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Review Article

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

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Mechanism of enzyme action

Enzymes are biological catalysts and although the reactions they promote are well known, it is only as the result of intensive studies within limited classes in recent years that their mechanism of action has been revealed. Current theories regard the enzyme as a surface on which a substrate(s) is held by interatomic forces and subjected to bi-functional or polyfunctional catalytic processes such as those previously described.

The major part, or in some cases the whole, of the enzyme consists of a polypeptide chain of amino-acid units arranged in a definite conformation such as a helix and supported in such an arrangement by hydrogen bonds between adjacent parts of the chain. Somewhere along the chain is an area known as the active-site where the substrate is complexed and the catalytic processes occur. Defined in space this site may embrace one or more adjacent parts of the chain. The functional groups responsible for catalysis are present on those parts of the chain making up the active-site and are correctly orientated to perform their functions on the substrate. Hydrogen bonding, electrostatic interaction, van der Waals' forces or hydrophobic forces, enable the substrate to bind at the active site in the correct three dimensional arrangement for "intramolecular" catalysis to occur. The dimensional requirements of the substrate are probably defined by groups on the protein chain adjacent to the active site which perform a steric function.

Enzyme reactions may be differentiated from the normal catalytic processes occurring in chemical reactions such as hydrolysis or oxidation, by two features: substrate specificity and the speed of the reactions which occur in the pH range 2-10. These features become understandable if we consider that enzyme reactions proceed by an "intramolecular" mechanism involving a combination of the processes previously described where correct fit of a substrate at the enzyme surface will invoke the tremendous power of such reactions. Furthermore, hydrogen or hydroxyl ions present in the medium are only required to provide a suitable ionising medium for the catalytic functions and do not themselves participate in

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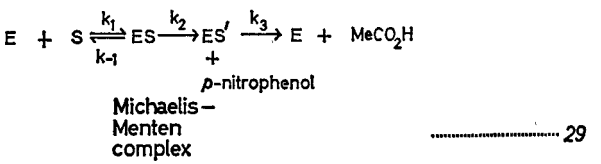
the reactions. The mapping of the active-sites of enzymes is still in its infancy, but it seems reasonable to assume that all enzymes incorporate similar catalytic processes although additional refinements such as the presence of oxidation-reduction systems as co-enzymes are necessary to explain the mechanism of action of enzymes such as the dehydrogenases. The mechanism of enzyme action outlined above will now be illustrated in some detail for the hydrolytic enzymes, α-chymotrypsin and acetylcholinesterase.

α-CHYMOTRYPSIN

α-Chymotrypsin is an enzyme capable of hydrolysing amide and ester linkages in certain substrates and is found in pancreatic juice where it is stored in the form of a precursor, chymotrypsinogen. The enzyme consists of a polypeptide chain of amino-acid units only, and does not require a co-enzyme for its activity. Chymotrypsin has been isolated



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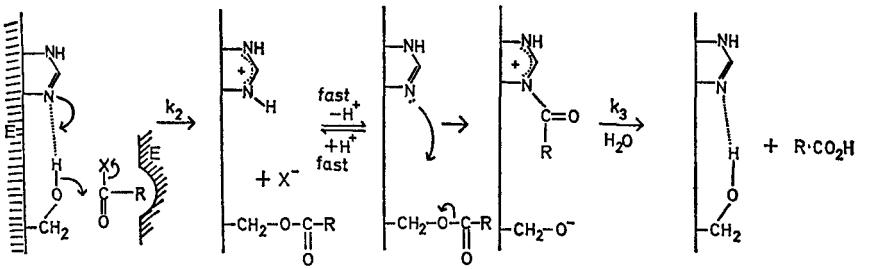
in crystalline form, and has a molecular weight of 23,000 (Rao & Kegeles, 1958). Inhibition studies using dyflos indicate that there is only one active site present in each molecule (Jansen, Nutting, Jang & Balls, 1949; Jansen, Nutting & Balls, 1949).

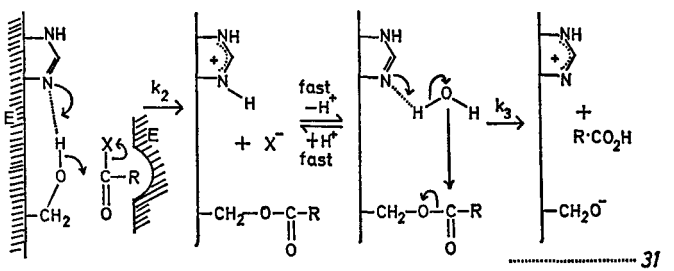
Chymotrypsin hydrolyses *p*-nitrophenyl acetate to acetic acid and *p*-nitrophenol and kinetic measurements indicate preliminary complexing between the substrate and enzyme in accordance with the Michaelis-Menten scheme for enzyme action. The complex then rapidly breaks down into an acyl-chymotrypsin (ES') with the release of *p*-nitrophenol, and the acyl-chymotrypsin is then slowly hydrolysed to acetic acid with regeneration of the enzyme (Hartley & Kilby, 1954) (eqn *29*, above).

