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The Modelling of Biological Catalysts

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A historical survey of studies on the modelling of biological catalysts is presented. Pioneering investigations, investigations which have led to new concepts, hypotheses, or principles, or those which have led to results of practical importance are examined. Attention has been concentrated on the modelling of oxidoreductases.
The bibliography includes 124 references.

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Biological catalysts (enzymes) are specific protein catalysts present in all living cells. The study of non-protein catalysts, which accelerate the same reactions as enzymes, has been established as a field of research into the modelling of biological catalysts.

Any model reflects a particular characteristic feature of the original. If a high catalytic activity is regarded as the characteristic feature of enzymes, then colloidal solutions of metals must be regarded as the first models. Investigation of these systems dates back to the beginning of this century; the first object modelled among enzymes was catalase, which catalyses the decomposition of hydrogen peroxide under the conditions of the living cell.

I. THE FIRST MODELS OF METALLOENZYMES

Bredig and his co-workers investigated colloidal solutions of platinum and other highly dispersed noble metals and showed that these simple "models" do indeed resemble enzymes.[1,2] The study of the kinetics of catalytic processes in the presence of highly dispersed metals led to the observation of definite similarities between the kinetic characteristics of the models and the originals. Like enzymes, Bredig's models acted at very low concentrations and the dependence of the rate on the hydrogen peroxide concentration in the model systems was extremely similar to that characteristic of enzymes. This was observed not only for colloidal metals but also for metal oxide sols. The situation was the same for the influence of the pH on the catalytic activity; it was found to be similar in the model and modelled systems. An increase of the pH in both cases led to the attainment of a maximum in the rate of decomposition of the peroxide. The maintenance of solutions at elevated temperatures resulted in a decrease of the activities of both catalase and the model systems.

Comparison of the activities of specific substances (catalyst poisons) on enzymes and models is especially convincing. It was found that the models, i.e. metal sols, are inactivated by the same substances as catalase, namely by mercury compounds, cyanides, hydrogen sulphide, iodine, etc. After poisoning the platinum sol with hydrogen cyanide, the activity of the sol can be restored by the prolonged passage of air through the solution; catalase behaves in exactly the same way. Such high similarity in the dependence of various factors was to some extent due to the fact that the modelled enzyme contained a metal (iron) as part of the composition of the active group.

Metal salts have attracted the attention of many investigators,[3,4] but on the whole comparative study of the properties of sols and enzymes in fairly primitive systems has not been able to accelerate significantly progress in the modelling of biocatalysis.

It has been established[5,6] that platinum metals accelerate the decomposition of formic acid and also decompose formaldehyde (into CO and H_2). These may be seen as functions resembling the activity of oxidoreductases. The transfer of hydrogen from formaldehyde to Methylene Blue under the influence of the "Schardinger" enzyme from raw milk is modelled, according to Bredig and Sommer,[7] by the reduction of the dye with formic acid in the presence of platinum, iridium, gold, and palladium sols. For these reactions, it has been possible to demonstrate kinetic analogies with enzymes and to establish that enzyme poisons such as hydrocyanic acid and mercury salts have a poisoning effect.

In terms of activity, the sols are greatly inferior to enzymes. Thus, according to Hennichs' data,[8] a preparation of catalase from the liver is almost a thousand times more active than colloidal platinum. Admittedly, it is fairly difficult to carry out a comparison since neither their number nor the character of the distribution of the active centres on the sol particles nor the legitimacy of applying homogeneous kinetics to the microheterogeneous systems investigated are known.

There was a tendency to explain the catalysis by dispersed metals of the hydrogen peroxide decomposition reactions by the formation of intermediate peroxide compounds. Mercury peroxide compounds have been detected in the catalytic decomposition of H_2O_2 by mercury.[9]

Later studies of metal salts were concerned with the properties of tungsten,[10] colloidal silver,[11] and iron(III) hydroxide.[12]

Platinum metal salts are very unstable and their employment for preparative purposes would require an increase in their stability. This has been achieved by introducing into the colloidal metal solution the so-called protective colloids,

forming on the surface of the sol particles an adsorption layer which prevents their coagulation.[13,14] The products of the alkaline hydrolysis of egg albumin, namely the sodium salts of lysalbinic and protalbinic acids, were used as the protective colloid in 1904.[13] These acids are precipitated in an acid medium and they can be used only in neutral or weakly alkaline solutions. Using gum arabic as the protective colloid, active salts suitable for the hydrogenation of a large number of compounds over a wide pH range were obtained in 1912.[14]

Despite serious doubts about the usefulness of attempts to understand the mechanism of enzyme action by studying metal salts, careful investigation of this historical stage in the development of the science of catalysis has shown that it was by no means fruitless. If not the metals themselves then metal ions do indeed have a direct bearing on enzymes.

Progress in biological research, which had led to the discovery of the chemical nature of the active groups in metal-containing enzymes, has attracted attention to the catalytic properties of a surprising group of compounds containing a porphyrin nucleus and different metal ions coordinated to the system of pyrrole rings of the nucleus.

In 1926 the first step was taken in the study of the activities of groups characteristic of enzymes. Kuhn and Brann[15] investigated the catalytic activity of haemin—a complex of iron and a protoporphyrin ring. Compounds of this type constitute the active groups in many enzymes catalysing oxidation processes and the problem of the properties of haemin was therefore important from the standpoint of modelling. In enzymes, haemin is linked to the protein and measurement of the activity of isolated haemins threw some light on the role of the protein moieties of the molecules of biological catalysts (see Nikolaev's review[16]).

