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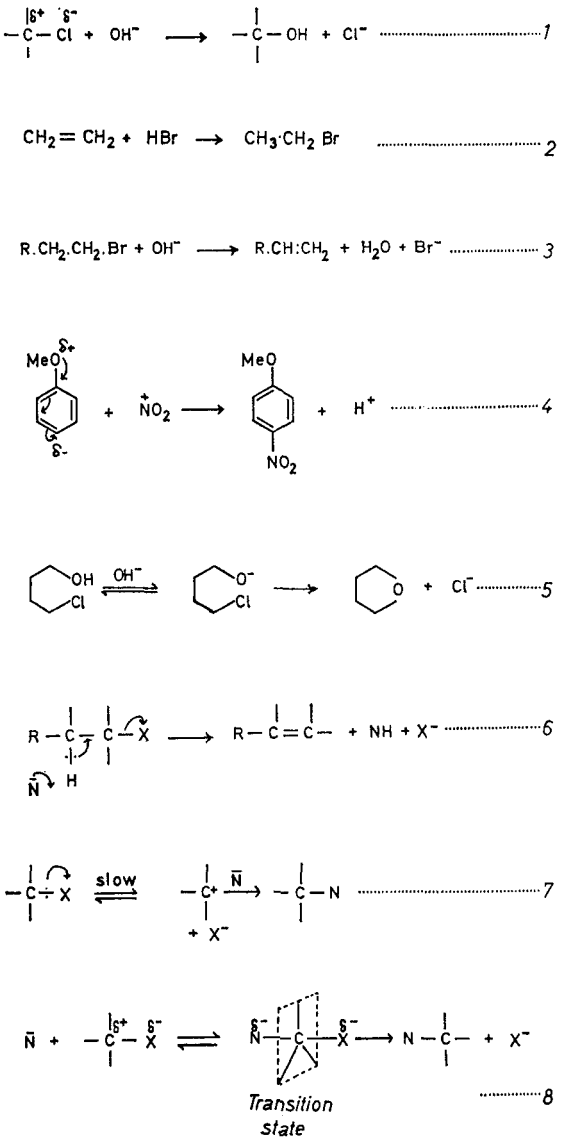
1962; Miller, 1962); (ii) polarisability (Jencks & Carriuolo, 1960; Parker 1961a); (iii) carbon-basicity (Bunnett, Hauser & Nahabedian, 1961; Parker, 1961b); (iv) nature of the electrophilic centre (Hudson & Green, 1962); (v) partitioning of the intermediate in attack at unsaturated carbon



(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*).

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

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

C=catalytic function

INTRAMOLECULAR CATALYSIS

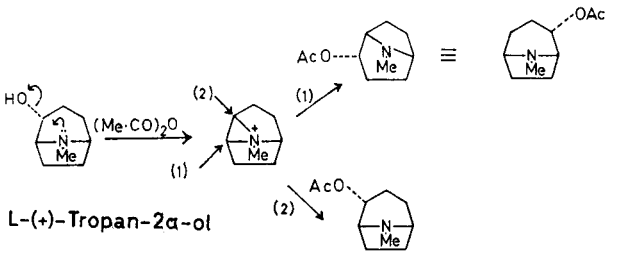
When neighbouring group participation stabilises a transition state or produces a reactive intermediate (eqn *9*) it increases the rate of an intermolecular reaction. This constitues intramolecular catalysis in which the neighbouring group is said to provide anchimeric assistance to the reaction.

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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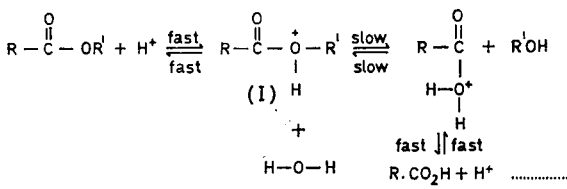

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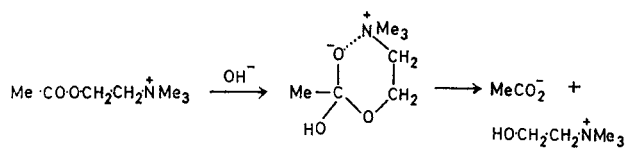

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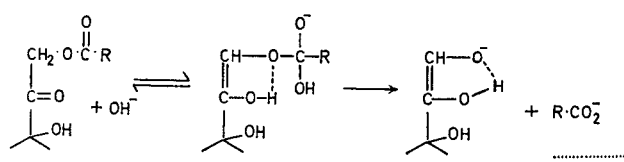