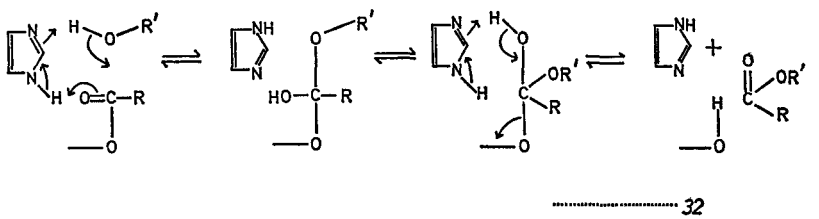
The acyl-chymotrypsin formed is stable in acid solution and has been isolated (Balls & Wood, 1956). The values for k_2 and k_3 for *p*-nitrophenyl acetate hydrolysis have been determined at various pH values and found to be pH dependent. Calculation shows that the catalytic groups involved in these steps have pK_a values of 6·7 (Gutfreund & Sturtevant, 1956) and 7·4 (Spencer & Sturtevant, 1959) respectively. Bender, Schonbaum & Zerner (1962b) have since shown that the same de-acylation rate (k_3) is obtained for five different esters of *trans*-cinnamic acid with chymotrypsin, indicative of the formation of a common intermediate *trans*-cinnamoyl α-chymotrypsin. These results may be interpreted as participation by an imidazole group (present in a histidine moiety) in the catalytic process since this has the required pK_a.

The presence of a second functional group necessary for the catalytic process was shown by Dixon, Dreyer & Neurath (1956), who found that the reaction of acetyl-α-chymotrypsin with hydroxylamine to give the hydroxamic acid at pH 5·5, was abolished by 8M urea. The reactivity of the

acyl-enzyme was restored when the concentration of urea was decreased by dilution. These results may be interpreted as an alteration by the urea solution of the conformation of the helix and thus the proximity of the functional groups on adjacent chains at the active site so that the deacetylation reaction is prevented. The second functional group involved in the catalysis is probably the hydroxyl group of a serine moiety since inhibition of chymotrypsin by dyflos followed by acid degradation gives serine phosphate (Schaffer, May & Summerson, 1953).







Various workers have attempted to show that in the acylated enzyme, the acyl group is attached either to imidazole or the serine hydroxyl, since this knowledge is essential for any proposal of mechanism of action of the enzyme. Dixon & Neurath (1957) concluded from a spectroscopic investigation that the acyl group initially resides on serine and the acyl-enzyme formed is stable at low pH values but at higher pH values it undergoes nucleophilic attack by the imidazole ring nitrogen atom to give

an acetylimidazole which is slowly hydrolysed to acetic acid with regeneration of the enzyme. Their scheme for the mechanism of action of chymotrypsin is shown in equation *30* (page 603). The imidazole nitrogen atom behaves as a general base catalyst in (i) and (ii) and then as a nucleophilic catalyst in (iii), (iv) and (v).

Spencer & Sturtevant (1959) re-examined the work of Dixon & Neurath and concluded that the observations made by these workers were due to changes occurring in the enzyme itself in being raised from pH 4·8 to pH 8·2. These changes result in light scattering as a consequence of protein precipitation occurring during the alteration in pH (Wootton & Hess, 1960). Spencer & Sturtevant consider that the imidazole functions as a general base for the acylation and de-acylation reactions, and their scheme is shown in equation *31*.

Bender & others (1962b), have detected spectrophotometrically *trans*-cinnamoyl-α-chymotrypsin formed during hydrolysis of *o*-nitrophenyl-cinnamate, indicating that the acyl-enzyme is an intermediate rather than an artifact. Furthermore, although the spectral changes noted did not differentiate between an acyl-imidazole or acyl-serine intermediate they were compatible with acylation on a serine hydroxyl which is in the environment of an aspartate carboxylate anion. Additional evidence is now available which, with these findings, identifies the group acylated as a serine hydroxyl group (see Bender & Kézdy, 1964, for review).

