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THE EVOLUTION OF BIOCATALYSTS

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I. INTRODUCTION

There are a number of different aspects to the evolution of biological catalysts which are of interest to a wide circle of chemists. The first appears from a comparison of the effects achieved by our ordinary catalysts with the virtually unlimited possibilities of the highly organised enzymic systems of the living cell.

Anyone familiar with the difficulties involved in the selection of a suitable catalyst and accustomed to using catalysts at high pressures and temperatures is bound to be impressed by the performance of the biological catalysts, which can oxidise sulphur directly to sulphuric acid at low temperatures, fix nitrogen at atmospheric pressure, synthesise methane, oxidise naphthalene, induce the reaction of hydrogen with oxygen, and form penicillin, polypeptides and proteins. What is more, these catalysts both originate and sustain the processes which we understand by the term "life".

However, our knowledge of enzymes falls far short of that of catalysis, especially so far as the general theoretical principles are concerned. For this reason the theoretical study of the action of biocatalysts deserves close attention.

The organic catalysts and the "models" of enzymes are obviously less difficult objects of study, and the advances achieved with a series of models provide the foundation for a development of theoretical concepts. Such concepts will be different from those inferred from a study of non-enzymic catalysis; and, in particular, it should be kept in mind that the theoretical analysis of the action of catalytic systems, characteristic of the living cell, is still in the embryonic stage.

Another aspect is their immediate relation to biochemical evolution. These catalysts, functioning so effectively in nature, are the result of a specific "selection", of whose mechanism we have very little knowledge. For this reason a comparison between enzymes and those compounds whose properties are similar to those of the biological catalysts merits especial attention. Such a comparison shows at once that although there is a very large number of "similar" substances, the types of structures inherent in enzymes are considerably fewer.

The purpose of the present review is not so much to describe different pathways of varying plausibility for the chemical evolution of biocatalysts, but rather to analyse the physicochemical properties of the relatively small number of the biocatalysts "selected" to be a basis for the catalytic mechanisms of life.

We shall attempt to characterise the possible catalytic reactions of the non-biogenic stage — processes which prepared organic compounds for a higher form of organisation and which represent a transition towards the biological evolution proper. We shall then consider the properties of the catalytic processes in biological specimens as we find them today. After briefly describing the most important aspects of protein biocatalysts, we shall deal with those innumerable compounds which can model the functions of proteins and enzymes, in particular the metal complexes.

A comparison of the properties of the two types of compounds can bring out certain important features concerning the selection factors.

We shall conclude by analysing the general tendencies in the development of catalytic systems united by different kinds of bonds, and their thermodynamic aspect.

Information about the actual pathways of the synthesis of biocatalysts is very sketchy, but even more so is our knowledge of their changes in the course of time.

Since life began to develop some 10^9 years ago[1], the structures that the biocatalysts acquired in the course of evolution can be taken to reflect the general principles governing the totality of processes in an open system — a system involving a "flux" of energy throughout the long period of its existence. Thermodynamics gives no general answer to the question as to how a system which is continuously subjected to the different energy influences of the outside world and which changes its mass would evolve. Biological specimens provide experimental material (obtained without the participitation of the experimenter) from which we can judge what exactly is happening in such a system. At this point the question may be asked: why are we so concerned only with enzymes? The answer is that all the processes which the cell uses to sustain its existence, or, from the chemist's viewpoint, to provide the required level of stability, are catalytic; in the words of I.P.Pavlov, the enzymes are the very "originators of life". The development of catalytic and autocatalytic functions is one of the most characteristic signs of biochemical evolution. If the equilibrium in isolated thermodynamic systems is described by extremal values of the characteristic functions, then its analogue for the constantly developing systems is the establishment of stable and reproducible structures with low entropy, which are capable of producing entropy by means of catalytic mechanisms. Thus, the quasi-equilibrium in an open system is characterised by the reproduction and development of a structure which is protected from chaotic decomposition by systems of co-ordinately functioning catalysts.

In his report at the symposium on the origin of life Calvin[1] pointed out that autocatalysis was one of the selection factors operating throughout the period of chemical evolution. As an example he cited the system consisting of bivalent copper ions and molecular hydrogen; this system is stable as long as it does not contain an ion of monovalent copper; if by any chance this ion appears, it catalyses the reduction of bivalent copper by hydrogen with practically complete conversion. This is undoubtedly autocatalysis — one of the most manifest agents of selection.

Nevertheless, autocatalytic processes are incomparably less frequent than catalytic ones, and primary process may be postulated to account for the preservation of certain types of molecules. Out of a multitude of reactions which take place in a mixture of compounds, a particular compound A is not affected or changed by precisely that process which it catalyses. This is true even if A catalyses several reactions. Hence, the molecules which are least changed in a complex of reactions are those catalysing the largest number of processes; they "outlive" the others, and ultimately form a catalytic system capable of warding off practically all the chemical attacks inherent in a given medium, *i.e.* a system compatible with the maximum number of reactions. This is exemplified by the development of the enzymes of the oxidase type, which are stable to the action of oxygen, and yet catalyse its use in the synthesis of the compounds which are necessary for the reproduction of the whole system.

These organisms are well adapted precisely to those media in which autocatalysis and "compatibility" initiated their development. Put in completely hostile media, they perish as a result of non-catalytic processes. Organisms can survive if they are capable of building new types of catalysts which can eliminate the destructive influences. Thus, most insects die under the effect of certain chlorine derivatives, *e.g.* DDT, but some resistant species are capable of producing an enzyme which destroys DDT by splitting off hydrogen chloride; in other words they owe their survival to a catalytic mechanism. This is the general principle of all protective processes which organisms use for keeping their structure unchanged, and for counteracting all noxious substances.

At the outset of chemical evolution, catalysis played only a subsidiary role. The main factors were radiation and electrical discharges, and the reactions probably propagated by means of radical diffusion. Calvin estimates that chemical evolution began about 2.5×10^9 years ago[1].

According to Rubey, the earth's first atmosphere consisted of carbon dioxide, nitrogen, carbon monoxide, and water vapour; Oparin thinks that in the early stages of development the earth had a reducing atmosphere, containing methane, ammonia, water, hydrogen sulphide, and hydrogen[2,3].

It is extremely difficult to assess what sort of reactions were possible in the earth's atmosphere and on its surface in the pre-biological period. Our only criterion is the thermodynamic possibility of a particular process; but even there we are very often limited by our ignorance of the temperatures and pressures which existed at the time. It is even more difficult to compare the number of the most probable non-catalytic reactions with the processes which probably took place in the presence of catalysts.

The non-catalytic processes which undoubtedly played an important role in the pre-biological period were the following: (1) the photochemical decomposition of water followed by the oxidation of ammonia to nitrogen, and of hydrocarbons to alcohols, aldehydes, and acids; (2) the photochemical decomposition of ammonia resulting in the formation of free radicals and hydrazine, and probably of a whole series of nitrogen-containing organic compounds; (3) the photochemical decomposition of methane and its conversion to other hydrocarbons, notably olefines; (4) the photochemical formation of formaldehyde from carbon dioxide and water; (5) the formation of methylamine from methane and ammonia (at 500°); (6) the condensation of acetylene with formaldehyde, and with other organic compounds, such as alcohols, esters, etc.; (7) the reaction of methane or ethylene with carbon monoxide in the presence of an electric discharge, forming aldehydes; (8) the reaction of nitrogen with acetylene in the presence of an electric discharge, to give hydrogen cyanide; (9) the reaction of hydrogen cyanide with acetylene, yielding pyridine; (10) the reaction of carbon monoxide with ammonia in the presence of an electric discharge, forming urea; (11) the formation of mercaptans from olefines and hydrogen sulphide; (12) the formation of phosphoric acids as a result of the chain oxidation of phosphine; (13) the formation of cyanamide; (14) the formation of phosphoguanidine from cyanamide and ammonium phosphite[3].

Miller carried out a series of experiments which attracted considerable interest. He subjected a mixture of hydrogen,

methane, ammonia, and water vapour to the action of an electric discharge generated by a high-frequency Tesla apparatus. In some experiments he also used a silent discharge. The analysis of the products obtained gave considerable quantities of glycine, alanine, sarcosine, aspartic, glutamic, and formic acids, together with a series of other organic compounds. The addition of iron ammonium alum to the system caused no essential change in the qualitative or quantitative composition of the reaction products[4].

In an attempt to verify Oparin's view that ultra-violet radiation from the sun was one of the fundamental sources of energy in the pre-biological period, Pavlovskaya and Pasynskii irradiated a mixed solution of formaldehyde, nitrates, and ammonium chloride for 20 h with the light of a PRK-2 lamp, and obtained a series of amino-acids[5].

In Urey's opinion, organic compounds were formed in the atmosphere as a result of the action of ultra-violet radiation and electric discharges[6]. Miller thinks that hydrogen atoms, formed by the photolysis of methane, ammonia, or water, reacted with carbon monoxide yielding formaldehyde or glyoxal; active nitrogen was able to react with methane and other hydrocarbons, yielding hydrocyanic acid. It seems that this process involved the participation of H atoms and NH_2 radicals. High-energy radiation probably played a subsidiary role. In 1951–1952 some organic compounds were synthesised from carbon dioxide and water by the action of fast α-particles from a 40 MeV cyclotron. Formic acid was produced in small amounts; formaldehyde was formed only in the presence of iron ions, which, in Miller's view, served as reducing agents; oxidative conditions, apparently, are not favourable for the synthesis of organic compounds[7].

We shall not consider the other more complicated processes whose non-catalytic character is doubtful. Instead, we shall deal with a number of reactions which proceed in the presence of catalysts. The hydration of ethylene and acetylene is catalysed by the oxides of aluminium, tungsten, mercury, and by other catalysts. The reaction of acetylene with hydrogen sulphide or water yields heterocycles. For instance, furan can be obtained in the presence of aluminium oxide. This reaction proceeds at 425°, but is thermodynamically possible up to 800°; the formation of thiophen is possible up to 250°. Aluminium oxides (bauxites) catalyse the condensation of acetylene with ammonia; at 300° the oxides of aluminium, chromium, and iron catalyse the formation of pyrrole, as well as pyridine, from acetylene and ammonia.