These experiments demonstrated particularly clearly that the active group, separated from its carrier, has a much lower activity and an extremely weak selectivity. The activity of haemin in the hydrogen peroxide decomposition reaction was increased by its adsorption on charcoal.[17] The adsorption activates haemin in relation to specific but by no means all the reactions in which it behaves in general as a catalyst.

Iron ions are also activated by adsorption on charcoal.[17] Such systems can be regarded as the simplest and very rough models of enzymes in which the active group and the carrier are already clearly outlined. At the same time it was shown that compounds which do not contain any metal and have a comparatively simple structure are capable of catalysing reactions characteristic of enzymes. Thus Shilov, who developed the concept of the cyclic transition complex in organic reactions,[18] studied the catalytic functions of amines and aminoacids;[19] Swain and Brown[20] showed that the catalytic effect can be determined by the simultaneous action on the substrate of two different functional groups contained in the catalyst molecule. This effect ("bifunctional catalysis") was subsequently shown to play an important role in the mechanism of enzyme action.

In the subsequent research into the modelling of enzymes, two trends were established: (1) the study of models of the active groups of enzymes in order to elucidate the characteristics of the structure and mechanism of their action as part of the composition of the enzyme; (2) the synthesis of highly active catalysts based on the existing information about the structure and specific mechanism of enzyme action.

The first of these trends outgrew in the course of its development the initial aims of the investigations and led to the formulation of a new branch of chemistry—metal complex catalysis. In our country, studies on these lines were initiated by one of the authors of this review.[21]

II. METAL COMPLEXES AS MODELS OF ACTIVE GROUPS

Nikolaev[16,22,23] carried out a series of investigations of models of the active groups of metal-containing enzymes and chose copper as the model metal. In contrast to the ions of iron, whose hydroxide $Fe(OH)_3$ has a very low solubility product, copper ions form many complexes which are stable in an aqueous medium in the presence of an excess of the ligand in solution and permit a wider variation of the nature of the ligands. Other metal ions were later also studied as catalysts of various reactions (decomposition of hydrogen peroxide, oxidation of polyphenols, oxidation of ascorbic acid, etc.) and the first relations between the catalytic activity and the nature of the ligand were formulated.

The results obtained are summarised in a monograph[16] and reviews.[22,23] The main idea, which stimulated the study of the catalytic properties of complexes, consisted in the hypothesis that the properties of the active centre in heterogeneous catalysts can be interpreted in terms of data concerning the influence of the environment of the metal ion on its activity and the complex is to some extent an analogue of the active centre "extracted" from the solid and transferred to the solution. This way of "creating bridges" between heterogeneous and homogeneous catalysis has since become promising, but progress along this pathway requires more effort than seemed to be the case at the beginning. Nevertheless, even the first steps have already shown that the variation of the activity and selectivity of catalysts of the type of metal complexes is entirely feasible and leads to extensive and interesting trends in the theory and practice of catalysis. The study of the influence of the nature of ligands, the nature of the metal ion, and the geometrical characteristics of metal complexes threw light on many aspects of the mechanism of enzyme action and clarified the problems which must be solved in theoretical enzymology.

In the first place it was found that the condition which must be fulfilled for the complex to exhibit an appreciable catalytic activity at all is the limitation imposed on the coordination number, namely the latter must not be greater than four. Complexes of the "closed" type with a coordination number of six or more are as a rule inactive.

The presence in the coordination sphere of amine nitrogen is also important for metal complex catalysts of redox processes and in particular of the decomposition of hydrogen peroxide—the introduction of oxygen into this sphere lowers the activity. American investigators[24] have shown that these conditions are to a large extent valid also in the catalysis of the hydrolytic reactions of organic substances by transition metal complexes.

The investigation of the catalytic properties of the iron complex of triethylenetetramine, which has come to be called "inorganic catalase", belongs to this series of investigations.[25,26] As in the early studies by Bredig, these investigations emphasise the similarity in the nature of the catalytic poisons of catalase and the complex investigated and also the similar isotope effects. Among all the metal complexes investigated hitherto, this system has shown the highest catalase activity (subject to the same stipulations concerning the difficulty of the correct comparison of the catalytic activities of systems in terms of their turnover number determined at different pH). It was later shown[27] that the complexes of iron ions with triethylenetetramine model an important feature of the catalase process. The mechanism of the catalysis of the decomposition of hydrogen peroxide by this complex involves a sequence of two two-electron transfers, i.e. the catalysis has a non-radical mechanism as in the case of catalase.

The properties of metal sols containing on their surfaces compounds of different composition, have been described from the standpoint of catalysis and colloid chemistry.[28] Micellar catalysis, which can be regarded as one of the aspects of the modelling of biological catalysts, should be treated as a further development of this trend.

A review of metal complexes in which the ligand is a molecule or ion of the substrate can be found in a monograph.[29]

III. MODELS OF METAL-FREE ACTIVE GROUPS OF ENZYMES

Investigations into model structures without metal ions developed in parallel with the studies devoted to the metal complex models of the active groups of enzymes. Researches into the latter led to the establishment of a new field of science—that of metal complex catalysis. Studies on the former compounds laid specific foundations for another branch of science—catalysis by organic compounds.

