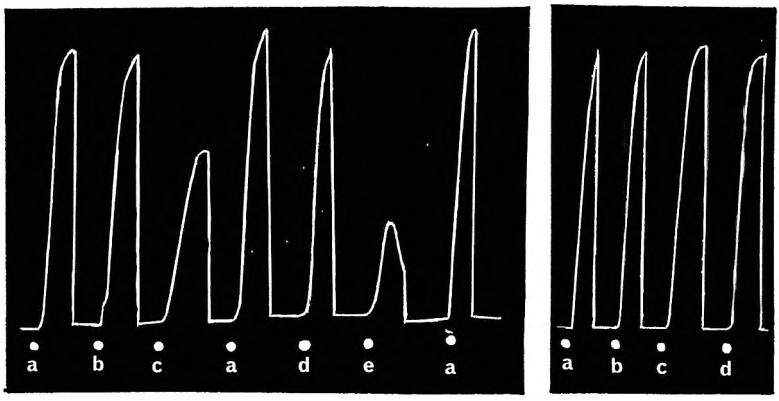

FIG. 5. Effect of macusine B on the rat uterus stimulated with carbachol. Response of uterus shown to (a), 0·66 μg/ml carbachol; (b), 0·66 μg/ml carbachol + 1·30 μg/ml macusine B; (c), 0·66 μg/ml carbachol + 0·01 μg/ml adrenaline; (d), 0·66 μg/ml carbachol + 1·3 μg/ml macusine B; (e), 0·66 μg/ml carbachol + 1·3 μg/m macusine B + 0·01 μg/ml adrenaline. Second trace shows response to (a), 0·66 μg/ml carbachol; (b), 0·66 μg/ml carbachol + 1·0 μg/ml pronethalol; (c), 0·66 μg/ml carbachol + 0·01 μg/ml adrenaline; (d), 0·66 μg/ml carbachol + 1·3 μg/ml macusine B + 0·01 μg/ml adrenaline.

Rat isolated colon, uterus and phrenic nerve diaphragm. Macusine B partially blocked the depressant effect of adrenaline on the acetylcholine-stimulated colon (Fig. 3) and this effect was easily reversible. The alkaloid blocked the response of the uterus to 5-HT (Fig. 4). The effect was readily reversible. It had no effect on the responses of the uterus to acetylcholine or vasopressin.

In a dose of 1·3 μg/ml, the alkaloid potentiated the inhibitory effect of adrenaline on the carbachol-stimulated uterus (Fig. 5). This effect was readily reversed by washing. The inhibitory effect of macusine B was blocked by the β-receptor blocking drug pronethalol (Black & Stephenson, 1962).

The alkaloid had no effect on the electrically stimulated contractions of the diaphragm when doses of up to 80 μg/ml were added to the bath.

Rat heart rate and ECG. Macusine B had no effect on the ECG of the rat in sublethal doses other than to cause a small increase in the heart rate. This increase began 1 min after the intraperitoneal injection of the alkaloid and lasted for approximately 3 min.

Guinea-pig vas deferens—hypogastric nerve. Macusine B blocked the electrically stimulated contractions of the vas deferens in a dose of 10 μg/ml. The effect was reversible.

Rabbit isolated duodenum and heart. Macusine B reduced the muscle tone when added to the bath in a dose of 0·5 μg/ml (Fig. 6), and this

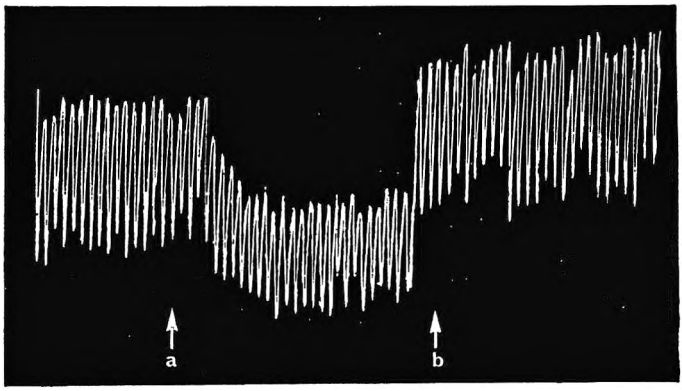

FIG. 6. Effect of macusine B on the rabbit duodenum. Duodenum suspended in oxygenated Tyrode solution at 37°. Response of duodenum to 0·5 μg/ml macusine B shown. Alkaloid added at (a) and the bath washed out at (b).

effect was easily reversible. The alkaloid had no effect on the pendular movement of the duodenum and at 2·5 μg affected neither the amplitude of contraction nor the heart rate when injected into the perfusion fluid. However, it did block the effect of tyramine on the isolated heart (Fig. 7) and slightly potentiated the increase in the heart rate due to an injection of 0·5 μg of adrenaline. The alkaloid did not affect the increase in amplitude of contraction of the isolated heart caused by adrenaline.

Chick isolated rectum. Macusine B caused a decrease in the muscle tone and also potentiated the depressor effect of adrenaline on this preparation, when added to the organ bath in a dose of 1·3 μg/ml. When added to the bath alone, this concentration of alkaloid was equiactive with 0·005 μg/ml of adrenaline.

Test for local anaesthesia. There was no significant difference between the group treated with macusine B and the control group. The time for the onset of plexus anaesthesia for the experimental and the control group

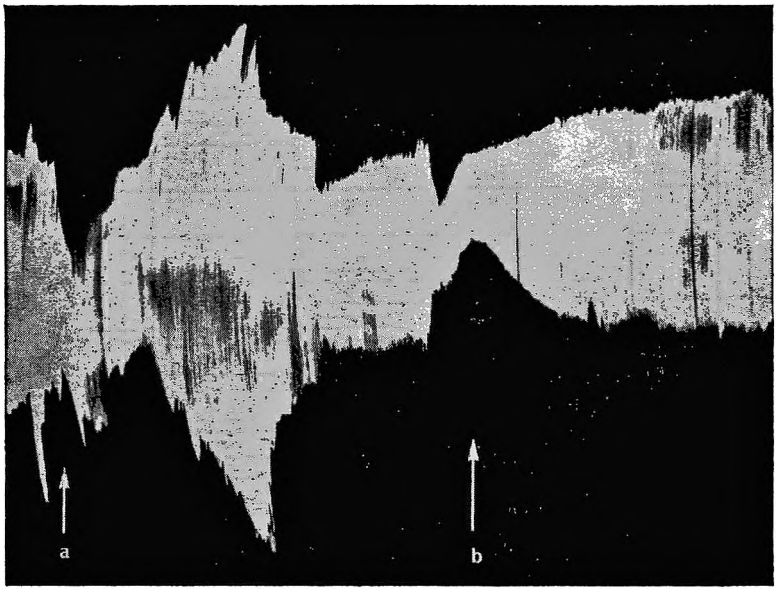

FIG. 7. Effect of macusine B on the rabbit heart. Heart perfused with oxygenated Ringer solution at 37°. At (a) 20 μg tyramine (as hydrochloride) added to perfusion fluid and at (b) 20 μg tyramine + 25 μg macusine B added.

was 14·0 $\pm$ 0·35 min ($\pm$ s.e.) and 13·5 $\pm$ 0·50 min respectively. In contrast, the time of onset of anaesthesia for the group treated with 0·2% lignocaine was 5·2 $\pm$ 0·77 min.

Cat blood pressure. Macusine B had a marked hypotensive effect which was readily blocked by pronethalol. The alkaloid also reversed the pressor response to adrenaline and in most instances completely blocked the pressor response to noradrenaline, but only partially blocked that due to tyramine. These effects lasted for up to 30 min after intravenous injection. Pronethalol blocked the effect of macusine B on the pressor response to adrenaline, noradrenaline and tyramine, but approximately 45 min after the administration of pronethalol it was found that, although the hypotensive response to macusine B was still blocked, the alkaloid could once more reduce the pressor response to adrenaline. In the experiments in which the electrocardiogram was recorded, macusine B alone was found to increase the heart rate for 5–10 min after injection.

In some experiments the alkaloid not only reversed the effect of adrenaline but also reversed that of noradrenaline. This was repeated several times on 3 of the 8 cats showing the noradrenaline reversal.

Macusine B did not affect the electrically stimulated contractions of the nictitating membrane. It also had no effect on the depressor response to acetylcholine and vagal stimulation nor on the pressor response which follows the occlusion of the carotid arteries for 10 sec.

In experiments in which the respiration was also recorded, the alkaloid depressed the respiratory rate and caused irregular breathing.

Blood glucose and plasma non-esterified fatty acids. Macusine B in a dose of 25 mg/kg (approximately half of the LD50 dose), completely blocked the hyperglycaemic effect of adrenaline. The alkaloid had only a slight effect on the blood glucose level when injected alone (Fig. 8).

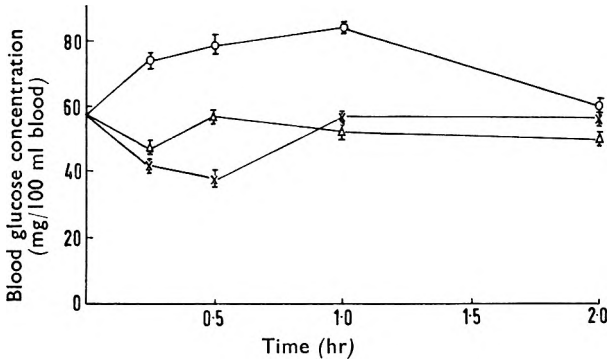

FIG. 8. Effect of macusine B on the blood glucose level of mice. Mice injected with 1 mg/kg adrenaline (s.c.). O; 25 mg/kg macusine B (i.p.) alone △ and 1 mg/kg adrenaline + 25 mg/kg macusine B (i.p.) ×. Blood samples withdrawn at 15, 30, 60 and 120 min after injection. Each point shows the mean ± standard error.

It significantly reduced the rise in plasma free fatty acids caused by the subcutaneous injection of adrenaline (Table 1). The dose of alkaloid used had no effect on the plasma free fatty acid level of the group injected with the alkaloid alone.

TABLE 1. EFFECT OF MACUSINE B ON THE NON-ESTERIFIED FATTY ACID LEVEL OF RATS

Group	NEFA μ-equiv./litre
Controls	535 ± 7·2* (7)
Adrenaline alone	725 ± 37† (4)
Macusine B alone	531 ± 40 (4)
Adrenaline + macusine B ..	567 ± 9·3 (4)

Controls given saline (i.p.). Groups given adrenaline alone (1 mg/kg s.c.), macusine B alone (15 mg/kg i.p.) or adrenaline (1 mg/kg s.c.) and macusine B (15 mg/kg i.p.).

* Mean ± standard error.

† Difference from controls significant ($P \geq 0.001$). Number of animals in each group indicated n parentheses.