Bender, Schonbaum & Zerner (1962a) have recently shown that the pK_a values for acylation and de-acylation for each of three ester substrates studied are in reasonable agreement. This evidence, together with the similar effects of deuterium oxide, solvent composition and substrate structure on the overall catalytic and de-acylation rate constants, leads Bender (1962) to consider that the acylation and de-acylation steps are equivalent and carried out by the same catalytic functions, thus ruling out the mechanism proposed by Dixon & Neurath (1957) (eqn *30*, page 603) since the two steps are not equivalent. One of the rate-dependent steps involves proton transfer since, when the catalysis is carried out in deuterium oxide, the rate is decreased two to three-fold. This rules out proposed mechanisms which involve only a nucleophile (Westheimer, 1957; Rydon, 1958). The latest mechanism proposed by Bender & Kézdy (1964) is summarised in equation *32* (page 603) which embraces the equivalent steps of acylation (R′-OH = serine) and de-acylation (R′-OH = water). The imidazole functions as a general acid-general base catalyst through its two nitrogen atoms in a concerted process which gives a tetrahedral intermediate.

Some substrates of α-chymotrypsin give bell-shaped pH-rate profiles for the overall catalytic rate constant where the rate determining step is the acylation reaction, whereas a sigmoid profile is obtained when de-acylation is the rate determining step (Bender, Clement, Kézdy & Zerner, 1963; Bender, Clement, Kézdy & Heck, 1964). In addition to the imidazole group involved in acylation and de-acylation, it is proposed that there is present in acylation an acidic group ($pK_a \sim 9$) which is either absent or not concerned in the bond-changing processes of de-acylation.

These workers initially reached the same conclusion as Erlanger, Castleman & Cooper (1963) by regarding this acidic group as a serine hydroxyl in a "perturbed" state. However this acidic group has now been identified as the α-ammonium group of the *N*-terminal isoleucine residue (Labouesse, Oppenheimer & Hess, 1964; Oppenheimer, Labouesse, Carlsson & Hess, 1964). The function of this group is to induce conformational stabilisation of the active site in acylation, a function which is not necessary in de-acylation because of the covalent link of the acyl-enzyme. This implies that the conformation of the free enzyme is pH dependent and evidence has been presented to support this (Bender & Kézdy, 1964). The induced conformation is possibly due to an electrostatic bond between those parts of the enzyme surface at the active site carrying the ammonium group and a carboxylate ion.

In the proposed scheme *32*, the steric requirements between the imidazole molecule, the serine hydroxyl and the substrate are not fulfilled in a model. Recent evidence indicates that the two histidine residues in chymotrypsin are spatially very close, the actual sequence being His–Phe–Cys–S–S–Cys–His (Brown & Hartley, 1963; Hartley, 1964). It is suggested that conformation is important in the enzyme in order to ensure the close relationship between these two imidazole groups so that both participate in proton transfer in accordance with equation *32*, one acting as a general-base and the other as a general acid (Bender & Kézdy, 1964).

Bender, Kézdy & Gunter (1964), have analysed the kinetic factors responsible for the differences between the hydroxyl ion and α-chymotrypsin catalysed hydrolysis of *N*-acetyl-L-tryptophan amide. They were able to predict the enzymatic rate from a quantitative consideration of the intramolecular character of the general acid and general base catalysis by imidazole, together with a freezing of the substrate at the enzyme surface in a conformation where the ground state resembled the transition state for the reaction (i.e. energy barrier for reaction low). They concluded that the activity of the enzyme α-chymotrypsin could be discussed on a straightforward chemical basis where each factor involved was based on firm chemical analogy.

ACETYLCHOLINESTERASE

Two hydrolytic enzymes closely related to chymotrypsin are "pseudo"- and "true" (acetyl-) cholinesterase. Acetylcholinesterase hydrolyses acetylcholine (70) (page 607) faster than any other choline ester whereas "pseudo" cholinesterase hydrolyses other choline esters equally well. Furthermore, pseudocholinesterase shows saturation by excess substrate in accordance with the Michaelis-Menten scheme but not inhibition (Wilson, 1954), whereas acetylcholinesterase is inhibited by excess acetylcholine above a certain substrate concentration and the rate of hydrolysis falls off (Zeller & Bissegger, 1943).

Adjacent to the active site of acetylcholinesterase is a negatively charged site known as the anionic site where the quaternary nitrogen of the substrate is considered bound (Zeller & Bissegger, 1943). Evidence

for this view is provided from a study of the inhibition of the enzyme by tertiary bases and quaternary ammonium compounds which prevent the hydrolysis of acetylcholine. A tertiary base such as physostigmine (71) is far more effective as an inhibitor in acid solution where it exists in the protonated form than as the free base in alkaline solution, whereas quaternary ammonium compounds such as neostigmine (72) are equally effective over a wide pH range (Wilson & Bergmann, 1950a). The possibility that acetylcholinesterase has two anionic sites has been proposed; this would conveniently explain the inhibition noted with excess substrate (Bergmann, 1955).

