CLXIII. EXPERIMENTS ON BACTERIA IN RELATION TO THE MECHANISM OF ENZYME ACTION.

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INTRODUCTION.

It may be urged that resting or non-proliferating bacteria do not form suitable material for experiments on the mechanism of enzyme action; for the reason that the reactions to which such bacteria give rise may be due, not so much to the enzymes as to the circumstance that they form organised systems capable of effects not brought about by constituent members of the systems.

In fact, it may be urged, a living, though not proliferating, cell may possess some property which is expressed by the reactions we are investigating and which would not be possessed by the cell were it disorganised or dead. That this criticism is groundless is shown by the following evidence.

1. If a suspension of *B. coli* be shaken with toluene or ether, the organism is no longer capable of reproduction on the usual nutrient media and therefore, so far as we can ascertain, is dead. But it can still give rise to a large number of activations, *e.g.* with formic, lactic, and succinic acids, at rates not markedly different from those induced by the normal or untreated organism.

2. The effect of exposing *B. coli* to some abnormal treatment (resulting usually in the death of the organism) is to produce, as exposure is prolonged, a step by step elimination of the various activating mechanisms, as though each mechanism were differently susceptible to the treatment. Now, were the activations of the cell due to the latter acting as an organised whole, we should expect that at some particular point the cell would become disorganised and its activations would cease. Each mechanism would be eliminated at the same time and there would be no step by step degradation. Since this is contrary to what actually occurs we cannot suppose that the activations of the cell depend upon the survival of the latter as an intact organised system.

We have indeed *no* evidence to support a contention that the reactions of the organism are dependent upon its survival as a living unit. It is true that when an organism is killed certain of its activations (usually those of the sugars in the case of *B. coli*) disappear, but the simple interpretation is that the agent responsible for the death of the organism is also responsible for the disappearance of the activating mechanisms—not that the disappearance occurs because the organism has been killed.

There can be no doubt, however, that the power of the cell to proliferate is dependent upon the intactness of (among other things) many of the activating mechanisms with which we have to deal. This is entirely to be anticipated, for we cannot expect a cell to survive, whose cellular or intracellular structures have been so changed, by exposure to abnormal conditions, that they can no longer perform activations (which, on our views, are simply an expression of the nature of these structures).

In a previous communication by one of us [Quastel, 1926] the theory was advanced that the dehydrogenations¹ effected by bacteria are primarily due to polarisations of substrate molecules induced by electric fields which characterise particular centres—the “active centres”—of cellular and intracellular surfaces. The effect of polarisation may be to activate the molecule, *e.g.* it may become a hydrogen donator or acceptor, but since activation necessitates the uptake of a critical amount of energy, it follows that not all molecules which are polarised will become activated. This will depend on the nature

¹ Such dehydrogenations do not refer to reductions brought about by glutathione or substances which are active *in vitro*; they refer primarily to substances which must be activated by the cell before their oxidation can occur.

and intensity of the polarising field and upon the electrical and chemical nature of the substrate molecules. The mechanism by which a substrate, when activated, becomes a hydrogen acceptor or donator is discussed in earlier papers [Quastel, 1926; Quastel and Wooldridge, 1927] and will not be discussed further here.

The view there put forward makes it possible to interpret in a consistent manner such phenomena of biological oxidations as β -oxidation, asymmetric oxidation of the double bond, the α -oxidation of propionic acid, the mode of oxidation of branched chains and so forth. It also indicates how it is possible to regard specificity of behaviour as belonging to the molecules themselves; it being unnecessary to postulate the existence of numerous specific enzymes related to oxidations. Thus one active centre may be able to effect the activation of a number of substrates, the chance of one molecule rather than another being activated depending upon its structure. But specificity of behaviour must depend not only on the chance of the molecule being activated at a particular centre but also on its power of obtaining access to that centre. If the structure of the molecule is such that, although it may become activated by the centre, it cannot reach the centre, or remain there for a sufficient period of time for activation to occur, it is clear that the molecule will not react. Specificity, then, is also a function of the accessibility of a molecule; adsorption and appropriate orientation are factors which help the access of a molecule to a centre.

The "active centre" is a part of, or a property of, cellular and intracellular surface structures; it is inseparable from surface structure, as it is a function of it. Some conception of its properties and mode of formation will be given in Section V. It is not necessary to believe that only surfaces of considerable magnitude are capable of possessing such centres. Any surface structure, however small, may possess such centres; this will depend upon the nature of the surface. This view is discussed more fully later.

If a surface structure be attacked by chemical or by physical means a change in the number and type of the active centres distributed over the surface will take place. The change will be dependent upon the extent to which the surface structure is changed. The centres which are least resistant to "shocks" (though the resistance must vary, we expect, to some extent with the type of "shock") will be the first to disappear as the surface structure is attacked. But it does not follow that an active centre must *entirely* disappear as a result of change. It may form a new centre, according to the nature of the change, with a smaller strength of field, or perhaps a field of quite different nature. As a surface structure is attacked, the general effect, we expect, will be that the least stable active centres are first eliminated; then come those relatively more stable and finally the most stable. Thus there will result a definite sequence of eliminations which will proceed step by step. The sequence will be the same, as a whole, for a large number of methods of treatment of the organism—but that it is unlikely *always* to be so is clear,

for individual variations, due to particular effects of an attacking reagent, will on certain occasions make themselves manifest. Thus a certain reagent may react vigorously with a certain constituent group of the active centres, rendering inert the substrates which are made accessible to the centres by means of this group. Yet the elimination of this group may not influence very markedly the activating power of these centres, and so substrates whose accessibility is unaltered are still activated. Such a reagent would be liable to give a different sequence of eliminations from that produced by another reagent.

That the general effect of reagents is to give a sequence of eliminations which remains as a whole the same is illustrated by experiments described in our last paper [Quastel and Wooldridge, 1927]¹ where it was noted that a variety of different treatments of *B. coli* gave the same general order of eliminations. Experiments described in Section III will further illustrate this.

The importance of accessibility—*e.g.* adsorption or orientation—of a substrate as a factor in the determination of specificity will be discussed later, but it is as well to point out now that although a molecule may become activated at a centre it may not, in this activated state, undergo the reaction in question until it has passed through a particular phase of internal (electronic) structure or through a particular phase of stability. Various factors, such as orientation or adsorption, may influence the time necessary for the activated molecule to reach this phase. This interval of time is of importance, for it is possible that the molecule may become deactivated by collision before it has time to react. If the substrate molecule, through some property of its own structure or of that of the active centre, is unable to remain in contact with the centre for a sufficiently long period of time, its chance of reacting becomes small, in spite of the fact that the centre may have sufficient power to produce an activation. On the other hand, if there be present in the centre a grouping (or set of groupings) which will hold the molecule for a sufficient period of time reaction will ensue. This grouping is the “active group” of an enzyme. On our views it is simply one of the factors influencing accessibility of a substrate and hence influencing the specific behaviour of the active centre. The centre, *as a whole*, activates; one or more of its groupings is responsible for the access of the substrate. It may be possible to modify or destroy the centre, without necessarily altering the groupings responsible for access, *i.e.* the substrate may still be adsorbed without activation occurring. Or again, it may be possible to eliminate a group responsible for access without reducing the field strength to such an extent that it can no longer activate the molecule. The importance of this point lies in the fact that we should be able to differentiate between two molecules, activated at the same centre but each requiring a different group to make it accessible. By eliminating such a group one

¹ The references [Quastel, 1926] and [Quastel and Wooldridge, 1927] will, from this point, be represented by the signs [1] and [2] respectively. These papers should be consulted for full details of the experimental technique adopted in this communication.

substrate will be rendered inert whilst the other molecule, whose access is unaltered, may still be capable of activation by the centre. Thus the centre would have the appearance of being composed of two specific enzymes.

Specificity, then, is a function of the following factors.

1. Nature of the polarising field at the active centre.
2. Structure of the substrate molecule.
3. Orientation and adsorption of the substrate molecule at the centre; these depend also on the chemical structure of the centre itself.