Bakhadur showed that the action of sunlight on solutions of paraformaldehyde containing potassium nitrate and iron chloride produces valine, lysine, serine, and other amino-acids[8]. It is possible therefore that iron ions served as catalysts in photochemical processes. The same author observed that colloidal molybdenum oxide could catalyse the formation of amino-acids by the action of electric light upon a mixture of water and paraformaldehyde. Air was the source of nitrogen in these experiments.

The present author's work with Barshchevskii[9] has demonstrated that zinc oxide contaminated with copper, lead, and other heavy metals, is an active photocatalyst in processes involving hydrogen transfer†. Oparin expressed the opinion that rocks and minerals[3] could act as catalysts in polymerisations of acetylene — one of the first products formed during the cooling of the earth (from water and carbides).

In 1955 Akabori put forward the hypothesis that the precursors of proteins could have been formed by a reaction between formaldehyde, ammonia, and hydrogen cyanide, yielding aminoacetonitrile, followed by polymerisation and hydrolysis of the latter compound on the surface of clays. In subsequent reactions side-chains would be introduced into the polyglycine molecule formed. Akabori[11] showed that heating of aminoacetonitrile with acid clay yielded a product with positive biuret reaction; on the other hand, treatment of polyglycine deposited on kaolinite by formaldehyde or acetaldehyde in the presence of a basic catalyst (pyridine) resulted in the formation of serine and threonine groups. The author thinks that the methylene groups in polyglycine adsorbed on kaolinite are more reactive in the condensation with aldehyde than they are if no carrier is used. In Bernal's opinion, life could originate on the surface of clays as a result of an accumulation there of organic compounds[12]. In a reducing atmosphere, silicates, sulphides, and oxides could catalyse polymerisations and a series of other reactions, connected with the conversion of hydrocarbons. Interesting model experiments in this field were carried out by Frost, who in his theory on the origin of petroleum ascribed a major role in the formation of this product to the catalytic action of clays[13].

It should be remembered that ions of copper, manganese, iron, and other metals are capable of catalysing hydrogen transfer from donors, which thereby undergo oxidation, to acceptors, which include, apart from oxygen, reversibly reducible compounds such as some dyes. Processes of this type did not develop until later, and led to the formation of metalloflavoprotein systems. The present author has recently established that hydrogen transfer from cysteine and ascorbic acid to various dyes is catalysed by copper ions[14]; it is likely that hydrogen transfer in a reducing atmosphere was also catalysed by metal ions.

A characteristic feature of biocatalysis is the formation of energy-rich phosphorus compounds and their subsequent utilisation in synthesis. As one of the forerunners of the highly developed mechanisms in the living organisms, Calvin considered the orthophosphate–pyrophosphate system containing iron[1]. He emphasised the fact that the hydrolysis of pyrophosphates was generally a very slow process, and that organisms gradually developed catalytic systems for accelerating it and for making effective use of the energy liberated. The participation of metal ions in oxidative phosphorylations has been proved. The conversion of ferrous orthophosphate to ferric pyrophosphate sets free electrons which can be transferred to oxygen; this process combines the oxidation of the ferrous ion (which is exergonic) with the conversion of orthophosphate to pyrophosphate (which is endergonic). In Calvin's opinion, the effectiveness of this process increases if iron forms a compound with an organic molecule which makes its action specific. Organisms containing these efficient complexes have a better chance of survival than those which do not contain iron in the catalytically active form.

To sum up this short outline of the function of catalysts in the early stages of chemical evolution, we conclude that the initial accumulation of simple compounds was achieved mainly through the effect of non-catalytic factors. Catalysis played only a limited part in some photochemical reactions, in the formation of hydrocarbons and heterocycles, in the

† According to the data of Shub, Tyurikov, and Veselovskii, the photoexcitation of iron oxide, which is a semiconductor, enables it to initiate the decomposition of hydrogen peroxide[10].

formation and polymerisation of hydroxynitriles, in the transformations of amino-acids, etc. But in subsequent periods the importance of its role in the evolution of organic compounds began to increase at a fantastic rate.

A large number of the new species had catalytic properties; metal ions in the oceans acted as catalysts for an ever-increasing number of different chemical processes; even relatively simple organic compounds possessed catalytic functions. Amines can catalyse the decomposition (decarboxylation) of keto-acids, and, as shown by Langenbeck[15], the activity of some of them can be very high; the present author has shown amines and amino-alcohols to be capable of catalysing the oxidation of polyphenols[16].

It is well known that amino-acids can be dehydrogenated in the presence of hydrogen acceptors (oxygen or various dyes) under the action of isatin and its derivatives[17]; acetamide accelerates the conversion of cyanogen to oxamide; cyanide ions catalyse the condensation of benzaldehyde to benzoin; many transformations involving the addition or the loss of a proton are catalysed by acids and bases. With the development of an oxidative atmosphere oxidations, catalysed by metal ions of variable valency, became increasingly important, etc.

A number of schemes, some soundly, some less soundly based, have been and are being suggested for the formation of compounds which played an important role in biological evolution. Thus, the formation of carbohydrates was undoubtedly influenced by the ability of aldehydes to polymerise and condense in the presence of catalysts — *e.g.* the hydroxides of calcium and barium oxides, or calcium carbonate. Euler and Euler[18] established that glycolic aldehyde and pentoses can be formed from formaldehyde in the presence of calcium carbonate. Hexoses were formed by the condensation of glyceric aldehyde, with the intermediate formation of dihydroxyacetone, in the presence of barium or calcium hydroxides. In all probability processes of this sort took place in the non-biogenic stage. According to Bernal[19], lipoids did not appear till much later; Oparin, however, thinks that they might have been formed before the appearence of life[3].

Amino-acids could have resulted from a number of various reactions. In addition to the work of Bakhadur mentioned above, Fox *et al.*[20] also showed that amino-acids can be formed from malic acid and urea in weakly alkaline media in the presence of calcium salts.

From the moment the "selection" mechanism came into action, the evolution of catalysts in nature began to follow two essentially different paths. Metals, oxides, sulphides, and aluminosilicates formed stable crystal lattices, in which, in the course of time, the stabilisation of new catalytic forms took place, partly through the elimination of defects, partly through the development of more regular structures, and partly through the lowering of the catalytic activity.

The difference between the catalytic activity of the new catalysts and that of the "old" inorganic ones is well known. Natural inorganic catalysts, consisting of oxides, salts, sulphides, etc. evolved into compounds with weakened catalytic activity. This could result in the partial exclusion of such catalysts from the processes which previously they accelerated. They were replaced by another type of catalysts, which we shall call the *biochemical* type.

Some 2 milliard years separate the beginning of the accumulation of organic compounds from the appearance of the first forms of life. This phase of the chemical history of the earth's surface is at once one about which we are most uncertain, and at the same time the most important for an understanding of the progress of biochemical evolution. In that time organic compounds were not accumulating at an accelerating rate — they were formed and decomposed, taking part in the self-reproduction processes characteristic of aperiodic structures.

It is remarkable that the period of the "chemical preparation" — a period of powerful and diverse transformations, about which we can only form cautious hypotheses — should come to a halt when biochemical evolution began. The development of organisms had on the whole little effect on the basic features of the structure of proteins and enzymes. When Prolone analysed the haemoglobin from the Crossopterygian fish, he found nothing unusual in its amino-acid composition, and indeed it proved to be close to the haemoglobin composition of the vertebrates. Synge thinks that chemical evolution occurred in the past and that the only organisms which survived were those which attained a "standard" chemical composition[21].

Actually, small variations in the amino-acid composition of proteins, which retain a basic structural plan, can apparently give rise to different species of organisms. In this paper we cannot discuss this interesting problem, since we are mainly concerned with the basic structural plan of the most widely distributed biocatalysts.

We conclude that during the period discussed above certain factors led to a selection of the systems which were subsequently to become the structural elements of an infinite variety of living creatures. The majority of the other systems which involved organic reactions had disappeared. At present, the number of organic reactions occurring in nature without the presence of biocatalysts is so small, that it was completely natural for the pioneers in organic chemistry to make an error in so terming this science.

II. PROPERTIES OF PROCESSES ACCELERATED BY BIOCATALYSTS

It can be assumed, as a result of careful analysis, that all the many different metabolic processes occurring in the presence of biocatalysts consist in fact of but a small number of reactions, each involving different substrates, but each with completely distinct general characteristics. The most general characteristic of biochemical processes is that they involve transfer reactions. The group of the so-called transferring enzymes can transfer phosphoric acid radicals, acetyl, methyl, and mercapto groups, as well as the large radicals such as the glycine one (which can be transferred from any peptide to an amino acid). In this way glycyltryptophan is obtained from glycylglycine and tryptophan, glycylphenylalanine from glycylglycine and phenylalanine, etc.

The diversity of the products of protein biosynthesis is undoubtedly connected with enzymes of that type. There are grounds for the assumption that the capacity to catalyse transfer reactions is a characteristic of the hydrolytic enzymes; for instance the transfer of amino-acid radicals is catalysed by papain and chymotrypsin, which are typical hydrolases. It is impossible to find analogues of these biocatalysts in the field of ordinary catalysis, but there is no doubt that transferring enzymes played a large part in the adaptation, selection, and perfection of the enzyme systems;

it was through their activity that different materials were formed for the synthesis of proteins and sugars, and, in the long run, of enzymes themselves.

The transferring enzymes played an important part in the biosynthesis of sugars, particularly in the interconversion of polyfructosides and sucrose. In artichoke tubers there is an enzyme which catalyses the transfer of fructose radicals from inulin to sucrose and free fructose[22].