The possibility of creating simple organic models of enzymes was foreseen in an early stage by Ostwald[1] (p.161). The first attempts to demonstrate the similarity of the stereochemical specificities of enzymes and simple organic models were undertaken in 1908. It was shown that nicotine resolves the optical isomers of camphorcarboxylic acid at different rates[1] (p.161).

In 1927 Langenbeck investigated isatin as a model of the enzyme dehydrase and established the mechanism of its action, which has features in common with the mechanism of the enzyme action.[30] Three years later Langenbeck, Hutschenreuter, and Jütteman proposed the principle of systematic activation[1] (p.96). A number of rules were formulated whereby one could be guided whilst modifying in succession a simple organic compound, chosen as the model of the enzyme, in order to increase its catalytic activity.

A striking demonstration of the effectiveness of the principle of systematic activation is provided by the study of methylamine and its derivatives as catalysts of the decarboxylation of phenylglyoxylic acid. By successive modification of methylamine to form 3-amino-1-methyloxindole, it has been possible to increase its decarboxylase activity a thousandfold. Using this procedure, investigators approached the elucidation of the structure of the active group in decarboxylase. The results of studies by Braunstein and Shemykin[31] and by Metzler and co-workers[32-34] constituted a major advance in the employment of the modelling method in order to establish the mechanism of enzyme action. These studies were concerned with the investigation of the models of transaminases and other pyridoxal transamination, racemisation, etc. enzymes. Pyridoxal or its phosphate, i.e. a relatively simple organic compound without its protein component, served as the model system.

It is necessary to consider studies which led to the elucidation of the activating role of the macromolecular component linked to the model of the active group of the enzyme. The activation of haemin and iron ions by their adsorption on charcoal has already been mentioned above. Model systems of this kind without metal ions were described in 1932 and were called faser-catalysts.[2] Faser-catalysts are derivatives of cellulose in which the massive molecule of the cellulose carrier is linked to diethylamine, modelling the small active group of decarboxylase. The model reaction used in this study was that involving the elimination of carbon dioxide from bromocamphoric acids. Similar studies have also been carried out on the decarboxylase activities of various amines linked to lecithin present in solution in the form of an emulsion.[1]

The intrinsic contribution of the modelling method to enzymology at the beginning of the 1960s can be summarised as follows. It was shown that (1) a high activity and definite specificity of action can be reproduced also with the aid of materials of non-biological origin; (2) the activity of enzymes is associated with a small group of atoms which is extremely sensitive to any structural rearrangement (as a rule, these groups contain nitrogen forming part of a heterocycle or an aminoacid group, i.e. amino-nitrogen); (3) the coordination of atoms in the active group of the complex must leave a certain "reaction space"[16] which the substrate molecule can enter; (4) the "carrier" (in enzymes this is a protein) alters both the activity and the specificity of the action of the catalyst.

The question of the role of the macromolecular component in enzymes (the protein) remains, however, unanswered. What physicochemical factor or factors cause such influence by the protein component of the enzyme molecule on the reactivity of the active group? An answer to this question is of fundamental importance for the problem of creating synthetic highly active and selective catalysts. When the protein nature of the macromolecular component of the enzyme is in fact essential for the operation of the kinetic properties of enzymes, the prospects for creating enzyme-like catalysts of practical importance are extremely remote.

The report that the protein component of the enzyme papaine can be shortened almost by 90% by treatment with proteolytic enzymes without an appreciable loss of catalytic properties attracted much attention. Although this result did not throw light on the role of the protein in the enzyme, it undoubtedly "inspired" investigators concerned with the modelling of enzymes. Subsequent studies showed, however, that the specific activity characteristic of papaine is retained following a decrease of the number of aminoacid residues only from 187 (in papaine) to 75–80 (in the active enzyme).[35]

Tests of the results of studies on the fragmentation of papaine showed subsequently that this result was also erroneous.[36] In many of the other enzymes investigated it was likewise impossible to observe the retention of activity following a significant shortening of the protein chain. The question of the possible role of the protein component in enzymes as a factor determining their catalytic properties is discussed below.

IV. METAL COMPLEXES AS MODELS OF CATALASE AND NITROGENASE

The development of the thermodynamics of successive complex formation and the rapid accumulation of quantitative data concerning the stability constants of various metal complexes in the 1960s made it possible to carry out systematic investigations of a series of complexes of copper(II) ions, ranging from Cu^{2+} to the copper-containing protein haemocyanine, and iron(III) ions, ranging from Fe^{3+} to catalase. These investigations have been summarised[37] and have been partly described in another communication.[38] The level of development of the kinetic methods for the investigation of complex processes made it possible to operate not only with the concept of the effective catalytic activity but also with the characteristics of the process mechanism, including the rate constants for the individual stages in the most thoroughly investigated systems.[39-44]

It was shown in these studies that mononuclear complexes of copper and iron ions induce a radical-chain decomposition of hydrogen peroxide with participation of the metal complex in the initiation, propagation, and termination stages. Depending on the concentrations, the chain length varies from 3 to 50.

Thus, when more stringent requirements are imposed as regards the similarity of the properties of the enzyme and model in terms of the isotypical nature of the fundamental process mechanism, the mononuclear copper(II) and iron(III) complexes cannot be regarded as models of catalase.