It will be seen that a centre may easily differ from a neighbouring centre. Each has an individuality of its own—each may, indeed, be regarded as a specific enzyme but with a certain limited range of specificity. The two fundamental ways in which an active centre differs from a truly specific enzyme are:

- (a) a range of specificity which is limited—characteristic of each centre:
- (b) the identity of an active centre with a surface structure.

Willstätter [1927] states: "It seems that we must consider an enzyme to be composed of a specifically active group and a colloidal carrier.... The colloidal carrier seems to vary somewhat in its nature, but to be necessary for the stability of the active group." This statement can be regarded as an approximation to the view of active centres, it being emphasised once more, however, that on this view activation is due to the centre as a whole, probably to a composite of groups, and not necessarily to a specifically active group. The latter, whilst forming an essential feature of the centre, may be important primarily in securing the access of the substrate to the activating field.

Let us now consider the evidence which leads us to suppose that activations (dehydrogenations) due to bacteria are associated with surface action.

I. EVIDENCE FOR SURFACE ACTION IN THE DEHYDROGENATIONS PRODUCED BY *B. COLI*.

Whilst the evidence from work on enzymes points very clearly to the participation of surface action it is difficult to prove rigidly, by one particular experiment, that in the activations due to bacteria surface activity is fundamentally involved. It is only when the evidence as a whole is taken into account that there seems to be no escape from this conclusion, except by postulating a number of assumptions which are usually highly questionable and for which we have no evidence. By surface activity is meant activity by surfaces, either cellular or intracellular.

Quantitative measurements [1] have already indicated that the main site of reduction of methylene blue by a hydrogen donor is at the cell surface and for reasons given in that paper it was shown that a site of activation of hydrogen donors is also at the cell surface.

If a suspension of *B. coli* be shaken with toluene [2] a number of activations (notably those of the sugars and glutamic acid) are eliminated, whilst others (succinic acid, lactic acid, formic acid) are retained. We may try to explain

this purely on permeability considerations. The argument would be, presumably, that the effect of the toluene is to act selectively on the cell membrane, rendering it impermeable to the sugars but not to succinic acid, etc. Now this selectivity of action cannot be attributed merely to mechanical blocking for whereas glycerol is made inactive, α -glycerophosphoric acid is as active after as before treatment; again, methylene blue must still be freely permeable for the rates of action due to formic acid, etc., have not changed. We may then suppose that the selectivity of action is due to the formation of a film, at the surface, possessing selective permeabilities, but this cannot be true, for the formation of such a film would not allow methylene blue to penetrate it. Methylene blue does not diffuse, under the conditions of our experiments, from aqueous solution into toluene and we should expect a film of toluene at the cell surface to act like a lipid layer which, as we know from the work of Loewe [1912], would adsorb but not dissolve the dyestuff, *i.e.* the latter would not penetrate into the cell.

We may suppose finally that there is a specific entrance into the cell for the sugars, glycerol and glutamic acid and another one for succinic acid, lactic acid, methylene blue, etc., and that toluene blocks the specific entrance for the sugars and not the other entrances. Now from the results of other experiments we can demonstrate differences between succinic acid and lactic acid, between glycerol and glucose, etc., so that if the interpretation of our results is simply to be that of specific attacks on pores specific for substrates we would have to assume a specific pore for almost every substrate—a conclusion which seems to be entirely out of the question.

Since the problem does not find a satisfactory solution simply in the consideration of changes in permeability, we may take the view that the toluene penetrates the cell and there affects certain specific enzymes, *i.e.* those related to the sugars, etc. But ether, benzene, chloroform, propyl alcohol, all have extremely similar effects to toluene, and since it is scarcely conceivable that such substances as these form definite chemical compounds with the enzymes, it is reasonable to suppose that they would act in the manner we would anticipate from such capillary active substances. We expect that they would form films upon the specific enzymes, rendering the latter inaccessible to methylene blue or to the substrates or to both. This clearly indicates, as we would naturally expect from other work on enzymes, that the specific enzymes for the dehydrogenations are associated with surfaces of some description. This does not apply only to the sugars, for experiments with propyl alcohol indicate that the other specific enzymes—those for succinic acid, lactic acid, etc.—are equally associated with surface action.

If the toluene does *not* enter the cell, thus affecting certain enzymes therein, we must imagine that it forms a film upon a particular patch or series of patches on the cell surface, these patches being particularly associated with the activations of the sugars, etc. If these patches were not so associated there seems to be no reason why the sugars and methylene blue should not

enter the cell at parts of the surface not affected by toluene and so react with enzymes in the cell.

Were we to ascribe the effects of treatment of *B. coli* by nitrites [2] or by high concentrations of salts [2] or by the halogens or by allyl alcohol or by sodium acrylate (Tables I and II) to changes in permeability of the cell membrane alone, we should be placed in the difficult situation of having to account for the fact that whilst a variety of donators are prevented from entering the cell, methylene blue appears to be freely permeable always. For if it were not so, formic acid could not react at its normal rate when many of the other donators have been rendered inert. Again, it seems inconceivable that such a variety of attacks should change the permeabilities of the substrates in almost exactly the same order. The simple interpretation of these facts is that the main site of reduction of methylene blue is at the cell surface, supporting the conclusion drawn from quantitative evidence.

It seems, therefore, from various lines of evidence that surface activity is intimately associated with the activations of many hydrogen donators. The evidence is based upon experiments carried out with methylene blue, and *with this dye-stuff* it appears that the outer cell surface of *B. coli* is the main site of reduction; hence this surface must constitute *one* important site of the activation of hydrogen donators. Methylene blue has been valuable in demonstrating this fact and hence in indicating clearly the importance of surface action. But it would be absurd to consider that because, with methylene blue, the outer cell surface is the main site of reduction, activations cannot be performed within the cell. The facts that mechanical disintegration of bacteria appears to have a destructive action on certain activating mechanisms [1] and that lysis of the organism by bacteriophage ultimately removes the power of the cell to reduce methylene blue [Shwartzman, 1927; Gozony and Surányi, 1925] support our conclusion that the cell surface is a site of activations, but we cannot expect that the breakdown of the cell surface will necessarily involve the destruction of all intracellular surfaces and the elimination therefore of all activating power. Our thesis has been to show that surface activity, whether it be cellular or intracellular, is fundamentally connected with the activations we are investigating and it should therefore be borne in mind that when reference is made to surface activity, the term surface includes both cellular and intracellular surfaces.

Let us now consider briefly the phenomenon of activation and the conception of active centres.

II. ACTIVATION.

It has been necessary for several reasons to consider that in homogeneous reactions a molecule cannot undergo transformation and dissociation unless it possess a certain minimum internal energy. "By internal energy is meant all the energy it possesses over and above what it would possess at very low temperatures except energy of translation, which, by the principle of relativity,

can have nothing to do with the possibility of an internal change. Any molecule containing an amount of energy equal to or greater than a critical amount may be called an activated molecule. It may not be in a position to undergo the particular transformation we have in mind for this may depend upon the orientation of the several parts of the molecule, upon the location of the energy within the molecule and upon other factors of a similar nature" [Lewis and Smith, 1925].

Two types of chemical union are recognised: the electrostatic attraction between oppositely charged ions, this is a polar linkage; and the attraction which is produced through the sharing of an electron by two atomic nuclei, this is a non-polar linkage. We suppose that for the dissolution or formation of a non-polar linkage there must be primarily a shift of the electrons from their original orbits. This necessitates the uptake of energy, the process being termed activation. A critical amount of energy of activation must be taken up by a molecule for reaction to be possible.

Ions do not require activation in ionic reactions with which we are familiar, for such reactions consist simply of rearrangements of polar linkages. There is no necessity, for instance, to postulate activation of hydrogen or hydroxyl ions.

At present two fundamental methods of activation are recognised, collision and radiation. It is unnecessary, for the purposes of this paper, to go into the processes in detail, but it is important to note the following points.

1. An activated molecule may be deactivated by collision.
2. An activated molecule may not react until a particular phase of the internal structure of the molecule has been reached [Lindemann, 1922; Thomson, 1927]. We may expect a definite time interval between time of activation and time of transformation, the interval depending on the way in which activation has been effected.

Active centres.

Much of the work on surface catalysis carried out within recent years¹ has gone to show that catalytic activity occurs at particular areas of the surface and that it varies in nature according to the nature of these areas. The areas, or active centres, constitute as a whole the active surface of the catalyst.

