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Zelinskii Institute of Organic Chemistry, USSR Academy of Sciences, Lomonosov Moscow State University

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THE PRINCIPLES OF BIOCATALYST MODELLING

L. A. Nikolaev

CONTENTS

I. Introduction 275

II. Short historical survey 276

III. Some models of important enzymes 278

IV. Comparison of the properties of models and biocatalysts 283

I. INTRODUCTION

One hundred and thirty years ago a compound was discovered in malt extract that catalysed the conversion of starch to sugar. Payen and Persoz, the discoverers of this natural catalyst, called it "diastase" ("separating"), because it separates dextrin from the starch grain envelope[1]. These workers could never have surmised that it had fallen to them to reach the boundary of a field in which the activity of amazing chemical "machines" is related to life by elusive threads. The increasing attractive force of the problems of enzymology directed the attention of chemists and physicists to this subject, and none of them found in this new and mysterious world anything that could be regarded as trivial from the point of view of their own sciences.

Biocatalysts are products of a long evolution that appears to have extended over several thousand million years. During this time the compounds generally used in technology as catalysts (oxides, salts, sulphides, etc.) were to a large extent deactivated; it is well known that freshly prepared compounds are more active than the naturally occurring forms. Thus, catalytically active systems in the biological field developed in a manner fundamentally different from the evolution typical of inorganic products.

Biocatalysis is one of the facets of life. When it was shown that nearly all chemical processes occurring in cells are catalytic, biocatalysts became particularly important in speculations on the fundamental laws of biochemistry. The development and perfection of forms of life, as well as its origin, have thus been found to be due to the combined action of catalysts. The biological activity of various organisms was a most important channel for the reduction of chemical potentials even in geologically remote times. Therefore, the appearance and development of biocatalysts may be considered as the expression of a general law, according to which potential-equalisation processes in some way set up the mechanisms they require in nature.

In practice circumstances are such that nearly any energy-rich compound formed on the earth as a result of radiation or heating may become the substrate for the nutrition of some organism, heterotrophic or autotrophic. The catalytic systems are found to be unusually versatile. Enzymes, or more accurately aggregates of enzymes, can produce effects that are difficult, and sometimes impossible, to achieve with ordinary catalysts.

Enzymes are virtually the same in both the simplest and most complex organisms, which underlines their versatility as chemical tools. Moreover, the evolution of forms of living organisms has had hardly any effect on the chemical nature of the catalysts required for biological activity.

One of the most striking conclusions that evolutionary biochemistry has led to is that the formation of biochemical systems responsible for metabolic processes was largely completed in remote times[2]. Thus, whatever stimuli acted up to that time ceased, for unknown reasons, to play the part of chemical rearranging factors, as if the function they performed at some stage of development was no longer necessary.

It would be interesting to discover why a certain factor ceased its "creative" activity, and why under certain conditions chemical evolution of the most important catalytic systems was greatly retarded and virtually ceased. Obviously, in our present state of knowledge an answer to this question can be given only in the most general form. If we consider any part whatever of a complex chemical system and examine how the effect of a given chemical factor is reflected in the structure of the chosen part of the system, we observe a number of successive changes in composition and also in macrostructure. The operating factor may be, for example, oxidation by means of hydrogen peroxide, or phosphorylation leading to the formation of pyrophosphate bonds. These factors may cease to operate for two reasons. First, molecules, or groups of molecules, that are unaffected by the given factor, may appear. In this case the resistance may not be constant, since chemical evolution may be resumed as a result of the accumulation of matter. Second, some catalyst may appear in the part of the system under consideration: for example, the oxidative effect and corresponding chemical rearrangement of the system under the influence of hydrogen peroxide became impossible as soon as evolution led to the emergence of catalase. The appearance of enzymes that transfer phosphoric-acid residues, of course, introduce a controlling element into the processes by which phosphoric esters are formed, and so on.

One may imagine a rough sequence in which external chemical attacks lost their random deforming character and, thanks to the development of catalytic systems, became trigger systems for coordinated processes, maintained by the same catalysts.

Processes associated with the transfer of energy-rich groups, and also hydrogen-transfer processes, are probably among the oldest, that is, the earliest to be subjected to control. Much later, processes in which molecular oxygen took part were subjected to control and ceased their deformative activity. This also appears to apply to photosynthetic processes. Kamen[3] points out that anaerobes

which synthesise chlorophyll, differing from that required for the photosynthetic formation of oxygen, cannot exist in an atmosphere containing even traces of oxygen. All living organisms were once anaerobes. Different forms of these organisms that had a photosynthetic apparatus disposed of a catalyst system very similar to that which provides for the respiration of aerobes; the absence of cytochrome oxidase, which is characteristic of anaerobes, evidently plays an important part in their fate.

The macromolecular masses of proteins and the complex structure of the active centre of proteolytic enzymes suggest that these catalytic systems arose much later. All these conjectures are doubtless very debatable; however, it seems useful to look for a relation between the causes that led to cessation of the chemical rearrangement of cellular matter and the action of biocatalytic systems. The main difficulty is that internal as well as external factors must be taken into account in its analysis. It would therefore perhaps be more correct to seek a solution of similar problems in model structures.

However, the cessation of activity of a given external chemical factor as a result of catalyst formation is, of course, by itself insufficient to explain important facets of the activity of catalyst systems in nature; in particular it does not supply an interpretation of the development process.

If we wish to understand the significance of the laws to which the development of biocatalytic systems is subject, we may assume that these laws also manifest themselves in structures much less complex than the living organism. This consideration leads to the problem of modelling both the individual centres of biochemical "machines" and the catalytic systems as a whole.

To-day the term "modelling" is probably much more common than in any other epoch of scientific development. It is striking that modelling has become the subject of research, on the one hand, by biochemists comparing the chemical nature of enzymes with less complex compounds, and on the other, by physicists and engineers interested in the problems of cybernetics. The young branch of science, bionics, is concerned with the setting up of mechanisms that reproduce communication systems in complex aggregates of cells. The chemist turns his attention mainly to molecules, within which he hopes to find keys to the secret behaviour of cellular material, but the engineer, assembling relays and other components that simulate the functions of neurons, has little interest in the material they are made of. However, this separation of points of view can only be temporary.

In fact, the course of biochemical evolution has led to the formation of systems in which their "material" satisfies the demands of further development in the best possible way. This development is no longer related to significant rearrangement of molecules of the material; purely chemical evolution, as has been noted, was largely completed a very long time ago. Since that time, the course of development of more and more complex forms of life actually began to resemble the combination of manufactured relays in the hands of the "constructor". However, if we confine ourselves, on the one hand, to copying communication systems of organisms, and on the other, to models of individual enzymes, we shall lose sight of the necessity of investigating the law by which this "independence of the material" arises.

The question of modelling the process of self-reproduction has been considered by Neyman, Penrose, and Krol'[4]. In all cases the "medium" consisted of radio components, which replaced the nutrient medium of organisms and served as the raw material for self-organising machines. Krol' has noted that, since such artificial media do not exist in nature, we are not witnessing the evolution of machines, not having prepared the necessary media artificially. This observation is extremely characteristic; it indicates a situation in which the problem is being solved "from both ends". Clearly, the problem at the "junction" of two approaches — at the molecular and machine levels — is more complicated; it is also of exceptional interest.