Only the copper complexes whose oxidation-reduction potentials are appreciably positive, permitting the Cu(II) ↔ Cu(I) transitions in the rate-limiting chain initiation and propagation stages proved to be catalytically active. The incorporation of an oxygen-containing ligand into the coordination sphere or the formation of a copper(II) complex with four nitrogen-containing ligands lead to an unfavourable alteration of the oxidation-reduction potential and a loss of catalytic properties by the metal complexes. When the latter interact with the peroxide anion, there is hardly any generation of radicals.

The molecular mechanism of catalysis characteristic of catalase and many other metalloenzymes operates only in the case of Cu^{2+} complexes with triethanolamine[45] and Fe^{3+} complexes with ethylenediaminetetra-acetic acid[46] incorporating two metal ions in close proximity, which act as a cooperative acceptor-donor of an electron pair.

The study of catalase[47] led to the conclusion that the four ferriporphyrin groups present in catalase are also involved in the decomposition of H_2O_2 cooperatively and not autonomously. The molecular mechanism of the catalysis of the decomposition of H_2O_2 by haemocyanine is also determined by the presence in its structure of pairs of proximate copper ions,[48] jointly participating in two-electron oxidation-reduction transitions.

It is noteworthy that the first hypothesis concerning the joint action of a pair of iron ions in catalase was put forward in 1932,[49] while studies which demonstrated the transformation of the catalase activity into the peroxidase activity on dissociation of catalase into four subunits containing one iron ion each have played a significant role in reaching the conclusion that the active centre of catalase includes not less than two iron ions.[50]

In 1967–1968 Purmal'[51,52] formulated the hypothesis of a special role in oxidation-reduction catalysis of dinuclear and polynuclear complexes capable of acting in accordance with the mechanism of molecular catalysis. In these studies attention was also drawn for the first time to the polynuclear character of the majority of oxidation-reduction metalloenzymes.

In the above hypothesis it was assumed that in the case of a pair of metal ions in close proximity, each of which is a one-electron oxidation-reduction agent, there is a possibility of the simultaneous transfer of an electron pair between the substrate and the catalyst, which determines the molecular mechanism of catalysis. The two-electron oxidation-reduction corresponds to the transition from one stable valence-saturated state of the substrate to another. For the vast majority of substrates, such a process is therefore energetically more favourable than the transition of the substrate from a stable form into a reactive radical formed by a one-electron transfer. Thus the mechanism of molecular catalysis can compete successfully with the mechanism of radical reactions when the latter is not a chain mechanism with a fairly long chain length. In the case of metal ions which are characteristic two-electron agents such as thallium, for example, acting in accordance with a two-electron transfer mechanism in their interaction with organic or inorganic substrates, there is no necessity for the cooperation of two metal ions. An analogous behaviour obtains for the iron(III) complex of triethylenetetramine, already mentioned, the mechanism of the catalysis by this complex involving Fe(II) ↔ Fe(IV) transitions. The catalysis of the decomposition of H_2O_2 by nickel(II) complexes of monoethanolamine apparently takes place similarly, involving Ni(I) ↔ Ni(III) transitions.[53,54] For this complex, the radical component of the process is virtually absent and monoethanolamine is not oxidised. (This system was subsequently used in laboratory practice for the non-radical decomposition of hydrogen peroxide in the presence of organic compounds.) Cooperation in the form of the formation of binuclear or polynuclear complexes with metal ions in close proximity is essential for ions whose stable oxidation states differ by unity. The polynuclear nature of metalloenzymes determines the possibility of catalysis by the latter of oxidation-reduction reactions via mechanisms not involving the intermediate formation of free radicals.

Thus the study of an enzyme and model systems with various structural and electrochemical characteristics led to the idea that dinuclear and polynuclear complexes play a special role in the chemical and biochemical catalysis of oxidation-reduction reactions. This idea was subsequently confirmed and developed in further investigations.

One should note that studies of the catalase process constituted a major stage in the history of the development of research into the modelling of enzymes. Another process whose modelling is likely to have great practical value is that involving the fixation of molecular nitrogen. Here too, although at a higher level, the evolution of studies on the modelling of catalase, beginning with the investigation of very simple models, was repeated. For a long time, it seemed that the ability to fix inert molecular nitrogen under mild conditions is characteristic solely of the biochemical systems of microorganisms. The detailed mechanism of the enzymic nitrogen fixation has not been ultimately elucidated even today although it has long been known that transition metals (molybdenum and iron) play an important role in this enzyme system. Together with the hypothesis that the transition metal complex ions can incorporate the N_2 molecule in the coordination sphere, this fact has served as a basis for the search for systems activating molecular nitrogen.