Hydrogen transfer has an unusual place among the transfer processes. It seems that the mechanism of hydrogen transfer consists generally in the transfer of an electron along a chain of donors and acceptors, whereby the molecule, which finally becomes negatively charged, acquires a hydrogen ion from its surroundings: Kluyver[23] has suggested the name transhydrogenation for this reaction. There are data, however, which support the theory of direct transfer of hydrogen atoms[24]. The significance of hydrogen transfer is so great, that Kluyver[23] considers the transfer reactions to occupy a dominant position amongst the fundamental processes which "constitute biochemistry". These processes can be represented by the following equations:

$$AH \rightarrow A + BHB,$$

$$AHB \rightarrow ABH,$$

$$AHB \rightarrow A + BH,$$

$$AH + B \rightarrow ABH.$$

The role of proteins in the processes connected with electron transfer was partly explained in the work of Geissman and Blyumenfel'd[25] which showed that electrons can be transferred from one polypeptide chain to the other along hydrogen "bridges".

All the catalytic processes, both now and in the early stages of biochemical evolution, can clearly be divided into two classes. The first consists of all the transformations which utilise the differences in the chemical potentials existing in nature to synthesise compounds which contain energy-rich bonds. The second class consists of those processes in which this energy is used for the formation of highly organised self-reproducing entities, namely living beings.

Given a constant potential difference, the chosen route of the energy transfer is the one corresponding to the smallest changes in each intermediate stage. Thus, the difference in the energy levels tends to be broken down into small intermediate steps, each transition requiring a specialised catalyst. The large number of intermediate steps involved in the course of fermentation, for instance, or in the similar process of respiration, is clearly demonstrated by the Embden–Meyerhof scheme.

The most important way in which energy may be captured for the synthesis of proteins is by the oxidation of glucose. This involves a series of catalytic reactions such as the phosphorylation of glucose. Adenosine triphosphate (ATP), containing energy-rich bonds, is obtained in further transformations, and its energy is utilised for synthetic purposes. The coupling of the energy of a series of reactions is extremely complicated, and is carried out in a large number of steps. Our knowledge of some of these steps is very limited.

Pyruvic acid, obtained by the oxidation of glucose, is involved in a complicated cycle of tricarboxylic acids — the Krebs cycle. Hydrogen split off from pyruvic acid begins a long journey along a chain of catalysts, till it finally reacts with oxygen or some other final acceptor. The chain begins when the substrate transfers a hydrogen atom to nicotinamide-adenine dinucleotide or nicotinamide-adenine dinucleotide phosphate, which is a dehydrogenase coenzyme. The reduced form of the coenzyme then passes hydrogen on to the flavoprotein, and is thereby regenerated. The reduced flavoprotein reacts with cytochrome, by passing an electron to it and thus reducing the cytochrome iron to the divalent state. Hydrogen passes into solution as an ion. Cytochrome reduces cytochrome oxidase, by the transfer of an electron, and finally, cytochrome oxidase transfers the electron to an atom of oxygen, forming an oxygen ion, which reacts with hydrogen ions to give water.

In his study of the transport of hydrogen from the metabolite to oxygen, Lipmann[26] pointed out that three phosphate bonds were formed in the process, averaging 12 kcal per bond, which is equivalent to 0.25 V for a two-electron system. The difference between the potential of oxygen and that of the "average" hydrogen donor is about 1.2 V. Consequently, it can provide energy for four phosphate bonds. The transfer of hydrogen from the metabolite to oxygen is carried out by a series of catalytic mechanisms, involving in succession nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate, flavoproteins, and cytochromes. At potential differences of 0.1, 0.5, and 0.9 V (referred to the hydrogen electrode at pH 7) catalysts transform the 0.25 V energy units into energy-rich phosphate bonds. Lipmann stressed further that these energy-transforming systems, which are used for the transfer of electrons (taken from hydrogen) hardly depend at all on the nature of the hydrogen donor. He thinks that energy-rich phosphate bonds can be formed in the most diverse oxidative processes. According to the principles of biochemical evolution, the energy transformation mechanism selected should have been one consistent with the largest number of individual steps; the advantage of phosphorus compounds in this respect is obvious.

The basic biosynthesis of amino-acids, elucidated in the well-known work of Braunstein, is also closely connected with transfer reactions[27]. The carbon skeleton of amino--acids is built from phosphoglyceric and pyruvic acids and from other products of the sugar biosynthesis. The amino group enters the molecule either as a result of hydrogenative amination, or during an earlier stage of its genesis, remaining in it while it becomes more complicated. Braunstein points out that proline, hydroxyproline, ornithine, and arginine can be prepared from glutamic acid; cysteine, tryptophan, and alanine from serine, etc.

The biosynthesis of amino-acids is catalysed by the pyridoxal group of enzymes. According to Braunstein, only ketoglutaric acid itself can be subjected to direct reductive amination; all the other amino-acids are obtained by transamination, which is sometimes combined with a rearrangement of the carbon skeleton of the acid. Thus, glutamic acid is the first organic product of nitrogen assimilation.

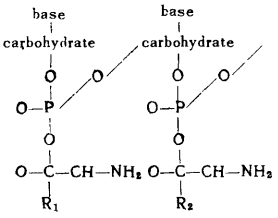
We shall now consider the most important group of enzyme-catalysed synthetic reactions.

A great deal of work has been devoted so far to the biosynthesis of proteins, which has recently become the central problem in biochemistry. Borsook[28] introduced the concept according to which the synthesis of proteins took place in three stages; first, activation of the amino-acid carboxyl groups; second, transfer of the amino-acids to the nucleic acid which serves as a "templet" for their arrangement in a predetermined sequence, and where they

combine *via* peptide bonds; and finally, removal of the completed protein molecules from the templet. After this the protein molecule curls up into the characteristic "spiral". Borsook thinks that the carboxyl group is activated during its interaction with adenosine triphosphate. The latter, by splitting off one phosphate group, is converted to the diphosphate, liberating the free energy required for the synthesis.

The mechanism of peptide formation consists, apparently, in the reaction of the enzyme with the amino-acid, *e.g.* with glutamic acid, and ATP, yielding a ternary complex in which glutamic acid is not phosphorylated; this complex subsequently reacts with ammonia, to give glutamine, ADP, phosphate, and the enzyme. Magnesium ions catalyse this synthesis. According to Borsook it is the activated amino--acids (through interaction with ATP), not the peptides, which are transferred to the templet. He bases his opinion on the work of Work and collaborators[29], where it was shown that the synthesis of casein is achieved directly from amino-acids.

The activated amino-acid is transferred to the templet by means of an activating enzyme or nucleotide, whose exact nature we do not know. The fixation of the amino--acid on the nucleic acid templet can be represented by the following scheme:



In the opinion of Haurowitz[30], the first step in the synthesis of proteins is the formation of a species-specific peptide model, which subsequently curls up into a three--dimensional globular molecule. The function of ribonucleic acid is to stretch this protein model, and to keep it in the stretched condition. Hammarsten[31] thinks that protein synthesis is of secondary importance compared with that of ribonucleic acid (RNA). According to Brachet[32], nucleoproteins or RNA provide a specific framework upon which enzyme systems are formed. The framework also serves for the formation of RNA itself, so that this is a case of autocatalysis as regards RNA.

The templet hypothesis seems to be soundly based, and it will certainly play an important role in the study of biochemical evolution. Indeed we must try to visualise a whole series of transformations leading from the very imperfect mechanisms of the amino-acid interactions, either on clays or by irradiation in a homogeneous medium, to the highly specialised reproduction of protein molecules on templets consisting of phosphorylated ribonucleic acid. Such mechanisms, though in much simpler form, probably existed and were used to form the precursors of proteins through the precursors of RNA; they changed continuously as long as the factors causing the change existed.

The synthesis of proteins proceeds extremely slowly — at a rate a hundred, and even a thousand, times slower than that of the usual enzymic reactions, for it involves the formation of the catalysts required for the reproduction of the protein molecule in finest detail.

Thus the characteristic features of the processes accelerated and controlled by biocatalysts are the following: (1) the coupling of the catabolic reactions (decomposition of food) with the synthetic processes (this is accomplished by means of compounds with high-energy bonds); (2) the breaking up of the general process into separate stages with small potential differences between each stage; (3) the change of the structure by means of the transfer electrons, hydrogen atoms and larger entities; (4) the reproduction, by means of the templet mechanism of the protein and enzyme molecules, *i.e.* those structures which can secure all the required stages, and, finally, (5) coupling of reactions in which the differences in the chemical potentials of the reactants are employed, irrespective of their nature.

Data regarding the metabolism of bacteria (see Stephenson[33]) suggest that whenever there is a potential difference between various compounds, an organism can be found which is capable of using this difference for its development and reproduction. In this respect the unusual universality of the biocatalyst systems is not only evidence of the formative action of the selection factors, but also an indication of the development of low-entropy structures capable of increasing the entropy of the surroundings. We shall come back to this point later. It is fully understood that the two groups of catalytic reactions must be coupled and must have a control mechanism.

III. CHARACTERISTIC STRUCTURES OF THE MOST IMPORTANT BIOCATALYSTS AND POSSIBLE WAYS OF THEIR FORMATION

To understand the nature of the biochemical evolution of catalysts, we have to look at the present stage of their development, and examine the results of the operation of selection factors over 2 thousand million years. The fear that we might have to deal with a host of types of catalysis adopted by life on earth, is readily proved to be groundless. The remarkable and very significant unity of the biochemical structure of living organisms is well established.

The prominent biochemist Florkin[34], who studied the problems of biochemical evolution, analysed the dry residues of different organisms; some of his data are collected in Table 1. The resemblance does not by any means end at the quantitative composition. Synge, in his report to the Symposium in Moscow in 1957, emphasised that the proteins, which are present in considerable quantities in all the living organisms studied, are – significantly — a compact chemical group. We encounter precisely the same amino-acids in the proteins of fungi, higher plants, algae, viruses, heterotrophic bacteria, and apparently, autotrophes. Precisely the same enzyme systems are invariably found in the most diverse organisms — and at different stages of development[35].

The means for carrying out different operations -- oxidation by oxygen, hydrogen transfers, energy storage, etc. — are present in the same form in bacteria, plants, viruses, and vertebrates. The resemblance of the enzyme mechanisms in different forms of life is so great that the individual differences appear at first sight to be unimportant.