The acetylcholine molecule with its three methyl groups attached to the cationic nitrogen is bound to the enzyme only as tightly as the corresponding dimethyl compound. However, with the former compound there is a marked decrease in entropy of the acetylcholine-enzyme complex (Wilson & Cabib, 1956) and this has been associated with the occurrence of a profound structural change in the enzyme-substrate complex (Belleau & Lacasse, 1964).

The kinetic scheme proposed for the hydrolysis of acetylcholine by acetylcholinesterase closely follows that proposed for chymotrypsin and other esterases, where an acyl-enzyme is initially formed which is decomposed by water to give products (eqn *33*, page 607) (Wilson, Bergmann & Nachmansohn, 1950).

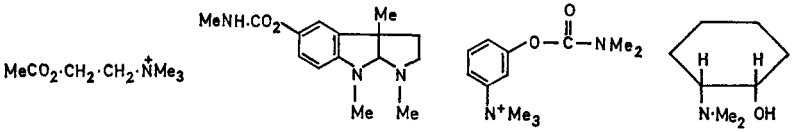
The acetylated function in the acyl enzyme is protected from hydrolysis by water as a result of excess substrate complexed at the anionic site. This accounts for the inhibiting effect of excess substrate (Wilson & Cabib, 1956; Krupka, 1963).

An examination of the rate-pH profiles obtained for the hydrolysis of a number of substrates by the enzyme shows that a basic and acidic group with pK_a 6·2–7·2 and pK_a 9·0–9·6 respectively, participate in the reaction at the active site (Wilson and Bergmann, 1950b; Bergmann, 1955; Bergmann, Segal, Shimoni and Wurzel, 1956; Krupka & Laidler, 1960). There is general agreement that the groups concerned are imidazole and the phenolic hydroxyl of tyrosine.

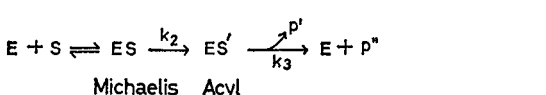
Phosphorylation of acetylcholinesterase leads to inactivation of the enzyme (see page 545) and this process proceeds in a manner analogous to substrate hydrolysis, except that the phosphorylated enzyme is stable to hydrolysis by water although it can be reactivated with stronger nucleophiles (Wilson, 1951). It seems very likely that phosphorylation (and by analogy, acylation) occurs on serine since inhibition of the enzyme with organophosphorus compounds containing ^{32}P followed by degradation gives serine ^{32}P-phosphoric acid (Schaffer, May & Summerson, 1954).

An early mechanistic scheme for the action of esterases including acetylcholinesterase proposed by Cunningham (1957) and later slightly modified for chymotrypsin by Dixon & Neurath (1957) (eqn *30*, page 603) assumes catalysis of the acylation and de-acylation steps by imidazole. An alternative scheme has been proposed by Krupka & Laidler (1960) who have included the acidic group at the active site (eqn *34*, page 607). The Michaelis complex has no ionisable groups present at the active site,

whereas the free enzyme and acyl-enzyme have the basic and acidic groups previously noted. This suggests that the substrate and enzyme in the complex are bound through these groups and that these groups are liber-



(70) (71) (72) (73)



Michaelis complex Acyl enzyme

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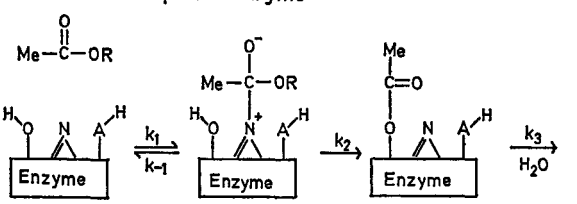

ENZYME + $MeCO_2H$

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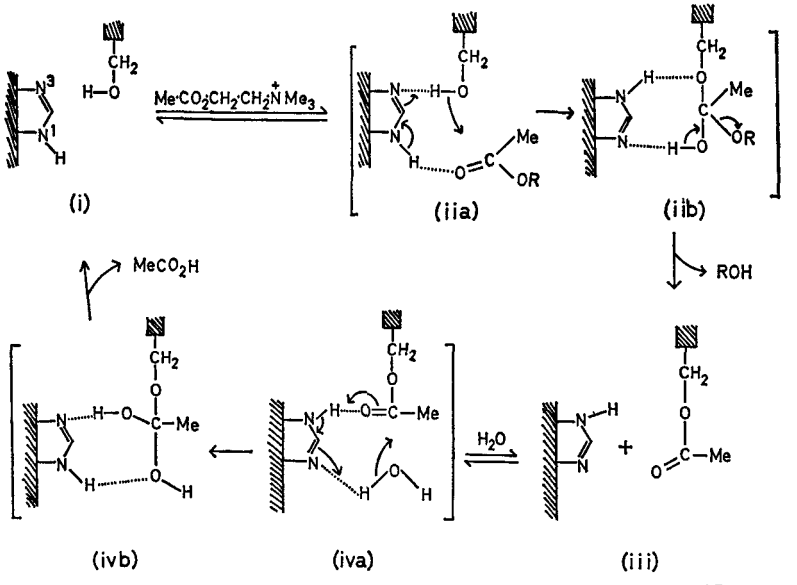