The first study by Vol'pin and Shur,[55] who achieved the chemical fixation of N_2, was published in 1964. They showed that various transition metal (titanium, chromium, molybdenum, iron, etc.) compounds are capable of effectively binding nitrogen at room temperature in aprotic media in the presence of reductants (organolithium, organomagnesium, or organoaluminium compounds, aromatic radical-anions, and powerful inorganic reductants). In the subsequent hydrolysis of these intermediates, ammonia was formed.[56] Later this kind of fixation was achieved also under catalytic conditions.[57,58] Under the influence of reductants, compounds of transition metals in low-valence states [titanium(II), vanadium(II), and molybdenum(II)], which coordinate N_2, takes place. In the subsequent stages of the reduction of the coordinated N_2, reduction products are formed: metal complex derivatives of hydrazine and ammonia.[59] The metal-nitrogen complex was first synthesised in 1965 by Allen and Zenoff from a complex incorporating N_2H_4 as the ligand.[60] The formation of complexes containing N_2 on direct interaction between molecular nitrogen and metal complexes was first established by Shilov and co-workers.[61,62] The studies enumerated above have disproved the idea that chemical fixation of N_2 under mild conditions is impossible and, although the chemical difference between the enzyme nitrogenase and metal complex systems discovered by Vol'pin and Shilov is great, the foundations for the modelling of nitrogenase were laid.

Subsequent researches into the modelling of nitrogen fixation were prosecuted from two directions: Shilov and co-workers investigated simple inorganic and organometallic models;[63–65] Likhtenshtein and co-workers[66–69] studied the structure of the active centre of the enzyme and

developed certain general approaches to the search for and the synthesis of model systems on the basis of information obtained in the investigation of the real enzyme. The situation described above, where simple compounds, down to metal hydroxides, i.e. compounds just as simple as Bredig's first models of catalase, served as models of a complex protein compound, has been repeated. However, as in the modelling of catalase, the outward dissimilarity of titanium hydroxides and nitrogenase conceals a fundamental similarity. This similarity consists in the possibility of achieving the cooperative step in the multielectron reduction of N_2. Likhtenshtein and Shilov[70] analysed the energetics of consecutive transitions in a series of reactions:

$$N_2 \rightarrow N_2H \rightarrow N_2H_2 \rightarrow N_2H_3 \rightarrow N_2H_4 .$$

As a result of the high strength of the first bond to be dissociated in N_2, successive one-electron reduction of N_2 cannot proceed at an appreciable rate under the mild conditions of biological fixation. The energetic difficulties in the reduction of nitrogen are eliminated in the four-electron synchronous reduction of N_2 to a hydrazine derivative. The dissociation of the two bonds in the N_2 molecule is compensated by the formation of new bonds with the metal centres of the catalyst, i.e. the transition

$$M \ldots N \equiv N \ldots M \rightarrow M = N - N = M$$

implies a definite rearrangement of the electron density within the metal complex. An obligatory condition governing the possibility of such a process is the involvement in the latter of at least two metal ions.

The study of the structure of the active centre of nitrogenase led to the conclusion that it contains a pair of low-valence molybdenum ions in close proximity.[66,67] On the other hand, in the hydroxide, which is a polyhydroxo-complex according to the conditions of its preparation, there is a fairly large number of metal ions close to one another.

Thus both nitrogenase and the simple models discovered in these studies are polynuclear metal complexes despite all the differences between them. A system has been found which is capable of reducing nitrogen homogeneously in protic water–alcoholic media.[71] The conditions for the formation of this system, namely the vanadium(II) complex of catechol, and the manifestation of its activity show that a polyhydroxo-complex incorporating several vanadium ions functions in this instance. An interpretation of the mechanism of the action of nitrogenase taking into account the characteristics of its structure has been described.[66,67] Its essential features reduce to the following.

Since nitrogenase is coupled to a one-electron reductant system, in particular ferredoxin, the structure of the nitrogenase active centre constitutes a kind of device for "switching" the one-electron reduction mechanism to a four-electron mechanism. The mechanism of the enzyme action includes stages involving electron transport from the surface sections towards the iron-containing cluster in the bulk of the macromolecule, the storage by the iron-containing cluster of the electrons obtained from the one-electron reductant, and the four-electron attack on the nitrogen molecule in the nitrogenyl complex with participation of at least two metal atoms (molybdenum atoms in all probability).

The discovery of the possibility of nitrogen fixation by simple model compounds has stimulated studies on these lines. These researches underwent a rigorous and complex development in England. Studies by English researchers also developed in two directions, involving the investigation of metal complexes (their structure and the relation between structural features and reactivity in relation to nitrogen fixation) and the investigation of various aspects of nitrogenase, including gene engineering. Information about the character of the bonds involving molybdenum atoms in the active centre of nitrogenase was obtained by the modelling method. The similarity of the exafs spectra (the spectra of the fine structure of the edge of the X-ray absorption) of nitrogenase and molybdenum–sulphur complexes capable of binding and activating nitrogen confirmed the hypothesis of the presence of sulphur bridges between the molybdenum atoms in the active centre of the enzyme.[72]

The chemical investigations were directed towards the study of the reactivity of the coordinated nitrogen. The formation of complexes of N_2 with various Mn, Fe, Co, Ni, Mo, and W complexes was established. The attempt to protonate the coordinated N_2 led to the decomposition of the complexes with evolution of N_2. The subsequent reaction of the coordinated N_2 with formation of $M{=}N{-}NH_2$ was observed only for the Mo and W complexes with phosphorus-containing ligands.[73,74] The formation of two moles of NH_3 was observed on subsequent protonation.[74] It was not possible to determine the intermediates in the transition from the hydrazo-complex to NH_3,[75] i.e. an independent confirmation of the multielectron mechanism of the reduction process was obtained. These results stimulated interest in the study of the mechanism of the electrochemical reduction of nitrogen complexes with metal ions bound in one way or another to the electrode surface.[76]

Exceptionally interesting results have been obtained recently in this field. The molybdenum complexes of phospholipids, adsorbed on a mercury amalgam, proved such effective reductants for nitrogen that it was possible to follow the process at room temperature and atmospheric nitrogen pressure.[77] The above principal characteristics of the mechanism of the action of nitrogenase were reflected in this model system.