Discussion

Structurally, macusine B resembles the ergot and rauwolfia alkaloids and the toxiferines, but its pharmacological activity more closely resembles that of the ergot alkaloids than that of the other groups. It is probable

that because of the high toxicity of toxiferine I, the activity of macusine B has been masked in previous investigations of the crude extracts of *S. toxifera.*

From this study it appears that the main effect of macusine B is on both α- and β-adrenergic receptors. The α-adrenergic-blocking effect of the alkaloid is suggested by its reversal of the pressor response to adrenaline and to its blocking the pressor response to noradrenaline in the cat. This effect is further suggested by its blocking the action of adrenaline on the acetylcholine-stimulated contractions of the rat colon. The β-sympathomimetic effect of macusine B is suggested by the fact that its hypotensive action is blocked by pronethalol. Since the alkaloid has no direct effect on the isolated heart, the hypotension is presumably due to vasodilatation in the vascular bed. Further evidence for the sympathomimetic effect is provided by the observation that the alkaloid relaxes the rabbit isolated duodenum and chick rectum and potentiates the depressor effect of adrenaline on the carbachol-stimulated rat uterus, an action blocked by pronethalol.

Macusine B blocks the action of tyramine on the rabbit isolated heart and this can explain the reduction in the pressor response to tyramine by the alkaloid *in vivo.* Burn & Rand (1958) suggested that tyramine causes its pressor response by liberating noradrenaline from endogenous stores and therefore the alkaloid could partially block the tyramine response *in vivo* by blocking the α-adrenergic receptors. In doing this, macusine B resembles the action of the ergot alkaloids (Swaine, 1963). There is now some evidence to suggest that tyramine has a direct sympathomimetic action which is independent of its ability to release noradrenaline (Vane, 1960; Nasmyth, 1962; Varma, Gillis & Benfrey, 1964) and therefore the effect of macusine B on the tyramine response may not be due entirely to its α-adrenergic blocking action.

Macusine B in a dose that causes hypotension does not depress the electrically stimulated contractions of the nictitating membrane. Furthermore it only depresses the contractions of the electrically stimulated guinea-pig vas deferens-hypogastric nerve preparation in a dose which is approximately eight times greater than any that is effective on other isolated organ preparations. It seems unlikely therefore that the alkaloid causes hypotension by ganglionic blockade.

The metabolic effects of adrenaline have been variously ascribed to stimulation of both the α- and β-adrenergic receptors or to the activation of some other type of receptor (Furchgott, 1959). If the glycogenolytic action of adrenaline can be explained in terms of the stimulation of α-receptors then it is apparent that macusine B blocks the hyperglycaemic effect of adrenaline by blocking these receptors and in so doing resembles the action of other compounds which block the α-adrenergic receptors. Thus Levi & McCutcheon (1964) in their study of the effect of several sympathomimetic amines and adrenergic blocking drugs, found that the hyperglycaemia and hyperlacticacidaemia caused by adrenaline was mainly due to stimulation of the α-receptors and that stimulation of the β-receptors had a much smaller effect. Similar results were reported

by Sutherland & Cori (1948) and Ellis (1956), while Ellis, Anderson & Collins (1953) found that dihydroergotamine effectively blocked the glycogenolytic acitvity of adrenaline *in vitro*. Nevertheless, these findings are in disagreement with those of Van der Pol (1956), McCutcheon (1962), Pilkington, Lowe, Robinson & Titterington (1962) and Hornbrook & Brody (1963) who showed that the glycogenolytic effects of adrenaline can be inhibited mainly by blocking the β-receptors.

Besides blocking the hyperglycaemic effect of adrenaline, macusine B also inhibits its lipid mobilising effect. Since the alkaloid only blocks the α-receptors it seems likely that the hyperlipaemia produced by adrenaline is due to the stimulation of the α-receptors, as has been suggested by Gordon & Cherkes (1956) and Jeanrenaud (1961) and not by the stimulation of the β-receptors as has been reported by Love, Caar & Ashmore (1963). Further evidence for the view that lipid mobilization is not due to stimulation of the β-receptors is provided by the observation that macusine B does not affect the plasma lipid levels when injected alone, even though it stimulates the β-receptors in several isolated organ and whole animal preparations. The seemingly irreconcilable findings reported in the literature for the type of receptor upon which adrenaline acts to produce its metabolic effects might be due to differences in the species or strain of animals used, to the metabolic effects being mediated by different receptors under different physiological conditions or in some way not involving receptors.

Macusine B is a competitive inhibitor of 5-HT on the guinea-pig isolated ileum, yet it reversibly inhibits the responses of the ileum to nicotine, which suggests that it is not a specific antagonist of 5-HT. Gaddum & Hameed (1954) suggested that 5-HT acts on specific receptors in the guinea-pig isolated ileum which are distinct from the nicotine receptors. Later, Gaddum & Picarelli (1957) advanced evidence suggesting that there were two types of tryptamine receptor in the terminal ileum of the guinea-pig, one type being associated with smooth muscle (D-receptor) and the other type associated with the ganglia (M-receptor). This view has been challenged by the investigations of Day & Vane (1963), who found that the effect of 5-HT on the guinea-pig ileum was primarily due to the agonist stimulating the receptors in the nervous tissue while the receptors associated with the smooth muscle were of little significance in eliciting the usual response. Brownlee & Johnson (1963) also showed that 5-HT contracted the ileum by activating receptors situated in the intramural parasympathetic ganglion cells and that these receptors were pharmacologically distinct from those activated by dimethylphenylpiperazinium and nicotine. It is possible that macusine B may inhibit the responses of the ileum to these agonists by acting on ganglia and that it may also have ganglion blocking or local anaesthetic activity but so far no direct evidence has been found for either activity in concentrations used to produce the pharmacological effects already described. The action of macusine B on the ganglia therefore resembles that of another α-adrenergic blocking drug phenoxybenzamine which inhibits the responses of the ileum to 5-HT and nicotine (Brownlee & Johnson, 1963). However,

the mode of action of macusine B and phenoxybenzamine on the responses of the ileum to these agonists must differ, for, whereas phenoxybenzamine depresses the responses to nicotine and 5-HT at concentrations which also depress the responses to acetylcholine and histamine (Brownlee & Johnson, 1963), macusine B only inhibits the 5-HT and nicotine induced responses. Macusine B also inhibits the effect of 5-HT on the isolated rat uterus presumably by acting on the tryptamine receptors which occur on the smooth muscle. Thus macusine B, while resembling the action of some of the ergot alkaloids in blocking the 5-HT—induced responses of the rat uterus, differs from these alkaloids in its ability to block the 5-HT and nicotine elicited responses of the guinea-pig ileum.

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References

Armitage, A. K. & Vane, J. R. (1964). *Brit. J. Pharmacol.*, **22**, 204–210.
Battersby, A. R., Binks, R., Hodson, H. F. & Yeowell, D. A. (1960). *J. chem. Soc.*, 1848–1854.
Birmingham, A. T. & Wilson, A. B. (1963). *Brit. J. Pharmacol.*, **21**, 569–580.
Black, J. W. & Stephenson, J. S. (1962). *Lancet*, **2**, 311–314.
Brownlee, G. & Johnson, E. S. (1963). *Brit. J. Pharmacol.*, **21**, 306–322.
Bülbring, E. (1946). *Ibid.*, **1**, 38–61.
Bülbring, E. & Wajda, I. (1945). *J. Pharmacol.*, **85**, 78–84.
Burn, J. H. & Rand, M. J. (1958). *J. Physiol.*, **144**, 314–336.
Day, M. & Vane, J. R. (1963). *Brit. J. Pharmacol.*, **20**, 150–170.
Duncombe, W. G. (1964). *Clin. Chim. Acta*, **9**, 122–125.
Ellis, S. (1956). *Pharmacol. Rev.*, **8**, 485–562.
Ellis, S., Anderson, H. L. & Collins, M. C. (1953). *Proc. Soc. exp. Biol., N.Y.* **84**, 383–386.
Furchgott, R. (1959). *Pharmacol. Rev.*, **11**, 429–441.
Gaddum, J. H. & Hameed, K. A. (1954). *Brit. J. Pharmacol.*, **9**, 240–248.
Gaddum, J. H. & Lembeck, F. (1949). *Ibid.*, **4**, 401–408.
Gaddum, J. H. & Picarelli, Z. P. (1957). *Ibid.*, **12**, 323–328.
Gordon, R. S. & Cherkes, A. (1956). *J. clin. Invest.*, **35**, 206–212.
Herring, D. A. & Marsh, D. F. (1951). *Fed. Proc.*, **10**, 309.
Hornbrook, K. R. & Brody, T. M. (1963). *J. Pharmacol.*, **140**, 295–307.
Huggett, A. St. G. & Nixon, D. A. (1957). *Lancet*, **2**, 368–370.
Jeanrenaud, B. (1961). *Metabolism*, **10**, 535–581.
Levi, B. W. & McCutcheon, R. S. (1964). *J. pharm. Sci.*, **53**, 503–506.
Love, W. C., Caar, L. & Ashmore, J. (1963). *J. Pharmacol.*, **140**, 287–294.
McCutcheon, R. S. (1962). *Ibid.*, **136**, 209–212.
Nasmyth, P. A. (1962). *Brit. J. Pharmacol.*, **18**, 65–75.
Paton, W. D. M. & Perry, W. L. M. (1951). *Ibid.*, **6**, 299–310.
Pilkington, T. R. E., Lowe, R. D., Robinson, B. F. & Titterington, E. (1962). *Lancet*, **2**, 316–317.
Schomburgk, R. (1879). In: *On the Urari; the deadly arrow poison of the Macusis, an Indian tribe in British Guiana, Adelaide, Australia,* and quoted in: *Curare its history, nature and clinical use,* McIntyre, A. R., p. 7–9, Univ. Chicago Press.
Sutherland, E. W. & Cori, C. F. (1948). *J. biol. Chem.*, **172**, 737–750.
Swaine, C. R. (1963). *Proc. Soc. exp. Biol., N.Y.*, **112**, 388–390.
Timms, A. R. (1956). *Brit. J. Pharmacol.*, **11**, 273–281.
Van der Pol, M. C. (1956). *Acta physiol. pharmacol. Néerl.*, 541–546.
Vane, J. R. (1960). In *Ciba Foundation Symposium on Adrenergic Mechanisms.* Editors, Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M., p. 356–372, London: Churchill.
Varma, D. R., Gillis, R. A. & Benfrey, B. G. (1964). *J. Pharmacol.*, **144**, 181–185.
Weil, C. S. (1952). *Biometrics*, **8**, 249–263.