(i) (iia) (iib) (ivb) (iva) (iii)

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ated when transfer of the acyl function to serine occurs (Krupka & Laidler, 1960). Inhibitors of acetylcholinesterase may be divided into two classes; small molecules which block de-acylation by combining with the anionic site and acidic site, e.g. (73), and larger molecules such as

neostigmine (72) which are bound only at the anionic site and do not block de-acetylation (Krupka & Laidler, 1961). Krupka & Laidler have deduced from these facts that the anionic site is 5·0 Å and 2·5 Å from the basic (imidazole) and acidic sites respectively.

A recent scheme by Brestkin & Rozengart (1965) introduces the idea that acetylcholine participates in activation of the catalytic site and that the reactive form of serine does not exist in the molecule before interaction of the enzyme with the substrate (eqn *35*, page 607). Hydrogen-bonding between the carbonyl oxygen atom of the acetylcholine molecule, bound at the anionic site, and N–1 of the imidazole ring increases the basicity of N–3 which bonds with the serine hydroxyl (iia). This bonding increases the nucleophilic nature of the serine oxygen which attacks the acetylcholine carbonyl group with formation of a cyclic Michaelis complex (ii b). Electron rearrangement in (ii b) with expulsion of choline gives the acyl-enzyme (iii), which reacts with water through (iva, b) with regeneration of the enzyme and formation of acetic acid. The role of the acidic group at the active site is probably to transfer a proton from imidazole to either the choline or serine oxygen anions formed in (iib) and (ivb) respectively.

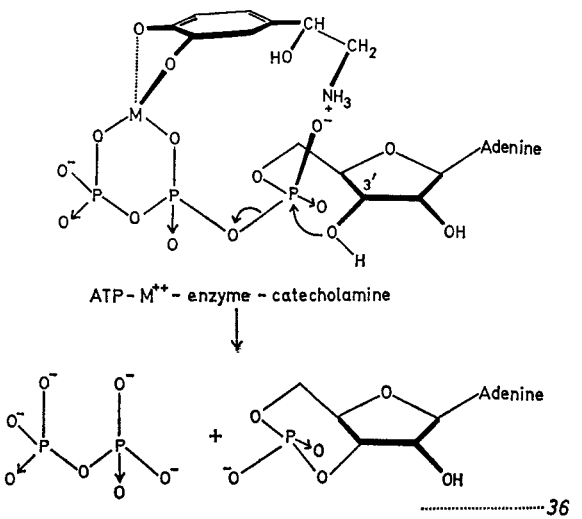
In conclusion it can be said that the proposed mechanisms for the action of α-chymotrypsin or acetylcholinesterase have not yet been universally accepted. However, from the foregoing discussion it is clear that the workers in this field consider that enzyme action can be explained in terms of the simple intramolecular processes which constitute the basis of this article.

Hormones

Whereas much is known about the general actions of hormones on organs *in vivo* and in tissues *in vitro*, very little information is available concerning the biochemical mechanisms which are influenced at cellular level. There has been some speculation about the nature of the receptors involved (Schwyzer, 1963). Some hormones may act upon the deoxyribonucleic acid (DNA) of chromosomes enabling genes to initiate the synthesis of ribonucleic acid (RNA), specific proteins and enzymes. Others may also interact with enzyme systems or with cell membranes (including membranes of subcellular structures).

Evidence has been forthcoming about the nature of the biochemical carrier of the phosphate group at the adrenergic receptor (Rall & Sutherland, 1959; Haynes, Sutherland & Rall, 1960). Adrenaline was shown to have a catalytic effect on the cyclisation of adenosine triphosphate (ATP) to adenosine-3′,5′-phosphate (3,′5′-AMP) which by activating phosphorylase increases the rate of glycogenolysis. The catecholamine is thought to interact with the system ATP-Mg^{++}–enzyme leading to 3′,5′-AMP as a result of (*a*) electrostatic attraction between the ammonium ion of the hormone and the phosphate ion, and (*b*) the formation of a co-ordination complex involving the phenolic groups of the hormone and the metal ion. The cyclisation process involves nucleophilic attack

by the 3′-OH group of the ribose moiety on the phosphorus, resulting in the ejection of pyrophosphate and 3′,5′-AMP, (eqn *36*, below) (Belleau, 1960). Under physiological conditions the oxygen atom on the phosphorus bears a negative charge which, as a result of interaction with the positively charged nitrogen atom of the hormone, becomes more effectively distributed. This increases the electrophilic nature of the



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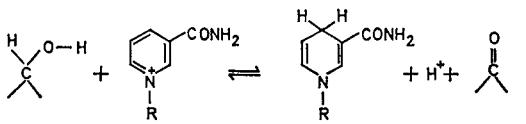
phosphorus reacting centre and consequently facilitates the process of 3′,5′-AMP formation and pyrophosphate ejection. Thus the hormone, in assuming the role of a general acid catalyst, supplements the activity occurring at this enzymatic site. [The blocking of the adrenergic receptor by dibenamine (554) can now be explained in terms of the esterification of the phosphate anion.]