The set of studies on nitrogenase and model systems led to the formulation of ideas and principles of a general character important for the theory of selection and construction of models of polynuclear metalloenzymes with an oxidation-reduction activity. The concept of multielectron transitions involving considerable changes in the nuclear configurations was put forward.[78] Likhtenshtein suggested the principle of the "optimum movement".[79] Its essential feature is that a synchronous process, accompanied by appreciable nuclear rearrangements, is favoured by the energy factor, but, when the elementary step involves many nuclei, the rate of reaction may become vanishingly small even for significantly exothermic reactions. In other words, the number of atoms involved in the reaction should be sufficiently large to ensure favourable energy relations but sufficiently small to retain a high probability of the synchronisation of the movement of nuclei along the reaction coordinate.

Theoretical procedures for the estimation of the probability of synchronisation in processes involving many nuclei have been developed.[80]

V. MODELS OF OXIDASES, OXYGENASES, AND OTHER ENZYMES

An important set of enzymes involved in terminal oxidation processes includes oxygenases and oxidases which catalyse the reactions of molecular oxygen with various organic substrates. In 1954 Udenfriend et al.[81] proposed a hydroxylating system which leads to the same hydroxylation products as certain mono-oxygenases. Udenfriend's model system includes iron ions, ethylenediaminetetra-acetate as the ligand, and ascorbic acid or its analogues as the electron donor. The identification of the chemically reactive species leading to the reaction products in this multicomponent system was for a long time difficult. The development of kinetic research methods made it possible to demonstrate that these

species in Udenfriend's system are metal–oxygen complexes having specific reactivities.[82]

Thus, in relation to this system, it has been possible to identify the intrinsic elementary step in the catalytic action in the activation of oxygen, which can be regarded as a new stage in the modelling of enzymes—the determination of the elementary mechanism of the chemical steps in the active centre of the enzyme and their reproduction in simpler systems.

The extent to which the elementary oxidation-reduction reactions of the metal complex ions are of a general character can be seen from a comparison of the catalytic functions of copper ions in various ligand environments in the oxidation of ascorbic acid by atmospheric oxygen. It has been established[83,84] that the reactive intermediate species is the oxygen complex CuO_2^+, which interacts with the substrate via a two-electron mechanism; an analogous result has been obtained also for the oxidation of methanol.[85] This elementary step models the action of a series of so-called colourless oxidases containing a copper(I) ion in the active centre. Under certain conditions, the cooperation of two copper ions is observed in the elementary step involving the two-electron oxidation of the ascorbate anion[86] or in the elementary step involving the two-electron reduction of oxygen.[87] These reactions model the non-radical cooperative processes occurring in the case of enzymes (the so-called blue oxidases) containing in the active centre two and more copper ions in close proximity.

The results of kinetic studies on relatively simple catalytic systems for the oxidation of ascorbic acid by oxygen[88] showed that the elementary step in the model and enzyme systems is in essence of the same type.

A specific feature of the enzyme system consists apparently in a three-dimensional structure of the immediate environment of the metal ion which limits the possibility of side reactions.

A number of investigations have been devoted to models of peroxidase. We shall not consider them because only a detailed kinetic study will make it possible to distinguish the "peroxidase-like" mechanism of the formation of a complex with H_2O_2 and its subsequent reaction with the substrate from the mechanism involving $H\dot{O}_2$ and $\dot{O}H$ radicals, which is characteristic of the majority of metal complexes.

A large series of comparative studies of the spectroscopic, thermodynamic, electrochemical and catalytic properties of various metal complexes and metalloenzymes, incorporating the same metal, have been carried out by Williams,[89,91] who arrived at conclusions significant for the search for syntheses of active models and also for the understanding of the specific properties of metalloenzymes. Thus, it has been shown that zinc complexes with tetrahedral but not octahedral coordination environments exhibit a carbonic anhydrase activity. The study of model systems made it possible to propose a structure for the active centre of carbonic anhydrase, where the zinc ion is present as a complex with a deformed tetrahedral structure in which two ligands consist of the sulph-hydryl groups of the protein. Comparison of the spectroscopic and thermodynamic properties of the zinc complexes of phenanthroline and the complex formed as a result of the addition of phenanthroline to the zinc atom of the active centre of alcohol dehydrogenase made it possible to demonstrate the presence of two or three vacant sites in the coordination sphere of zinc, available for substitution by the substrate. Williams[89] was the first to put forward the hypothesis, which was subsequently confirmed, that the specificity of the reactions of any metalloenzymes with small molecules (O_2, CO_2, H_2O, H_2O_2, and N_2) is determined almost completely by the substituted coordination sphere of the complex-forming metal.