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The compaction of some solid lubricant materials‡

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Samples of −100 mesh powders of stearic acid, palmitic acid, sodium stearate, potassium stearate, lithium stearate, calcium stearate, magnesium stearate, zinc stearate, zinc oleate, boric acid and a synthetic wax, were compacted and the die reaction determined using a "moving-die" technique. The shear strength of the compacts measured in a punch penetration test, with the exception of boric acid and zinc stearate, was independent of the compaction pressure above 500 kg/cm². Solid discs of stearic acid and palmitic acid possessed the same shear strength as the powder compacts; discs of synthetic wax and zinc stearate gave higher values than the corresponding powder compacts. Values of shear strength calculated from measurements of die reaction were higher than values obtained in the shear strength test, but show good agreement with the results calculated by other workers from sliding friction experiments. Shear strength values for compacts of boric acid and discs of synthetic wax, talc crystal, and graphite, indicate that these materials are unlikely to be such good lubricants as stearic acid or its salts.

IT has been shown (Hersey, 1960) that friction theory as propounded by Bowden & Tabor (1954) can be applied to a compacting system. Hence the force lost to the die wall, F_d, will be equal to the product of the true area of compact-die interface, A, and the shear strength of the friction junction, S.

$$F_d = A.S \quad .. \quad .. \quad .. \quad .. \quad (1)$$

Perfectly clean surfaces of similar or dissimilar materials will adhere with a strength equivalent to the bulk strength of the material (Biewend, 1842; Tomlinson, 1927; Bowden & Hughes, 1939). Almost any contaminant at the interface, e.g., an oxide layer, moisture or grease, will reduce the adhesional forces and hence the sliding friction.

Lubricants, as distinct from glidants, are used in compaction processes to facilitate consolidation under pressure and reduce ejection forces, the shear strength of the friction junctions being effectively reduced by interposing materials of low shear strength between the sliding surfaces. Talc, stearic acid, boric acid, paraffin wax and salts of stearic acid, are solid materials listed (Little & Mitchell, 1963) as being in frequent use.

In the concentrations normally used in pharmaceutical tabletting, sufficient lubricant is present to maintain a film on the surface of the die. The effectiveness of the film will depend on its shear strength, the force with which it adheres to the metal of the die, its resistance to penetration by the material of the compact, and its resistance to wear.

The object of the present work was to study the consolidation of some solid lubricant powders into compacts, the shear strength of the compacts being measured in a punch penetration test and correlated with friction measurements.

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‡ This work formed part of a thesis (C.J.L.) accepted for the degree of Ph.D. in the University of London.

Experimental

MATERIALS AND APPARATUS

The materials examined were stearic acid, palmitic acid, sodium stearate, potassium stearate, lithium stearate, calcium stearate, magnesium stearate, zinc stearate, zinc oleate, talc, boric acid and synthetic wax. All materials were sieved for 15 min using B.S. sieves on an Inclyno machine, and −100 mesh fractions were used.

Where possible the materials used were of B.P. or B.P.C. quality, but in the absence of official standards the best quality technical grade material was used.

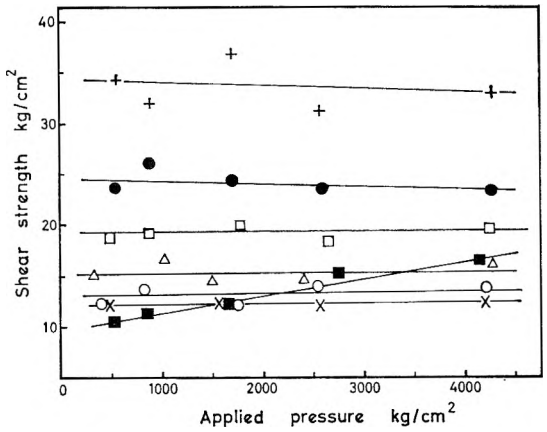

FIG. 1. Effect of applied pressure on shear strength of compacted lubricant powders. + Sodium stearate. ● Potassium stearate. □ Zinc oleate. △ Calcium stearate. ■ Zinc stearate. ○ Magnesium stearate. × Stearic acid.

Synthetic wax (Synthetic wax flake W.C. 5956; Wilkins, Campbell and Co.) consists of straight chain hydrocarbons of average molecular weight 750, and has a melting point of 105°. The material was reputed to have the pressing characteristics of a brittle substance, and was supplied in large flakes; a sample was ball-milled to obtain −100 mesh powder.

The apparatus used for compressing the powders has been described (Lewis & Train, 1965a). The shear strengths of the compacts were measured using a punch penetration test originally used with solid homogeneous discs (Train & Hersey, 1960), and subsequently applied to compacts of crystalline materials (Lewis & Train, 1965b).

METHODS

Samples of boric acid powder (5 g) and samples of the other materials (4 g) were compacted at various pressure levels up to 4,300 kg/cm² using the moving-die technique under standardised operating conditions (Lewis & & Train, 1965a). The applied pressure, P_a, the die reaction, F_d, and change in length of compact with pressure were recorded for each pressing. The compacts were ejected from the die and the shear strength measured.

To assess more accurately the shear strength of boric acid compacts, five replicate determinations were made on material pressed to 4,000 kg/cm². A similar number of replicate measurements were made on compacts of the other materials pressed to 1,725 kg/cm². Values of die reaction were not required from these pressings and the die remained stationary during the compaction.

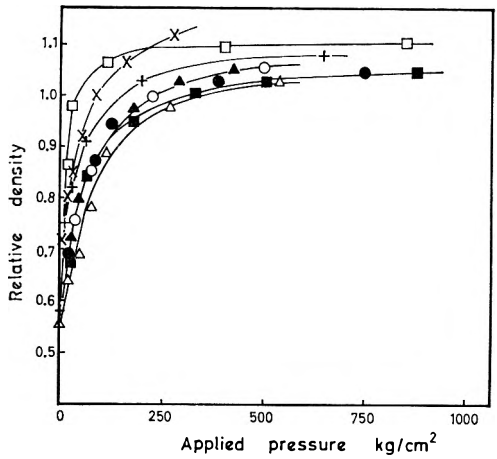

FIG. 2. The relationship between applied compaction pressure and relative density of compact. + Sodium stearate. ● Potassium stearate. □ Palmitic acid. △ Calcium stearate. ○ Magnesium stearate. ■ Zinc stearate. × Stearic acid. ▲ Lithium stearate.

Stearic acid, palmitic acid, synthetic wax, hard paraffin and zinc stearate, were melted with the minimum of heat and cast into cylindrical blocks approximately 3·5 cm diameter. Solid blocks of talc crystal and graphite were available. From these blocks of materials, discs 2·42 cm diameter and approximately 0·6 cm long were cut on a lathe. The shear strengths of these solid discs were estimated under the same conditions as those used for the compacts.

Results and discussion

The shear strengths of compacts made from powdered stearates of magnesium, sodium, potassium and calcium are virtually unaffected by the magnitude of the compacting pressure above approximately 500 kg/cm² (Fig. 1). Stearic acid and zinc oleate behave in a similar manner but zinc stearate shows a small but steady increase in shear strength with pressure. It was observed that all compacts of stearic acid and the stearates tended to laminate as they were ejected from the die. The measured length of the compact thus depended on the way the micrometer gauge was handled during the measurement, the laminated compact possibly being compressed. This led to some variation in measurements of length of compact, which probably accounts in part for the observed variations in strength.

That shear strength values are essentially constant over a pressure range 500–4,250 kg/cm² is explained by the fact that these materials attained zero porosity at pressures less than 500 kg/cm² (Fig. 2), so that an increase in pressure above that figure does not produce any greater

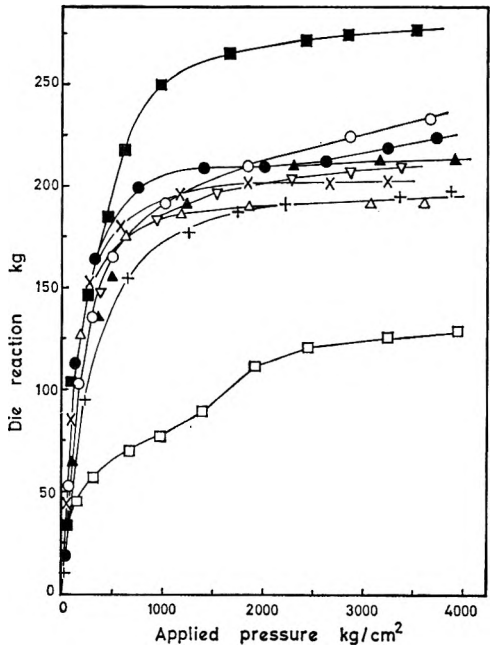

FIG. 3. Effect of applied compaction pressure on die reaction. + Sodium stearate. ● Potassium stearate. ▽ Palmitic acid. △ Calcium stearate. ■ Zinc stearate. ○ Magnesium stearate. ▲ Lithium stearate. × Stearic acid. □ Zinc oleate.

densification and only serves to aggravate lamination of the compact. Apparent values of relative density ρ_R (ratio of apparent density of compact to density of solid) greater than unity may be explained by the extrusion of material past the punch tips, this being particularly noticeable with stearic acid and palmitic acid. The difficulty of containing the experimental material was so great with zinc oleate that calculated values of ρ_R were meaningless.

Frictional losses for the above materials increase with pressure initially (Fig. 3) and then approach a constant value at pressures greater than 1,500 kg/cm². Except for zinc stearate and zinc oleate the maximum values of die reaction, F_d, lie within the range 190–225 kg.

Boric acid produces a greater die reaction (Fig. 4) than the soap lubricants, and it is found that the shear strengths of the compacts are also higher. In addition the shear strength of the compact increased with increased compaction pressure to a maximum at a pressure of 4,000 kg/cm² (Fig. 5). Although of a waxy nature, synthetic wax produced die wall frictional forces similar to boric acid (Fig. 4) indicating the unsuitability

of these two materials as die wall lubricants when a constraining load is applied.

Table 1 lists values of shear strength for the compacted powders and the solid discs. Using values of shear strength for powder compacts, and calculating the area of compact-die wall interface from experimental

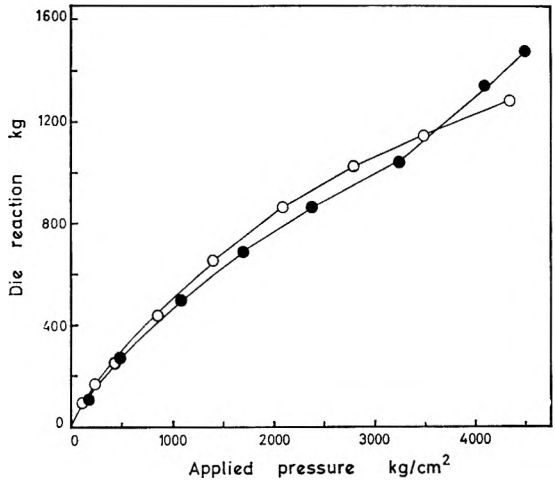

FIG. 4. Effect of applied compaction pressure on die reaction. ● Synthetic wax. ○ Boric acid powder.

measurements of length of compact at a given pressure, values for F_d were calculated from equation (1) and are compared with the maximum experimental values, also in Table 1. Experimental and calculated values were only compared for those materials attaining an approximately constant value of F_d as the compacting pressure increased.