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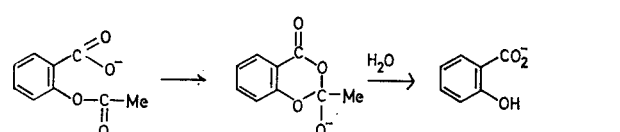

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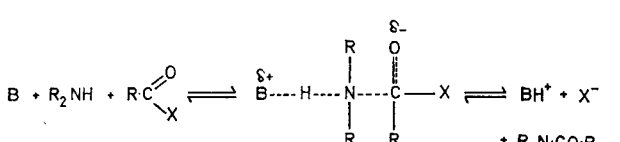

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Nucleophilic catalysis. A nucleophilic catalyst is a nucleophile which enters into either intermolecular or intramolecular reaction with an electrophilic *carbon* centre (cf. base catalysis). The reactive intermediate thus formed then undergoes *intermolecular* 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,

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

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

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

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

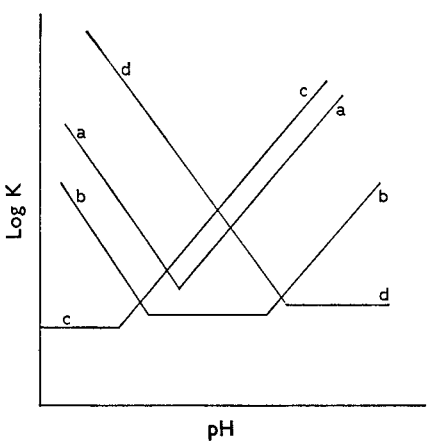


FIG. 1. Rate - pH profiles for combinations of acid and base catalysis and "spontaneous" reaction.

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,

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

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,

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

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.

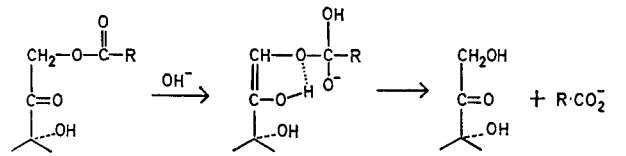
Intramolecular catalysis in pharmaceutical systems

GENERAL ACID CATALYSIS

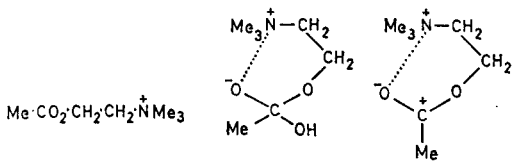
The spontaneous hydrolysis of hydrocortisone hemisuccinate may be attributed either to nucleophilic catalysis (see later) or general acid catalysis by the hydroxyl group of the enol form of the C-20 carbonyl function in the undissociated acid (eqn *19*, page 537) (Garrett, 1962a). The alkaline hydrolysis 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

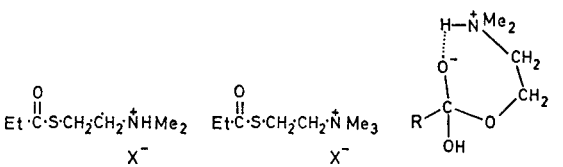
attachment of a quaternary nitrogen atom to a β-carbon atom. Acetylcholine (2), below, for example, is readily hydrolysed in alkaline solution and this is attributed to general acid catalysis by the positively charged nitrogen atom which can either stabilise the intermediate by distributing the charge 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

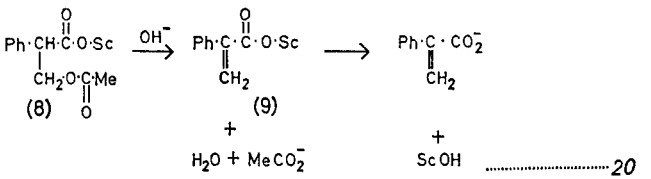
* Zaslowsky & Fisher (1963).
† Davis & Ross (1950).