In his analysis of the spectroscopic and magnetic properties of the metal complexes and metal-containing oxygen transferring agents haemocyanine and haemerythrin, Williams[91] proposed a general mechanism of their action:

$$\mathrm{Cu(\bar{I})\ \ldots\ Cu(\bar{I}) + O_2 \rightarrow Cu(II)O_2^{2-}Cu(II)},$$
$$\mathrm{Fe(II)\ \ldots\ Fe(II) + O_2 \rightarrow Fe(III)O_2^{2-}Fe(III)}.$$

He emphasised a characteristic feature of the functioning of these oxygen-transferring agents which is difficult to achieve in model systems: in the absence of oxygen the reduced metal ions are located in the protein structure sufficiently far from one another not to influence their magnetic and optical properties and their reactivities. Oxygen is bound as a result of the mutual approach of a pair of metal ions, which is accompanied by a change in conformation and "strain" in the protein component of the transferring agent. He put forward similar ideas concerning the nitrogen fixation mechanism. He explained the presence of molybdenum in all the nitrogen fixing system by the fact that the following process can be formulated only for two molybdenum(III) ions:

$$\mathrm{Mo(III)N_2Mo(III) \rightarrow Mo(VI)(N^{3-})_2Mo(VI) \rightarrow 2NH_3}.$$

The difficulty of modelling this system arises from the fact that two molybdenum(III) atoms which have approached one another must not form a strong Mo–Mo bond, retaining at the same time a vacant site in the coordination sphere for the formation of the complex with N_2.

One should note briefly that, simultaneously with the development of studies on the modelling of metalloenzymes, research into organic catalysts modelling the action of particular metal-free enzymes has developed fairly vigorously. Extensive information about these problems has been published.[92,93]

VI. THE MODELLING OF ENZYMES AS A PATHWAY TO NEW CATALYSTS

The development in the USSR of the second of the trends specified above (the synthesis of catalysts on the basis of the existing information about the structure and properties of enzymes and the principles of enzyme catalysis) was initiated successfully and logically in the USSR at the beginning of the 1960s by Khidekel'.[94–102] There is no doubt about the initial general postulate—that of the characteristics of the action of coenzymes and enzymes can be explained in principle on the basis of the fundamental postulates of organic, coordination, and polymer chemistry. However, the "unmapped regions" as regards information about systems of biological origin can pose stringent requirements on the intuition of investigators who choose this pathway.

The simplest systems, namely the complexes of copper ions with various amines, were investigated as analogues of copper-containing phenol oxidases in the initial stage of these studies.[94,95] With the aid of such catalysts, it was possible to vary widely the direction of the reactions as a result of the formation of complexes of the intermediate compounds or starting materials with the catalyst and by the variation of the composition of the catalysts. These results proved to be useful in carrying out oxidation reactions involving various phenols. It was possible to develop convenient methods for the synthesis of a number of quinonoid compounds, naphthols, and spirocyclic trimers.[96,97]

The retrospective analysis of the evolutionary pattern of the biogenesis of enzymes has been important for the selection of procedures for the synthesis of their analogues with different degrees of complexity in structure and organisation.[98,99]

Studies have been made on a series of dihydropyridine derivatives—analogues of the coenzymes nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate. New highly active reduction systems and catalysts for the hydrogenation of olefins, significantly superior to the existing liquid-phase hydrogenation catalysts, were obtained on the basis of these investigations.

It was possible to create new types of homogeneous and "heterogenised" complex hydrogenation catalysts—analogues of the enzyme hydrogenase. Catalysts based on the following systems were obtained: 1,4-dihydronicotinamide—a transition metal derivative, low-valence transition metal complexes with quinones and aromatic compounds, aromatic carboxylic acids, aminoacids and peptides, dyes of various types, hydroxyquinones, and aromatic hydroxyazo-compounds. Some of these catalysts proved to be suitable for a series of processes involved in fine and fundamental organic synthesis.[100] A series of studies have been devoted to the transfer of activated hydrogen by methods which are tentatively similar to the enzyme procedures.[101] Using these methods, it was possible to hydrogenate at a high rate ketones, aldehydes, and aromatic compounds and to achieve a series of selective hydrogenation processes (in particular, the reduction of the carbonyl group in the presence of the C=C double bond[102]). Systems incorporating a catalyst activating molecular hydrogen and a catalyst for the transfer of activated hydrogen have also been created.[101]

Catalytic systems of the above type make it possible to reduce at a high rate and with a high specificity a large number of organic and complex compounds.

The results concerning the catalytic hydrogenation of aromatic compounds and aromatic nitro-compounds were particularly valuable. Some of the new catalytic systems developed have been incorporated in industrial practice.[103-106]

Extensive information about systems in which homogeneous hydrogenation is effected by complexes of metals of all the Groups in the Periodic System has been published.[107] The catalysts described include those in the creation of which various natural hydrogenases served as the mobilising system.

A new interesting type of modelling of various enzymes was initiated following the development of the synthetic chemistry of polymers containing various functional groups (carboxy-, amino-, etc. groups), which are soluble in polar solvents.

The investigation of the catalytic properties of such polymers and their complexes with metal ions in the USSR was developed in the studies of Kabanov, Pshezhetskii, and Kirsh.[108-113] The activity of systems based on polyacrylic acids, polyethyleneimine, polyvinylpyridine, etc. relative to the catalase, peroxidase, ascorbic acid oxidase, catechol 1,2-oxygenase, and proteolytic functions exceeded by a factor of 10^3–10^4 the activity of the low-molecular-weight analogues, being inferior by a factor of 10^2–10^3 relative to the activities of the enzymes modelled.