The independence of the material probably becomes much more obvious when we are concerned with the higher functions of complex organisms of the same species. If the identity of the reactions on which phenomena such as muscular work in man and luminescence in insects are based is surprising, still more striking is the unusual diversity of forms of psychic activity in man, that is, of systems the chemical energetics and other properties of which are profoundly similar.

Investigation of the role of chemical structures in organisms shows that we are concerned with initial compounds that are capable of forming more highly ordered structures with a higher degree of organisation, entirely new properties, and unusual stability. But they themselves are not static; they arise and disintegrate in such a way that their appearance and disappearance are really forms of a process, or aggregate of processes, linked to the medium by feedback mechanisms. The most important condition for their existence is the wonderfully coordinated work of the catalytic systems of the very enzyme models which attract increasing attention every year. It must be emphasised that a study of enzyme models cannot be separated from the general problem of biochemical evolution.

II. SHORT HISTORICAL SURVEY

The problem of biocatalyst or enzyme modelling has a history of half a century. In the first attempts to reproduce the functions of enzymes the main effort was directed towards obtaining high activity, since this facet of enzyme action was particularly attractive to chemists.

In this period experiments were done with colloidal metals such as platinum, palladium, and others. Metal sols prepared by Paal and Skita at the beginning of the century showed high catalytic activity in a number of reactions, particularly in hydrogenations[5-7]. Experiments by Bredig and others showed that in some respects, such as high activity and sensitivity to poisoning, metal sols actually resemble enzymes. However, because of the great difference in chemical nature between a metal and the protein of an enzyme, sols cannot be regarded as biocatalyst models. In 1908 Bredig and Fajans found that nicotine has catalytic properties: it separates the optical isomers of the camphoric acids[8].

The work of Hofmann and Gotlob on organic vulcanisation accelerators[9] was done in 1912. In 1926 the first step was taken towards investigating the activity of groups characteristic of enzymes. Kuhn and Brann studied the dependence of the catalytic activity of iron on bond type; they examined haemin, an organic complex of iron with the protoporphyrin nucleus. Compounds of this type form the active groups of a large num-

ber of enzymes that catalyse oxidative processes; the properties of haemin were therefore important in modelling. In enzymes haemin is bound to the protein, and the measurements of the activity of isolated haemin to some degree threw light on the part played by proteins in biological catalysts [10].

The catalytic activity of haemin is dealt with in a fairly large number of papers [11]. Haemin is an active catalyst in the decomposition of hydrogen peroxide and in some oxidations. However, it was clearly shown in experiments on these reactions that the active group is much less active and not quite as selective when it is isolated from its carrier. The activity of haemin in the hydrogen peroxide decomposition reaction is increased by adsorption on charcoal; adsorption activates haemin in some, but not all, reactions in which it generally behaves as a catalyst.

Simple iron ions are also activated by adsorption on charcoal. Such systems may be regarded as the simplest, and very rough, enzyme models in which the active group and the carrier are both represented.

According to Warburg [12,13], iron-containing charcoal, produced by the charring of haemin, shows considerable activity in the oxidation of amino-acids. Examination of its magnetic properties and X-ray analysis led us to conclude that this charcoal contains complex organic compounds of iron and nitrogen firmly adsorbed on the charcoal [14].

In 1926 Kuhn and Brann noted the marked peroxidase activity of iron complexes [10]. A year earlier Baudisch studied the properties of a catalyst consisting of the sodium salt of a pentacyanoferrate(III) and found that this compound accelerated the oxidation by hydrogen peroxide of a mixture of phenylenediamine and benzidine [15].

In 1929 Euler and Jansson [16] showed that complexes of copper with hydroxy- and amino-acids are almost inactive in the hydrogen peroxide decomposition, but complexes with pyridine and ammonia are active. Probably because they used dilute solutions, these workers obtained an incorrect value for the activity of the pyridine complex; it is at least 15 times as active as indicated in their paper.

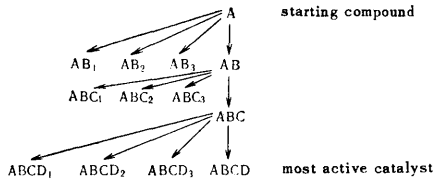
The study by Manchit and Pelann [17] of the $\alpha\alpha$-dipyridyl iron complex has attracted great attention. The catalase function of this complex does not appear to be related to a change in the ionic charge of the bivalent iron; the complex containing one or two dipyridyl molecules per iron ion is active.

In 1932 the work of Bredig and Gerstner on "faser--catalysts" appeared; these consisted of cellulose chemically bonded to diethylamine. The amine played the part of an active group and the cellulose that of a carrier. Faser-catalysts, which Langenbeck considers to be the first enzyme models, accelerated the elimination of carbon dioxide from bromocamphoric acid and showed signs of optical selectivity [18].

Shibata and coworkers studied the stereochemical selectivity of the optically active cobalt complexes. In particular, it was found that *d*-catechin is more rapidly oxidised under the influence of the *d*-forms of cobalt complexes [19,20].

The work of Langenbeck [21], who developed and applied the principle of "systematic activation" in studies on organic catalysts for certain reactions, is particularly interesting. The basis of the principle is that active compounds, selected from those that accelerate a given reaction, are modified chemically by the addition or substitution of new groups. From the derivatives thus obtained, the most active are selected and further modified by substitution. In this way Langenbeck achieved great success; he gradually increased the activity of catalysts for the decarboxylation reaction, until the best model was less active than the natural enzyme only by a power of ten. The initial link in the catalyst chain was methylamine, which is fairly weak, and the final link 1-methyl-3-aminooxindole. Phenylglyoxalic acid was used as a substrate.

The general scheme of Langenbeck's method may be briefly represented as follows:



Langenbeck also obtained a heterogeneous model of carboxylase, using methyl salicylate and lecithin as carriers; these dissolved the catalysts and formed an emulsion in aqueous solution.

The method of increasing the complexity of the molecule by replacing certain atoms in the organic catalyst by radicals also proved fruitful with the iron-porphyrin complex. Langenbeck showed that the addition of imidazole or its derivatives to haemin sharply increased its catalytic activity in the hydrogen peroxide decomposition, and in the oxidation of pyrogallol and cysteine.

In his study of dehydrogenase models, Langenbeck, using previous observations of Traube, found that isatin may be regarded as a model for amino-acid dehydrogenase. It is interesting that hydrogen given off by an amino-acid may be transferred not only to oxygen but also to another acceptor, for example, Methylene Blue.

The active groups of many important enzymes contain a metal in the form of a complex. It is therefore quite natural that the problems of modelling should include the examination of the possibility of using complexes as catalysts. However, the problem proved to be more complicated than might be expected from analyses of catalase and oxidases of different types. A large number of metals are essential for the functioning of biocatalytic systems; the mode of action of cations is often obscure, although there is no doubt about the presence of a given cation ("activator") in the catalytic system.

It has been established that in living organisms a deficiency of ions of one metal may be made up by the presence of an excess of ions of another.

Recently, Hewitt [22] published a review of the metabolism of microelements in plants. Calcium in plant cells may be partially or wholly replaced by strontium, molybdenum by tungsten or vanadium, potassium by rubidium, and so on. This is doubtless related to the activation of different enzymes by metal ions. Undoubtedly, a large number of enzymes, the active groups of which do not contain metals, show activity in the presence of metal ion activators.