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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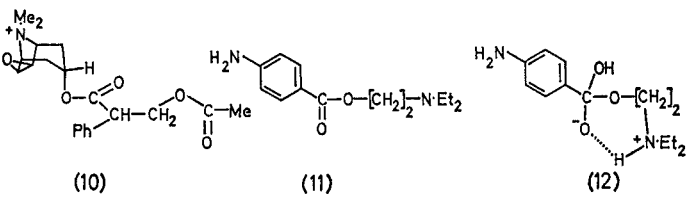

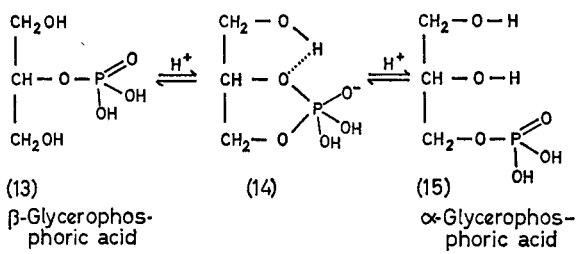

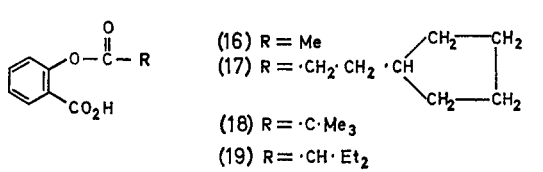

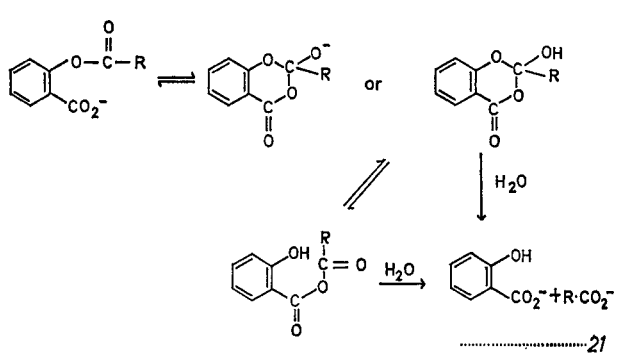

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in accordance with equation *20* (page 539). The rate constant for alkaline elimination of the acetyl linkage in the acetylscopolamine methyl bromide was five times greater than that for the corresponding base but the rate constant for the second ester 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).

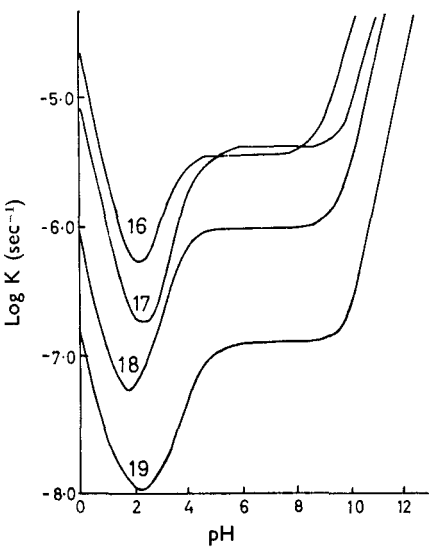


FIG. 2. Apparent first order rate constants for esters of salicylic acid. (16 = acetyl–; 17 = cyclopentylpropionyl–; 18 = trimethylacetyl–; 19 = diethylacetyl). (cf. page 539.)

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

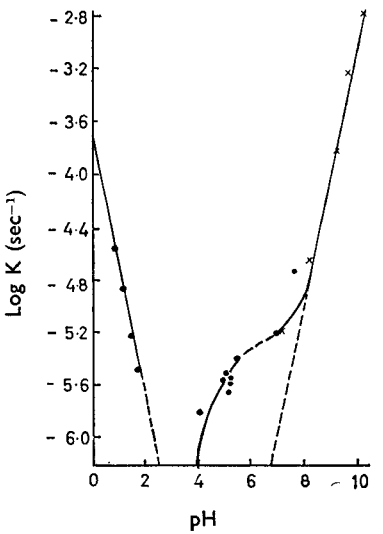


FIG. 3. Rate constants for the hydrolysis of hydrocortisone hemisuccinate at 70°, in 30% ethanol. ((●) = Rate constant as determined by colorimetric assay; (×) = rate constant as determined by constant pH titrations).

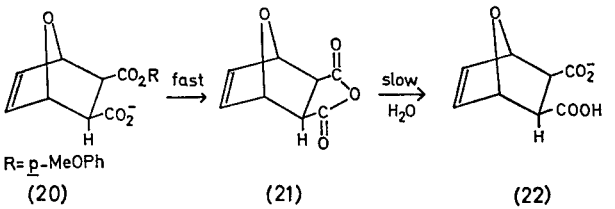
be attributed to either nucleophilic catalysis by carboxylate ion in a manner analogous to that noted previously with aspirin and similar esters (e.g. eqn *23*, page 543) or alternatively to a kinetically indistinguishable process involving general acid catalysis by the enolised C-20 carbonyl group in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