The formation of proteins, or of the hypothetical protein precursors, occupies a very special place in the biochemical evolution of catalysts. In Oparin's words, "proteins

TABLE 1.

Organism	Proteins, %	Fats, %	Carbohydrates, %
Sea urchin larva	63.7	19.5	5.4
Oyster	51.2	11.1	28.2
Silkworm egg	56.0	19.2	7.0
Silkworm larva	55.5	13.3	1.8

TABLE 2.

Enzyme	Groups essential for catalytic activity	Enzyme	Group essential for catalytic activity
Pancreatic amylase	amino	Papain	phenolic, mercapto
		Pepsin	phenolic
β-Amylase in barley	phenolic, disulphide	Phosphatase	amino
		Ribonuclease	"
Chymotrypsin	ditto	Trypsin	indolyl, amido
Lysozyme	amino, carboxyl, guanidine, amido, hydroxyl, disulphide		

have vast chemical potentialities"[3]. In fact proteins combine within themselves the properties which make them an indispensable component of all the most important biocatalytic systems.

The structure of proteins has been widely discussed in the literature and is not considered in the present review. Attention is drawn only to those properties of proteins which are of immediate importance to catalysis, and from that standpoint some physicochemical properties which are common to proteins in general are commented on.

Proteins are themselves able to catalyse a multitude of reactions. It has been established that the macromolecule as a whole is not required for the manifestation of the catalytic function, but only some particular groups in it, which may be regarded as analogous to the "active centres" in heterogeneous catalysis[36]. Porter generalised the results of a number of workers on the nature of these groups which are indispensable for the manifestation of the biological activity of proteins. His data are given in Table 2.

Sanger[37] showed that insulins of different origin, although differing in their structure, can nonetheless possess identical physiological properties; this fact suggests that a definite structure is required in only a small part of the molecule. Sumner[38] showed in the case of urease that even denaturation, which is accompanied by deep-seated structural changes and by an irreversible fall in solubility, does not involve the complete loss of activity. The present author studied the very pronounced catalytic effect of proteins (casein and albumin) on the oxidation of indigo-carmine with hydrogen peroxide, and found that denaturation of the proteins did not completely annihilate their catalytic functions[39].

The first proteins could have been formed, by the mechanism described by Akabori and Bernal, from simple nitrogen compounds on the surface of the clay particles or on rocks with similar properties. Their high molecular weight, capacity for hydration, and high viscosity facilitated their concentration in a limited space and the formation of phase boundaries.

Hydrolytic processes undoubtedly caused partial destruction of the polypeptide chains, but the amino-acids formed by hydrolysis could be used again under different conditions as material for synthesis; this led to a greater diversity of products. It can be supposed that various proteins were the first types of biocatalysts, in which the active group consisted of relatively simple combinations of atoms in the peptide chain.

Protoproteins probably did not possess very diverse catalytic functions, but the most active ones underwent selection in the process of evolution. The most important property, which subsequently made the proteins carriers of enzymic functions is "internal coupling". This means that their molecules contain aggregations of atoms along which energy can be transferred over long distances (see section IV).

The semiconducting properties of proteins determined the development of the protein molecule as a whole. If the chemical reaction proceeding at one point of a molecule has no effect whatever upon its other parts, then this molecule can be regarded as consisting essentially of two autonomous parts, and the properties of one (*e.g.* mass) need not affect the functions of the other. But the situation is different with proteins, whose huge mass seems to be indispensable for the performance of biological functions.

If the protein is treated as a "unified" system, we can explain both its fundamental role in the cell, and the fact that in the course of evolution it did not undergo disaggregation, but, on the contrary, became more complex.

The other property of proteins which makes them the basis of an enormous number of biocatalysts is their capacity to increase sharply the catalytic activity and the selectivity of metal ions, metal complexes, and certain organic compounds with catalytic properties.

Regardless of the presence in proteins of definite catalytic functions, the characteristic feature of the majority of biocatalysts is their "dual" structure. In addition to protein, the enzyme also contains a non-protein part, frequently called the coenzyme or the prosthetic group, which is more or less closely united with the protein. In some cases the stability of the bond between the two parts is so small that the enzyme is virtually completely dissociated into the protein "carrier" and the prosthetic group. In the presence of the enzyme, the substrate is subjected to the action of protein and the prosthetic group alike. All three unite to form an intermediate complex.

Prosthetic groups were the next structural elements to be formed during a long period which elapsed from the appearance of first organic compounds to the origin of life.

There is no doubt that metal ions played and are still playing a prominent role in the formation and functioning of even those enzymes whose prosthetic groups are believed not to contain a metal. New discoveries are made every

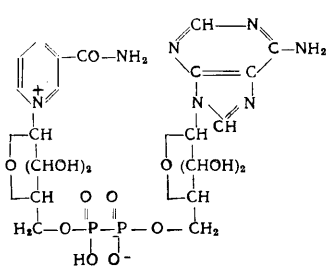
year concerning the dependence of the activity of enzymes on the presence in them of small quantities of this or that metal. Nitrate reductase contains molybdenum; carbonic anhydrase – zinc; flavoproteins require copper for their functioning. Pyridoxal enzymes seem to need iron and copper, etc.[40]

Metal ions manifest their catalytic activity in many instances. At one extreme is the case where it is uncertain whether the metal is combined with the enzyme or with its substrate; at the other, the case where the metal is firmly united with the protoporphyrin nucleus, and both are fixed on to the protein. For this reason, the role of the metals in the genesis of complex prosthetic groups can hardly be underestimated. Even in the early stages of biological evolution, metal ions acted as centres of attraction for groups of atoms in organic molecules which were capable of complex formation. This resulted in the mutual approach of different molecules, followed by a change in their electronic state, various redox processes involving the central ion, and finally, the synthesis of new types of highly active biocatalysts in which the metal ion remained in a stable complex. We shall discuss later the various types of metal-ion functions. For the moment we shall consider the structural properties of the most important prosthetic groups.

In the early stages of the development of life the atmosphere was reducing and the biocatalysts controlling hydrogen transfer undoubtedly played an important part. The anaerobic dehydrogenases are an ancient type of biocatalyst.

The oxidative atmosphere, formed about 800 million years ago, brought in new possibilities for the synthesis of catalysts. The difference between the chemical potentials of oxygen and oxidisable compounds, on the one hand, and the products of oxidation on the other, was utilised by different mechanisms of aerobic dehydrogenation and electron transfer; the most efficient of these mechanisms involved the protein-porphyrin complexes.

The metalloporphyrin complexes containing iron, vanadium, or copper, were included in the transfer processes, forming the prosthetic groups of oxidases. They were present in catalases, which decompose hydrogen peroxide, in haemoglobin and haemocyanin, which bind oxygen reversibly, in polyphenol oxidases, in cytochromes, and probably also in a series of other biocatalysts. Whereas the mechanism of catalase action is still controversial, the function of cytochromes (*a*, *b*, and *c*) has been established beyond doubt, namely to transfer electrons along a chain of hydrogen-transfer reactions. These complexes have also played a decisive part in the development of photosynthesis.

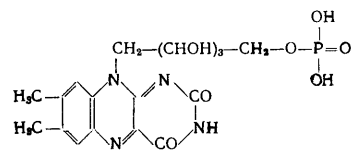


The prosthetic group of anaerobic dehydrogenases is the complicated compound nicotinamide-adenine dinucleotide (Coenzyme I). These dehydrogenases transfer hydrogen to acceptors other than oxygen. They are characterised by their great activity and the ease with which they dissociate into the prosthetic group and the protein carrier. The nature of the protein is the determining factor in the specificity of dehydrogenases. The structure of the prosthetic groups is shown above.

Nicotinamide-adenine dinucleotide and nicotinamide--adenine dinucleotide phosphate (Coenzyme II) is the prosthetic group of a series of dehydrogenases, differing from NAD in having an additional phosphate radical.

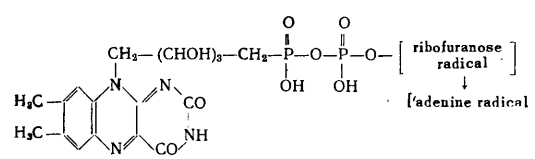
In aerobic respiration the hydrogen removed from the substrate by means of dehydrogenases is transferred to flavin enzymes, the cytochrome system, or "the respiratory pigments". The substrates of these dehydrogenases are lactic, malic, succinic, and glutamic acids, as well as glucose, glucose monophosphate, etc.

The prosthetic group of the flavin enzymes – aerobic dehydrogenases – consists of riboflavin:



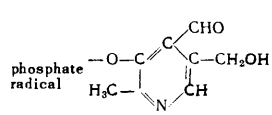
The flavin enzymes can transfer hydrogen directly to oxygen. The reversible oxidation and reduction of the enzyme is accompanied by a change in colour; the oxidised form is yellow, and the hydrogenated one is colourless. The well-known yellow respiratory enzyme belongs to this group; one of its substrates is hexose monophosphate, which plays an extremely important role in metabolism.

The oxidation of amino-acids is catalysed by a dehydrogenase whose prosthetic group is flavin adenine dinucleotide:



Different dehydrogenases, adapted to particular substrates involved in metabolism, are remarkably uniform in the structure of their prosthetic group, in spite of their own relatively complicated structure. The same applies to porphyrin catalysts which control oxidations and electron transfers. There is a growing belief that "adaptation" to specific substrates is invariably carried out by the protein.

The prosthetic group of transaminases, which catalyse the transfer of the amino group, is pyridoxal phosphate:



Amino-acids form a complex with this compound, which subsequently decomposes into a keto-acid and a product differing from pyridoxal in having a CH_2NH_2 group instead of CHO. This phosphopyridoxamine form of transaminases reacts with the keto-acid to give a complex which decomposes into the regenerated initial enzyme–pyridoxal transaminase- and the amino-acid, with the amino group in a different position.

Ideas about the possible chemical pathways for the formation of one or other biocatalyst have a bearing on the evolutionary theory of the origin of life. Equally interesting is the question why precisely these, and not other catalysts, came to be used so universally by different organisms to solve their metabolic problems.