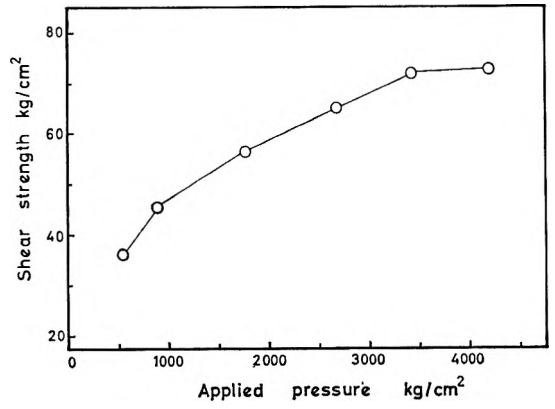

FIG. 5. Effect of applied pressure on shear strength of compacts of boric acid powder.

Most of the salts of stearic acid investigated decompose at high temperatures without melting, so that it was not possible to manufacture solid discs. Compacts of talc powder suffered gross lamination and fracture on ejection from the die so that the shear strength of a compact could not be measured for comparison with solid discs.

Only the calculated values for the sodium and potassium stearates are comparable with the observed practical results. Maximum die reaction values in the range 190–225 kg would require a material possessing a shear strength of 28–34 kg/cm², i.e., approximately twice the measured value for compacts of stearic acid, palmitic acid, calcium stearate and magnesium stearate, three times larger in the case of zinc stearate, and five times larger in the case of lithium stearate.

TABLE 1. THE SHEAR STRENGTH OF LUBRICANT MATERIALS AND VALUES OF DIE REACTION, F_d

Material	1 Shear strength powder compacts* kg/cm²	2 Shear strength solid discs** kg/cm²	3 Max. exptl. die reaction F_d, kg	4 Calculated die reaction† F_d, kg
Lithium stearate	6·0	—	212	41
Zinc stearate	9·3	20·2	275	61
Palmitic acid	12·3	12·7	208	89
Stearic acid	13·7	13·3	200	99
Calcium stearate	15·0	—	190	101
Magnesium stearate	20·0	—	230	135
Potassium stearate	31·3	—	210	212
Sodium stearate	33·9	—	195	229
Synthetic wax	50·5	65·0	—	—
Boric acid	73·0	—	—	—
Hard paraffin	—	19·0	—	—
Talc, with grain	—	63·2	—	—
Talc, cross grain	—	80·0	—	—
Graphite	—	75·0	—	—

* Mean of five determinations. ** Mean of three determinations. † From data in column 1.

Shear strengths calculated from F_d values show good agreement with the results of Bailey & Courtney-Pratt (1955), who found that bimolecular films of calcium stearate possessed a shear strength of 25 kg/cm², and that multi-layers gave a lower value. Wilson (1955) reported shear strength values for stearic acid in the range 27–35 kg/cm², and indicated that the friction observed was mainly due to shearing of the lubricant film. Correlation is probably the more remarkable when it is realised that the results of Wilson, and of Bailey and Courtney-Pratt, were obtained in sliding friction experiments using very refined techniques.

Comparison of the present results with those of other workers gives rise to three considerations.

(1) The punch penetration shear test produces shear in multilayers and not bimolecular layers; the low values of shear strength obtained can then be explained (cf. Bailey & Courtney-Pratt, 1955).

(2) Experimental values of die reaction, F_d, are due to shear of bimolecular, or very thin films.

(3) If the shear strength of the compact is the true value for the materials used, the higher values calculated from F_d measurements may be due to the material possessing a higher shear strength when it is subject to a

compressive load. This would be consistent with the findings of Bridgman (1946), Boyd & Robertson (1945), and Cameron (1960).

Further work on the measurement of shear strength of lubricant powder compacts subjected to a compressive load will help to clarify the matter.

Solid discs of zinc stearate have twice the shear strength of the powder compacts (Table 1), although the two sets of values for stearic acid and palmitic acid demonstrate single values within the limits of experimental error. It may reasonably be assumed that the film of compacted lubricant powder on the die wall will behave in the same manner as the solid material under conditions of shear. The results for zinc stearate seem to indicate that for salts of stearic acid the shear strength of the solid material may be greater than that of a powder compact. This would account for the difference between calculated and experimental values of F_d (columns 3 and 4, Table 1).

Shear strength values for talc and graphite discs show how less desirable these materials may be as lubricants compared to the fatty acid and soap materials, especially when subject to a constraining load (Train & Hersey, 1960). Present results for the shear strength of talc discs are higher than those obtained by Train & Hersey, and are almost certainly due to the increased rate at which the shearing load was applied.

If the shear strength of the lubricant is the main factor affecting its efficiency during normal pelletting and tabletting procedures, then the material with the lowest shear strength should be the most efficient. The materials used in this study have been evaluated as lubricants on an instrumental tablet machine and it is hoped to publish the results elsewhere.

References

Bailey, A. I. & Courtney-Pratt, J. S. (1955). *Proc. roy. Soc.*, **A227**, 500–515.
Biewend, E. (1842). *Ann. Physik.*, **57**, 164–165.
Bowden, F. P. & Hughes, T. P. (1939). *Proc. roy. Soc.*, **A172**, 263–279.
Bowden, F. P. & Tabor, D. (1954). *The Friction and Lubrication of Solids*, Oxford: Clarendon Press.
Boyd, J. & Robertson, B. P. (1945). *Trans. Amer. Soc. Mech. Engrs.*, **67**, 51–56.
Bridgman, P. W. (1946). *Rev. Mod. Phys.*, **18**, 1–93.
Cameron, A. (1960). *Amer. Soc. Lubric. Engrs. Trans.*, **2**, 195–198.
Hersey, J. A. (1960). Ph.D. Thesis, University of London.
Lewis, C. J. & Train, D. (1965a). *J. Pharm. Pharmacol.*, **17**, 33–41.
Lewis, C. J. & Train, D. (1965b). *Ibid.*, **17**, 1–9.
Little, A. & Mitchell, K. A. (1963). *Tablet Making*, 2nd ed., Liverpool: Northern Publishing Co.
Tomlinson, G. A. (1927). *Proc. roy. Soc.*, **A115**, 472–483.
Train, D. & Hersey, J. A. (1960). *J. Pharm. Pharmacol.*, **12**, *Suppl.* 97*T*-104*T*.
Wilson, R. W. (1955). *Proc. Phys. Soc.*, **B65**, 625–641.

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Effect of some spasmolytic drugs on the isolated human myometrium

The late D. N. LEHRER

Spasmolytic drugs possessing a musculotropic action appear to be the most effective in inhibiting the motility of the human myometrium *in vitro*. Substances whose action is mediated via a predominently β-receptor mechanism have relatively little or no effect on this preparation.

A NUMBER of substances which possess an excitatory action on the human uterus exist, but there is a paucity of drugs which can inhibit its spontaneous motility *in vivo*. Such drugs would be of potential importance in the management of certain obstetric and gynaecological problems such as dysmenorrhoea (Bygdeman & Eliasson, 1964), and premature labour (Hendricks, Cibils, Pose & Eskes, 1961), prematurity being a leading cause of perinatal mortality (Johnson, McGaughey, Scoggin, Wilson & Thornton, 1963).

The object of the work described here was threefold; firstly, to re-investigate the action of some drugs of known activity on the isolated human myometrium preparation; secondly, to establish if possible the type of activity required of a compound for it to inhibit the isolated human myometrium; and thirdly, to investigate the action of some drugs not previously tested on this preparation.

Experimental

Human uteri were obtained from patients aged 35–45 years, having undergone hysterectomy, usually for menorrhagia, and all uteri were postpartum. Each uterus was stored in a salt solution of the following composition: g/litre NaCl, 9·0; KCl, 0·4; $CaCl_2$, 0·24; $NaHCO_3$, 0·4; glucose, 0·1; at 4° for 12–24 hr before experiment. Segments were prepared as described by Chambers & Pickles (1958) and suspended in the aerated physiological solution in a 50 ml bath at 38°. Isotonic contractions were recorded with frontal writing lever, at a tension of 1 g and magnified ten times.

Drugs. Noradrenaline hydrochloride, adrenaline hydrochloride, phenylephrine hydrochloride, isoprenaline hydrochloride, papaverine hydrochloride, orciprenaline (Alupent), isoxsuprine, Efosin (Hoechst) [a mixture of 1-(3,3-diphenylpropyl) piperidine and αα-diethyl-1-piperidine butyramide], fencamfamin, (*m*-methoxy-α-methylphenethyl) (*m*-methoxyphenethyl)amine hydrochloride (AH.1101), 1-(3,4-dihydroxyphenyl)-2-hexylaminoethanol hydrochloride (AH.2139).

Results

The effects of noradrenaline, phenylephrine, adrenaline, isoprenaline, orciprenaline, isoxsuprine, papaverine, Efosin, fencamfamin, AH.2139 and AH.1101 on the isolated human myometrium preparation are summarised in Table 1.

The predominantly α-receptor stimulant sympathomimetic amines noradrenaline and phenylephrine and the αβ-receptor stimulant adrenaline all produce a contraction of the isolated human myometrium (Fig. 1).

TABLE 1. THE EFFECT OF SOME COMPOUNDS ON THE ISOLATED HUMAN MYOMETRIUM

Drug	Class	Dose μg/ml		Effect on human myometrium *in vitro*
Noradrenaline ..	α-predominant adrenergic stimulant	2·5	(3)	Marked rise in tone
Phenylephrine ..	"	25·0	(1)	Rise in tone
		250·0	(2)	Rise in tone
Adrenaline ..	αβ-adrenergic stimulant	0·04	(2)	Rise in tone
		1·50	(2)	Rise in tone
Isoprenaline ..	β-predominant adrenergic stimulant	2·0	(1)	No effect
		2·5	(1)	No effect
		6·25	(1)	No effect
		12·50	(1)	No effect
		25·0	(4)	Variable effect: (*a*) No effect (*b*) Fall in tone (*c*) Slight rise followed by slight fall
Orciprenaline ..	β-predominant adrenergic stimulant	12·0	(1)	No effect
		25·0	(1)	Very slight fall in tone.
Isoxsuprine ..	β-adrenergic stimulant + musculotropic activity	50·0	(1)	No effect
		100·0	(1)	No reduction in tone but abolition of contractions Long duration of action
AH. 2139 ..	β-predominant adrenergic stimulant	25·0	(2)	No effect
Papaverine ..	Musculotropic spasmolytic	2·0	(1)	Fall in tone
		2·5	(3)	Fall in tone
		6·25	(1)	Very marked fall in tone
		10·0	(2)	Marked fall in tone
		12·5	(1)	Very marked fall in tone
		20·0	(1)	Very marked fall in tone
Fencamfamin ..	Musculotropic spasmolytic	25·0	(2)	Fall in tone
Efosin	Musculotropic spasmolytic	6·25	(2)	Fall to very marked fall in tone
		10·0	(1)	Marked fall in tone
AH.1101 ..	Musculotropic spasmolytic	6·25	(1)	Fall in tone
		15·0	(1)	Marked fall in tone
		18·8	(1)	Marked fall in tone

Figures in parentheses indicate the number of experiments.