Bifunctional catalysis

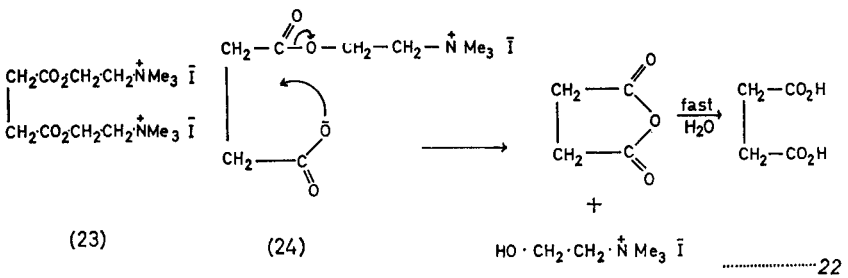
The three catalytic processes so far discussed have been illustrated with examples where only one type of catalysis is involved. This section deals with simple systems where two different but complementary catalytic processes are occurring. The simultaneous occurrence of two such processes each of which 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

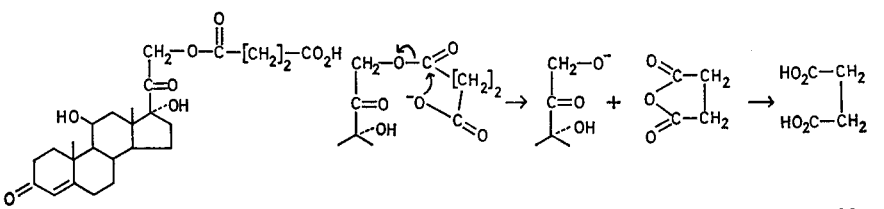


(20) (21) (22)



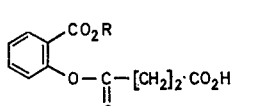
(23) (24)

22



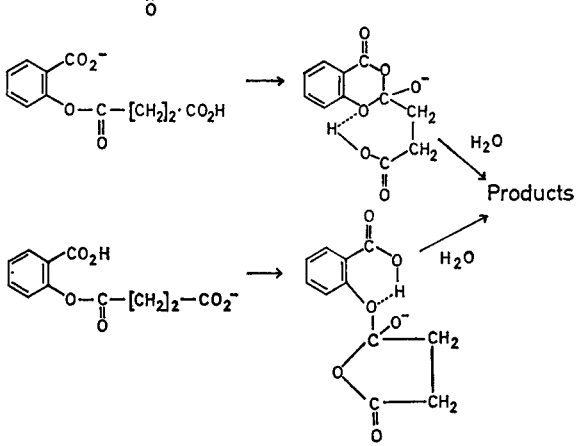
(25) (25)

23



(26) R=H

(27) R=Me



24

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.

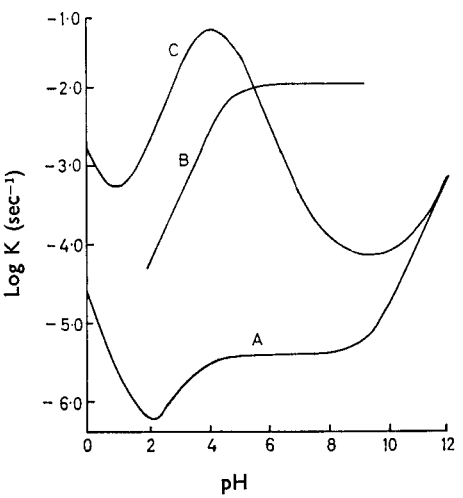


FIG. 4. Rate constants for hydrolysis of esters of salicylic acid, at 25° A = Acetylsalicylic acid; B = methyl succinylsalicylate; C = succinylsalicylic acid.

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

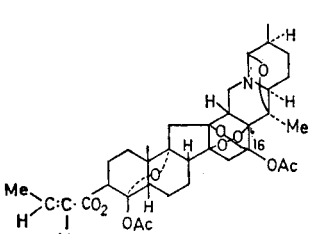
In the reaction between a nucleophilic and electrophilic centre in a biological system, one of the functions constitutes part of an enzyme at whose surface the inhibitor or substrate molecule, bearing the second function, is held by the relevant forces. This enables the second function to become orientated 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