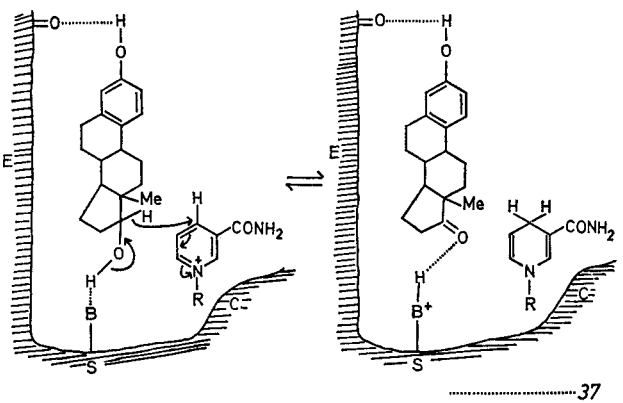
The formation of a complex between the catechol ring and the metal ion greatly increases the affinity of the hormone for the enzyme. Furthermore, molecular models reveal that chelation and ion pair formation can occur without the development of strain in the complex. As a means of obtaining further information about the β-adrenergic receptor site a series of tropolones biologically isosteric with the catechol system was shown to be effective as blocking agents (Belleau, 1963; Belleau & Burba, 1963). This clearly further implicates chelation as an important factor in the interaction of catecholamines with these receptors.

In attempting to correlate the structure and biological activity of 5-hydroxytryptamine (5-HT), Csötöstök, Per-enyi & Földes (1963) have found that the dissociation constant of the amino-group of 5-HT is 16 times greater than that of 4-HT which compares well with differences in their biological activities. Since ionisation is a prerequisite for the biological activity of 5-HT the possibility exists that this hormone, like adrenaline, becomes actively involved in the chemical changes taking place at an important receptor site. In this respect, the possibility

that 3′,5′-AMP acts as a mediator in the activity of 5-HT has already been contemplated (Haynes & others, 1960) to account for the stimulating effect of the hormone on the rate of glucose uptake by certain tissues. These authors contend that the adrenocorticotrophic hormone (ACTH) acts by controlling the phosphorylase level in the adrenal cortex. This suggestion follows from the discovery that ACTH causes an increase in the intracellular concentration of 3′,5′-AMP. Finally, as a result of these many observations, it is postulated that 3′,5′-AMP may function extensively as an agent of hormonal control.



VIII



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The occurrence of reduced and oxidised forms of many hormones in the body, e.g. hydrocortisone and cortisone, oestradiol and oestrone, suggests that this may be related to their physiological activity. Thus the action of oestradiol may be that of a catalyst involved in the transfer of hydrogen between reduced and oxidised forms of pyridine nucleotides. The net effect is the transfer of hydrogen from nicotinamide-adenine dinucleotide phosphate (NADP) to nicotinamide-adenine dinucleotide (NAD) (Talalay & Williams-Ashman, 1958).

$$NADPH_2 + NAD \rightleftharpoons NADP + NADH_2$$

Hagerman & Villee (1959) believe that oestradiol combines with the enzyme activating it, enabling it to effect a direct transfer of hydrogen between the NADP and NAD systems.

In addition to this transhydrogenation there are thought to be two oestradiol dehydrogenases catalysing reactions (1) and (2) respectively.

(1) NAD + oestradiol $\rightleftharpoons$ oestrone + NADH + H^+

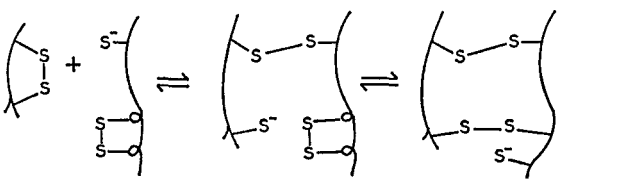
(2) NADP + oestradiol $\rightleftharpoons$ oestrone + NADPH + H^+