Activation by metal ions is much less specific than the function of ions that form complex catalysts of constant composition. Thus the enzyme tyrosinase is activated by iron, cobalt, and manganese ions; leucineaminopeptidase

is activated by magnesium and by manganese; arginase by manganese, cobalt, nickel, and iron; and carboxylase by manganese, cadmium, zinc, lead, nickel, iron, magnesium, barium, and copper. Activators of some types of phosphates are equally numerous.

Thus, the catalytic functions of transition metal ions are fairly clearly divided into two groups with different mechanisms of action.

The first group comprises cases of catalytic activity in which the metal forms definite compounds with closely limited functions. It includes, of course, such complexes as catalase, peroxidase, and other haemin enzymes, and vitamin B_{12}, a cobalt complex. As a rule, in this group one metal cannot be replaced by another without profoundly changing the catalytic properties.

Processes in which metal ions are only activators must be assigned to the second group. The basic features of this division is that ions of the first group are "non-replaceable", and therefore the mechanisms of action in both groups cannot be entirely identical. Although many properties of individual complexes of the first type have been thoroughly investigated, the mechanism of action of most activators is unknown and sometimes appears quite puzzling[23,24,25].

A particularly important type of active combination with a metal ion is one in which increased activity of the ion is achieved by means of certain ligands bonded to it, which are not substrates for the reaction; the metal porphyrins are a classical example of such complexes. The ammine complexes of copper and some complexes of iron, cobalt, and other metals show high activity in oxidation–reduction reactions.

A still more complicated system is that in which high activity is achieved by bonding of the metal–ligand complex to a protein carrier. Representatives of this class include enzymes and some enzyme models. A well-known review[26] of this problem published by Schwab and Rost about a quarter of a century ago gives information on model catalysts; the authors did not separate models simulating the active group proper from those that included a high-molecular-weight carrier. The models were divided into five groups. To the first the authors assigned "inorganic enzymes", that is, colloidal metal hydroxides and hydroxides having hydrolase (phosphatase) functions. The second group included the "respiratory models" of Warburg and Krause, that is, charcoal and a mixture of hydroxides of transition metals. This was followed by organic bases and the complex catalysts (compounds of cobalt) studied by Shibata. The fourth group included catalysts of the metal–quartz type and faser-catalysts. The fifth group included haemin and Langenbeck's "main valency" catalysts.

III. SOME MODELS OF IMPORTANT ENZYMES

At the present time the number of model catalysts has increased and the progress of knowledge in the field of enzymology has enabled us to find criteria better than activities for assigning a given catalyst to the class of "models". Obviously, metal sols and quartz cannot be regarded as models. Similarity of the model's general type of chemical structure to that of the true active centre of the enzyme is gradually becoming the starting point for researches in this field.

In a paper presented at the Fifth International Biochemical Congress, Westheimer pointed out the successes in the field of modelling that have led to the setting up of catalytic systems that operate under conditions close to those existing in cells by mechanisms similar to those of enzymes. He was confident that we shall soon be able to consider analogues of enzymes as well as models[27]. It was made clear that the mechanism of action of enzymes shows some distinctive features for which we cannot find analogies in ordinary catalysis. For this reason, it would be more rational to model the catalysis itself in the highly organised systems in which enzymes operate rather than the catalysts.

As for the individual compounds having similarities in structure and activity to enzymes or their cofactors, without exhausting the whole literature on the subject, several typical models of the most important enzymes will be mentioned. Oxidation–reduction enzymes have been modelled very frequently; catalase, the active group of which is a metal porphyrin, has been a favourite object for modelling; models of its active group have been sought amongst complexes of various metals.

In the author's laboratory[28-31] investigations have been made of the catalytic activity of complexes of copper, iron, cobalt, nickel, zinc, lead, and other metals in the hydrogen peroxide decomposition reaction, and in the oxidation of polyphenols, benzaldehyde, phenylenediamine, ascorbic acid, hydrogen sulphide, and other substrates. Compounds of copper have been studied in particular detail, since the catalytic function of the copper ion can be enhanced about a millionfold by complex formation with ammonia or amines. The complexes may therefore be regarded as "copper" models of catalase. By varying the ligand, we can find how such factors as chelate formation, substitution of other atoms, in the coordination sphere instead of nitrogen atoms, pH, complex stability, etc. affect the catalytic activity. For most of the complexes we studied, the order of reaction with respect to hydrogen peroxide was close to unity, that is, the picture as a whole is very similar to that observed with ammines.

The presence of a maximum on the curve showing the dependence of activity on the concentration of the free ligand in solution is a regular feature. The concentrations of amino-compounds corresponding to the activity maxima depend, of course, on the nature of the amine; sometimes they are fairly high[32]. This is due to the formation of more or less unstable six-coordinated complexes in the presence of an excess of ligand. Free positions in the coordination sphere are essential for catalysis. In practice the activity can often be conveniently evaluated as the number of moles of hydrogen peroxide decomposed per minute in the initial period of the reaction (for example, in the first five or ten minutes), calculated per gramme-ion of copper. If the plot of the amount decomposed against time departs appreciably from linearity, the slope at the origin is found by extrapolation. It is useful to maintain the amine concentration at a level corresponding to maximum activity. From the results of experiments with different buffers, a correction for pH can be introduced, and the activity can then be expressed at a single pH. Table 1 lists the most characteristic models of catalase. The activity of some of these is lower than that of the enzymes by only one or two powers of ten. It should be remembered that catalase is quite exceptional as a biocatalyst; for this reason, the setting up of such working models is of interest from the point of view of the theory of catalysis, irrespective of the problem of modelling.

TABLE 1. Some catalase models containing metal.*

Metal ion	Ligand	Activity	Literature references
Cu^{II}	amino-acids, piperidine, peptides, proteins	weak	28, 32, 33
Fe^{III}	salicylic acid	weak	34
Cu^{II}	amides, amino-alcohols, pyridine, picoline	average	28, 35
Fe^{III}	haem, its derivatives, phenanthroline, phthalocyanine	average	21, 36
Cd^{II}, Zn^{II}, Pb^{II}, Fe^{III}, Co^{II}	Schiff bases	average and weak	37, 38
Cu^{II}	ammonia, amines, diamines *N*-substituted diamines, biuret	high	28, 30
Fe^{III}	triethylaminotetramine	high	39

* The activity of a catalyst is termed "weak" if it is close to unity, "average" if it is ~ 10, and "high" if it is greater than 100.

It is interesting that piperidine complexes show low activity (1.2); this indicates the importance of amine nitrogen for the catalase function of the complex.

The catalytic functions of complexes formed by transition metal ions with biuret, triuret, peptides, and proteins[37] deserve mention. Biuret complexes show the highest activity at the limit of their decomposition; if the concentration of biuret in the solution is raised so as to make the complex stable, the activity falls sharply[34].

The author has studied the decomposition of hydrogen peroxide by a biuret complex at different temperatures. The temperature coefficient of the reaction is very low; the activation energy is 1500–2000 kcal mole^{-1}. Here the high catalase activity is associated with a low activation energy. The biuret complex is more active than the copper ammines and is undoubtedly a powerful catalase catalyst.