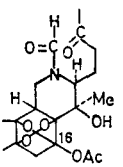
The enzyme cholinesterase, which is responsible in the body for the hydrolysis of acetylcholine produced during humoral transmissions at the nerve endings, is readily inhibited by organophosphorus compounds such as tetra-ethylpyrophosphate (TEPP) (32) (page 546), di-isopropylphosphonofluoridate (dyflos, 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 ($R{\cdot}CO{\cdot}NHOH$) (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



(28)



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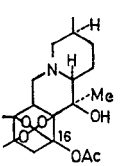

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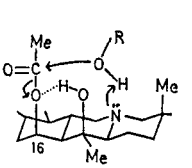
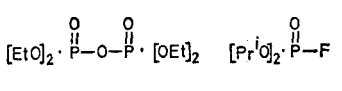
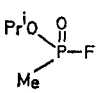


(31)

Relative rates of solvolysis of C_{16}-acetate: 1 (28); 40 (29); 1000 (30)



(32) (33)



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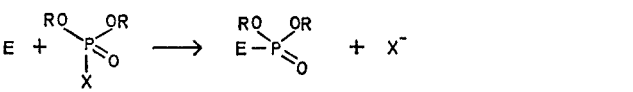
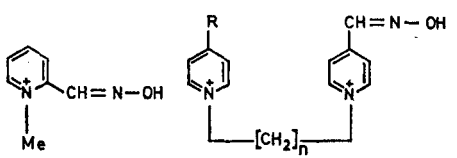
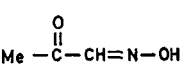


(DFP; X=F, R=Pr^i)

25



(35) (36) R=CH:N·OH; n=2-5 (37) R=H; n=3-5



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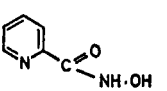

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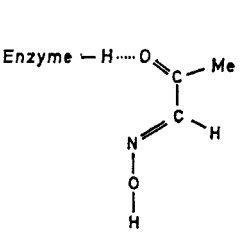
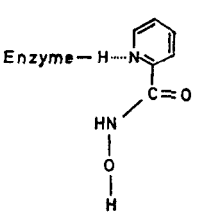


(40)



(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).

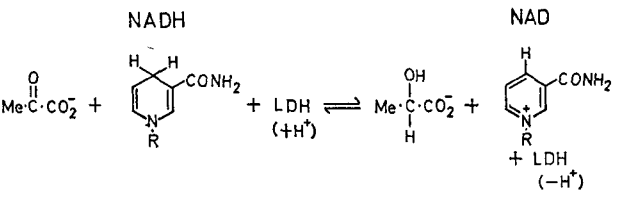
ACTIVE-SITE DIRECTED IRREVERSIBLE INHIBITION

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.

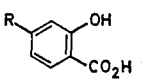
A closely related enzyme to LDH is glutamic dehydrogenase (GDH) and the behaviour of potential inhibitors to the two enzymes has been studied since GDH represents an enzyme system necessary to the host. It is considered that this approach will provide information about the specificity

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.



26



(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).

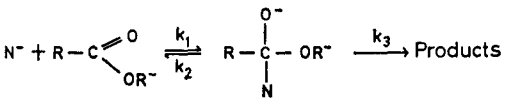
NATURE OF THE NUCLEOPHILIC GROUP

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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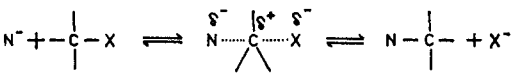

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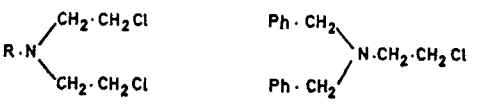
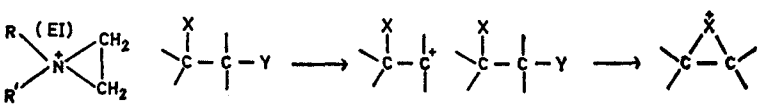
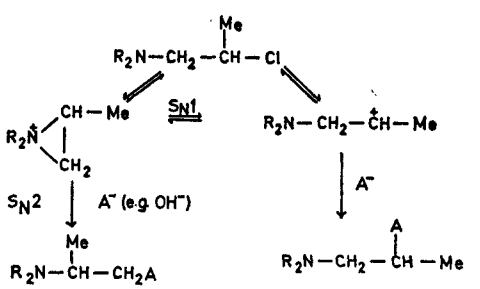






Reaction between a nucleophile and an ester is considered to proceed through a transition state to give an intermediate of similar structure (Hudson & Green, 1962) (eqn *27*, above). The energy term for the dissociation energy of the C–N bond in the intermediate contributes to the

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.