Laidler & Krupka (1961) have discussed reaction (1) in detail (see VIII, page 610) as an example of a molecular mechanism by which hydrogen transfer may occur in enzyme systems. This reaction is similar to the oxidation of lactic acid by NAD for which considerable kinetic information is available (*vide infra*). The rate-pH profile for the reaction shows a maximum, which suggests that a basic group –B and an acidic group –A–H play important roles. Studies involving isotope exchange (Loewus, Ofner, Fisher, Westheimer & Vennesland, 1953; Vennesland, 1955) suggest that this transfer of hydrogen is a direct one from substrate to coenzyme. A mechanism is proposed in which the rate-determining step for the enzyme-oestradiol-NAD system is visualised as in equation *37*. The non-polar oestradiol is probably attached to the enzyme surface by "hydrophobic bonds" arising as a result of the contact between non-polar groups leading to an increased degree of hydrogen bonding between solvent molecules. These "bonds" may also be supplemented by a hydrogen bond involving the oestradiol hydroxyl group. The transfer of hydrogen from the steroid to the NAD is facilitated by the active participation of group –B (enzyme site S; eqn *37*) as a general base catalyst. Oestradiol is converted to oestrone which now becomes attached by hydrogen bonding to the newly formed $-{}^{+}$B–H group. It is possible that other acidic and basic groups on the enzyme surface are involved (i) in the binding of NAD to site C and (ii) in the activation of NAD to facilitate hydrogen transfer. This action of oestrogen on a specific enzyme system causes a marked increase in the cellular energy available for directing chemical reaction towards synthesis.

More recent studies using spectrophotometric and polarographic methods (Allison, Poever & Gough, 1962) have demonstrated the electron donor and acceptor capacity of several hormones including 5-HT, indoleacetic acid, prolactin and natural and synthetic oestrogens. Consequently, hydrogen bonding and the formation of charge transfer complexes could be involved in the activity of many hormones. Villee (1963) provides further evidence for this view by demonstrating that both corticosterone and progesterone increase the rate of reduction of nicotinamide adenine dinucleotide (NAD) by glutamic dehydrogenase.

It has been shown that insulin, ACTH and vasopressin all affect the permeability of cell membranes. A possible mechanism to account for this alteration in permeability has been suggested as a result of studies with vasopressin (Fong, Silver, Christman & Schwartz, 1960; Rasmussen, Schwartz, Schoessler & Hochster, 1960; Schwartz, Rasmussen, Schoessler, Silver & Fong, 1960). The increase in permeability to water of the isolated toad bladder was found to be related to the amount of tritium-labelled vasopressin bound to the bladder protein. Since a large proportion of the bound material could be liberated by incubation with thiol compounds it is suggested that the hormone combines with membrane protein by a thiol-disulphide interaction. This view is supported by the observed inhibition of hormone action when the pH of the solution falls below 7. Under these conditions the nucleophilic mercaptide ion becomes more highly protonated and less able to react with the hormone S–S

bridge. This SH, S–S interchange between hormone and receptor, which proceeds readily as a result of an initial electrostatically induced alignment of the relevant surfaces, could induce profound conformational changes in the protein of the diffusion barrier by breaking critical disulphide cross-linking (eqn *38*, below) with the formation of channels through which water, urea and Na^+ could flow. It is also possible that the hormone induces a separation of disulphide-linked fibrillar structures (eqn *39*, below) which could initiate a series or wave of SH, S–S interchanges as shown. Wave-like reactions of SH, S–S interchange



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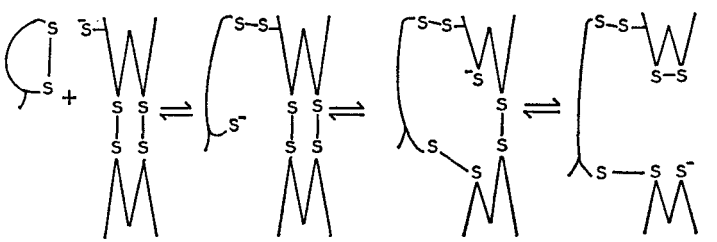

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have also been implicated in a number of other important biological systems such as blood clotting and the changes involved during the mitotic cycle (Jensen, 1959). Disulphide and thiol groups occur widely in protein molecules and are potentially among the most reactive of protein functional groups. Normally, their reactivity is restricted by the conformation of the protein molecule. However, the control of many important physiological processes may depend on factors which establish conditions under which "intramolecular" nucleophilic interaction between protein SH and S–S groups can take place.

An attempt has been made to interpret the activity of insulin in terms of such an interaction involving S–S linkages of the peptide chain and SH groups of a cellular receptor (Cadenas, Kaji, Park & Rasmussen, 1961). This interpretation has, however, been questioned by Carlin & Hechter (1962) who were unable to demonstrate any inhibition of insulin activity after exposing the receptor tissue to thiol blocking agents.