It seems that the first catalytic mechanisms were concerned with hydrogen transfer and hydrolytic processes. Metalloporphyrins, so important for oxidations involving oxygen, were included in biocatalysis later, and it is quite possible that they were added to proteins by virtue of their being the most effective complex catalysts. In fact, whereas the structural elements of dehydrogenases have a definite resemblance to desoxyribonucleic acid, and the prosthetic groups of protein catalysts are often simply parts of the amino-acid molecules constituting the given protein, metalloporphyrins, as well as chlorophyll, are characterised by great specificity of their structure and are altogether different from polypeptides. The same seems to apply to complex specific biocatalysts of the cobalamin type, which appeared later, at a period when living forms became more complicated.

The evolution from the iron ion to the iron-porphyrin complex took place, according to Calvin, during the period lasting about two thousand million years, which he called chemical evolution. In this author's opinion the synthesis of porphyrin proceeded as follows: the action of radiation on water and carbon dioxide produced formic and oxalic acids; C_2 compounds yielded C_4 compounds, in particular succinic acid, on irradiation; amino-acids were formed by the action of ultra-violet light on nitrate solutions containing formaldehyde and iron salts (this has now been established experimentally); glycine obtained in the previous stage reacted with succinic acid to give porphyrin rings [1].

The formation of haemoproteins marked the beginning of biological evolution. Granik [41] studied the biosynthesis of chlorophyll and came to the conclusion that the similarity of chlorophyll to haem was a result of the fact that they followed identical synthetic pathways up to the protoporphyrin stage; they subsequently divided into the "iron" and "magnesium" branches. He believes that the chain of biosynthetic reactions was a repetition of the different stages in the evolutionary development of the pigment systems.

According to Krasnovskii porphyrins were probably the earliest photosensitisers. Porphyrins were present in the oldest autotrophes; they existed in a reducing atmosphere and contained the reduced form of porphyrins. Owing to the peculiarities of their spectra, the free porphyrins cannot fully utilise the visible part of sunlight [42]. Further evolution led to the formation of chlorophyll; the introduction of magnesium into the porphyrin molecule increased the activity of the latter and led to the combination of the complex with the histidine of the protein carrier. Krasnovskii thinks that the complexes of proteins with carotenoids are more recent photoreceptors than porphyrins.

The syntheses of products such as porphyrins, purines, polypeptides, and nucleic acids proceed along very complicated paths. Buchanan and Wilson [43] showed that the biosynthesis of purines and pyrimidines proceeds *via* a series of intermediates such as oxaloacetic, ureidosuccinic, and orotic acids; formic acid, CO_2, and glycine can be incorporated into the purine part of inosinic acid.

It does not, of course, necessarily follow that the synthesis of those precursors of organic bases which are required for the formation of the simplest analogues of nucleic acids took precisely that course. But the relative simplicity of these compounds, and the possibility of the formation of similar products by the action of radiation on simple inorganic compounds, suggest that the first forms of RNA, DNA, and the associated protoproteins might have originated in this way on the earth's surface.

According to Shemin the starting materials for the synthesis of purines were glycine and succinic acid. Ammonia, glycine, and the formyl radical appear to be necessary for the formation of purine and pyrimidine compounds [44]. However, as Oparin correctly pointed out, these complicated processes, which consist of a series of consecutive transformations, take place only if the separate stages are arranged in the proper sequence with regard to time and space [3].

This is even truer for the synthesis of nucleic acids, which, according to Roka, proceeds through the following stages [45]. Polyphosphoric acid, whose formation presumably took place in the early stages of the development of life, reacts with glyceric aldehyde to yield polyphosphoglyceric aldehyde. This complex molecule reacts in succession with acetaldehyde, ammonia, oxaloacetic acid, glycine, and the formyl radical, and forms desoxyribonucleic acid. The acccumulation in one place of all these products depends on a particular combination of specific conditions.

The formation of polypeptides from amino-acids requires free energy (from 400 to 3000 cal mole^{-1}). It is supposed that the reaction proceeds using adenosine triphosphate as a source of energy. Akabori, on the other hand, proved experimentally that the polypeptides could be formed through the polycondensation of aminonitriles, catalysed by clay particles.

Thus, any hypothesis about the origin of the compounds which are characteristic of biological systems must allow for some sort of organisation, whether it be a mechanism of the coupling of exergonic and endergonic processes, an adsorbent possessing catalytic properties, or, as in Oparin's opinion, a coacervate drop.

Taking into account all these considerations, the following approximate stages in the evolution of biocatalysts can be postulated.

(1) The formation of amino-acids on the surface of clay or in homogeneous media under the influence of radiation or by catalytic reactions at interfaces.

(2) The formation of polypeptide chains, occurring in conjunction with reactions involving rupture of high-energy bonds — possibly simple compounds of the iron phosphate type. Alternatively, this stage might have given rise to a special mechanism whereby amino-acids underwent activation and combined into a protoprotein, the formation of a templet for the reproduction of definite amino-acid combinations having followed gradually.

The question remains open whether the templet was pure protein, or a phosphorylated combination of bases with carbohydrates, *i.e.* a prototype of RNA. But either way, the synthesis of first templets from polyphosphates and, probably, aldehydes, could lead to their successful functioning only within the framework of a specific organisation. This first organisation consisted simply in the formation of colloidal particles and the development of boundary surfaces.

There is no doubt that structures of the coacervate types played a most important role in the functioning of these

first, inefficient catalysts. It is probable that, owing to selection, only those mechanisms were preserved which were capable of best utilising the existing differences in chemical potentials and of reproducing themselves. Dehydrogenases and the transferring enzymes of the pyridoxal type could possibly have been the earliest biocatalysts.

(3) The selection of catalysts which were activated by proteins was an important stage in the development of enzymes. The proteins with a wide range of catalytic functions were in a privileged position. Thus, proteins evolved containing, in Baldwin's words, "built-in" prosthetic groups, closely bound to the protein molecule. At the same time proteins combined with low-molecular compounds which had catalytic properties, and increased these properties through this combination.

The study of a number of models of prosthetic groups shows that groups which would be activated on any selected protein are rare. The strength of the attachment to protein is not a decisive factor. In many cases the combination protein (prosthetic group) — substrate arises in the presence of metal ions, which play the role of a "coupling link" in a catalytic reaction.

(4) The development of the oxygen atmosphere introduced both a new source of energy and a number of new types of enzymes, with a very different structure from that of dehydrogenases. At the same time photosynthesis began to develop. The same porphyrin complexes served for the formation of oxidases and of chlorophyll.

For reasons which we shall discuss later, porphyrins were the metal complexes selected from a large number of others. This was the end of the development of a series of catalysts which have since been used for the transfer of hydrogen from the substance undergoing oxidation to atmospheric oxygen.

(5) With increasing complexity of the structure of catalysts and their carriers each new reaction, joining the number of those required to reproduce a given structure, either destroyed that structure, or became subjected to its controlling influence. The latter case is observed with the surviving structures. Along with the development of the controlling mechanisms the structure also developed and the "feedback" mechanism, which has now achieved a high degree of perfection, was established.

IV. METAL IONS AND BIOCATALYSIS. MODEL STRUCTURES

The role of metal ions, especially those of transition metals, was very important in the evolution of biocatalysts. There is now evidence that metal ions can catalyse hydrogenation by molecular hydrogen, decarboxylation, decomposition of bicarbonates, and hydrolytic reactions. Catalytic properties are not confined to the transition-metal ions, for the ions of potassium and sodium, like those of hydrogen, can accelerate condensations in homogeneous media[46]. Metal ions act as activators for a number of enzymes, *e.g.* amino-peptidases (manganese and magnesium ions). They can form very active complexes which are a part of prosthetic groups of some enzymes.

The characteristic features of the functioning of metal ions and the probable stages in the development of metal-containing biocatalytic systems can be understood either by the study of the function of metals in specimens available at present, or by the assessment of results obtained from biocatalyst models. It appears that all the modes of the functioning of metal ions in biological systems through the different stages of development have retained some of their importance to the present day. By studying the effects of the catalytic properties of ions, we might therefore be able to interpret correctly their role in the evolution of biocatalysts.

We begin our systematic discussion of these properties with cases in which the ion merely initiates a chain process. The most widely distributed, and the least specific effect is the initiation of chain processes by ions of variable valency (iron, copper, cobalt, etc.). The problem of the distribution in biological systems of catalytic processes involving chain reactions, *i.e.* those involving free radicals, is very complicated. The action of a number of catalysts, of catalase in particular, is based on the formation of intermediate products, and not on the development of chains. However, according to Mikhlin[47], the question of a chain mechanism is still open in the case of lipoxidases.

The generation of free radicals has undoubtedly played an important role in the early stages of the development of biocatalysts (see above, section I). The term "orientation" is most appropriate for describing a very weak ion-substrate interaction when the presence of a definite compound cannot be proved and the catalytic effect of the ion is not very specific. It is true that a detailed study of the "orientation" effect would probably reveal unstable intermediate compounds, but the acceleration, for instance, of the oxidation of indigo-carmine with hydrogen peroxide at the benzene–water interface, in the presence of manganese ions in a weakly acid medium (observed by the present author), is difficult to account for in terms of definite intermediate compounds.

In plant cells calcium can be totally or partially replaced by strontium, molybdenum by tungsten or vanadium, potassium by rubidium, etc. This fact is undoubtedly connected with the activating action of metal ions on different enzymes. The non-specific effect of ions on enzyme activity, described in Calvin and Martel's monograph[46], may well be based on such phenomena.

A closer ion — substrate interaction has a different effect. This is the case with innumerable redox processes in which the ion changes its valency and is used as an electron carrier. The oxidations of ascorbic acid, cysteine, glutathione, etc., in the presence of copper ions are some examples of a large number of reactions catalysed by metals[46]. This category probably also includes the interesting phenomenon of the catalytic action of copper ions on hydrogen transfers. Dehydrogenases, till recently believed not to contain a metal, do in fact require the presence of transition metal-ions for activation.