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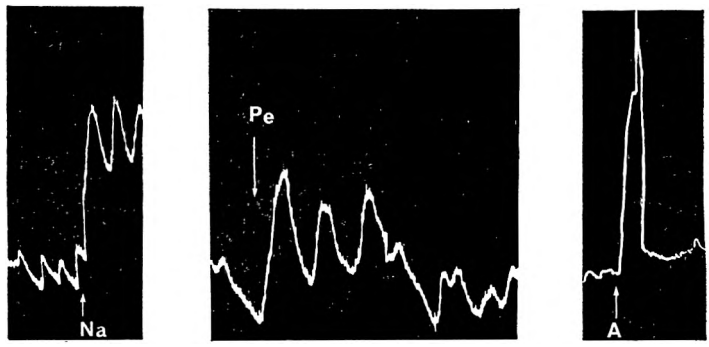

FIG. 1. Isolated human uterus. The effect of some sympathomimetic amines. A Na, noradrenaline, 2 μg/ml; Pe, phenylephrine, 200 μg/ml; A, adrenaline, 1 μg/ml

Phenylephrine showed less activity than either adrenaline or noradrenaline. The predominantly β-receptor stimulant isoprenaline had no effect on the preparation below the high concentration of 25 μg/ml. At this dose it gave variable effects; two administrations of the drug produced a fall in tone, one produced a very slight rise followed by an equally slight fall and a fourth administration had no effect at all. No effect was also obtained with the predominently β-receptor stimulant orciprenaline.

In contrast to these results, papaverine, at concentrations ranging from 2–20 μg/ml, consistently caused a decrease in tone and abolished the spontaneous contractions of this preparation (Fig. 2). A similar effect

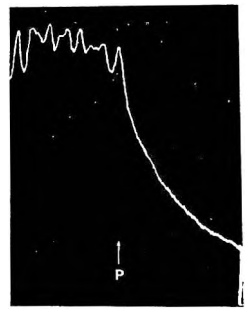

FIG. 2. Isolated human uterus. The effect of papaverine, a musculotropic spasmolytic drug. At P, 20 μg/ml papaverine was added to the organ bath.

was seen with fencamfamin or Efosin, both of which possess a predominently musculotropic action.

Isoxsuprine, which has a β-receptor excitor action (Lish, Dungan & Peters, 1960) and a papaverine-like action (Lish, Hillyard & Dungan, 1960), occupied an intermediate position in that although the drug was effective in inhibiting the motility of the human myometrium, the dose necessary was greater than that required for the other effective spasmolytics under test (Fig. 3).

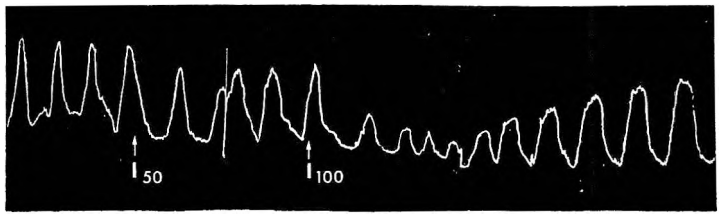

FIG. 3. Isolated human uterus. The effect of isoxsuprine, a drug possessing both β-stimulant sympathomimetic and musculotropic spasmolytic properties. At I_{50}, 50 μg/ml; I_{100}, 100 μg/ml of isoxsuprine was added to the organ bath.

During the evaluation of a number of new compounds, including two phenethylamines AH.2139 and AH.1101, it was found that AH.2139 possessed potent β-stimulant activity whilst AH.1101 was direct acting in the manner of papaverine. Of these two compounds, AH.1101 had marked spasmolytic activity on the isolated human uterus whilst AH.2139

was without effect. One experiment with AH.1101 is illustrated in Fig. 4.

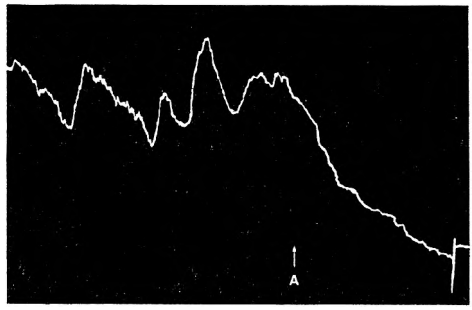

FIG. 4. Isolated human uterus. The effect of AH.1101, a musculotropic spasmolytic. At A, 20 μg/ml AH.1101 was added to the organ bath.

Discussion

Neurotropic agents such as atropine (Quadros & Sinha, 1960; Sandberg, Ingelman-Sundberg, Lindgren & Ryden, 1961) and hyoscine *N*-butyl bromide (Quadros & Sinha, 1960) are relatively ineffective in relaxing the isolated human myometrium preparation. Isoprenaline, which is a predominantly β-receptor activator has been reported to be inactive in relaxing the isolated human uterus and has, in fact, been shown to stimulate this preparation at high dosage (Quadros & Sinha, 1960). The action of adrenaline has been shown (Adair, 1935) to stimulate the excised human uterus, and this occurs on both gravid and non-gravid uteri. *In vivo*, however, adrenaline may either relax or contract the uterus depending on the contractile state, and on whether the uterus is in the pregnant or non-pregnant state (Garrett, 1959). Noradrenaline stimulates the human uterus under all these conditions (Bourne & Burn, 1927; Kaiser, 1950; Alvarez & Caldeyro-Barcia, 1954; Garrett, 1954, 1955). Rudzik & Miller (1962) have stated the activity of relaxin to be mediated by the release of catecholamines, in particular adrenaline, but relaxin was found not to affect the motility of the isolated human uterus.

With those substances whose action is mediated through a musculotropic papaverine-like activity, there is evidence which suggests that the motility of the human uterus is always inhibited. Jung (1962) showed that the musculotropic spasmolytic drugs Efosin, Erantin (α-2-dimethylamino-1-methylethyl)-α-phenylphenethyl propionate) and AD.205 [(2-benziloyloxyethyl)dimethyl octylammonium bromide] markedly inhibited the spontaneous contractility of the isolated human uterus preparation. The high antispasmodic effect of Efosin had been reported earlier by Quadros & Sinha (1960). Additional drugs of this category which have been shown to possess relaxant activity on the isolated human uterus preparation include papaverine and Monzal [1-(3,4-dimethoxyphenyl) -4-phenylbutyldimethylamine] (Wagner & Kessler, 1958) and papaverine and Spasmaverine [ethyldi(3-phenylpropyl)amine] (Sandberg, Ingelman-Sundberg, Lindgren & Ryden, 1961).

Bygdeman & Eliasson (1964) have shown that isoxsuprine has a spasmolytic action on the *in vivo* human uterus, and Lish & others (1960) reported it to have a direct musculotropic action in addition to activating β-receptors. It seems that the former mechanism is the more important. Recently bradykinin has been reported as having a powerful spasmolytic activity on the human uterus *in vitro* (Landesman, Campbell & Wilson, 1963), and once again this seems to be a direct action since Khairallah & Page (1961) have shown bradykinin to act directly on intestinal smooth muscle.

There is therefore some evidence to suggest that compounds likely to relax the isolated human myometrium preparation must possess a predominently musculotropic spasmolytic activity. Additional support to this evidence is given in this paper. Substances whose effects are mediated through a predominantly α-receptor mechanism, such as phenylephrine and noradrenaline, and an α- and β-receptor mechanism such as adrenaline, cause an increase in tone of the isolated human myometrium preparation. Substances like isoprenaline, orciprenaline and AH.2139, whose actions are mediated through a predominantly β-receptor mechanism, have no effect on this preparation unless very high concentrations are used, when a fall in tone may occasionally be observed, but these effects could not be reliably repeated. On the other hand, papaverine and drugs under investigation which possessed a predominantly musculotropic action consistently produced a marked fall in tone of the isolated human uterus preparation and abolished its spontaneous motility.

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References

Adair, F. L. & Haugen, J. A. (1939). *Am. J. Obstet. & Gynaec.*, **37**, 753–762.
Ahlquist, R. P. (1948). *Amer. J. Physiol.*, **153**, 586–600.
Alvarez, H. & Caldeyro-Barcia, R. (1954). *Gynaecologia, Basle*, **138**, 190–212.
Bourne, A. W. & Burn, J. H. (1927). *J. Obstet. Gynaec. Brit. Emp.*, **34**, 249–272.
Bygdeman, M. & Eliasson, R. (1963). *Experientia*, **19**, 650–651.
Chambers, P. L. & Pickles, V. R. (1958). *J. Physiol.*, **144**, 68–79.
Garrett, W. J. (1954). *J. Obstet. Gynaec. Brit. Emp.*, **61**, 586–589.
Garrett, W. J. (1955). *Ibid.*, **62**, 876–883.
Garrett, W. J. (1959). *Ibid.*, **66**, 927–938.
Kaiser, I. H. (1950). *Surg. Gynaec. Obstet.*, **90**, 649–654.
Hendricks, C. H., Cibils, L. A., Pose, S. V. & Eskes, T. K. (1961). *Amer. J. Obstet. & Gynaec.*, **82**, 1064–1078.
Johnson, W. L., McGaughey, H. S., Scoggin, W. A., Wilson, L. A. & Thornton, W. N. (1963). *Ibid.*, **86**, 296–302.
Jung, H. (1962). *Geburtsh. Frauenheilk.*, **22**, 635–648.
Khairallah, P. A. & Page, I. H. (1961). *Amer. J. Physiol.*, **200**, 51–54.
Landesman, R., Campbell, W. L. & Wilson, K. H. (1963). *J. Obstet. & Gynaec.*, **22**, 102–107.
Lish, P. M., Dungan, K. W. & Peters, E. L. (1960). *J. Pharmacol.*, **129**, 191–199.
Lish, P. M., Hillyard, I. W. & Dungan, K. W. (1960). *Ibid.*, **129**, 438–444.
Quadros, M. & Sinha, Y. K. (1960). *J. Obstet. & Gynaec. Brit. Emp.*, **67**, 659–662.
Rudzik, A. D. & Miller, J. W. (1962). *J. Pharmacol.*, **138**, 82–87.
Sandberg, F., Ingelman-Sundberg, A., Lindgren, L. & Ryden, G. (1961). *J. Obstet. & Gynaec. Brit. Common.*, **68**, 1000–1007.
Wagner, H. & Kessler, R. (1958). *Gynaecologia*, **146**, 459–460.