Adsorbed molecules at an active surface are regarded as in a distorted condition, or in a state of strain, the amount of distortion depending upon the molecular structure.

It has been shown that if a reaction catalysed by a surface occurs between two reactants both must be adsorbed and activated [see Hinshelwood, 1926].

¹ The subject is reviewed in the *Annual Reports of the Chemical Society*, 22, 17. Owing to the existence of this report we refrain from quoting the literature concerned with surface catalysis and active centres. But fully to understand this section, the report should be read by those unacquainted with the literature.

The value of adsorption, of some description, at an active centre is obvious when it is considered that we cannot expect activation at the centre, in general, to be instantaneous. When it is considered, too, that deactivation by collision may occur it is sufficiently patent that appropriate orientation of a substrate molecule (which may lessen the time for activation to occur) and adsorption (which may give time for the activation) are factors of utmost importance in the consideration of the specificity of behaviour of active centres.

It seems to be fully established that the active areas occupy only a small fraction of the surface and that they vary in capacity both to adsorb reactants and to promote reaction.

Taylor [1925] conceives that the activity of metal surfaces is due to incomplete surface crystallisations, so that there are produced occasional groups of atoms associated with high energy and chemical unsaturation relative to the atoms in the regular crystal lattices. [In this connection see also Kistiakowsky, Florsdorf and Taylor, 1927.]

In this paper we shall adopt the view put forward previously by one of us [1] that the activations at biological surfaces occur at the active centres of these surfaces, the mechanism of activation being a polarisation of the substrate due to electric fields at the centres. The specificity of action at the centre depends upon the accessibility of the substrate to the centres, as well as upon the electrical nature of the substrate and the nature of the polarising field.

We are aware that a purely "chemical" view of activations is possible but we find such a view inadequate to interpret our results and unsatisfactory in its inability to give us a rational picture of the mechanism whereby activations are effected. Our own view can be regarded as quite definitely chemical, yet it has the considerable advantage that one is enabled to perceive more clearly the extent to which various factors can operate upon specificity of action, than by a view which calls for a new enzyme related to, and for the formation of a new unstable intermediate compound with, every new substrate which is found to be activated.

We will now enquire into some of the properties of the active centres concerned with the dehydrogenations effected by bacteria.

III. BIOLOGICAL ACTIVE CENTRES.

Treatment with the most diverse reagents has the effect of eliminating the activating mechanisms of *B. coli*. This leads to the conclusion that different groupings may be involved in the activation of substrates and since any one enzyme, or active centre, can be eliminated by any of the treatments, if the latter be carried out for a sufficient period of time, we must conclude that an active centre is made up of a number of groupings.

Or, if we take the attitude that the effect of various reagents is not to attack specifically certain groups in the active centres but to alter surface

structure as a whole, so that the active centres are changed or eliminated, we must still conclude that such centres are of a somewhat complex nature and not composed simply of one grouping, as, for instance, the amino- or hydroxyl-group.

It is an interesting fact that approximately the same order of eliminations occurs on submitting *B. coli* to the action of

- (a) varying concentrations of different salts (Table II);
- (b) bromine and iodine (Table I);
- (c) formaldehyde (Table I);
- (d) allyl alcohol and acrylic acid (Table I).

The action of salts on B. coli.

An examination of Table II, which gives a summary of the experimental results, will show how the same order of elimination of the substrates occurs with all the salts investigated. There are several points of interest arising out of these results.

(a) The reduction time for formates appears to keep almost constant in spite of large changes in the reduction times for succinate or lactate or glucose.

(b) The iodate ion is more effective than the chlorate ion, and the barium ion far more than the calcium, strontium or magnesium ion in accomplishing inactivations.

The fluoride ion is far more effective than the other halogen ions in attacking, particularly, the glucose-activating mechanism.

(c) Relatively low concentrations of salts, with the exception of barium chloride and potassium iodate, do not affect the organism. As concentration is increased the activating mechanisms are selectively eliminated, the bivalent calcium, barium and magnesium ions being more effective than the univalent potassium and sodium ions. The chlorate, bromate and iodate ions are also much more powerful in action than the chloride, bromide and iodide.

The action of bromine and iodine on B. coli.

Very small quantities of these halogens are required to effect the destruction of activating mechanisms of *B. coli*. As shown in Table I, the least resistant are those for glycerol, glutamic acid and succinic acid. Then come those for the sugars, then those for α -hydroxybutyric acid and lactic acid and finally, the most resistant, those for formic, acetic and butyric acids.

The action of formaldehyde and acetaldehyde on B. coli.

Both aldehydes have a powerful action on the activations of *B. coli*, formaldehyde at lower concentrations than acetaldehyde (Table I). The order of elimination is similar to that noted with the halogens, etc. (see Table III), but the sugar-activating mechanisms appear to be rather less resistant.

Table II.

Reduction times of 1 cc. 1/5000 methylene blue in presence of donators (succinic acid, etc.) by *B. coli* submitted to the action of various salts at various concentrations at p_H 7.4. Organism well washed after each treatment. Conditions as in Table I.

Treatment <i>coli</i> 1	t_{succinic}	t_{lactic}	t_{formic}	t_{glucose}	Treatment <i>coli</i> 2	t_{succinic}	t_{lactic}	t_{formic}	t_{glucose}
<i>M/5</i> NaCl	9'	6.25'	3.5'	4.25'	<i>M/2</i> NaCl	16.3'	11'	3'	56'
<i>M/5</i> KCl	12.5	9.25	4	7	<i>M/2</i> KCl	20	11	3	55
<i>M/5</i> KBr	12	7.5	4	5.3	<i>M/2</i> KBr	14.5	10.3	3	11
<i>M/5</i> KI	17	11.75	4.25	9	<i>M/2</i> KI	24.5	13.3	3.3	38.5
<i>M/5</i> NaF	29.5	18	3.25	24.5	<i>M/2</i> NaF	21	16	3.5	∞
<i>M/5</i> KClO ₃	19.5	10.75	4.25	10.5	<i>M/2</i> KClO ₃	160	27	3	∞
<i>M/5</i> NaBrO ₃	22.5	10.5	4	14.25	<i>M/2</i> NaBrO ₃	150	18	3.7	∞
<i>M/5</i> KIO ₃	∞	35.5	4.5	∞	<i>M/2</i> KIO ₃	∞	∞	5.3	∞
<i>M/5</i> NaNO ₃	13.5	17.75	4	7.25	<i>M/2</i> NaNO ₃	23.5	13	3	∞
<i>M/5</i> NaNO ₂	30	18	4.5	15	<i>M/2</i> NaNO ₂	51	19	5.5	∞
<i>M/5</i> Na ₂ SO ₄	11.5	7	3	6	<i>M/2</i> Na ₂ SO ₄	15.5	13.7	3.5	∞
<i>M/5</i> (NH ₄) ₂ SO ₄	11	7.3	3.3	5.7	<i>M/2</i> Na citrate	15	13.7	3.7	∞
<i>M/5</i> CaCl ₂	25	11.7	4.7	105	<i>M/2</i> CaCl ₂	52	37	11	∞
<i>M/5</i> SrCl ₂	27	10.3	4.5	11.7	<i>M/2</i> SrCl ₂	67	26	5.3	43
<i>M/5</i> BaCl ₂	∞	∞	165	∞	<i>M/50</i> BaCl ₂	15	11	4.3	7.5
<i>M/5</i> MgCl ₂	36	16	5.5	18	<i>M/2</i> MgCl ₂	100	31	6.3	∞
Control	7.5	6	4	5	Control	16.5	11	3.3	6.7
<i>M/1</i> NaCl	18	12.5	3.7	∞	Dist. water	24.5	12	3.3	9.5
<i>M/1</i> KCl	29	14	4	28	for 1 hour				
<i>M/1</i> NH ₄ Cl	28	13	4	∞					

Table III.

Showing the general order of eliminations produced by various treatments which are described horizontally. The least stable mechanisms are eliminated first. When two or more mechanisms are eliminated together they are given the same number, and that which is next eliminated is given the corresponding number allowing for those which were previously eliminated.