Although weak, the activity of protein–biuret complexes depends on the nature of the protein. Globulins form the most active complexes, followed by gelatin, casein, and albumin.

We shall now consider the catalytic properties of iron complexes, and, to begin with, we should emphasise that the iron–porphyrin complexes, which form the active part of a number of oxidative enzymes and electron carriers, may show considerable activity even when isolated from the protein carrier. The activity of these complexes is increased by the addition of some compounds, including pyridine and imidazole and its derivatives, which greatly increase the activity of haem. It is particularly interesting that if we select different derivatives of imidazole, we can alter the specific catalytic action: the compound of haem with sodium imidazole-4,5-disulphonate has pronounced peroxidase properties but does not catalyse the decomposition of hydrogen peroxide. Compounds of haem with imidazole, on the other hand, are four times more active in this reaction than haem itself[21]. Similar compounds may evidently be formed in organisms and perform definite biological functions; they may nevertheless be regarded as models, since the selectivity is controlled not by the protein part of the catalyst but by a much simpler group.

The introduction of ammine nitrogen into the coordination sphere of the complex activates the catalase function of the iron ion. When solutions of sodium nitroprusside are treated with ammonia the compound $Na_3[Fe(CN)_5NH_3]$ is obtained. The author has compared the catalytic activity of solutions of potassium ferrocyanide, nitroprusside, and this ammonia complex. The hexacyano-complex and nitroprusside show very low activity, but the introduction of ammonia sharply increases the catalytic activity; exactly the same effect is observed as that which was so clearly established in the discussion of copper complexes.

Complexes of metals with Schiff bases, especially those obtained from *o*-aminophenol and salicylaldehyde (SAP), are of interest, because, although they are unstable to hydrogen peroxide, in non-aqueous solutions and mixtures they give rise to effects that cannot be observed in purely aqueous solutions.

Kudryavtsev, Savich, Kundo, and Nikolaev studied the decomposition of hydrogen peroxide in water and in borate buffer. At pH 6–7 in aqueous solution only the compounds of cobalt, copper, and nickel were active; other ions do not form active complexes with SAP. In borate buffer at pH 9.8 all the ions showed much greater activity; the order of increasing activity was different: nickel, cadmium, copper, zinc, lead, iron, cobalt; the activity of the cobalt complex was increased by a factor of 3, and the activity of the iron compounds was sharply increased[37,38,40].

The activity of the zinc compounds in particularly interesting: the zinc ion shows practically no catalase activity. Here the change from the ion to the complex gives rise to a qualitatively new catalytic function.

Still more striking results are observed in pyridine solutions. The catalase activity of lead ions in the form of a complex with SAP increases nearly 300 times (calculated on the metal) compared with the activity of the simple ion. The same order of activation is observed with iron, cobalt, and nickel. The latter is noteworthy since the nickel ion and many of its complexes are virtually devoid of catalytic activity. The following is the order in which the activity increases in pyridine–water (1 : 9) mixtures: zinc, cadmium, copper, nickel, cobalt, iron, and lead. From a comparison of data on ligand structure with the activity of different complexes, more or less general conclusions can be drawn, at least for aqueous solutions. Catalytic activity is favoured by the presence of amino--nitrogen in the coordination sphere, particularly by the presence of four nitrogen atoms and a chelate structure. Calculations of the electron density distribution made for several active copper complexes by Goudot[41] showed that high activity corresponds to the presence of a negative charge on the copper ion, that is, there is a marked donor effect.

The catalase activity is reduced if oxygen enters the coordination sphere or if a saturated coordination complex is obtained. Compounds with high activity usually show relatively low complex stability.

The author's results indicate that a radical mechanism is unlikely for the decomposition of hydrogen peroxide by these complexes. There are grounds for believing that the complexes form intermediate products containing HO_2^- and H_2O_2. In most cases these products form loose transition

states, and the kinetic equations contain large entropy factors and comparatively large activation energies. However, this is not a general rule; some complexes, for example Cu-biuret, are characterised by low activation energies.

The oxidase functions of complexes present a picture of greater diversity than the catalase functions, although usually such a sharp increase in activity on complex formation is not observed as occurred, for example, with the copper ion. In oxidation reactions catalysed by metal ions complex formation, in particular chelate formation, may reduce the characteristic catalytic activity of the ion. A similar phenomenon usually occurs when the ion combines with ligands to give stable complexes[46].

Chalk and Smith[42] showed that copper, manganese, and iron ions accelerate the oxidation of cyclohexene, copper by a factor of ~100, iron by a factor of 10, and manganese by a factor of 200. Bonding with disalicylideneethylenediamine increased the activity of the iron by a factor of ~40; it also increased the activity of manganese slightly but reduced that of copper by a factor of ~10. This example is characteristic in that it shows how specific is the action of a given ligand on the quantitative and qualitative catalytic functions of the ion. Thuillier points out the obvious similarity of the structure of the iron–EDTA complex to that of cytochrome[49].

A similar example is to be found in the phthalocyanines. Structurally, the phthalocyanines are very similar to the porphyrins; for this reason, a comparison of the catalytic properties of the metal complexes of both types of compound is of interest. Sodium, potassium, calcium, barium, and cadmium phthalocyanines show the properties of salts, but the derivatives of other metals are complexes. In 1938 Cook[36] studied the catalytic properties of complex phthalocyanines of different metals in the decomposition of hydrogen peroxide and in oxidation reactions. Phthalocyanines are very insoluble in water; Cook therefore dissolved them in 75% pyridine, and in some cases even in concentrated sulphuric acid.

Cook examined the catalase properties of iron, zinc, chromium, and vanadium. Compounds of bivalent iron showed the greatest activity. In addition to studying the homogeneous decomposition of hydrogen peroxide, Cook attempted to compare the properties of haemin with those of the phthalocyanine complexes; he deposited the phthalocyanine compounds on wood charcoal. The activity of haemin was greatly increased by adsorption on charcoal, and iron phthalocyanine showed a similar effect. The adsorbed catalyst could be poisoned by cyanides.

Cook investigated the oxidation of organic compounds by atmospheric oxygen catalysed by metal phthalocyanines; the iron compound was found to be active, functioning as an oxygen carrier. Because of the poor solubility of the complex, the catalysis was heterogeneous in most experiments. Moreover, in some oxidations considerable decomposition of the complex occurred.

Paquot[50] used phthalocyanine complexes to accelerate the atmospheric oxidation of olefinic hydrocarbons.

Myer[46] showed that the formation of complexes with diamines increased the catalytic activity of iron ions by 300% and of manganese ions by 480% in the oxidation of linseed oil; it is interesting that complex formation with these ligands had practically no effect on the catalytic activity of the cobalt ion. With manganese the activating ligand was disalicylideneethylenediamine and with iron, *o*-phenanthroline. The formation of a five- or six-membered ring, with the metal ion bonded to nitrogen, is important for activity.

Copper ions catalyse the oxidation of the most diverse compounds, including ascorbic acid, hydroquinone, cysteine, glutathione, etc. The author has found that some compounds to some extent enhance the oxidative function of copper; the activator–copper–substrate complex is oxidised more rapidly than the copper–substrate complex. Histidine activates the oxidase function, possessing a weak but well-marked activating effect on the copper ion[43]. The copper–histidine complex was found to be inactive in the oxidation of pyrogallol.