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A new and sensitive bioassay for catecholamines

I. S. DE LA LANDE AND JUDITH A. HARVEY

The isolated central artery of the rabbit ear is highly sensitive to catecholamines when perfused with Krebs solution containing 5-hydroxytryptamine. The preparation responds to 1 ng of noradrenaline and is extremely long lasting. It does not discriminate between noradrenaline and adrenaline.

IN the course of experiments designed to measure the output of catecholamines from the isolated rabbit ear (de la Lande, Paton & Waud, 1964) it was observed that segments of the central artery displayed high sensitivity to nerve stimulation and to catecholamines. The effects of nerve stimulation and vasoactive drugs are discussed elsewhere (de la Lande & Rand, 1965); the present report describes a simple and highly sensitive method of assaying catecholamines on the arterial segment. It is based on the finding that 5-HT, besides causing vasoconstriction, greatly enhanced the sensitivity of the artery to noradrenaline (de la Lande & Rand, 1965).

Methods

Lop-eared rabbits are anaesthetised with urethane, 1·76 g/kg intraperitoneally. The central artery of the ear is exposed, and a portion of the artery, 5 to 7 cm in length, is excised together with the central vein and closely adhering connective tissue. The artery is cannulated at its proximal end and suspended in an organ bath (Fig. 1). The lumen is

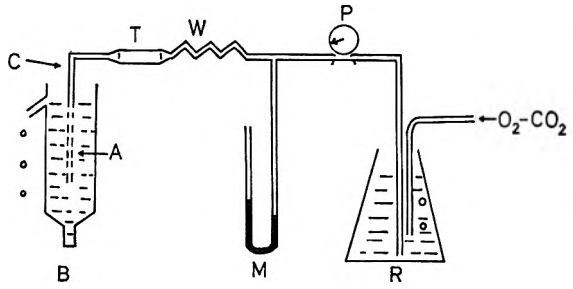

FIG. 1. Diagram of apparatus described in text. Symbols: A, isolated central artery; B, organ bath at 37°; C, cannula; M, mercury manometer; P, roller pump; R, reservoir of Krebs solution at 37°; T, rubber tubing; W, warming coil at 37°.

perfused at 37° with Krebs bicarbonate solution containing 5-hydroxytryptamine creatinine sulphate (5-HT), 0·04 μg/ml, and gassed with 95% oxygen and 5% carbon dioxide. The outflow from the artery is allowed to drain by upward displacement so that the preparation is completely immersed in the organ bath. Perfusion is by a constant-volume roller pump and the rate of perfusion is maintained at approximately 8 ml/min.

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Drugs, dissolved in normal saline, are injected into the system through rubber tubing attached to the proximal end of the cannula. Changes in the diameter of the artery cause a change in the perfusion pressure which is measured by a Condon mercury manometer. The resting perfusion pressure is usually about 30–40 mm of mercury.

Results

SENSITIVITY

The response of the preparation comprises a transient rise in perfusion pressure due to the injection volume, followed by a further rise in pressure, the magnitude of which is a function of the amount of noradrenaline injected. The relation between dose and response and the high sensitivity obtained is illustrated by the records of two preparations in Figs 2 and 3.

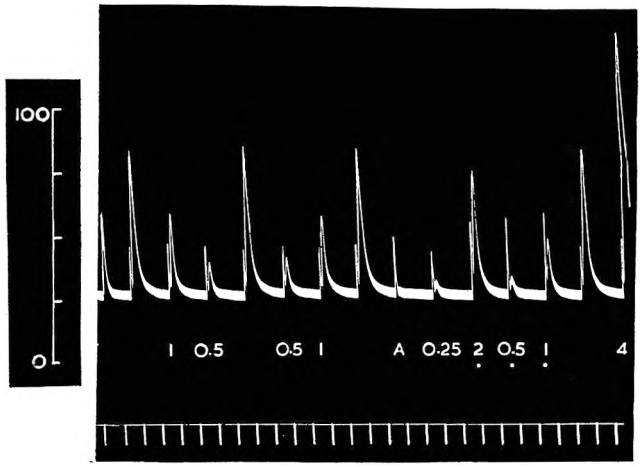

FIG. 2. The effect of graded doses of noradrenaline (ng) on the isolated perfused artery. The volumes injected are 0·2 ml except where the dose of noradrenaline is marked with a white dot, where 0·4 ml was used. Note that the response heights are depressed with the greater volumes. A is the injection of 0·2 ml of saline alone and illustrates the injection artifact. The interval between injections is 2 min. The scale on the left is perfusion pressure in mm Hg.

The sensitivity permits the detection of 0·5 ng and the precise assay of 1 ng of noradrenaline; this degree of sensitivity has been reproduced in each of eleven preparations. When expressed in terms of concentration, the limit of sensitivity is 1–2 ng/ml. The limit is imposed in part by the need to keep the volume of injection small (below 0·4 ml) to avoid interference by an excessive injection artifact (Fig. 2).

Features of the preparation are the length of time for which it can be used and the absence of major fluctuations in sensitivity over long periods. Fig. 3, upper frame, shows a portion of a record for a preparation which received a test dose of noradrenaline of 1 ng at 2 min intervals for 8 hr after commencing infusion; the lower frame shows the responses of the same preparation on the following day, after storage at 4° overnight.

SPECIFICITY

Comparison with other naturally occurring amines and polypeptides is shown in Table 1. The excitatory substances listed are those which produce a similar response to noradrenaline; of these, only adrenaline displays comparable activity. Dopamine, 5-HT, and angiotensin are less active than noradrenaline by factors of more than 30, 50 and 50 respectively. An estimate for histamine is not possible in view of the marked

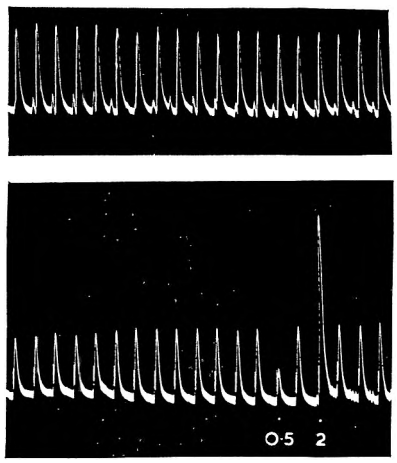

FIG. 3. Effects of cold storage. *Upper frame:* responses of a preparation to 1 ng of noradrenaline every 2 min 2 hr after commencement of infusion. *Lower frame:* responses of the same preparation after 6 hr infusion, storage at 4° for 15 hr, and infusion for a further hr. The doses are 1 ng except where otherwise shown. The vertical scale is as for Fig. 2.

TABLE 1. RELATIVE ACTIVITIES ON THE ISOLATED ARTERY

Substance	Action	Equipotent ratio (NA = 1)
Adrenaline	Excitatory	4, 1, 1, 1, 1, 0·8, 0·5
Dopamine	Excitatory	100, 66, 30, 30
Histamine	Excitatory	40, 20, 10, 10 (>100), 1·4, 1 (>1,000)
5-HT	Excitatory	250 (1,000), 250 (1,000), 100 (300), 40
Angiotensin	Excitatory	200, 125, 100, 50
Acetylcholine	Depressant	<100 (>100), >10, >20 (>150)
Bradykinin	Depressant	>100, >100, >50

With excitatory substances, a figure of 100 means that 100 ng of the substances produces a response equal to that of 1 ng of noradrenaline. The figures for the depressants refer to the amount of substance which depresses the response to noradrenaline (1 to 2 ng) by less than 10%. The figures in brackets refer to the ratios measured in the presence of the following antagonists: for histamine, mepyramine maleate 50 µg/litre, for 5-HT, methysergide bimaleate 50 µg/litre, and for acetylcholine, hyoscine hydrobromide 20 µg/litre.

variation in sensitivity between different preparations, which is some 40-fold. Acetylcholine and bradykinin depress the response to noradrenaline in concentrations of about 10 and 50 times greater, respectively, than those of noradrenaline. The vasoconstrictor effects of 5-HT, histamine and acetylcholine are substantially reduced by the addition of appropriate antagonists to the infusion fluid (Table 1). The antagonists

do not affect noradrenaline sensitivity. The absence of change to sensitivity after methysergide implies that antagonism by this drug extends only to the vasconstrictor effect of 5-HT.

PRECAUTIONS

Points of procedure which have been routinely followed in developing the method and applying it to the assay of catecholamines are as follows:

(1) 5-HT was added to the infusion medium shortly *after* commencement of infusion.

(2) The volumes of test and unknown solutions of noradrenaline, and also their concentrations, were adjusted wherever practical to provide approximately the same injection volumes (less than 0·5 ml) and response heights (less than 100 mm of mercury).

(3) A constant time interval between injections was rigorously followed. In most preparations 2 min proved a suitable compromise between the opposing requirements of speed of assay and the time required for recovery of sensitivity following the preceding response.

(4) During intervals between assays the preparation received a constant test dose of noradrenaline at 2 min intervals by automatic syringe.

Discussion

The most sensitive preparations available for the assay of noradrenaline are the pithed rat, and the superfused rat stomach strip sensitised by 5-HT (Armitage & Vane, 1964). The pithed rat is more specific for noradrenaline than the isolated perfused artery, which is also the least sensitive of the three preparations. The significant advantage of the arterial segment is that the preparation can be used for many hours. Test doses of noradrenaline can be given every 2 min by an automatic syringe and the preparation requires no further attention until required for assay. If necessary, it can be stored overnight and used the following day. The perfused artery is particularly useful when frequent assays of noradrenaline in perfusates are required, as may be the case in studies on catecholamine uptake or release from isolated tissues.

A limitation of the method is lack of specificity since it does not discriminate between adrenaline and noradrenaline. This is not likely to prove a serious drawback except with tissues such as mammalian adrenal gland which are rich in adrenaline; in those cases where the arterial segment is equally sensitive to adrenaline and noradrenaline, the lack of specificity may prove an advantage for the assay of the sum of activity of adrenaline and noradrenaline. Dopamine constricts the preparation but is unlikely to interfere significantly with a noradrenaline assay unless present in amounts ten times greater than noradrenaline. The tissues where this proportion of dopamine is exceeded are liver, intestine and lung (Schumann, 1960) and corpus striatum of the central nervous system (Carlsson, 1959). Interference by acetylcholine, 5-HT and histamine is virtually eliminated by the addition of appropriate antagonists

to the perfusion fluid. There remains, however, the possibility of interference by the vasoactive polypeptides, bradykinin and angiotensin, when these are present in high concentrations.

It must be emphasised that the success of the method depends on the presence of 5-HT in the perfusion fluid in the concentration stated in methods. The mechanism of the potentiation of catecholamine sensitivity by 5-HT is being further examined.

Acknowledgements. The work was carried out under the auspices of a University of Adelaide Research Grant. We wish to acknowledge the skilled assistance of Michael J. Tyler.