	Glycerol	Glutamic acid	The sugars	Succinic acid	Fumaric acid	α -Hydroxybutyric acid	Lactic acid	α -Glycerophosphoric acid	Formic acid	Acetic acid	Sodium nitrate
Increase of temperature	1	2	3	6	6	4	5	9	9	9	8
Increase of acidity	1	1	6	3	3	5	6	9	10	10	6
Increase of alkalinity	1	2	3	4	.	5	9	5	5	5	.
Hypertonic NaCl	1	2	3	6	1	4	8	7	8	8	.
NaNO ₂	4	3	6	1	1	4	8	7	9	9	.
Acetone	1	1	1	5	.	4	6	6	6	6	.
Benzene	1	1	1	5	.	4	6	6	6	6	.
Toluene	1	1	1	5	.	4	6	6	6	6	.
Ether	1	1	1	5	.	4	6	6	6	6	.
Phenol	1	1	1	5	.	4	6	6	6	6	.
Chloroform	1	1	1	5	.	4	9	8	6	7	.
Propyl alcohol	1	1	1	5	5	4	6	6	6	6	.
Cyclohexanol	1	1	1	1	1	1	1	8	8	8	.
Aniline	1	1	1	5	.	4	5	7	7	7	.
Methylene blue	1	1	1	5	.	1	6	7	7	7	.
Formaldehyde	1	1	1	1	1	6	7	8	8	8	.
Acetaldehyde	1	2	3	3	.	5	6	7	7	7	.
Allyl alcohol	1	1	1	1	1	7	8	1	9	9	.
Acrylic acid	2	1	3	3	3	7	8	6	9	10	.
Iodine	1	1	5	3	3	5	8	7	9	9	.
Bromine	2	1	5	3	3	6	8	6	9	9	.
KCN	3	4	6	6	6	9	10	5	1	1	.
KMnO ₄	3	3	6	7	.	8	9	5	1	1	.
H ₂ O ₂	1	1	1	8	8	10	11	7	4	4	4

The action of allyl alcohol and acrylic acid on B. coli.

Both these substances have very powerful effects (Table I). As usually observed, the mechanisms for lactic acid and the lower fatty acids are the most resistant to attack.

The order of elimination of activating mechanisms of B. coli.

The order of elimination noted above is similar to that already found on exposure of *B. coli* to nitrites at relatively low p_H or to different hydrogen ion concentrations [2]. See also Table III which gives a complete summary of these results on eliminations.

It would be natural to suspect that the same *order* of eliminations in such a variety of treatments is consequent upon the operation of some common factor underlying all the eliminations. This may be, as we have suggested elsewhere, a general change in the structure of the surface with the result that the most unstable centres disappear first, then those of greater stability and so on. But this view, though accounting for the same order of disappearances, cannot be entirely adequate. It does not take into account specific actions on the centres themselves, which by influencing one or more groups at the centre, may remove accessibility of certain substrates without necessarily affecting, in a marked degree, the activating powers of the centre. Were such groupings affected there would probably be a departure from the usual order of eliminations.

Such a departure from the usual order is noted particularly in the effects of exposing *B. coli* to dilute solutions of potassium permanganate or to relatively strong solutions of potassium cyanide.

The action of potassium permanganate, potassium cyanide and hydrogen peroxide on B. coli.

Here the mechanisms for formic acid and acetic acid, which are usually the most resistant to attack, are among the least resistant (see Table I). Some particular grouping, very sensitive to attack with $KMnO_4$ or KCN , would seem to be particularly associated with formic acid or acetic acid. A somewhat similar effect is produced by treating the organism with H_2O_2 ¹ (Table I). It may be an aldehyde or carbonyl group, but treatments with sulphites, phenylhydrazine, semicarbazide or aminoguanidine (Table IV) do not exhibit this anomalous behaviour of permanganate or cyanide.

Dilute concentrations of potassium cyanide are almost without action on *B. coli* and relatively strong solutions must be used to exhibit the eliminating action. Potassium permanganate solution, on the other hand, must be used in extremely dilute solution to show its selective effects; relatively strong solutions simply eliminate the entire activating action.

¹ The effect of H_2O_2 appears to vary somewhat with the strain of the organism, a fact which may be explained by variation in catalase content.

Table IV.

Conditions as in Table I.

Treatment	t_{glucose}	t_{succinic}	t_{lactic}	t_{formic}
Control (normal organism)	5	7.5	6	4
10 % NaCl (control), 1 hr., p_H 7.4	∞	15.5	11.5	3.7
5 % Semicarbazide, 1 hr., p_H 7.4	12	10	6	3
10 % Semicarbazide, 1 hr., p_H 7.4	∞	24	19	4.7
5 % Semicarbazide, 17 hrs, p_H 6.0	∞	∞	60	5.5
10 % NH_2OH , 1 hr., p_H 7.4	∞	∞	30	5.2
5 % NH_2OH , 17 hrs., p_H 7.4	∞	136	34	9
10 % Na_2SO_3 , 1 hr., p_H 6.0	36	21	12	4.7
5 % Na_2SO_3 , 17 hrs., p_H 6.0	∞	∞	31	8.7
1 % Phenylhydrazine, 17 hrs., p_H 6.0	∞	∞	50	∞
1 % Phenylhydrazine, 1 hr., p_H 7.4	>60	>60	8	>60
1 % Aniline, 1 hr., p_H 7.4	∞	63	33	6
2 % Aminoguanidine, 1 hr., p_H 7.4	32	33	19.5	4.2

Reversibility.

The action of cysteine and sodium hydrosulphite. So far as we can ascertain at present, the majority of the eliminations effected by the various treatments *B. coli* is made to undergo, are irreversible. Yet it may be that the correct experimental conditions to effect regeneration of the activating mechanisms, apparently irreversibly eliminated, have not yet been obtained and it would be unwise at present to conclude definitely that true irreversibility of action occurs. We can, however, show that some of the mechanisms attacked by potassium permanganate and particularly by potassium cyanide are capable of being regenerated. Exposure of KCN-treated *B. coli* to sodium hydrosulphite solution or cysteine (see Table V) brings about a marked recovery in the time of reduction due to formic and acetic acids. A slight recovery has been observed with the KMnO_4 -treated organism. As noted also in our previous paper [2] the formic acid or acetic acid mechanism after treatment of *B. coli* with nitrites can be partially regenerated by subsequent treatment with sodium hydrosulphite. Such a recovery does not occur with the succinic acid or lactic acid mechanism.

Table V.

Conditions as in Table I.

- Col. A. Reduction times due to *B. coli* treated with 1 % KCN soln. for 1 hr. at p_H 7.4 and 37° and finally well washed.
 Col. B. Reduction times due to KCN-treated *B. coli* which has been suspended in 2 % cysteine soln. for 1 hr. at p_H 7.4 and 37° and finally well washed.
 Col. C. Reduction times due to KCN-treated *B. coli* which has been suspended in 5 % sodium hydrosulphite soln. for 1 hr. and finally well washed.

Donator	A	B	C
M/28 Succinic acid	13.2	11.7	80
M/28 Lactic acid	6.2	7.2	16.5
M/28 Formic acid	60	9.5	23
M/7 Acetic acid	45	7.7	22
M/14 Glutamic acid	18.5	15.7	60
M/28 α -Hydroxybutyric acid	11.2	12	29
M/28 α -Glycerophosphoric acid	17	11.7	25.5
M/28 Glucose	5.7	6.5	17.5

The action of copper sulphate solution on B. coli and the reversing effect of H_2S .
 Exposure of *B. coli* to dilute solutions of copper sulphate (see Table VI) with

subsequent thorough washing, by centrifuging, results in the elimination of activating mechanisms of the organisms. If H_2S be passed through a suspension to the copper-treated organism and this again well washed, the organism, which is coloured brown, is now capable of activating its substrates. The phenomenon is similar to that described by Myrbäck [1926] in the case of invertase inactivated by silver. It was thought that possibly the copper would protect the activating mechanisms from the action of toluene, aniline or phenol or methylene blue but, as the results in Table VI indicate, no such protective action occurs. It is particularly interesting in this connection that H_2S not only regenerates the activating mechanisms of *B. coli* which have been eliminated by treatment with copper, but it causes a considerable number of the treated cells which are apparently dead to become capable of proliferation. Most cells of *B. coli* are incapable of proliferation after treatment with dilute copper sulphate solution and subsequent thorough washing, but if H_2S be passed through a suspension of such treated cells, many of them now become capable of growing. We have never failed to obtain this result, though if treatment with copper has been too prolonged it is difficult to obtain regeneration of many cells by H_2S . Typical figures are shown in Table VII.