Mahler points out that flavoproteins, which can be oxidised by cytochromes and which contain metals have been recently discovered. He thinks that the function of the metal in catalysis by flavoproteins is to increase the stability of the intermediate semiquinone; on the other hand, the metal serves to unit the flavin molecule with two acceptor molecules into one complex, and to make possible an intramolecular electron transfer. The presence of the metal ion, which acts as a "coupling" between the donor and the acceptor, imparts to the reacting system the necessary internal "flexibility". Flavoproteins contain copper, iron, or molybdenum (or other metals), depending on the nature of the enzyme[48].

The present author undertook the study of the transfer of hydrogen from ascorbic acid to methylene blue. This reaction proceeds very quickly in the presence of light; it proceeds also in the dark, but much more slowly. In order to find out the possible catalytic role in this reaction of small amounts of metal salts, solution of methylene blue was treated with a small quantity of a substance capable of forming stable, sparingly soluble complexes with the ions of heavy metals. Ethylenediamine tetraacetic acid is especially suitable; it is used as an additive to oxidising agents [oxidisable substances? (Ed. of Translation)] for the elimination of traces of metals which could catalyse oxidations. Another such compound is cupferron, which also forms stable complexes with no catalytic property. The experiments showed that the addition of EDTA or cupferron to methylene blue sharply inhibits the discoloration of the dye by ascorbic acid; the effect is observed in the presence of light and in the dark alike. It follows therefore that the transfer of hydrogen from ascorbic acid to methylene blue is catalysed by metal traces present in the dye. An attempt was made to accelerate the reaction of methylene blue with ascorbic acid by adding to the solution salts of different metals, such as aluminium, sodium, magnesium, copper, iron, cobalt, nickel, titanium, and chromium. A noticeable acceleration of the reaction was observed only in the presence of copper. Thus, copper catalyses hydrogen transfer not only in metalloflavoproteins, but also in far simpler systems, and is responsible for effects usually ascribed to the direct reaction between oxidising and reducing agents [49].

Metals with only one valency can function as catalysts by strongly polarising the molecule with which they are united. In this connection we should mention the action of aluminium ions, investigated by Kornberg and his collaborators [50]. The reaction is accomplished in the zone surrounding the ion, whose radius depends on the nature of the reactants.

Goudot thinks that in such cases electron transfers also occur. She studied different enzyme–metal–substrate systems [51,52] and discovered the following properties, depending on the nature of the ion and the ligand. The redox potential of the cation which polarises the molecule of the substrate must be sufficiently high for the substrate to be able to undergo oxidation; the number of donating groups should correspond to the number of valency electrons which the metal ion is capable of accepting. This explains the failure of the attempts to decarboxylate oxaloacetic acid by means of the free magnesium ion. This ion can form octahedral complexes with three molecules of the acid, but cannot accept more than two electrons, which rules out a catalytic reaction between the divalent metal ion and three molecules of the substrate: for the electron transfer to take place the charges of the reacting groups in complex-forming agents should not all be equal. For instance, the aluminium ion does not catalyse the decarboxylation of acetoacetic acid, as all the six reactive groups in three molecules of the chelating agent have essentially equal negative charges.

The next type of active compound containing a metal ion are complexes in which an increased activity of the ion is obtained by its combination with certain ligands, which are not substrates in the reaction.

The study of the catalytic activity of relatively simple complexes opens up interesting prospects. The number of stable compounds which contain a metal and are attached to enzymes as prosthetic groups, is in fact extremely limited, and it is especially interesting that the very same molecular structure, namely the protoporphyrin ring, serves as a base for a number of completely different biocatalysts. At the same time the number of catalytic metal complexes is extremely large.

It is hardly likely that all other complexes proved to be unsatisfactory simply because they were not sufficiently active. We shall show later that some of them have a very high activity, approaching that of the enzymes. It is clear, therefore, that the catalysts selected in the course of evolution possessed a special combination of properties, all of which were required for the functioning of the chemical system of the cell.

The copper complexes, studied for a number of years in the author's laboratory, are especially suitable for modelling the prosthetic groups of enzymes — particularly those of catalase. The activity of such "copper models" is so great that it is almost a million times higher than that of the simple copper ion. The author studied the catalase activity of copper complexes with amino-acids, amides, amines, diamines, di- and tri-peptides, biuret, triuret, heterocyclic compounds, some proteins, etc. [53,54,55]. The study of these models revealed a series of general principles. First, for the appearance of very high activity four nitrogen atoms are required, co-ordinately bound to the copper ion. The introduction of oxygen atoms into the co-ordination sphere causes a sharp decrease of activity. Thus, chelation, whereby two polar groups of the ligand are co-ordinated to the metal, results, as a rule, in an increase of activity. The nature of the ligand is very important. The complexes with ammonia, biuret, trimethylene diamine, and propylene diamine are especially active.

The active complexes are easily decomposed by concentrated peroxide solutions. To study their activity 0.05–0.1 M solutions of peroxide should be used and a slight excess of free amine maintained in solution. In the presence of a large amine excess, the activity drops owing to the formation of complexes with co-ordination number 6. In such complexes there is no "reaction zone" round the ion where a molecule of peroxide or an HO_2^- anion could enter. The usual free-radical acceptors, *e.g.* methyl methacrylate, have no effect on a reaction catalysed by complex ions; this is the essential difference between the catalytic action of complexes and that of simple ions, which function according to a radical-chain mechanism. The present author believes that in the former case the catalytic reaction proceeds *via* a number of intermediates.

Goudot studied the question why some of the ligands used by the author gave especially active copper complexes. Using the method of molecular orbitals she calculated the charges carried by the atoms in ligands. Her ideas about the reaction mechanism generally agree with the author's — the reaction occurs within an intermediate complex, which consists of the copper complex and two molecules of peroxide (or one molecule and an HO_2^- ion). The metal ion is an intermediary in the electron transfer. Goudot thinks that when the diamagnetic copper ion forms complexes by sp^2 hybridisation (like iron in catalase), it has little chance of attracting electrons to its only unfilled orbital. The ligand–metal complex must be formed by dsp^2 hybridisation, leaving one of the p orbitals free, so that the metal ion may react with the first molecule of the substrate. For the reaction with the second molecule to take place, the ligand has to delocalise an electron pair from the metal ion.

Ligands should be "donors" and contain a considerable number of π electrons (this is the case with biuret, which

forms a very active complex). When the electrons are delocalised in the ligand, the ionisation potential of the atoms which react with the ion should increase[56]. The overlapping of wave functions probably occurs also in cases where the complex is activated further as a result of adsorption. The other important feature of the functioning of catalase copper models is the constancy of the activation energy of the reaction. Despite the enormous acceleration of the decomposition of peroxide by complexes, the activation energy remains practically unchanged[57]. Consequently, the acceleration proceeds at the expense of the entropy factor. Porphyrin complexes, and haemin in particular, have another significant feature, in being capable of undergoing further activation. Langenbeck[15] showed that heamin activity can be increased by combining it with imidazole and pyridine. Whereas haemin itself is not a fully specific catalyst (it catalyses the decomposition of hydrogen peroxide as well as oxidations by hydrogen peroxide), the compound of haemin with sodium imidazolesulphonate accelerates only the latter reaction, and 12 times faster than does haemin. Thus, the addition of new ligands both increases the activity of, and renders more specific, catalysts of the metal-complex type. This, according to Langenbeck, accounts for the extraordinary effect of the protein carrier on the properties of the prosthetic group.

Formation of complexes is not the only means of activation in the synthesis of biocatalysts. Activation and increased selectivity are achieved, as a rule, by fixation of the prosthetic group (*e.g.* metalloporphyrin complex) on the protein carrier. In his brilliant experiments Langenbeck showed that the actual structure of the prosthetic group of an enzyme can be determined by studying models. Attempts are being made to use the same method for studying the role of the carrier. Lautsch and his collaborators[58] showed that by fixing different porphyrin compounds on polymers, it is possible not only to increase their activity but also to obtain changes in their absorption spectra similar to those observed in the fixation of porphyrins on proteins.

Iron complexes (haemin) are greatly activated when adsorbed on charcoal. It is notable that the order in which different charcoals increase the activity of haemin is the same as for the iron ion[59]. Thus, adsorption activation, characteristic of ions, is retained in complexes. The above properties of the iron porphyrin complexes have together placed them in an exceptional position relative to other complexes. Copper ions are not activated on charcoal and the selection of activating carriers for this ion is very difficult. Finally, all the attempts to activate the "copper models" of catalase by adsorption gave precisely the opposite result — the loss of catalytic activity. Consequently, the instability of the active copper complexes towards peroxides, their failure to be activated on adsorption, the need to keep an excess of the ligand in solution during the catalysis, all render the copper compounds unsuitable for the formation of enzymes of the catalase type.

Let us now consider the models of oxidases. The present author has studied the oxidation of ascorbic acid and pyrogallol by copper compounds. Of all the complexes studied, only copper–histidine and copper–insulin complexes were more active than copper ions themselves. Even greater activity was obtained by the adsorption of the copper–histidine complex on insulin[60].

Karpusova and the author[61] made a detailed study of the oxidation of pyrogallol by oxygen. Copper complexes were found to be very active catalysts; complexes with monoethanolamine, ethylene diamine, and propylene diamine were the most active of all. The activation energy in this reaction depends a good deal on the nature of the ligand, although here too the entropy factor plays a decisive role. The ligands themselves have a catalytic activity in this reaction; but the activity is increased on combination with the copper ion. Especially interesting is the fact that in this reaction copper ammine complexes can be activated by fixation on proteins.

Table 3 summarises the different types of the catalytic action of metal ions which could have been, or are at present, important in biological systems.

What properties are required of a compound for it to be used in the formation of a biological catalyst? With due caution we can lay down the following physicochemical properties as criteria of the biochemical value of a catalyst:

1. Activity.
2. Capacity for activation, namely the ability to change greatly their activity by slight changes in structure.
3. Capacity for adsorption activation — which is only the extreme case of the property cited under 2. Adsorption activation is the change in activity caused by weak interactions involving the catalyst molecule.
4. Ability to lower the activation energy of the reaction catalysed, particularly when adsorbed on a specific carrier.
5. Ability to preserve the interaction between its separate structural elements — "internal coupling" — after increase in structural complexity.