A study of models of the enzymes that oxidise polyphenols is of considerable interest. The classical investigations of Bakh led to the conclusion that the enzyme that oxidises pyrogallol contains copper. Korpusova and Nikolaev[47] studied various complexes of the copper ion from the point of view of their ability to accelerate the oxidation of pyrogallol solutions with gaseous oxygen. It was shown that in many cases the formation of complexes greatly enhanced the catalytic activity of the copper ion. The monoethanolamine and ethylenediamine complexes were the most active compounds, but the isopropanolamine and propylenediamine complexes were also very active.

It is particularly interesting that the amines themselves show appreciable catalytic activity. This effect may be explained on the assumption that a hydrogen bond is formed between the hydroxyl hydrogen and the amine group, which facilitates the oxidation. In aqueous solution the water molecules and the pyrogallol compete for the electron pairs of the amine nitrogen atoms. It therefore follows that the stronger the bond between the hydroxyl hydrogen and the amine the more active will be the corresponding amine.

TABLE 2. Models of various oxidases and peroxidase.

Metal ion	Ligand	Reaction	Literature references
Mn Fe^{II}	disalicylideneethylenediamine	oxidation of cyclohexene	42
Fe^{II}	catechol	oxidation of ascorbic acid	43
Fe^{II}	antipyrinetoluenesulphonic acid	oxidation of ascorbic acid	43
Fe^{II}	sodium nitroprusside	oxidation of pyrogallol	44
Fe^{II}	EDTA	oxidation of tetralin, octalin, pinene, methylcyclohexene, pyrogallol, benzaldehyde, olefins, etc.	45
Cu^{II}	histidine, insulin	oxidation of ascorbic acid	43
Mn^{II}	disalicylideneethylenediamine	oxidation of linseed oil	46
Cu^{II}	amino-alcohols, pyridine, ethylenediamine, propylenediamine, morpholine	oxidation of pyrogallol	47, 48
Cu^{II}, Co^{III}, Ni^{II}	Schiff base	oxidation of ascorbic acid and pyrogallol	40
Co^{III}	ethylenediamine, ammonia, chlorine	oxidation of catechin	19

Thus, if the reaction between a proton and the amine is represented by the equation:

$$HAm^+ \rightleftarrows H^+ + Am,$$

where HAm^+ is the protonated form of the amine, then the lower the equilibrium constant of this reaction (*i.e.* the higher the concentration of the protonated form of the amine) the greater is the activity of the amine. Table 3 shows that there is a strong correlation between the equilibrium constants and the activities of different amines.

Comparison of the activities of these ligands with those of the complexes shows that the increase in activity due to complex formation depends on the nature of the amine; with mono- and di-ethanolamine the increase is well marked, but the activities of ethylenediamine and propylenediamine are virtually unchanged by complex formation.

Thus, the inclusion of a metal in the unstable amine–polyphenol system does not increase the rate of oxidation in all cases, but, as far as can be judged from limited results, only when the amine does not form a chelate structure. In experiments with different amines the author showed that the difference in entropy between the catalysed and uncatalysed reactions depends on the nature of the amine; the sign as well as the magnitude of the difference changes. This difference is positive for isopropanolamine and monoethanolamine, and the entropy of the transition complex containing the catalyst is larger than that of the transition complex in the normal reaction. On the other hand, catalysts containing picolines as ligands give rise to a transition complex with reduced entropy, and acceleration of the reaction is associated with a fall in activation energy.

The catalytic activity of complexes of copper with morpholine in reactions of diphenol compounds has been studied by Brackman and Havinga. They proposed a reaction scheme in which conversion of quinones[48] played an essential part. These workers also found that the activity of copper is increased by complex formation. Many complexes with Schiff bases show catalytic activity. In 1956 Langenbeck and Koechler[37] described the catalytic properties of complexes with Schiff bases prepared from sulphosalicylic aldehyde. Parallel studies were done on chelates formed from pyridoxal, the structure of which is somewhat similar to salicylaldehyde. Both types of chelates showed activity in the oxidation of pyrogallol and purpurogallin; cobalt chelates are very active. The Schiff base prepared from salicylaldehyde increased the activity of the cobalt ion 10 000 times, and that prepared from pyridoxal increased it 1000 times.

TABLE 3.

Amine	Activity, cm^3 O_2/3 min	$K = \frac{[H^+][Am]}{[HAm^+]}$
Pyridine	0.8	$1.6 \cdot 10^{-6}$
Triethanolamine	1.1	$1.2 \cdot 10^{-8}$
Diethanolamine	1.4	$1.1 \cdot 10^{-10}$
Monoethanolamine	3.2	$2.5 \cdot 10^{-10}$
Propylenediamine	8.5	$1.1 \cdot 10^{-10}$
Ethylenediamine	9.5	$3.0 \cdot 10^{-10}$

TABLE 4. Some hydrolase models.

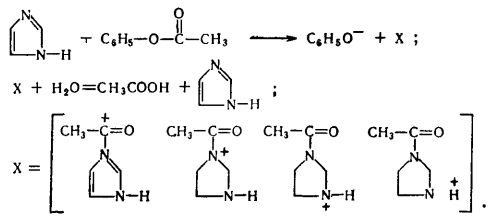
Compound	Reaction	Literature references
Complexes:		
Copper with ligands: ethylenediamine, dipyridyl, histidine, phenanthroline, imidazole	hydrolysis of diisopropyl fluorophosphate	52
Imidazole and its derivatives	hydrolysis of phenyl acetate	53

Kudryavtsev *et al.* have shown[40] that complexes of copper, cobalt, and nickel with Schiff bases prepared from salicylaldehyde and *o*-aminophenol (SAP) have an appreciable oxidase activity. In studies on the dependence of the catalytic functions of complexes of this type on the nature of the metal, aqueous and aqueous pyridine solutions of cobalt, copper, nickel, zinc, cadmium, lead, and iron complexes were examined. The high activity of the complex containing copper is noteworthy; this is a specific catalyst in the oxidation of ascorbic acid. Iron is second in order of activity, followed by cadmium and cobalt. This suggests that the oxidase function of slightly active constant-valency ions such as cadmium can be activated. The cobalt complex showed the greatest activity in the oxidation of pyrogallol in pyridine; all the other ions are considerably inferior to it in oxidase activity. The cadmium ion is second in order of activity, but the activity of the iron ion is extremely weak.

The problem of modelling hydrolytic enzymes is much more difficult. Models of these enzymes have been obtained, from both typical chelate complexes and from some amino-acids (Table 4). Most theories of enzymic hydrolysis allow for some kind of polarisation effect[51], which may be due either to metal ions or to the action of different polar groups. The active groups of hydrolase consist of a combination of structural elements of the polypeptide chains. The active group of chymotrypsin, trypsin, and other proteases and esterases contains a definite combination of amino-acids, with histidine playing the greatest part. The mechanism of action appears to be based on acylation of the enzyme and hydrolysis of the acylated form; apart from histidine, the sequence Gly–Asp–Ser–Gly is important. Several workers[54,55] have shown that trypsin and chymotrypsin hydrolyse nitrophenyl acetate.