References

Armitage, A. K. & Vane, J. R. (1964). *Brit. J. Pharmacol.*, **22**, 204–210.
Carlsson, A. (1959). *Pharmacol. Rev.*, **11**, 493–493.
de la Lande, I. S., Paton, W. D. M. & Waud B. (1964). *Proc. Aust. physiol. Soc.*, May, p. 39.
de la Lande, I. S. & Rand, M. J. (1965). *Aust. J. exp. Biol. med. Sci.*, **43**. In the press.
Schümann, H. J. (1960). In *Adrenergic Mechanisms*, Editor, Vane, J. R. Ciba Foundation Symposium, p. 11.

Letters to the Editor

Sensitivity of aggressive mice to centrally acting drugs

SIR,—Aggressiveness in normal laboratory mice can be induced by isolating them in individual cages for a suitable period of time (Yen, Stanger & Millman, 1959). Recently it was demonstrated that the toxicity of dexamphetamine is much increased in aggressive mice compared with normals, whether the animals are grouped or isolated (Consolo, Garattini & Valzelli, 1965).

Dexamphetamine, beside its activity on the central nervous system, is known to exert peripheral sympathomimetic cardiovascular effects. By contrast, fencamfamin, an amphetamine-like drug, while centrally stimulant (Hotovy & others, 1961), is without any peripheral sympathomimetic activity (Brittain, Jack & Spencer, 1964). We now report that this substance also is more toxic in aggressive animals.

Male Swiss albino mice, about 20 g, were kept six per Makrolon cage with a floor surface of 40 cm^2 at room a temperature of 22° and a relative humidity of 60%. Aggressive mice were obtained by individual isolation for a period of 4 weeks in single cages of the same size with opaque walls.

The drugs were given intraperitoneally to 48 normal mice grouped and to 48 aggressive mice grouped and the toxicity was calculated after 24 hr according to the method of Litchfield & Wilcoxon (1949). The LD50 (95% confidence limits) mg/kg were for dexamphetamine* 9·0 (8·0–12·0) and 3·7 (2·6–5·3) for the normal and aggressive mice respectively. Corresponding figures for fencamfamin were 52·2 (37·3–73·1) and 8·7 (5·1–14·7).

It is evident that fencamfamin is more toxic in agressive mice; the toxicity of amphetamine increases 2·4 times and that of fencamfamin about six fold.

A similar sensitivity is seen with a barbiturate drug (pentobarbitone) in aggressive mice. Table 1 presents the results obtained measuring the sleeping time after pentobarbitone alone or in combination with chlorpromazine.

TABLE 1. POTENTIATION OF BARBITURATE SLEEPING-TIME (PENTOBARBITONE 55 MG/KG/I.P.) BY CHLORPROMAZINE (2·5 MG/KG/I.P.) IN GROUPS OF 16 NORMAL AND AGGRESSIVE MICE

Treatment	Sleeping-time (in min ± s.e.)	% of sleeping animals
Controls (normal mice)	23′ ± 2′.30″	83
Controls (aggressive mice)	10′ ± 3′.25″	16
Chlorpromazine (normal mice) ..	84′ ± 1′.50″	100
Chlorpromazine (aggressive mice) ..	39′ ± 2′.15″	100

Pentobarbitone alone is less effective in aggressive than in normal mice and chlorpromazine induces a prolongation of pentobarbitone sleeping time more marked in normal than in aggressive mice.

The results reported here show that aggressive animals have a peculiar sensitivity to drugs acting on the central nervous system.

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July 23, 1965

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* Data from Consolo & others (1965).

References

Brittain, R. T., Jack, D. & Spencer, P. S. J. (1964). *J. Pharm. Pharmacol.*, **116**, 565–567.
Consolo, S., Garattini, S. & Valzelli, L. (1965). *Ibid.*, **117**, 53–54.
Hotovy, R., Enenkel, H. J., Gillisen, J., Hoffman, A., Jahn, U., Kraft, H. G., Muller-Calgan, H., Sommer, E. & Struller, R. (1961). *Arzneimitt.-Forsch.*, **11**, 20–24.
Litchfield, J. T., Jr. & Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99–106.
Yen, C. Y., Stanger, R. L. & Millman, N. (1959). *Arch. int. Pharmacodyn.*, **123**, 179–185.

The nature of the antagonism of bronchospasm in the guinea-pig by ascorbic acid

SIR,—In a Communication presented to the British Pharmacological Society in July 1964, we showed that ascorbic acid exerted a protective action on anaphylactic shock in the guinea-pig (Dawson & West, 1965). This result has recently been confirmed by Guirgis (1965). We have now found that this protective action seems to be a direct effect of ascorbic acid on the bronchial muscle.

Guinea-pigs were anaesthetised with chloralose (100 mg/kg) intraperitoneally, and artificially ventilated with a constant volume pump through a tracheal cannula. Bronchoconstriction was measured from changes in ventilation pressure in the trachea using a transducer system, and arterial blood pressure was recorded from the external carotid artery. Drugs were injected into the exposed jugular vein. Similar degrees of bronchoconstriction were produced by 5-hydroxytryptamine (5 μg), bradykinin (10 μg), and histamine (5 μg), and all these actions were prevented by previously injecting ascorbic acid (500 mg/kg) in neutral solution within 10 min of the injection of the spasmogens (see Fig. 1).

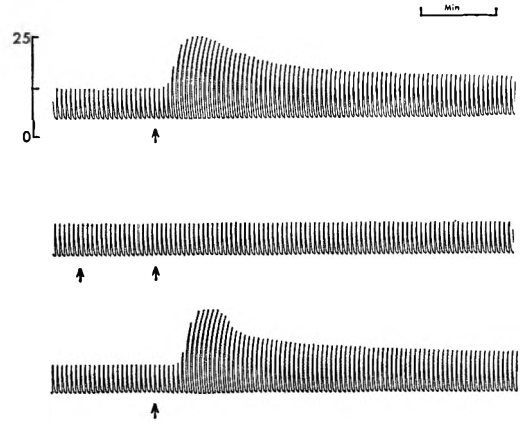

FIG. 1. Record of tracheal pressure of an anaesthetised guinea-pig. Upper tracing, response to histamine (5 μg); middle tracing, effect of ascorbic acid (500mg/kg) given at the first arrow one min before the next histamine dose; lower tracing, response to histamine 30 min later. Time in min. Pressure in mm Hg.

Smaller doses of ascorbic acid (100 and 200 mg/kg) proportionately reduced the actions. Doses of adrenaline (1–5 μg) also abolished the bronchoconstrictor action of the three spasmogens, but adrenaline raised the arterial blood pressure whereas ascorbic acid did not. Pretreatment of the animals with pronethalol

(10 mg/kg intravenously) abolished the inhibitory effect of adrenaline on the bronchial muscle but not that of ascorbic acid. Reserpinisation of the animals with four daily doses of reserpine, 2 mg/kg, intraperitoneally, did not modify the action of ascorbic acid on the spasmogen response. Dr. Collier & Mrs. Piper also tell us that adrenalectomy does not reduce the protective effect of ascorbic acid on bradykinin bronchospasm.

The present results show that the inhibition of bronchospasm by ascorbic acid is not mediated by catecholamines, does not involve β-adrenergic receptors since its action is not prevented by pronethalol, and is probably a direct effect.

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July 20, 1965

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References

Dawson, W. & West, G. B. (1965). *Brit. J. Pharmacol.*, **24**, 725–734.
Guirgis, H. M. (1965). *J. Pharm. Pharmacol.*, **17**, 387.

Anorexigenic drugs and lipid mobilisation

SIR,—As reported previously (Santi & Fassina, 1964), dexamphetamine, a typical anorexigenic substance, strongly elevated the plasma level of free fatty acids. Effectiveness of anorexigenic sympathomimetic drugs, may thus be considered to be based, not only on modification of psychic function, but mainly related to an interference with the central mechanisms of regulating food intake (Andersson & Larsson, 1961).

TABLE 1. EFFECT OF SOME ANOREXIGENIC DRUGS ON PLASMA FREE FATTY ACIDS (FFA) IN RATS

Treatment and dose	Time of death min	Plasma FFA μequiv./litre mean ± s.e.
Saline	30	424 ± 30 (11)† —
Chlorphentermine hydrochloride 12 mg/kg	30	710 ± 10 (4) < 0·001*
Methylphenidate hydrochloride 14 mg/kg	30	713 ± 28 (7) < 0·001
Pipradol hydrochloride 16·5 mg/kg	30	847 ± 10 (4) < 0·001
Saline	60	403 ± 33 (19) —
Dexamphetamine sulphate 2 mg/kg	60	672 ± 26 (17) < 0·001

* P versus saline treated controls.
† number of animals.
Adult non-fasted female rats (Wistar strain, 200–250 g) were given drugs or saline intraperitoneally and decapitated under ether anaesthesia. Time of death corresponded to the maximum activity for each drug. Free fatty acids were titrated by the method of Dole (1956). Doses of chlorphentermine, methylphenidate and pipradol are equimolar (54 μmoles/kg); dose of dexamphetamine (10·8 μmoles/kg).

We have now investigated the effects of additional anorexigenic drugs on plasma free fatty acids in rats. The three different classes investigated show a particular pharmacological and structural interest. Chlorphentermine is closely related chemically to dexamphetamine, but is almost completely devoid of the typical central nervous system (CNS) stimulant activity (Holm, Huus, Kopf, Möller Nielsen & Petersen, 1960; Gylys, Hart & Warren, 1962). Methylphenidate and pipradol (Karczmar & Howard, 1959; Spengler & Waser, 1959) are CNS stimulants and differ from amphetamine in structure (piperidine-like compounds) and in adrenergic properties (Krueger & McGrath, 1964).

Finally, desipramine, imipramine and amitriptyline were tested because they have been claimed to have anorexigenic action (Waser & Spengler, 1963).

As shown in Table 1, chlorphentermine, methylphenidate and pipradol, injected in rats in equimolar doses (54 μmoles/kg) all induced an increase of plasma free fatty acids. The amount of such increases was comparable for the three drugs (+ 70–100%). Dexamphetamine was about five times more active (on a molar basis) than the other drugs.

Desipramine (82 μmoles/kg), imipramine and amitriptyline (160 μmoles/kg) strongly increased plasma free fatty acids (60–80%, Table 2). Desipramine was more active than imipramine and amitriptyline. The lipomobilising action of these drugs is less rapid in onset than the action of amphetamine or piperidine-like compounds: the maximum value of plasma free fatty acids was reached 30–60 min after injection by using chlorphentermine, methylphenidate, pipradol or dexamphetamine, and 150 min after desipramine, imipramine or amitriptyline.