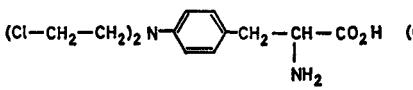
"INTRAMOLECULAR" ALKYLATION

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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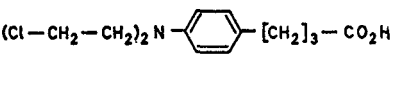

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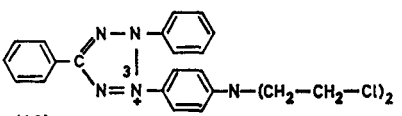

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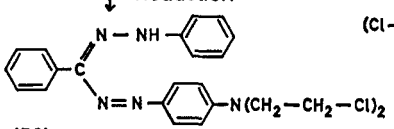
Reduction



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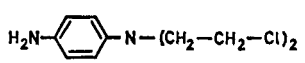

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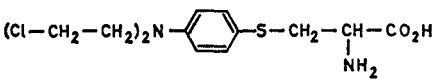

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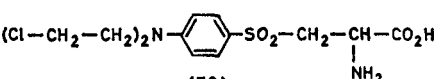

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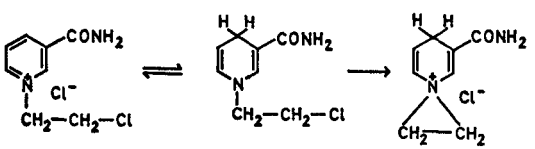
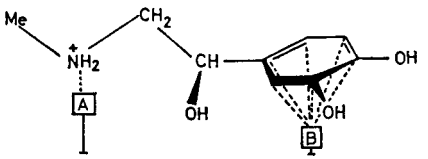


(54) (55) (56)

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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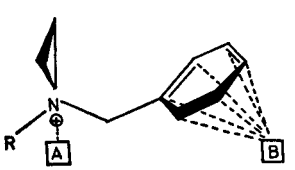

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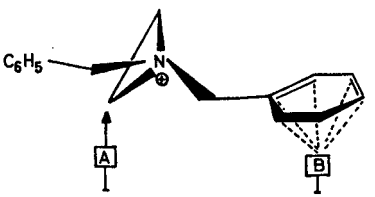

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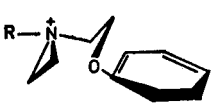

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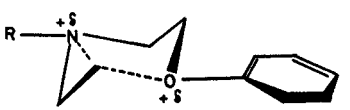

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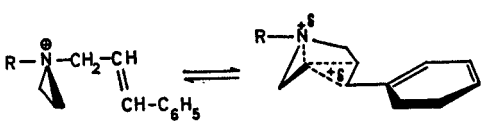

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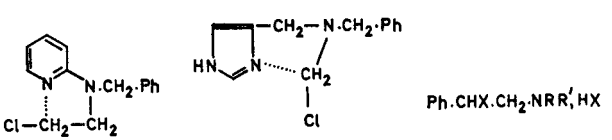


(63) (64) (65)

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.

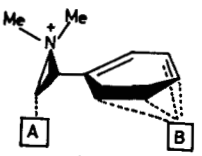
The formation of the required hybrid state (61) is also facilitated by the substitution in the aromatic nucleus of +E or +I groups which increase the nucleophilic character of the ethereal oxygen atom. Thus, a 2-methyl group in the phenyl ring increases adrenergic blockade activity (Ullyot & Kerwin, 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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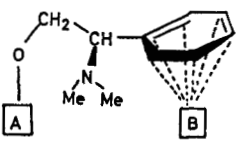

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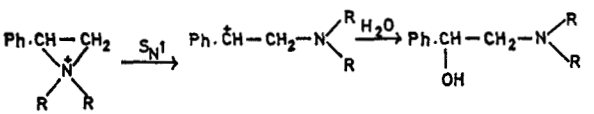
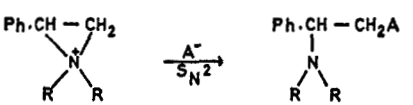
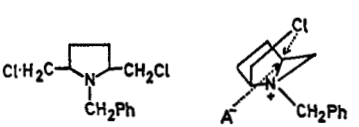


V



VI



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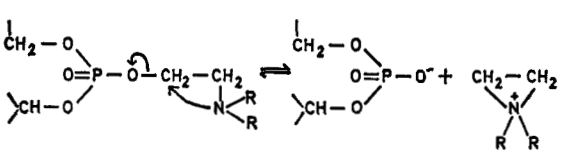

VII

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)