Free imidazole and its derivatives also have this property. The intermediate product is *N*-acetylimidazole. However, the activity of the imidazole "model" is lower than that of the enzyme chymotrypsin by a factor of 10^5. The catalytic activity of L-histidine in this reaction has also been examined; it has been found that blocking of the amino- and carboxyl groups increases the activity 3–4 times[54]. Histidine peptides have also shown hydrolytic activity. Bruice and Schmir[53] studied the catalytic properties of imidazole in the hydrolysis of phenyl acetate. These workers had in mind the part that the histidine imidazolyl groups play as components of the active sections of hydrolytic enzymes. They examined the mechanism of hydrolysis in the presence of imidazole and concluded that the most likely mechanism was one in which the imidazole reacts directly with phenyl acetate, forming phenol and some product that subsequently reacts with water to give acetic acid

and the regenerated catalyst. The product is probably an acylo-imidazole complex stabilised by the superposition of several structures:



Metal ions undoubtedly play an important part in natural hydrolytic systems. The necessity of the presence of magnesium for the hydrolysis of ATP has been interpreted by Szent-Györgyi[56] on the basis that magnesium has a polarising action on the twisted ATP molecule. It is to be expected that complex ions would produce hydrolytic effects. Wagner-Jauregg and coworkers[57] showed that some copper chelate complexes catalyse the hydrolysis of diisopropyl phosphorofluoridate (DFP). The complexes containing ethylenediamine, $\alpha\alpha$-dipyridyl, histidine, *o*-phenanthroline, and imidazole as ligands are the most active. The active complexes are unsaturated coordination compounds. In aqueous solutions the sites in the coordination sphere that form the reactive space of the complex (positions 5 and 6) are occupied by hydroxy-groups or by water molecules. Further studies showed the same regular features that the author observed in the catalase-active complexes. In particular, increased stability reduced the catalytic activity; the activity is a function of pH and depends largely on the presence of a chelate ring.

Investigation of the complexes formed by di- and tri-amines has shown that ligands of this type may be arranged in decreasing order of activity of the complexes: tetramethylethylenediamine, dipyridyl, ethylenediamine, hydroxyethylethylenediamine, dihydroxyethylethylenediamine, diethylenetriamine. It is suggested[58] that hydrolysis occurs as a result of the simultaneous action on the substrate of OH^- ions and diaquo-metal chelate ions. A complex with the substrate may first be formed, and is then attacked by OH^-. This model is of interest because the substrate (DFP) appears to play the part of "organiser", bringing the OH^- ion and the catalyst proper, copper chelate, into a suitable conformation. In enzymology it has often been noted that the substrate can have an organising influence on the components of the active centre. Anfinsen[56] has pointed out the part that the tertiary structure of proteins of hydrolytic enzymes plays in the mechanism of their action and in the level of their activity[59]. The active centre is said to be stabilised by a complex system of interactions, as a result of which secondary and tertiary structures arise; complete information necessary for the formation of these structures is contained in a definite amino-acid sequence in the extended polypeptide chain.

Anfinsen's reasoning refers to ribonuclease. Whether this picture is a general one is still undecided, but there are grounds for assuming that the active centres of enzymes could be formed at "a higher level", that is, when the course of evolution had already led to significant development of higher-order structures. It is extremely important that this fact was discovered in the investigation of protein-splitting enzymes. The gradual development of polypeptide systems led to the formation of the active centre, built up from different fragments of the twisted polypeptide chain or chains, and the emergence of this centre initiated hydrolysis processes that probably limited chain growth.

The modelling of dehydrogenases presents no formal difficulties, since a large number of reversibly oxidisable dyes are known to function by the same mechanism as the enzyme systems. The dye abstracts hydrogen from some substrate, is converted to the hydrogenated form, and then gives off its hydrogen to oxygen or another acceptor. Thus, Methylene Blue acts as a catalyst in the oxidation of many organic compounds, in particular ascorbic acid; this reaction is accelerated by light[60,61].

It is interesting to find to what extent a particular model of this type is specific for the substrate. In nature the active groups of dehydrogenase coenzymes, NAD and NADP, interact with the substrate and the corresponding protein to form dehydrogenase systems with specific activity. Therefore, before dehydrogenase models are examined, it is useful to consider the more general problem of modelling enzyme systems containing a high-molecular-weight carrier (Table 5).

The sharp increase in catalase activity produced when iron ions are adsorbed on charcoal may serve as a simple example. The porphyrin complex haemin and iron phthalocyanine are also activated by this carrier. As the activity of charcoal itself is low, the deviation from additivity is very marked. The specific effect is emphasised by the failure of charcoal to activate most other ions; also, many carriers (stannic oxide, barium sulphate, and alumina gel) inhibit the catalase activity of the ions. "Charcoal models" are obtained not only by adsorption of ions from solution, but also by charring materials containing metal compounds. It would be fairly difficult to determine the active centre in such catalysts or even to evaluate approximately the "ballast" that plays the part of an activator. Warburg[12] found that charcoals prepared by charring haematin had considerable catalytic activity in oxidation reactions. In 1932 the author found[66] that blood charcoal had a strong catalytic action on the halogenation of organic compounds; dihalogeno-substituted products were obtained from benzene, and ethyl bromide from ethanol.

TABLE 5. Models containing a high-molecular-weight component.

Active group	High-molecular-weight component	Reaction	Literature references
Haemin	charcoal	decomp. of H_2O_2, oxidation of oxalic acid	12, 21
Phthalocyanines of heavy metals	charcoal	various oxidation reactions	36
$>CH-N(C_2H_5)_2$	cellulose	decomp. of bromocamphoric acid, formation of mandelonitrile	18
Methylene Blue, thionine	starch	oxidation of metol and pyrogallol	60
Copper – histidine	insulin	oxidation of ascorbic acid	43
Copper – amino - alcohols	albumin, legumin, casein	oxidation of pyrogallol	62
Polychelate metal complexes		decomposition	63, 64
Indigo carmine	cellulose	oxidation of hydrogen sulphide	65

To examine the form of iron in charcoal of the type obtained from blood, the author studied charcoal prepared by charring haematin. Magnetic and X-ray studies indicate that the iron in this charcoal is in the form of a nitrogen-containing complex, in which the iron is probably bivalent and bonded with no more than three or four atoms of nitrogen and carbon; there is very little activating ballast present. The author observed similar effects in the adsorption of iron ions on charcoal obtained by charring sulphanilic acid. This charcoal has a very large surface area (700 $m^2 g^{-1}$) and X-ray examination shows it to be amorphous.

The nature of the bond between the active group and the carrier is clearer in the "faser-catalysts" of Bredig and Gerstner[18]. These workers prepared diethylaminocellulose by the method of Karrer and Wehrle and found that it accelerates the elimination of carbon dioxide from bromocamphoric acid. The catalyst shows optical selectivity: in the synthesis of mandelonitrile it increased the laevo--rotation. The active group of this catalyst appears to have the structure of a diamino-derivative:

$$\rangle CH—NH\,(C_2H_5)_2\,.$$

Krause and coworkers[67] have carried out extensive investigations of the catalytic activity of mixed hydroxides. The activating action of copper hydroxide on the catalytic function of a mixture of the hydroxides of manganese and cobalt led these workers to conclude that copper hydroxide is an analogue of the active group of the enzyme. Catalytic systems of this type are very interesting in themselves, especially as hydroxides also catalyse ester hydrolysis, that is, they behave as esterase models[68]. However, they appear to have played an important part only in the first stages of biochemical evolution, having been later replaced by other systems.