The catalytic complexes should have a planar structure co-ordination number 4, and, in redox processes, a chelate structure, where the ligand is attached to the central metal ion through nitrogen atoms. Only those which were capable of activation by adsorption, and which lowered the activation energy of the reaction they catalysed, were selected in the course of biochemical evolution. The requirement

TABLE 3.

Type of ionic effect	Example
Initiation of the chain reaction	oxidation of benzaldehyde in the presence of cobalt ions
A weak orientation effect (the "coupling" effect)	acceleration by manganese ions of the oxidation of indigo-carmine by hydrogen peroxide in benzene emulsion. The possible activation of leucineamino-peptidase by manganese and magnesium ions
Redox processes	oxidation catalysis in the presence of copper, iron, etc., ions
Formation of unstable complexes between the ion and the substrate	hydrogen transfer catalysed by copper ions
Polarisation effect	decarboxylation of keto-acids in the presence of aluminium ions
Catalysis by metal–ligand complexes	decomposition of hydrogen peroxide by copper ammine complexes. Catalysis by porphyrin complexes
Catalysis by means of complexes fixed on carriers	oxidation of pyrogallol by the copper--ethanolamine complex on albumin carrier
Catalysis by complexes fixed on specific proteins	the action of enzymes of the catalase type

that the complex should be capable of adsorption activation and that it should function not at the expense of the entropy factor but by lowering the activation energy, is probably equivalent to the requirement that this complex should be energy-coupled with the activating carrier. It seems that this coupling can be established through a conduction band, or, to use the author's early terminology[62], by means of "conducting channels".

Szent-Györgyi[63] stressed the importance of internal coupling in biological structures. In his opinion, the available experimental material leaves no doubt as to the existence of energy migrations within the protein molecule. He does not attempt to explain the mechanism of this energy transfer. In the preface to Szent-Györgyi's book, Terenin refers to the plausibility of energy transfers along the triplet and singlet levels of the protein. Szent-Györgyi thinks that "the protein molecule constitutes a single integrated system, and that the energy imparted to it belongs to the system as a whole" (ref. 63, p. 26).

In this connection we may observe that the inclusion in biologically active compounds of chains along which interaction of atomic groups is relatively difficult, brings in new and more stringent selection factors. This is particularly true of chains such as $-CH_2-CH_2-CH_2-$. Shorygin and Alaune[64], in their work on the optical properties of molecules in relation to the interaction of atomic groups, concluded that CH_2 bridges "uncoupled" phenyl, amino, and C=C groups. The presence of such insulating chains in ATP, for instance, is the cause of the coiling up of the molecule, the purpose of which is to allow the purine and the phosphate ends to come into very close contact and so make energy transfer from the phosphate to the purine end possible. This remarkable property is exhibited in the presence of the magnesium ion, which seems to act as a bridge for the transfer of electrons. Therefore, metal ions serve to overcome the difficulties which stand in the way of the internal energy integration of the parts of the biological system which are separated by insulating chains. Of especial interest in this connection are the cases in biological systems where it is possible to destroy large regions of a complicated macromolecule without essentially changing its catalytic function. Thus, a considerable part of the urease protein can be destroyed almost without detriment to its enzymic activity. The papain molecule consists of 180 amino-acid radicals. In this report to the VIIIth Mendeleev Congress, Engelhardt[65] underlined the importance of the fact that in spite of the elimination of 120 amino-acid residues from this enzyme, 99% of its initial enzymic activity is preserved.

These phenomena are difficult to explain, but one of the possible interpretations lies in the assumption of weak energy-coupling between the active group and the remote parts. It is possible that in the course of evolution the biologically active compounds retained certain "rudimentary" parts, which once had a role but were later separated from evolutionary areas by a specific energy-insulating barrier.

V. SUCCESSIVE STAGES AND CHARACTERISTICS OF MOLECULAR ORGANISATION

When Bernal asked him which of the two was formed first, proteins or nucleic acids[3], Oparin considered the question meaningless. It seems that the two components of this reproductive combination developed side by side, although unfortunately we are unable to determine their original form, or follow with confidence the course of their evolution. The initial synthesis is of amino-acids, for example, occurring in homogeneous media under the influence of radiation, did not require a complicated specialised organisation. On the other hand, polypeptides and protoproteins did require it.

Bernal himself divided the process of the origination of life into seven stages: the first stage was characterised by the absence of all interactions, when micromolecular processes developed throughout the hydrosphere. In the second stage there was a fixation of "subvital" units on mineral aggregates; in the third stage coacervates appeared; the fourth stage was marked by the formation of nucleoprotein systems, capable of some sort of metabolism; the fifth and sixth stages are connected with the appearance of lipoprotein membranes and the origin of the cell, containing mitochondria; in the seventh stage the nucleus appeared[66]. This does not include the following processes; the selection of coacervate systems, which contained compounds capable of increasing the catalytic activity by adsorption; the development of new bonds by means of electron transfers; the organisation of the spatial structure of the component parts of the conjugated system, which was to secure the utilisation of high-energy bonds and the corresponding transfer of atomic groups; the appearance of patterns and of the apparatus for self-reproduction.

It is extremely difficult to establish how the increasingly more complicated forms of organisation arose, and why they arose in one particular way and not in any other. At present we still do not know the general principles underlying our hazy ideas about biochemical evolution.

It is obvious, however, that every reaction causes certain changes in the environment, which are not recorded in its equation: changes in temperature, diffusion, radiation, etc.; the appearance of phase boundaries; detonation effects; etc. In a given reaction these effects either facilitate the progress of a particular process, or hinder it. The former type of process has a better chance of survival since it sets up the "organisation" necessary for it. When the organisation itself has a catalytic effect on that reaction, we are dealing with self-reproduction and with what is known in cybernetics as the "feedback" effect.

This is the term used to describe the effect of a given process on the environment, which causes that environment to change the course of the process. Common examples of feedback action in mechanical apparatus are centrifugal regulators; in chemical systems it can be exemplified by the burning of a fuel generating a stream of air, which intensifies combustion.

Intensification or weakening by feedback action are called positive and negative feedback, respectively. A characteristic feature of feedback action in physicochemical systems, and, consequently, in biological objects, is the extreme diversity of the forms it can take. Indeed, a process in a given environment can affect its own development in many ways. The formation of catalysts for self-reproduction is also a particular case of feedback action in biochemical systems. Obviously, such a system must be very stable, a necessary condition for its stability being the presence of feedback in the broadest sense.

Consequently, the forms of organisation selected in the course of evolution were those which ensured the most efficient feedback and control. It seems that the simplest organisations were various phase boundaries where adsorption occurred, bringing specific regularities into the relative

disposition and concentration of molecules. Adsorption finally led to catalytic effects. As already mentioned, even adsorption at the water–hydrocarbon interface was the cause of the acceleration of some reactions. Aluminosilicates had far greater potentialities in this respect. Of all the polymerisation products formed on the surface of these adsorbents, the only compounds which underwent further development were those which, being catalysts themselves, ensured a certain amount of feedback.

These forms were probably based on the functioning of conduction bands in protoproteins. The compounds formed started by being catalysts only, and were preserved because their catalytic ability acted as a protection against the chemical influences which could destroy the existing structure.

In 1941 Lipmann[67] showed that the energy liberated in the transfer of hydrogen in cells was not lost or transformed into heat because of coupled phosphorylation, which yielded energy-rich bonds, *i.e.* increased the chemical activity of certain compounds. Here biocatalyst systems obviate the increase in entropy and the disorganisation of the structure, and help to maintain the organisation. The first stage of evolution was probably concluded by the selection of catalysts.

Coacervate drops, so important according to Oparin, were apparently the most suitable and promising form of the preservation of the selection and development of catalysts in the stage of incipient organisation. Oparin's opinion is corroborated by examples of the striking properties of coacervates, particularly their capacity for selective adsorption[3]. Thus, some coacervate drops were able to absorb selectively the active compounds such as porphyrins from the surrounding solution. Porphyrins reacted with iron increasing its catalytic activity. Oparin pointed out further that if this increase enhanced the biological stability of the drops, they enjoyed considerable advantage in the course of selection over other analogous systems.

Since effective catalysis consists of a series of steps, involving small changes in the chemical potential and low activation energy, it follows that some mechanism for the transfer of electrons and, probably, hydrogen atoms along donor-acceptor chains must have existed. Consequently, the catalysts must have developed not only a suitable apparatus, but also the proper spatial arrangement. Hence the idea of the specific "geometry" of biocatalysis.

Twenty years ago Talmud suggested the general features of a model for "metabolism". He considered that the protein molecule, in the form of a globule, must have a series of openings whose dimensions correspond approximately to those of the benzene ring. Through such openings simple amino-acids could enter the interior of the globule and, having combined there into larger protein fragments, were unable to escape. This theory also implies that the interior of the globule favoured condensations whereas the surrounding medium contained an enzyme able to decompose polypeptides. Talmud's experiments showed that proteins could entrain glycine ethyl ester from the solution; at a slightly higher temperature the ester was transformed into diketopiperazine and the portion of the compound retained by protein remained in solution[68].

The studies of Cramer and his collaborators[69] on interstitial compounds (where the "guest" molecule is occluded in the cavity, or crystal, of another which is a "host") showed that occlusion can lead to considerable changes in the molecular state of the "guest", namely shifts in redox potentials, colour changes, catalytic effects, etc.; it can therefore be concluded that processes occurring inside a macromolecule have played a part in the evolution of biocatalysts, and that they should be considered as a complex example of phase-boundary effects.

Nevertheless, this mechanism alone is insufficient to explain the development of protein structures; the forms of organisation which allow for the coupling of energy must also be taken into account. These forms have to secure the utilisation of high-energy bonds, or, as in the synthesis of protein-like substances, act as the original templet of the RNA type, able to orient amino-acids or to lengthen the appropriate templet. Embryonic forms of such organisation may have existed in complex coacervates.