TABLE 2. EFFECT OF SOME THYMOLEPTIC DRUGS ON PLASMA FREE FATTY ACIDS (FFA) IN RATS

Treatment and dose	Time of death min.	Plasma FFA μequiv./litre mean ± s.e.
Saline	150	563 ± 29 (12)† —
Desipramine hydrochloride 25 mg/kg	150	1044 ± 46 (12) < 0·001*
Saline	150	521 ± 30 (12) —
Imipramine hydrochloride 50 mg/kg	150	924 ± 42 (12) < 0·001
Saline	150	550 ± 15 (12) —
Amitriptyline hydrochloride 50 mg/kg	150	863 ± 21 (12) < 0·001

* P versus saline treated controls.
† number of animals.
Drugs and saline were injected i.p. in adult non-fasted male rats (Wistar strain, 200–250 g). Doses correspond to 82 μmoles/kg for desipramine and to 160 μmoles/kg for imipramine and amitriptyline.

These results support the hypothesis that the metabolic action of these drugs is related to their anorexigenic effect (Santi & Fassina, 1964). It may be recalled that the breakdown of a molecule of glucose makes available 24 molecules of adenosine triphosphate, whereas a molecule of a long chain fatty acid (for instance C18) could supply 144 moles of adenosine triphosphate, if completely metabolised. Whatever the mechanism, the test of lipid mobilisation may be regarded as an experimental tool for the further characterisation of some centrally acting drugs.

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References

Andersson, B. & Larsson, S. (1961). *Pharmacol. Rev.*, **13**, 1–16.
Dole, V. P. (1956). *J. Clin. Invest.*, **35**, 150–154.
Gylys, J. A., Hart, J. J. D. & Warren, M. R. (1962). *J. Pharmacol.*, **137**, 365–373.
Holm, T., Huus, I., Kopf, R., Möller Nielsen, I. & Petersen, P. V. (1960). *Acta pharm. tox. Kbh.*, **17**, 121–136.
Karczmar, A. G. & Howard, J. H. Jr. (1959). *Proc. Soc. exp. Biol., N.Y.*, **102**, 163–167.
Krueger, G. L. & McGrath, V. R. (1964). *Psychopharmacological Agents*, Editor, Gordon, M. Vol. I, p. 225–250, New York and London: Academic Press.
Santi, R. & Fassina, G. (1964). *J. Pharm. Pharmacol.*, **16**, 130–131.
Spengler, J. & Waser, P. G. (1959). *Arch. exp. Path. Pharmak.*, **237**, 171–185.
Waser, P. G. & Spengler, J. (1963). *Schweiz. med. Wschr.*, **93**, 90–92.

Pharmacopoeias and Formularies

THE NATIONAL FORMULARY OF THE UNITED STATES OF AMERICA. TWELFTH EDITION, 1965. (N.F.XII.) Published by the American Pharmaceutical Association, Washington, D.C., U.S.A. (pp. xliv + 618 (including Index). $10.00.

This New Formulary becomes effective on September 1, 1965. Responsibility for it rests with the Council of the American Pharmaceutical Association which appoints a Committee of Ten under Dr. E. G. Feldman as Director of Revision. This committee appoints its vice-chairman and secretary from amongst its members. The Director in turn nominates to Council an Advisory Panel not exceeding 50 (48 on this for N.F.XII) all of whom have special contributions to make in some pharmaceutical or therapeutic field. There is an Advisory Committee—largely medical—on "Admissions" and 26 Advisory Panels on subjects ranging from Arsenic Tests and Nomenclature to Dental Drugs, Veterinary Drugs and Vitamin Preparations. Apart from these panels two hundred other participants in the Revision Programme are listed—including advisers from Canada (3), England (2), Holland, Sweden and Switzerland (1 from each).

The need for revision within five years of standard pharmaceutical works of reference, as has been general since 1940, would seem to be supported by this volume with its 248 new "admissions" and 280 monographs of N.F.XI now deleted. Interestingly enough, in spite of this net loss of 32 monographs, N.F.XII is 100 pages larger than N.F.XI. The Committee started by striking out "Extent of Use" as a significant criterion in determining suitability of a drug for inclusion—recommendations for admission are now based solely on "Therapeutic Value". In this the Committee anticipated the requirements on efficacy of Congress in 1962. Since 1955 three editions have seen 729 deletions. In this country we would not support all these admissions or all these deletions but there are fashions and local fashions in prescribing, as in skirt lengths and trousers widths.

The nearest equivalent to N.F.XII in U.K. is B.P.C., the more impressive—and expensive—volume, partly because of its "Action and Uses" sections, whereas N.F. merely gives a "Category". Such categories as Anticonvulsant, Antiemetic, Antihypercholesterolemic, Antitussive—amongst many antis—Neoplastic Suppressant, Pharmaceutical Necessity and Solvent are helpful, but hardly justify the claim that the Formulary "has also assumed added importance as a therapeutic guide to pharmacists and physicians" except that the strict criterion of therapeutic value as the basis for admission gives a cachet to its monographs. B.P.C. also has the advantage of being comprehensive in that it includes modified monographs on B.P. preparations whereas N.F. supplements but does not cover U.S.P. With admissions and deletions at the rate of about one of each per week it is clear that any volume is a little out of date before it is published. Interim Revision Announcements (there were four to N.F.XI) are provided upon request while Supplements, covering a wider scope, are mailed without charge to every owner of the book.

The two column format of N.F.XI is here repeated but in narrower columns with wider margins. This may facilitate readability at the price of elegance The paper used is either not quite thick enough or not quite opaque enough to prevent some of the print from being quite noticeable from the other side of the page. There are pp. 53 of Introductory Matter, 418 of Monographs, 97 on General Tests, Processes and Apparatus, 20 on Reagents and Test Solutions,

43 on General Information, including a useful synopsis of the Federal Regulations on Narcotic Drugs, and 30 of Index. Tables of Atomic Weights, Metric Doses and Apothecaries' Equivalents usefully decorate the fly leaves at the back—"good measure-running over!" Gm. not G. is used for gram and L., ml. and μl. are retained with ml. taken as the equivalent of the cc.

Pharmacists will be specially interested in the tests and assays directed to quality control. There are many new assays and many improvements in standards for tablets which are now subject to individual assay. Half the tablets have had their disintegration times reduced—in most cases by at least 50%. Increasing use is made of modern and instrumental methods of analysis. N.F.XII is primarily a book of standards and introduces in this edition for the first time gas-liquid chromatography, flame spectrophotometry, thin-layer chromatography, polarography and delta-pH titrimetry amongst other techniques. Spectrophotometric Reference Standards have increased from 42 in N.F.XI to 91 in N.F.XII—59 of these are provided for the first time. Virtually all the spectrophotometric assay formulae have been converted to calculations based upon absorbance values.

Although the U.S.P. has recently deleted its monograph on meprobamate on the ground that this drug is not a "true tranquiliser," it is included here and categorised as such! Only one typographical error has been detected—or does U.S.A. refuse to differentiate between principal and principle? In his closing tribute to his committees and participants the Director of Revision refers to their "Contribution to the fulfilment of the obligation to advance the public welfare which was assumed by the National Formulary more than 75 years ago". Perhaps such dedicated volumes are of limited value here because of differences in names, standards, practices. But the pattern has its values.

A. D. MACDONALD.

Book Review

INTRODUCTION TO PHARMACOLOGY. By J. J. Lewis. Third edition. Pp. xvi + 1048 (including Index). E. & S. Livingstone, Ltd., Edinburgh and London, 1964. 63s.

This textbook is now well-established and its success is evidenced by ths appearance of this third edition barely four years since the first edition wae published in 1960. With thirty-seven chapters comprising nearly one thousand pages it is certainly a comprehensive "Introduction" to the subject. This edition is one chapter and over 100 pages longer than the 2nd Edition.

The first chapter deals with general aspects of the subject such as administration, absorption, distribution and fate of drugs and theories of drug action which are important but which students so often fail to appreciate or to enjoy at the beginning of the subject. This may be because an understanding of these topics in perspective depends so much on having studied what appears in the rest of the book. This section would probably have more significance for the student if it were placed at the end of the book.

The next three chapters deal mainly with the pharmacology of the autonomic nervous system and with drugs related to this topic. The beginning is rather abrupt as there is no introduction to the concept of humoral transmission. The treatment is immediately very specialised with a pronounced chemical bias. It is up-to-date, but beginner students will find it heavy going. In the bibliography there is no reference to the appropriate sections in "Goodman and Gil-

man," nor to Professor Burn's little book on the Autonomic Nervous System, which are so well done and would make excellent complementary reading.

Later there is an account of the functional anatomy and physiology of the sympathetic nervous system, essential as a background against which to consider the pharmacology, but no comparable consideration of the parasympathetic system.

There is a useful discussion of structure-activity-relations for the sympathomimetic amines and a full account of the formation and metabolism of the sympathetic transmitter amines. There is plenty of factual detail in the section on the sympathetic nervous system but more correlation of the mechanisms at work would make for easier understanding.

It is surprising that some of the errors should have persisted into a third edition. In the section on ergot alkaloids, medical students using this book would get unorthodox impressions if they learnt that ergometrine is used to induce labour (p. 221) or that oxytocic drugs are not used before the third stage of labour has begun (p. 221) or that ergotoxine is used in obstetrics to prevent or reduce post-partum haemorrhage (page 225).

There is a good account of the pharmacology of histamine and antihistamines. It is pleasing to see 5-hydroxytryptamine and some of the vasoactive polypeptides given a prominent place in a students' textbook.

The section on general anaesthesia is notable for an up-to-date discussion of theories of anaesthesia. It is strange that the metabolism of the barbiturate drugs is dealt with so scantily since a great deal is now known which can provide a basis for a discussion and understanding of their properties and uses. The anti-epileptic drugs are dealt with in a comprehensive manner and the up-to-date account of the tranquillizers is well based on chemical structures and relations. By comparison the section on antidepressives, a group of drugs likely to be of increasing importance, is brief. On p. 337 the word should surely be psychotropic not psychotrophic?

In several of the sections the emphasis is on *drugs* rather than *mechanisms*, this is of course unavoidable because so often the mechanism is unknown, but Lewis's book is better in this respect than many other textbooks. The best type of approach is seen, for example, in the section on Emetics and Anti-Emetics where there is first a full consideration of nausea and vomiting, then a discussion of the drugs which influence these processes. Such an approach would improve the account of the diuretic drugs which are more readily understood after a brief consideration of the normal ion-exchange and excretion mechanisms of the kidney.

The section on purgatives is old-fashioned and mentions too many of the out-of-date compounds at the expense of clarity and usefulness.

The last quarter of the book is devoted to the chemotherapy of bacterial, viral, protozoal and fungal diseases and is a comprehensive and useful treatment of a large subject. The book ends with an exhaustive and extremely helpful index which occupies nearly one hundred pages.

This is a book which is available to and likely to be studied by a wide range of students with differing requirements such as pharmacists, pharmacologists, medical students and veterinary students. It is not ideal for any one group but has a great deal to commend it as an introductory text for any of these students. Because of its comprehensive coverage of drugs and drug chemistry (the structural formulae are clearly reproduced) it will prove to be a useful book of reference for anybody who needs to know about drugs and how they act.

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Review Article

Research Papers

Letters to the Editor

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