The copper ion attached to insulin, and also the copper–histidine complex bound to this protein, are models of ascorbate oxidase, according to the author's results. Nikolaev and Korpusova[62] found that the proteins albumin, casein, and legumin considerably enhanced the polyphenol oxidase activity of copper complexes with amino-alcohols and other ligands. In all these cases, fixation on carriers had only a slight effect on the visible and ultraviolet spectra. The activation of amino-alcohols on proteins appears to be due to complex formation with the amino-groups of the protein, since it is reduced when the protein is treated with formaldehyde.

Considerable activation is observed when the bond between the active group and the carrier is very weak, in contrast to the bond in the examples given above. Dyes that can change reversibly into leuco-forms have undoubted features of similarity with the active groups of dehydrogenases and with those compounds that act as intermediate links in hydrogen transfer in biological systems.

The catalytic action of Methylene Blue and thionine on the oxidation of pyrogallol and metol with oxygen, and the activation of these compounds by their adsorption on starch have been studied by Yushina[61]. The bond between the dye and the starch appears to be affected by means of the negative charges on the colloidal starch particles and the dye cations. The carrier only shows activation when it is in the colloidal state. The loss of activity produced by adsorption on starch powder thus appears to be analogous to the deactivation of the enxyme that occurs in denaturation.

The author had also shown[65] that cellulose is a good activator of the dehydrogenase function of indigo carmine. This dye can change reversibly into the leuco-form, which is rapidly oxidised by atmospheric oxygen. In the presence of cellulose the reaction is greatly accelerated if hydrogen sulphide is used as the hydrogen donor. The reaction proceeds very readily when the hydrogen sulphide solution contains trace amounts of copper and iron ions. Thus indigo carmine adsorbed on cellulose is a model for the dehydrogenase catalytic system. It is known that many enzymes of this class are activated by traces of metals.

There are interesting possibilities in the investigation of the catalytic properties of chelate polymers containing metal ions separated by organic groups. Catalysts of this type were studied by Boreskov, Keier, Rubtsova, and Rukhadze[63,64], who found that the catalytic activity of polychelates depends mainly on both the nature of the metal ion and on the nature of the organic units. Obviously, variation of the structure of the organic units opens new possibilities in controlling the steric and energetic factors in the catalytic process.

The reaction between indigo carmine and hydrogen peroxide studied by Kitsenko shows that orientation effects may sometimes be important. The degradative oxidation of the dye accompanied by bleaching is accelerated by phase boundaries of a different nature[69]. Thus the rate of the reaction increases at the aqueous solution–benzene or aqueous solution–toluene boundary and also at the solution–air boundary, etc. Here a special chemisorption effect, or the formation of intermediate products, is unlikely, but there is no doubt about the kinetic effect. From this it follows that, in general, the appearance of a new phase in complicated organic systems often involves a large number of types of possible catalytic action, even when the intermediate active products cannot be characterised.

IV. COMPARISON OF THE PROPERTIES OF MODELS AND BIOCATALYSTS

It is obvious that the more rigid the requirements that models have to meet the smaller will be their number. When the conditions that the model and the enzyme should be similar in structure and operate by similar mechanisms were added to the requirement of high activity, the number of models diminished. However, even these limitations leave too large a number of models to be considered, and they do not remove the difficulties encountered in formulating selection principles. For this reason, attention must be directed to those facets of biocatalysis which, firstly, have no analogies in ordinary catalysis, and secondly, have not been modelled in any clear form. The following features of the catalytic action of biocatalytic systems apparently differentiate enzymic from ordinary catalysis.

1. Active centres in a number of reactions are obtained as a result of the development of higher-order structures — tertiary, and probably also quaternary protein structures (hydrolase). The active centre includes not only the group of atoms directly entering into the catalytic act, but also part of the protein molecule extending far beyond its limits. This zone ensures a suitable arrangement of substrate molecules and is related to the specific activity.

2. The substrate sometimes undergoes preliminary activation. Thus, sugars are first phosphorylated before they enter the cycle of conversions that leads to pyruvic acid; amino-acids are also phosphorylated in the synthesis of the polypeptide chain, and fatty acids combine with CoA.

3. The active centre often comprises a group of atoms weakly bound to the protein and displaceable from one protein to another. The protein–substrate complex and the active group nevertheless act together, the bond between them sometimes being due to the inclusion of a metal "coupling". The specific adsorption of the substrate on the protein is one facet of the catalytic system, and the action of cofactors proper is another. Entropy and energy factors are therefore partly "disconnected". A system that might serve as a model is one in which the substrate is prepared for reaction by adsorption on a carrier to obtain the necessary orientation of parts of the molecule (or of several molecules), and in which an active centre or active species effects the catalytic reaction itself in the system.

4. The catalysts are combined in a system by principles of energy or substrate coupling; the energy of one reaction is used to overcome the activation barriers of another, and the substrate of one process is the end product of another. This needs a special organisation of a high degree of complexity (mitochondria).

5. The synthesis of the catalysts themselves is inseparable from their functions; the two processes are related by a feed-back mechanism. It has been shown that an increase in the concentration of the products of the action of certain enzymes leads to suppression of the synthesis of the enzymes themselves. The structures characteristic of biocatalysis, of course, meet these requirements. The polymer chains of peptides, including active amino-acids (histidine, for example, in hydrolysis reactions), possess very wide possibilities for the formation of active centres and the creation of higher-order structures precisely adjusted to the substrate. The substrate is activated by combining with phosphoric acid residues, while phosphoric acid (energy-rich system of pyrophosphate bonds) also enters into the composition of the active groups of the catalysts themselves (ATP, NAD, etc.).

Fixation on the high-molecular-weight carrier groups, supplemented by the action of the catalyst proper at its extreme activity, when virtually the whole substrate molecule is bound to the carrier and subjected to change at many points, means that the structure of the carrier determines the arrangement of the product. This "matrix" principle, which is very characteristic of biocatalysis, requires precise structural matching of all the participants in the reaction and leads to the phenomenon known as self-reproduction†. It is not difficult to show from an examination of its properties that a particular model is deficient in some respects, and from this it may be concluded that the make-up of biocatalysts in cells is in some way "unique". It cannot be said that these catalysts have a special type of bond or mechanism of action. There is nothing fundamentally new in the types of bonds in biological systems; they include covalent, donor–acceptor, and hydrogen bonds, as well as interactions based on steric effects (for example, clathrate), polarisation effects, etc.

The mechanism of action is, as a rule, associated with the formation of intermediate lubile forms with steric characteristics close to those of the transition complex. With model compounds, the intermediate products are usually less stable and more difficult to identify than with enzymes, but there is no reason to suppose that the fundamental reaction steps in biocatalysis differ from the corresponding stages in catalysis by less complicated catalysts.

Peculiarities become manifest at a higher level; indeed, when a number of similar catalysts are being tested, the possibility of transition to a higher level of organisation is a selection factor.