It has often been stated that the overall effects of a hormone may well be due to its simultaneous action on a number of enzyme systems when the balance between the rates of reactions would be an important factor. Nevertheless, the exploration at cellular level of hormonal action may

continue to reveal common fundamental mechanisms underlying multiple biological activities. Recently published findings (Tata, Ernster, Lindberg, Arrhenius, Pederson & Hedman, 1963) related to possible biochemical mechanisms underlying the activity of thyroid hormone lend further support to this view.

Drug action

The fact that most biologically active substances are so constituted as to be capable of ionisation leads one to reflect on the role of such elements as nitrogen, oxygen and sulphur in drugs. This immediately brings to mind well established concepts such as those related to the penetration of membranes and the formation of drug-receptor complexes. When coupled with more recent information emerging from studies on the mechanism of enzyme and hormonal actions (*vida infra*) such reflections readily evolve into speculations which may suggest fresh concepts from which a further insight into the action of drugs at cellular level may be gained. It is the purpose of this section to examine these concepts in the light of those which are already familiar.

A large number of drugs contain a nitrogen atom whose "lone pair" of electrons can play a significant role in the biological activity of the molecule. Thus this "lone pair" may bond covalently with a hydrogen ion to form a salt in accordance with the equilibrium,

$$R_3N + H^+ \underset{}{\overset{k}{\rightleftharpoons}} R_3N^+H$$

and the ratio of ion to neutral molecule at physiological pH will depend on the ionisation constant. It is thought that many substances penetrate cell membranes as neutral molecules to exert their biological activity within the cell as ions.

A drug produces its biological response as the result of an interaction with a functional or organised group of atoms referred to as a receptor site. In order to facilitate such interaction the drug molecule must be correctly aligned at the site. To achieve this alignment, binding forces are invoked by the presence of groups on both drug and receptor surfaces which are complimentary to one another. In this respect, ionic "bonds," hydrogen bonds, ion-dipole and dipole-dipole interactions and van der Waals' forces all play their part. Ionisation at physiological pH occurs in aliphatic amino-, carboxyl, thiol and sulphonamido-groups so that potential ionic "bonds" are frequently found in drugs containing nitrogen, oxygen or sulphur. Each of these elements possesses at least one "lone pair" of electrons and is capable of forming hydrogen bonds. Differences in electronegativities between carbon and oxygen or nitrogen lead to dipoles which are able to form weak bonds with regions of low or high electron density, such as ions or other dipoles. Such dipolar functions as carbonyl, amide and ether are often located in drugs. Since, in most cases, it is essential for a drug to dissociate from the receptor site when the concentration in the extracellular fluid decreases, irreversible covalent bonds are undesirable.

On attaining a good "fit" to the receptor the drug may act either by initiating a response or by decreasing the activity normally associated with the site. In the latter instance, the drug blocks access to the site by the molecule responsible for producing a biological response. The view is firmly held that this effect explains the mode of action of drugs such as sulphonamides, antihistamines and neuromuscular, ganglionic and adrenergic blocking agents.

We would support a thesis which recognises that some drugs may fulfil an active role in biochemical mechanisms proceeding at or near a receptor site. It thus becomes possible to envisage a system whereby a drug, suitably ionised at physiological pH and correctly orientated at a receptor site, could influence a biochemical reaction occurring at a closely situated enzymatic site. A clinical condition could conceivably originate from a depletion of the required catalytic species at an important biochemical site of activity. A drug which alleviates this condition might possibly owe its effect to, say, a cationic group which as a result of general acid catalysis supplements the abnormally low activity at this site. Drugs which simulate the actions of adrenergic hormones may well behave in this manner.

Such speculations as these might tentatively be extended to include several types of drugs where the activity could depend upon the presence of nitrogen, oxygen or sulphur in anionic forms. Thus barbiturates, being ionised (N^-) at physiological pH, may influence an important enzymatic reaction as a result of nucleophilic reaction. The mechanism by which central nervous system depressants uncouple oxidative phosphorylation may well involve such considerations. Further conjectures could possibly include other substances such as salicylates, phenols and thyroxine which are also known to uncouple oxidative phosphorylation.

The pronounced activity of thiol groups in hormones has previously been cited. This group representing the tautomeric forms of thioamides, is found in several classes of synthetic biologically active compounds, e.g. dithiocarbamates, thiouracil. In its ionisable form it may enable some of these drugs to act in a manner previously envisaged.

To continue with these speculations may not be appropriate or even desirable at this present stage in our understanding of fundamental mechanisms occurring at cellular level. However, our survey of the extremely powerful intramolecular mechanisms which occur at enzyme surfaces as a direct consequence of the close proximity and correct orientation of groups, leads us to suggest that our hypothesis as outlined in this section, may well be within the bounds of possibility.

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