Table VI.

Reduction times in minutes with *B. coli*. ∞ = not reduced in 4 hours.

Conditions as in Table I.

Donator	Normal	After treatment with $CuSO_4$ soln. (1/1000)	H_2S	After exposing copper-treated organism to			
				toluene and finally to H_2S	1 % aniline HCl (p_H 7.4) and then to H_2S	1 % phenol (p_H 7.4) and then to H_2S	methylene blue 1/10,000 soln. and then to H_2S
Succinic acid	13	∞	164	∞	∞	∞	∞
Lactic acid	7	∞	21	20	40	134	117
Formic acid	3.5	∞	4	4	8	9.2	13
Glucose	6	∞	9	∞	∞	∞	∞

Table VII.

To 10 cc. of a suspension of *B. coli* were added 2 drops 10 % $CuSO_4$ soln. This was allowed to stand at room temperature for $\frac{1}{4}$ to $\frac{1}{2}$ hr., centrifuged and the deposit suspended in 10 cc. sterile saline. A drop of this was spread over an agar plate. The suspension was then submitted to a stream of H_2S for 30' at room temperature and a drop again spread over agar.

Exp.	Colonies obtained from one drop of suspension after exposure to copper	Colonies obtained from one drop after passing H_2S through the suspension of copper-treated organism
1	80	800
2	59	Prolific >3000

The nature of the active centres.

It seems, from the general results on treatment of *B. coli*, that we must regard an active centre as made up of a number of groupings each of which

plays its part in determining the access of a substrate to the centre. If the surface structure as a whole is altered by a number of reagents we expect each reagent to give the same sequence of eliminations. Reagents of this class, which give the same order of eliminations, modify the fields (at the active centres) or affect the stability of the centres by a general change in the entire surface structure; reagents of the second class, which give pronounced departures from the typical order of eliminations, act by attacking particular groups at the centres. It appears to us likely that the average reagent has properties belonging to both classes.

Each centre acts as if it were a separate entity, possessing individual characteristics and, in a word, acting as if it were a separate enzyme. It differs from our usual conception of an enzyme in that it has a limited range of specificity and is inseparable from a surface structure. On such a view it is clearly as impossible for us to state the constitution of a biological active centre as it is to give the constitution of an active centre of a catalytically active alloy from only a knowledge of the constitution of this alloy. Yet it is to be remembered that a relatively small structure or colloidal aggregate may possess an active centre and it would be legitimate to state that the constitution of the structure or aggregate as a whole would be the constitution of the enzyme.

Let us now consider a hypothesis which seems to us to be the most reasonable alternative to that of active centres. The activity of a biological surface may be considered to reside in the specific actions of particular molecules which depend for their stability on a colloidal carrier or on adsorption at a surface. Thus, as soon as surface structure is affected, the molecules become affected, lose their stability and the enzymes are destroyed. Or the effect of a particular treatment may be upon certain of the specifically active molecules adsorbed on the surface. The two views—that of adsorption of specifically active molecules at surfaces which are necessary for their stability and that of active centres—are extremely closely related. It is possible, however, to obtain data which will help us to decide between these views.

IV. SOME CONSEQUENCES OF A THEORY OF ADSORBED SPECIFIC ENZYMES.

On this view we have to imagine that there exist specifically active molecules whose stability or activity is greatly dependent upon a surface to which they are attached. That stability of adsorbed materials can be closely connected with the surface structure is sufficiently obvious from the work of Willstätter and his colleagues. The fact that the stability of an enzyme is so highly dependent on adsorption at a particular type of surface indicates that some particular association of groupings, which can easily be disturbed with resulting inactivity, is responsible for activation. The point we wish to emphasise is that activating power is unlikely to reside solely in one particular group, say $-\text{NH}_2$, which may only be primarily responsible for attaching the substrate to the molecule. Now if the specific activating power of the

enzyme molecule is dependent on the configuration of this association of groupings we must conclude that any change in the molecule which disturbs this configuration will eliminate its activity. For action to take place, the substrate must be adsorbed at the particular configuration in the molecule, and the amount of adsorption of a substrate at this configuration must be dependent on the nature of the latter. Hence, if the enzymic activity of the configuration remains intact, the power of adsorbing a substrate by the configuration must also remain intact. Any change which alters the adsorption of the substrate must necessarily change the nature of the configuration and hence the latter's enzymic activity. It should be remembered in this connection that the adsorption of a substrate at any part of the surface which is not occupied by the specifically active molecule, or even at a part of the enzyme molecule remote from the active configuration, can have little to do with the activity of the latter. The link must be between substrate and the particular configuration in the enzyme molecule.

Let us now consider that we have a system of two enzymes, **A** which is specifically related to substrate *A*, and **B** which is specifically related to substrate *B*, both *A* and *B* being hydrogen donators. To this mixture of **A** and **B** we add concentrations of *A* and *B* which are at the saturation points of their respective enzymes, *i.e.* at such concentrations that increase in concentration of either *A* or *B* makes no perceptible increase in the velocity of transformation of either of them when taken individually. Then the velocity of reduction due to a mixture of *A* and *B* will be the sum of the velocities due to *A* and *B* taken separately, unless there is preferential or reciprocal adsorption of the substrates on the enzymes. We assume that there are no complications¹ such as side reactions, etc.

$$\begin{aligned}\text{Let} \quad V_A &= \text{velocity of reduction due to } A \\ V_B &= \text{velocity of reduction due to } B \\ V_S &= \text{velocity of reduction due to } A + B \\ V &= V_A + V_B.\end{aligned}$$

Then, given no adsorption of *B* on **A** or *A* on **B**

$$V = V_S.$$

If $V_S < V$ it is possible to calculate the amount of adsorption of *B* on **A** or *A* on **B**.

1. Let there be only adsorption of *A* on **B**; *i.e.* none of *B* on **A**. Then $V_S = V_A + \text{velocity due to unadsorbed portion of } \mathbf{B}$.

Therefore velocity due to unadsorbed portion of **B** = $V_S - V_A$.

But velocity due to total **B** = V_B .

Therefore fraction of enzyme **B** which is unadsorbed

$$= \frac{V_S - V_A}{V_B},$$

since velocity is proportional to quantity of enzyme.

¹ In our experiments this is a legitimate assumption, for the amount of methylene blue used is extremely small compared with the amount of donator.

Therefore, the fraction of enzyme **B** which has been inactivated by adsorption of **A**

$$= \frac{V_B - (V_S - V_A)}{V_B} = \frac{V - V_S}{V_B}.$$

2. Similarly, if there be only adsorption of **B** on **A**, the fraction of enzyme **A** which has been inactivated by adsorption of **B**

$$= \frac{V - V_S}{V_A}.$$

3. If adsorption both of **A** on **B** and of **B** on **A** occurs, then

(a) fraction of enzyme **A** inactivated must be $< \frac{V - V_S}{V_A}$,

and (b) fraction of enzyme **B** inactivated must be $< \frac{V - V_S}{V_B}$.

Now if by experiment we can show that there is *no* adsorption of **B** on **A**, then the amount of adsorption of **A** on **B** must be given by the expression $\frac{V - V_S}{V_B}$. We can test this by further experiment.