Simple systems containing catalysts arose in large numbers at the early stages of chemical evolution. Most of these quickly ceased to exist, not having the means to increase their stability. For these, evolution to a more stable state corresponded to the normal thermodynamic evolution of open or closed systems. Those that acquired the means of maintaining the level of organisation by means of feedback mechanisms with the medium obtained new means of stabilisation, and what is particularly important, new stability conditions. These conditions are not associated with extremal entropy values, or extremal values of the derivative of the entropy with respect to time; they are determined by work done by control apparatus linking external stimuli with deviations from the parameters of the system.

Let us follow the course of increasing complexity of systems from the most elementary, such as a rarefied gas, to a highly organised system, such as man. We find that the conditions corresponding to stability of the system differ substantially at the ends of this sequence. For the gas, thermodynamics precisely indicate the exact functions and conditions that determine equilibrium, but for man, or even for a less complicated living organism, adherence to the formulations of thermodynamics would lead to destruction of the system, not to stabilisation. Stabilisation is achieved by entirely different means, the development of which cannot be predicted by thermodynamics. An organism consists of mechanism, at the basis of which are molecules in a state of interaction with one another. Consequently, the gradual increase in complexity of the systems will lead in certain cases to a state in which the new systems arising are stabilised by laws different from those that controlled the transition to the more stable state in the initial systems — the precursor systems.

In fact, transition to the equilibrium state, corresponding to maximum entropy in the isolated system, or the achievement of a steady state in "flow", differs from stabilisation due to feedback systems, although these feedback mechanisms developed in the same "material" that is stabilised by the simple method mentioned above. It therefore follows that only those elementary systems could become the starting points in evolution that satisfied the fundamental requirement that stability conditions for combinations of systems should differ from those for the initial systems. It is this principle which leads to the independence from the material which has been discussed at the beginning of this review. From this point of view, we may attempt to interpret certain special features of biological evolution. Actually, as organic compounds gradually increased in complexity, new stability factors arose when catalysts were formed. Few cases are to be found in biology in which chemical control and chemical feedback occur without the participation of a catalyst. On the other hand, every simple case of catalytic action, reversibly inhibited by reaction products, is an example of chemical control. It is quite reasonable that all subsequent development should have been based on the synthesis of diverse catalysts and the perfection of mechanisms for their coordinated action.

† Interesting information on the imprints that the substrate produces on the catalyst surface may be found in a paper by Balandin [70].

Thus the process that had become the basis for the formation of living structures involved most readily compounds that were converted to elements of catalytic system and were therefore withdrawn from the sphere of operation of stabilisation laws characteristic of the simplest aggregates of heterogeneous molecules. It is characteristic of feedback stabilisation mechanisms that they prevent the system reaching a state of thermodynamic equilibrium; this gives them an advantage over all other stabilisation mechanisms. The interrelationships of a system of this "higher" order with the medium are determined by the fact that both the medium and the system will each tend to its own stable state. For the medium this will be, for example, the state of maximum entropy, and for the system, the state in which the control apparatus is stable in operation, corresponding to the minimum change in the parameters of the system for given external random influences. In this way, the characteristic equilibrium of systems of different degrees is established, the increase in entropy of the medium being related, at least phenomenologically, to the decrease in this function for the system.

Further increase in the complexity of the controlling apparatus and the general growth in the degree of organisation of the system is only possible if the associations arising will increase the stability of all less complex apparatus, in the same way as the conscious intellectual activity of man is directed towards the stabilisation of all the mechanisms on which his existence depends in some way or another.

Evolutionary selection by the application of an immense number of tests to the enormous number of biocatalyst "models" has limited adoption to those that were found suitable for the synthesis of catalyst systems with feedback, capable of sustaining the course of evolution to systems of higher degree with still more diverse stabilisation possibilities. The modelling of chemical self-stabilising systems is one of the most interesting problems of the future science of catalysis.

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Moscow Institute of
Transport Engineering

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ANODIC OXIDE FILMS

N. G. Bardina

CONTENTS

I. Introduction 286
II. Growth of anodic films 286
III. Non-porous films: empirical relationship between ionic current, field, and temperature 287
IV. Theory of the growth of anodic films 288
V. Properties of non-porous oxide films 290
1. Electronic conductivity 291
2. Rectification 291

I. INTRODUCTION

Oxide films on metals are widely used in the national economy, in various electrochemical devices, in condensers, in rectifiers, in chemical sources of current, and also for protecting metals against corrosion.

The metal–oxide–electrolyte system exhibits an appreciable capacity and can rectify alternating current over a wide range of potentials. Therefore such a system finds a wide use in electrical devices.

Oxide films also have independent importance in studying electrochemical reactions on metals, since under specific conditions it is necessary to take into account that all metals are covered with oxide films.

There is now considerable material on the experimental and theoretical problems of anodic oxide films, which it is difficult to generalise in a single review. Hence the present article will consider only certain questions concerning mechanism in the formation of anodic oxide films and of their properties.

This review considers the kinetic mechanisms of the growth of oxide films during anodic oxidation and also certain properties of films on valve metals.

II. GROWTH OF ANODIC FILMS

Anodic oxide films on metals or semiconductors are formed in a solution of electrolyte on applying a potential more positive than the equilibrium potential. Afterwards as the metal becomes covered by a thin continuous layer of oxide, which is insoluble in the electrolyte, an electrostatic field is established in the oxide. This field causes ions of metal or oxygen to move through the oxide layer, which also leads to growth of the film. For the formation of each new layer of oxide an additional potential dv is required, which is added to the existing potential in the film.

It is found experimentally that the quantity dv/dx does not vary as the thickness increases, but for certain metals there is a departure in one direction or the other.

The growth of the film is greatly hindered by processes at the boundaries of the film with the metal and with the electrolyte[1-4].

The formation of centres of crystallisation and also the thickening of the films requires overpotentials of from 10 to 1000 mV. These are made up of; (1) the energy for the removal of a metal ion from the lattice to the metal–oxide boundary; (2) the kinetic energy of the metal ion through the film; (3) the energy for the exchange of charged particles between the electrode and the solution at the oxide–electrolyte boundary.

In their structure oxide films can be divided into porous and continuous (pore-free). The resistance of porous films is of the order of a few ohms, and their potentials at the time of growth are near to equilibrium, they are almost invariably crystalline, and their growth is essentially by diffusion of the ions through the oxides or through the electrolyte. Such films are formed on cadmium, zinc, and magnesium.

Vermilyea[5] considers that continuous films are formed during simultaneous oxidation of metal at all points of the surface.

Misch[6] thinks that the nature of the oxide film depends on the value of the ionic potential (the ionic charge divided by the radius), the lattice type of the metal and oxide, and also the bond energy. The adhesion of the oxide particles depends on the ionic nature of the oxide, the resistance to impregnation by water, and the extent to which the ratio of the molar volume of the oxide to the molar volume of the metal (the Pilling and Bedworth ratio) exceeds unity. Continuous growth of the film depends on the overall ionic current in the film, in which connection the following facts must be considered: (1) the overall electrical conductivity of the oxide, and (2) the transport numbers of the ions, electrons, and holes.

If the oxide is an electronic semiconductor, then all the current is used in the discharge and liberation of gaseous oxygen, the ionic current will be insignificant, and the thickness of the film will not increase — such a reaction takes place on titanium[7].

Another type of reaction is the processes taking place on tantalum[8], aluminium[9], zirconium, for which the effectiveness of the anodising of the pure metal is 100%. The