Roka's idea of a "primary templet" based on polyphosphates, was not confirmed experimentally. Energy coupling under suitable spatial conditions could mean the transition to the synthetic stage in the functioning of first catalysts. Naturally, those synthetic products which owing to their composition or form were able to destroy the system of catalysts (in a coacervate or in a complex of the clathrate compound type), did just that. Therefore the extremely slow-acting selection was completed only in cases where the synthetic system reinforced and reproduced itself.

Vorob'ev and Shapot[70] carried out interesting model experiments involving the reaction of globulins with macromolecular DNA and RNA. They obtained synthetic complexes, very similar in properties to protoplasmatic structures, which consisted of nucleoproteins and were accordingly destroyed by desoxyribonuclease activated by magnesium ions. The product obtained by the combination of globulins with DNA had an elasticity which is typical of protoplasmatic structures. The authors attribute great significance to such formations and believe that compounds of this type constitute the physicochemical basis of the protoplasmatic structure. In fact, the synthesis of complicated proteins requires not only RNA, but also a very highly developed organisation. It is now believed that the formation of energy-rich compounds is carried out in mitochondria, which have a specific fine structure and contain a series of enzymes, located in specific positions relative to one another. The synthesis of proteins takes place in other cytoplasmatic particles — the microsomes.

The results of Khesin and of Gale and Folkes throw some light on the "level" of organisation required for the synthesis of proteins and enzymes. Khesin[71] isolated the secretory granules from the pancreatic cells, and showed that they were the sites of the synthesis of amylase. Gale and Folkes[72] disintegrated bacterial cells by the action of ultrasonic waves; after careful elimination of intact cells they added to the suspension a mixture of the essential amino-acids, nucleic acids, and hexose diphosphate and adenosine triphosphate as a source of energy. Protein synthesis and increased enzymic activity were observed in the suspension. Of especial interest is the fact that, in experiments on the synthesis of enzymes, RNA can be substituted by a mixture of purine and pyrimidine bases, which are its components.

Oparin finds it difficult to accept that in the early stages of biochemical evolution a compound as complicated as ribonucleic acid could be formed at one stroke. It is however highly probable that less complex compounds, capable of self-reproduction, could have been formed in mixtures

consisting of amino-acids and organic bases, coupled with an energy source.

VI. GENERAL PRINCIPLES

To grasp the full significance of the development of the complex organic structures which preceded the formation of the first living organisms, one has to bear in mind that this development took place in open systems. Oparin emphasised the fact that the continued subsistence of protoplasm and the stability of its form are based on continuous motion, and not on invariability or rest[3]. Protoplasm is not, therefore, a static but a dynamic system.

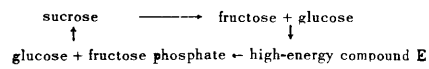
The thermodynamics of open systems has received considerable attention lately. Prigogine, an eminent worker in this field, divided bounded systems into open, closed, and isolated[73]. The first group comprises those systems which exchange both their mass and energy with their environment; in closed systems there is only energy exchange, while isolated systems exchange neither. Processes taking place in open systems differ essentially from those with which we usually deal in ordinary thermodynamics. Such systems, when containing a catalyst, are capable of changing the position of the equilibrium. Pasynskii points out that in an open system processes can take place in which the entropy falls at the expense of the entropy increase in the surroundings with which they are thermodynamically coupled[74]. This significant fact is illustrated clearly by the properties of any living organism, whose total activity consists in growth involving a local decrease of entropy, and in processes involving an increase in entropy, *i.e.* the transformation of compounds with high overall potential into products with a lower potential.

Pringl[75] remarked that all living beings are characterised by coupled chemical reactions in an open system, resulting in a fall of entropy at the expense of entropy increase in the environment. He regards this fact as of fundamental importance. He compares this localised drop in the entropy of living organisms with processes involving "autocatalytic" synthesis. In the opinion of Oparin, this last term only means the dynamic continuity of the evolution of all life. According to Pringl, the first open systems did not have definite boundaries, but represented localised increases in the concentrations of the reacting compounds in the first oceans. The Japanese scientist Sugita thinks that the fluctuations of molecular clusters, which eventually resulted in the formation of coacervates, were also a result of the development of irreversible processes in open systems[76].

Although, according to Frank[77], the capacity of all living matter for self-reproduction must be inherent in complex organic molecules, satisfactory models for the study of these phenomena are not available. A question of major significance is whether the development of self-reproducing systems is a *sine qua non* under specific conditions. Classical thermodynamics does not require this stipulation, but at the same time it is not contradicted by biological facts.

The point is that thermodynamics does not explain the different methods whereby a system achieves equilibrium. and, after all, so far as biochemical evolution is concerned, we cannot speak of equilibrium in the same sense as we do in the case of an isolated system. However, when we consider particular cases in biological specimens, we tend to look for features which are typical of spontaneous transformations, and assess the feasibility of any process by the sign of the corresponding thermodynamic function. We thus find that the concept involving consideration of an individual process is very inexact. In addition to spontaneous transformations accompanied by an increase in entropy, and in complete agreement with thermodynamic laws, numerous "active reactions"[78] occur; for example, when a compound is transported against the concentration gradient, when constant concentration ratios of the products of synthesis and decomposition, which are absolutely incompatible with equilibrium, arise, etc. Careful analysis of such cases always reveals a coupling mechanism whereby the energy required for its functioning is drawn from the energy-rich bonds, but the bonds themselves are obtained, for instance as in the oxidation of glucose, by means which obey the laws of thermodynamics.

Afanas'eva[79] analysed the causes of concentration ratios which do not obey thermodynamic laws, and suggested the following cycle to incorporate different pathways for the synthesis and for the decomposition. This difference is achieved at the expense of high-energy bonds, which are formed in other cycles:



At constant concentration of compound E, the ratio of sucrose concentration to the sum of the concentrations of its decomposition products is kept constant, and only at E = 0 is sucrose fully decomposed into glucose and fructose.

"Active reactions" are dealt with fully in the literature[77], and in all cases a coupling mechanism is postulated, which does not violate the laws of thermodynamics, but which is not envisaged by them. Are we right in concluding that the appearance of coupling mechanisms is another general principle which should be added to the other postulates of thermodynamics in order to make it fully effective as regards biological systems?

By following the evolution of biocatalysts, we get the impression that a relatively small number of structures which are typical of the living specimens of all known species is used for building up the immense multitude of mechanisms which are required for the development of the vast number of higher forms of life. When one considers, for instance, that the composition of the biocatalysts of the Crossopterygian fish is not very different from that of man, the difference between the two species must be ascribed to the different degree of complexity of their respective coupling mechanisms, which, in man with his capacity for creation, have produced a system with extremely low entropy. Consequently, evolution concentrated on the development and refinement of these mechanisms. From the viewpoint of cybernetics, such mechanisms are, simply, systems with feedback action.

There seems to be no reason for rejecting the view that the development of such coupled catalytic mechanisms represents a general principle which should naturally belong to thermodynamics, although it would mean overcoming the difficulties associated with the search for a strict formulation. This principle is of a statistical character inasmuch as of all the possible ways of equalising the existing potential differences, the ones finally selected are those which are associated with the development of self-reproducing structures. This means that in only one experiment the

appearance of such a structure is extremely improbable. Even if an immense number of experiments is carried out simultaneously under different conditions, the probability still remains very small. But in experiments repeated over a long time, only that path of equalisation of potential differences which results in the formation of self-reproducing structures is the most likely one, as the chance of all the others being repeated several times is small. This path, which has led to the formation of the cell, involved the smallest activation barriers and was so organised that the exergonic reactions had no opportunity to disrupt the structure or to increase its entropy; "protection against entropy" was effected by means of high-energy bonds, transfer of groups, and apparently, by the transfer of excitation energy to considerable distances along "channels" conduction.

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HYDROPEROXIDES OF ALKYLARENES AND THEIR DERIVATIVES

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INTRODUCTION

The main course of petrochemical synthesis is the oxidation of hydrocarbons[1,2]. Most petrochemical products can be obtained by direct oxidation of petroleum hydrocarbons, of products of the petroleum-processing industry, or of by-product and natural gases. At the present time higher paraffin hydrocarbons are transformed industrially into aliphatic alcohols and acids by catalytic liquid-phase oxidation[3]. During recent years investigators have shown exceptional interest in the liquid-phase autoxidation of hydrocarbons to hydroperoxides by means of atmospheric oxygen. The interest in autoxidation is explained by the fact that similar processes occur widely in nature and are employed commercially for the synthesis of products of great economic importance.

Many hydroperoxides are quite stable compounds, and have been isolated in a pure form and characterised. In the USSR important investigations on the liquid-phase oxidation of hydrocarbons to hydroperoxides have been carried out by Ivanov[4-9], Sergeev[10-15], Yurzhenko[16-21], Erofeev *et al.*[22,23] and others[24-26]. These investigations have provided methods for preparing concentrated hydroperoxides, analysing them qualitatively and quantitatively in solution, isolating them in a pure form, and converting them into products of economic value.

Organic peroxides and hydroperoxides have formed the subject of several monographs[4,27,28] and reviews[29-31], and have been included in special sections of textbooks of organic chemistry[32]. In the periodical literature many original papers are published annually on the autoxidation of hydrocarbons. For 1958 alone the Chemistry Section of the *Referativnyi Zhurnal* contained more than 60 abstracts of papers and patents. All this indicates the immense importance which is now attached to the autoxidation of hydrocarbons.

The present review deals only with the liquid-phase oxidation of aromatic hydrocarbons and their derivatives by molecular oxygen, *i.e.* the so-called autoxidation process. Such oxidation results in the formation of hydroperoxides. Depending on the catalyst, temperature, time, and rate of introduction of oxygen or air, these may be either the final or the intermediate products of oxidation.

LIQUID-PHASE AUTOXIDATION OF ALKYLARENES

The autoxidation of alkylarenes to hydroperoxides or so-called "Dioxes" (organic peroxy-compounds)[33] is becoming more and more an independent field in a state of broad and intensive development. Hydroperoxides of alkylbenzenes have already acquired great industrial importance both in their own right and as intermediates, *e.g.*