According to the theory of adsorbed specifically active molecules the expression $\frac{V - V_S}{V_B}$ must remain a constant, characterising a property of the enzyme **B** (*i.e.* its power of adsorption of substrate **A**) and should remain unaltered whilst **B** retains activity. Thus if a surface possessing a quantity of **B** be attacked, so that part of **B** is destroyed, the remainder of **B** should still give the expression $\frac{V - V_S}{V_B}$ as representing the adsorption upon it of substrate **A**. Or if a portion of the enzyme molecule itself can be attacked without altering the active configuration in it the value $\frac{V - V_S}{V_B}$ should still remain constant. These conclusions depend upon the underlying assumption that a change in the structure of the active configuration of the molecule must inevitably eliminate its highly specific relationship with its substrate. To prevent any misconception of this point, which is important for our argument, it would be well to indicate that an analogy between the enzyme molecule and, let us say, a toxin is fruitless in this connection. It may be urged, for instance, that the action of a toxin may differ in a quantitative way after chemical treatment from that which occurred before such treatment and that this is an argument for supposing that we may also change the activity of an enzyme molecule purely quantitatively by treatment. But the two molecules, toxin and enzyme, must differ in one very important aspect. Both possess, it may be agreed, certain particular groups or associations of groups whose presence is necessary for the demonstration of activity, and both molecules, we may agree for the purposes of argument, are capable of being changed chemically without necessarily altering the configuration of the active groups. But in a toxin molecule, much must depend on the chemical nature of the molecule apart from the presence of the active group, in determining its access to particular structures in the body where it makes its presence demonstrable, *e.g.* permeability of a membrane for the toxin, or the solubility

of the latter. Both factors depend on the chemical nature of the molecule as a whole and clearly such factors must influence the quantitative action of the toxin.

Again, the activity of a molecule such as adrenaline is in all probability centred in one or two relatively simple groups or atoms of the molecule, and it is the chemical structure of the molecule as a whole which determines the accessibility of these groups to the susceptible structure of the body.

With an enzyme molecule, however, if a change in the chemical structure does not affect the structure of the active configuration of groups (and this would be surprising in view of the fact that the molecule is very unstable when not attached to an appropriate surface) we would have to consider that the molecule is of such a nature that a disturbance or alteration at one portion of it does not at all affect the active configuration. If this is so it is extremely difficult to perceive how such an alteration could affect the access of a substrate to the configuration itself; it could certainly be argued that if the change were such as to affect the access of substrate to the configuration it must certainly have altered matters to such an extent that the perfect "fit" between substrate and active groups has been rendered less perfect, and hence have produced a cessation of activity. Such a view is fundamental to our understanding of the cause of the high specificity of action obtained between substrate and an enzyme molecule.

We may conclude, then, that, if we can demonstrate the constancy of the expression $\frac{V - V_s}{V_B}$ however the organism or enzyme systems be treated, this will be evidence in favour of the view of adsorbed specifically active molecules. If the expression $\frac{V - V_s}{V_B}$ varies considerably this will be evidence against the view and a more adequate explanation of enzymic activity must be sought.

Experimental results.

The velocities of reduction (reciprocal of reduction times) of mixtures of various substrates are noted in Table VIII, the velocities due to substrates when alone (V_A or V_B) also being given¹. Care was always taken that the concentration of any substrate was such that increase in concentration made no perceptible increase in velocity of reduction due to the substrate. The values $\frac{V - V_s}{V_A}$ and $\frac{V - V_s}{V_B}$ are worked out and, as explained earlier, represent the relative adsorptions of *B* on *A* or *A* on *B* respectively, assuming that reciprocal adsorptions do not occur².

Table IX gives the results obtained with *B. coli* which has been exposed to various treatments so that the mechanism for one or more substrates has

¹ Since it has been shown [1] that the rate of reduction of methylene blue is linear it is legitimate to take the velocity of reduction as the reciprocal of the reduction time. In our experiments a standard end-point (95 % reduction) is taken, to prevent error due to departure from linearity during the last 5 % of reduction.

² It is to be remembered in considering our experiments with *B. coli* that the main site of reduction is at the cell surface.

Table VIII.

Rates of reduction due to donators and mixtures of donators at their saturation concentrations.
 t_A =time of reduction in minutes due to A. t_s =time of reduction due to mixture of both
 A and B. Conditions as in Table I.

Concentrations of all donators = $M/28$, except glutamic acid $M/14$ and acetic acid $M/7$.

Exp.	A	B	$V_A = \frac{100}{t_A}$	$V_B = \frac{100}{t_B}$	$V_s = \frac{100}{t_s}$	$V = V_A + V_B$	$\frac{V - V_s}{V_A} \cdot 100$	$\frac{V - V_s}{V_B} \cdot 100$
1	Lactic acid	Succinic acid	18.2	4.3	19.6	22.5	16	67
			13.3	2.1	13.9	15.4	12	71
2	" "	Formic acid	8.3	7.3	13	15.6	31	35
			6.8	9.1	13.7	15.9	32	24
3	" "	α -Hydroxy- butyric acid	13.9	8.5	12.5	22.4	71	>100
			7.6	4.6	7.7	12.2	59	98
4	" "	Glucose	9.1	6.2	9	15.3	69	>100
			9.3	5	9.6	14.3	50	94
5	Succinic acid	α -Hydroxy- butyric acid	3.3	11.2	11	14.5	>100	31
			4.3	16.7	16.7	21	100	26
6	" "	Formic acid	2.1	14.3	13.5	16.4	>100	20
			2	13.9	13	15.9	>100	21
7	" "	Glutamic acid	5.6	6.6	8	12.2	75	64
			3.3	3.1	3.9	6.4	75	80
8	" "	Glucose	2.1	6.2	8.5	8.3	0	0
			2	3.3	5	5.3	15	9
9	Formic acid	"	10.5	6.2	15.4	16.7	12	21
			12.2	5.7	15.9	17.9	16	23
10	" "	α -Hydroxy- butyric acid	11.1	5.9	12.3	17	43	80
			10.9	5.3	12.5	16.2	34	70
11	" "	Glutamic acid	14.3	2.6	14.9	16.9	14	77
12	Lactic acid	" "	7.3	2.6	8.5	9.9	19	54
13	α -Hydroxy- butyric acid	Glucose	5.6	3	7.8	8.6	14	27
			5.9	3.7	8	9.6	27	43
14	Glutamic acid	"	2.6	6.9	9.1	9.5	15	6
			2.6	6.2	8.2	8.8	23	9
15	" "	α -Hydroxy- butyric acid	3.1	11.2	9	14.3	>100	47
			4.4	16.7	14.3	21.1	>100	40
16	Acetic acid	Formic acid	7.1	10	9.5	17.1	100	76
			8.9	14.3	13.3	23.2	100	62
17	" "	Lactic acid	7.3	6.6	9.8	13.9	56	62
			6	8.3	10.9	14.3	57	41
18	" "	Succinic acid	10.3	2	10.3	12.3	19	100
19	" "	Glucose	10	5	12.2	15	28	56
20	" "	Fructose	10.3	7.6	14.3	17.9	35	47
			9.5	6.3	13.3	15.8	26	40
21	Glucose	"	5.7	11.1	11.1	16.8	100	51
			3.7	6.4	5.9	10.1	>100	66
22	"	Mannose	5.7	5	5.2	10.7	97	>100
			8.2	11.5	9.5	19.7	>100	89
23	"	Galactose	5	6	5.3	11	>100	95
			5.4	5.1	5.1	10.5	100	>100
24	"	Mannitol	5	3.2	4.8	8.2	68	>100
			8.3	4.8	7.7	13.1	65	>100
25	"	Sorbitol	5.7	3.4	5.9	9.1	57	93
			5.4	2	5.5	7.4	35	95
26	Fructose	Mannose	11.1	5	9.8	16.1	57	>100
			13.3	11.5	14.3	24.8	79	91
27	"	Mannitol	9.1	4.8	8.3	13.9	62	>100
			10.3	3.3	9.8	13.6	37	>100
28	Mannitol	Sorbitol	2.5	2	2.6	4.5	76	95
			6.5	5.3	6.1	11.8	87	>100
29	Acetic acid	Glutamic acid	9.5	2.6	11.8	12.1	3	11

Table IX.

Velocities of reduction ($V = 100/\text{reduction time in minutes}$) due to donors and mixtures of donors with substrates made inactive (or partially so) by treatment of the organism. The treated organism is always washed after treatment and used under similar conditions to the normal organism. Concentrations as in Table VIII.

Exp.	Preliminary treatment of organism	A	B	V_A	V_B	V_S	$\frac{V - V_S}{V_A} \cdot 100$	$\frac{V - V_S}{V_B} \cdot 100$
1	With 0.005 % KMnO_4 soln.	Lactic acid	Formic acid	10	0	9.5	5	—
2	" " " "	" "	" "	8.3	0	8.2	1	—
3	With 1 % KCN soln.	" "	" "	11.8	0	11.8	0	—
5	Exposure to 57° for 1 hr.	" "	" "	0.7	6.4	7.1	0	0
6	With 0.005 % KMnO_4 soln.	Glutamic acid	" "	1.8	6.4	4.8	>100	53
7	With toluene	" "	" "	0	9.9	9.3	—	6
8	With 1 % KCN soln.	" "	" "	4.8	1.1	4.3	33	>100
9	" "	" "	Acetic acid	4.8	2.7	4.7	58	100
10	With toluene	" "	" "	0	8	8.5	—	0
	With 0.005 % KMnO_4 soln.	Succinic acid	Lactic acid	0.5	6.4	6.6	60	5
11	" "	" "	" "	1.5	10	9.8	>100	17
12	Exposure to 57° for 1 hr.	" "	" "	3.3	1.7	5.5	0	0
13	With 1 % KCN soln.	Glucose	Formic acid	10	0	10	0	—
14	With toluene	" "	" "	0	9.9	10	—	0
15	With 0.005 % KMnO_4 soln.	Lactic acid	Acetic acid	6.4	0	5.1	20	—
16	Exposure to 57° for 1 hr.	" "	" "	0.6	3.3	4	0	0
17	With 0.005 % KMnO_4 soln.	" "	Glutamic acid	16.7	1.8	13	33	>100
18	Exposure to 57° for 1 hr.	" "	" "	1.7	0	1.87	0	—
	With 2 % NaNO_2 soln. at p_H 6.0 for 1 hr.	Succinic acid	Formic acid	0	6.4	6.4	—	0
19	With 0.005 % KMnO_4 soln.	" "	" "	1.5	0	1.5	0	—
20	With 2 % NaNO_2 soln. at p_H 6.0 for 1 hr.	" "	Acetic acid	0	7.9	8	—	0
21	With 1 % KCN soln.	" "	" "	3.6	0	3.5	8	—
22	With 2 % NaNO_2 soln. at p_H 6.0 for 1 hr.	" "	Lactic acid	0	5.9	4.6	—	28
23	With 0.005 % KMnO_4 soln.	Lactic acid	Glucose	6.4	0	5.1	20	—
24	" "	Glucose	α -Hydroxybutyric acid	0	2.5	2.1	—	16

been eliminated either partially or completely whilst that for others has been retained. From these results it is possible to determine whether the expression $\frac{V - V_s}{V_A}$ or $\frac{V - V_s}{V_B}$ remains constant or not.

For instance, take the case of lactic acid and formic acid.

From Exp. 2, Table VIII,

$$\frac{V - V_s}{V_A} = 31 \%,$$

i.e. assuming no adsorption of lactate on the formic acid enzyme, adsorption of formate effectively eliminates 31 % of the lactic acid enzyme;

or $\frac{V - V_s}{V_B} = 30 \%$ (average),

i.e. assuming no adsorption of formate on the lactic acid enzyme, adsorption of lactate effectively eliminates 30 % of the formic acid enzyme.

Now from Exps. 1 and 2, Table IX, we find that when the organism is so treated that formate is no longer activated,

$$\frac{V - V_s}{V_A} = 2 \% \text{ (average),}$$

i.e. there is very little adsorption of formate on the lactic acid enzyme.

Therefore $\frac{V - V_s}{V_B}$ should be about 30 %.

But from Exp. 3, Table IX, we find that when the organism is so treated the lactic acid enzyme is almost completely eliminated,

$$\frac{V - V_s}{V_B} = 0.$$

This result is contradictory to the hypothesis of adsorbed specifically active molecules.

Similarly with the cases of

formic acid and glucose (Exp. 9, Table VIII; Exps. 12, 13, Table IX);

lactic acid and succinic acid (Exp. 1, Table VIII; Exps. 10, 11, Table IX);

lactic acid and acetic acid (Exp. 17, Table VIII; Exps. 14, 15, Table IX);

succinic acid and formic acid (Exp. 6, Table VIII; Exps. 18, 19, Table IX);

succinic acid and acetic acid (Exp. 18, Table VIII; Exps. 20, 21, Table IX).

All these results clearly are in opposition to a view which states that the enzymes involved are of highly specific nature and that the fact that substrates do not act quite independently of one another in presence of these substrates is due to adsorptions of inert substrates at the enzymes¹.

But the results are in harmony with the view that active centres on the surfaces are the responsible agents for activation.

Since an active centre has a limited range of specificity it follows that after treatment of an organism, when an alteration of the centre or a re-arrangement of its constituent groups has occurred, two effects will be manifest: (1) the configuration will have changed and this will result in some cases in

¹ It should be mentioned, in parenthesis, that if substrates are not used at their saturation concentrations, they appear to act independently of one another. It is highly important, therefore to perform preliminary experiments to ascertain the saturation concentrations.

the entire elimination of accessibility of substrates to the centre; (2) the nature of the activating field will have altered. This means that, after treatment, a centre may still retain activity for a particular substrate but yet the accessibility of other substrates to it may have entirely changed. The expression $\frac{V - V_s}{V_B}$ need *not* be constant, and on our views is unlikely to remain constant.

It is an interesting fact that the experiments of Table IX indicate that in many instances selective elimination of the activating mechanisms, or selective poisoning, reduces adsorption of the inactivated substrate at a centre, where it was previously adsorbed, to a value which is too small to be measured by our experimental method. This would certainly be expected if particular groups or group configurations were associated with the adsorption or orientation of certain substrates, and that these groups or configurations were selectively attacked by the various treatments the organism undergoes.

Table X.

Reduction times of 1 cc. 1/5000 methylene blue soln. due to donators (A) at their saturation concentrations in presence of other substances (B).
Conditions as in Table I. Normal *B. coli*.

A	B	t_A alone	$t_{\text{mixture of A and B}}$
Succinic acid $M/28$	Caprylic acid $M/14$	15	33
" "	Nonylic acid $M/14$	15	∞
" "	Adipic acid $M/84$	21.2	29.4
Formic acid $M/28$	Propionic acid $M/14$	9.3	9.7
" "	Caprylic acid $M/14$	9.3	9.0
" "	Nonylic acid $M/14$	9.3	14
Acetic acid $M/7$	Propionic acid $M/14$	10.0	10.5
" "	Caprylic acid $M/14$	10.0	9.5
" "	Nonylic acid $M/14$	10.0	64
Glucose $M/28$	Caprylic acid $M/14$	6.7	∞

It can be shown that retardations of velocities of reduction are effected by substrates which are *not* perceptibly activated, *e.g.* caprylic and nonylic acids (see Table X) so that it is sure that adsorption can occur at active centres of substances which are not activated by them. Again some inactivated donators are adsorbed at active centres as indicated in Exps. 22-24, Table IX. These facts considered in conjunction with the fact that an approximately similar order of eliminations occurs with many diverse treatments (Table III) point, as we have observed elsewhere, to some underlying factor, concerned with the energetics of the field, or with the stability of the centres at the surface, playing a significant part in the eliminations.

V. GENERAL CONSIDERATIONS.

Formation of active centres at biological surfaces.

There is, naturally, great vagueness at present as to the mechanism of formation of enzymes and their relationship to the remaining constituents of the cells, but it seems to be fairly widely held that the cell is able to elaborate at least two distinct classes of molecules, the very highly specialised molecules

which exhibit enzymic behaviour, and the enzymically inert substances which together make up the protoplasmic and histological structures of the cell. This view, which calls for a sharp line of demarcation between the architectural units of the cell, proteins, nucleotides, etc., and the specific enzymes, which are not only being synthesised themselves in the cell but which are regulating the course of metabolism and the growth of the cell itself, seems to be greatly strengthened by the fact that a large number of enzymes can be secreted by the cell. This gives the impression that enzymes are simply products of the cell in much the same way as, let us say, adrenaline.

When it is considered (*a*) that the number of highly specialised enzyme molecules, which the cell is presumed to contain, must be very large indeed and that they must vary considerably in their type and constitution, (*b*) that the evidence, presented in this paper, concerning the dehydrogenations effected by bacteria is contrary to the supposition that activity is due to the presence of many highly specific enzyme molecules, (*c*) that it is extremely difficult to understand how a cell is able to cope with material to which hitherto it has not been accustomed, if its content of specific enzymes, though large, is yet limited, it will be granted that the view stated above stands in need at any rate of some emendation.

The hypothesis we put forward, that enzymic activity may be regarded as the property of the active centres of cellular and intracellular structures (and this includes the smaller structures capable of extraction from or secretion by the cell) leads to a considerable simplification of the above view. Precisely what enzymic behaviour a particular structure or colloidal aggregate in the cell may possess depends on the nature of the active centres which form a part of the structures or of the colloidal aggregates. Enzymes, on this view, are themselves part of and cannot be dissociated from the architectural units of the cell. This does not imply, of course, that only the histological structures are involved; the smaller colloidal aggregates are just as much part of the architecture of the organism. The conditions existing in the cell are such as to bring about just that arrangement or juxtaposition of molecules which makes for the formation of active centres on the normal material of the cell. Thus we may imagine that the protein, nucleotides, etc., are not only so arranged as to form the various substances of the cell but that the arrangement is such that active centres are formed on these particular substances. Enzymes, therefore, and cellular structures are inseparably connected. It is necessary to consider, now, how the active centres are formed.

Let us consider the formation of molecular structures at an interface. This may be accomplished in either of two ways.

1. As new molecules are produced they will orientate themselves in a definite way determined by the forces at an interface and will tend to arrange themselves into a crystal lattice pattern. Now if a number of dissimilar molecules are engaged in forming a surface structure we expect that there will be produced points of weakness or of loose fitting. It is at these points that

stray fields occur, the fields being balanced by surface forces and constituting the active centres of the surface structure.

2. We may conceive of the production of a colloidal aggregate which is represented in a highly diagrammatic manner in Fig. 1. Here various molecules, represented by rectangles, are joined to each other by arrows which represent the connecting links between molecule and molecule. The link may be brought about by electron sharing, or the positions of the molecules relative to each other may be determined by orientating forces in the phases surrounding the molecules. The molecules will tend to take up positions so that the potential energy of the system is at a minimum.

Fig. 1. Illustrating the colloidal aggregate (sectional).

Now let us consider two particular constituents, *A* and *B*, of the aggregate. It may happen that were the molecule *A* free from the restraint of the neighbouring molecules of the aggregate, it would form a structure represented by the skeleton scheme (Fig. 2). Here the external field of the molecule would be at its minimum; "affinities" between atom and atom in it are saturated as far as possible. But when forming part of a structure the action of the other molecules might tend to strain the configuration. This is illustrated in Fig. 3 where we suppose that two constituent groups *M* and *M'* of the molecule *A* are held or attracted by neighbouring molecules in the aggregate, so that a strain occurs, pulling two other constituents *X* and *Y* considerably apart. Such a strain must cause the production of an external field between *X* and *Y*, much greater than that which normally occurs. To take a concrete example,

Fig. 2. Illustrating the normal character of molecule *A* with external field between *X* and *Y* at a minimum.

Fig. 3. Illustrating shift in relative positions of *X* and *Y* (with resultant production of increased external field) due to action of surface forces at *M* and *M'*.

in Fig. 1, X and Y are represented by the amino- and carboxyl groups. These would normally have considerable affinity for each other and tend to approach to a critical distance. The strain imposed by the neighbouring molecules of the surface structure and surrounding phase causes a widening of the distance between the two groups and it is here that we may expect the local production of an external electric field. The carboxyl group at A may link with, say, an $-SH$ group at B , to form continuity of the surface, but this link, being perhaps much weaker than that normally between carboxyl and amino-group, will not necessarily reduce the field at A to any considerable extent.

The active centre we would imagine in the molecule $-A-CO-S-B-$

to be made up at least of the groups $-NH_2$, $-CO-$ and $-S-$ and each of these groups will play its part in rendering a substrate accessible to the centre. The field due to these groupings may not be homogeneous and hence the orientation of a substrate at the centres, so that activation may occur, becomes a highly important consideration.

We have given this illustration of a possible formation of an active centre in order to make clear the difference between the centre and the usual conception of an enzyme. The actual composition of an active centre will in all probability be much more complex than in the illustration given. We may regard the entire aggregate as the enzyme, or the particular centre (at the link) as the enzyme. Each view is equally legitimate. But the residue of the aggregate, distinct from the region occupied by the centre, may be the seat of other active centres, so that the aggregate as a whole may have a much wider range of specificity than were the residue inert in this respect. Such an aggregate would be a relatively large colloidal particle and it would certainly be difficult to regard it as a *specific* enzyme. On the other hand, the residue may be of comparatively small dimensions and contain no other active centres. The specificity of action would be determined by the single centre and the range of specificity may be so small as to make the particle a highly specific enzyme. In this manner we regard the "soluble" enzymes¹ as related to the "insoluble." The distinction between them is simply one of degree.

Stability of active centres.

In the first place there can be little doubt that the centre, represented, let us say, by the illustration (Fig. 1), will have a limited range of stability. Its stability simply rests upon the operation of forces at the interface and we expect any factor which disturbs these forces to result in a disturbance of the links between A and B and other molecules of the aggregate with the consequent elimination of the centre. Any agent which has the effect of altering surface structure will have a "poisoning" action on the centres, though the

¹ It is to be remembered that only heterogeneous catalytic systems are being discussed.

poisoning may be selective according to the nature of the centres. We expect the most unstable centres to disappear first, the more stable next and so on. The second type of poisoning action will be on the groupings constituting the centre itself, *i.e.* one of these, say —CHO , may link up with the poison eliminating the access of some particular substrate to the centre. But the field, though changed, may still be capable of activating other substrates accessible to the centre. We expect that such a poisoning action may be reversible.

The actual size of the aggregate can give us little information on the stability of the centres. It may be small enough to be classed as a "soluble" enzyme and yet have relatively considerable stability; it may be large enough to be classed as "insoluble" and yet be extremely unstable. We can be sure, however, that any means which will strengthen the forces at the interface in preventing fission of $A\text{—}B$ will increase the stability of the centre. There appear to be at least the following ways of accomplishing this.

(a) By adsorption of the aggregate at an *appropriate* surface, which may tend to keep the molecules in position and prevent rupture by collision.

(b) By the adsorption or combination at the centre, itself, of some substrate, which is reversible in action and which may protect the centre. For instance, adsorption of sucrose by invertase has a protective action on the enzyme.

(c) By reducing the chance of rupture of $A\text{—}B$ by impact, *e.g.* by increasing the viscosity of the medium in which the aggregate is present.

Co-enzymes.

It is clear that any substance may be classed as a co-enzyme which (a) will tend to make a substrate accessible to the centre by combination with, or by facilitating a combination with, the substrate, the resulting combination possessing a lower energy of activation or increased adsorption or a more appropriate orientation at the centre, or which, (b) by some action at the centre itself, increases the activating power of the latter or the accessibility of the substrate. Such co-enzymes may not only make activity demonstrable where otherwise it could not be perceived but may increase the range of specificity of the active centre or enzyme.

Anti-enzymes.

We cannot expect that a molecule of the type $A\text{—}B$ (Fig. 1), whose enzymic activity is dependent upon surface forces and the nature of the union between A and B , *must* give rise to an antibody which will necessarily eliminate the activity of the enzyme. We may expect the production of antibodies which may act as precipitins, neutralising the charge of the aggregate as a whole, but such combination does not in the least indicate that the active centre will be destroyed.

SUMMARY.

1. An attempt based upon the conception of active centres is made to interpret the mechanism of enzyme action. This conception, which is a development of views put forward earlier [Quastel, 1926] on the mechanism of biological dehydrogenations, is discussed in relation to the specificity of enzyme action and to the behaviour of enzymes when exposed to various changes in chemical and physical environment.

2. Results are given which show that a hypothesis which claims that the activity of surfaces is due to the adsorption thereon of specifically active molecules is inadequate.

3. A suggestion is made as to the mode of formation of active centres (or enzymes) in the cell. It is shown upon what factors their stability depends and in what ways their activity is influenced.

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