VII. THE ROLE OF THE MACROMOLECULAR COMPONENT IN MODEL SYSTEMS AND OF THE PROTEIN IN ENZYMES

The study of the simplest model system has shown that their catalytic properties are "improved" when the low-molecular-weight active group is combined with a high-molecular-weight group. The stability of systems of the type of "active group–carrier" rises, while their catalytic activity as a rule also increases.

The modelling of enzymes by an independent procedure led to the conclusion that their high catalytic activity is associated with the protein, macromolecular nature of these systems. To a lesser extent, the study of model systems revealed the role of the protein as a factor determining the selectivity of the enzyme action. Nevertheless, this function of the protein component of the enzyme molecule was established initially on a speculative basis in terms of the "key–lock" model, and then also experimentally. Koshland's principle of induced correspondence gained wide recognition.[114-115] It became understood why the selectivity of enzyme action was associated with a high degree of steric correspondence between the substrate and the active centre in the enzyme structure. The factor determining this correspondence is the high structured organisation of the enzyme—up to a quaternary organisation. The absence of such organisation from model systems is sufficient to understand why the creation of a simple model with a high specificity of action in relation to polyatomic substrates is a problem unlikely to have a successful solution.

The choice of activity as the principal property of models constituted a psychologically understandable stimulus for calculations by investigators, who tried to show that the activity of their models is comparable or even superior to that of enzymes. In such calculations the activity is referred, for example, to the unit weight of the catalyst; the pH-dependent activity of the model is extrapolated to the pH range in which the enzyme functions, etc. On the other hand, when calculated data are disregarded, one may conclude that it has not been possible to create a model equal to the enzyme in its activity for their equal molar concentrations at similar pH, temperature, and substrate concentration.

The idea of the possible role of the protein as a factor determining the high activity of enzymes was put forward for the first time by Bauer in 1935,[116] who suggested that the protein can exist in equilibrium and non-equilibrium states; during the enzymic reaction, the protein passes from a non-equilibrium to an equilibrium state, while the energy of the transition is used to overcome the activation barrier to the reaction of the substrate. The energy evolved as a result of the enzymic reaction returns the protein molecule to the initial non-equilibrium state. The general principle of the possibility of employing the energy of a chemical reaction to accelerate the reaction under consideration, which appeared during the period when Semenov created the theory of branched chain reactions, was reflected in Bauer's hypothesis.

Kobozev examined from the same standpoint the problem of the relation between the catalytic activity of a low-molecular-weight compound and the properties of the high-molecular-weight substance attached to it.[117,118] His idea consisted in the hypothesis that the thermodynamically non-equilibrium mass should increase the catalytic activity of the species linked to it with respect to various reactions. He showed that in many instances a logarithmic relation is observed between the activity and the magnitude of the attached non-equilibrium mass. The specific question of the increase of catalytic activity of a species as a result of the addition of a protein to it has also been examined.[119,120] The role of the protein macromolecule is to participate in the recovery of the energy: the energy evolved in the course of the reaction is not scattered in the form of heat but is used to activate the catalytic centre.

A number of studies have been made whose aim was to specify in greater detail the problem of the possible forms of the accumulation of energy by the protein molecule.[121,122] Various approaches to the elucidation of the role of the protein in enzymes has been analysed by Blyumenfel'd.[123] His review can be supplemented only by the hypothesis of the entactic (extended, strained) state of the active centre of the enzyme put forward on the basis of the analysis of a series of model and enzyme systems.[91] According to this

hypothesis, the small activation barrier to the enzymic reaction is determined by the fact that, even before the enzyme combined with the substrate, the active centre of the enzyme contained a functional group with a high potential energy. The increased energy in the region of the active centre arises by virtue of the conformational energy of the entire protein molecule. Thus the zinc atom in carboxypeptidase or carbonic anhydrase is much more reactive than in its initial ionic form or in a complex with free low-molecular-weight ligands. When it is located in the active centre, the zinc atom is only partly bound to the protein ligands forming the strained configuration of a distorted tetrahedron. The possibility of such formations is a specific feature of the macromolecular polyligand (the protein), characterised by the presence of secondary and tertiary structures.

According to Blyumenfel'd, the conformational transformations of the protein are indissolubly linked to the elementary step in the enzymic reaction: the latter consists in a conformational change in the macromolecule (of the enzyme–substrate complex) and the rate of the substrate–product transformation is determined by the rate of this conformational change[123] (p.155). In contrast to the views presented above, in Blyumenfel'd's concept the protein molecule incorporating the active centre is in a conformationally equilibrium state before the start of the interaction with the substrate. In the region of the active centre the protein molecule passes to a conformationally non-equilibrium state only for the enzyme–substrate compound. The transition from the conformationally non-equilibrium state to the equilibrium state involves greater or smaller changes in the structural organisation of the protein—the dynamics of conformational changes. One of the most thoroughly developed dynamic models is that describing the functioning of the enzyme aspartate aminotransferase.[124]

Without considering in greater depth the current problems of modern theoretical enzymology, one can apparently speak of a relation between the high catalytic activity of enzymes and the structural-dynamic characteristics of the protein. Thus not only the selectivity of their action but also the catalytic activity of enzymes is associated with the fact that the protein consists of macromolecules with a structural organisation, the changes in the latter making the same contribution to the enzymic step as the redistribution of chemical bonds in the substrate species.

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The question arises of the prospects for the creation of model catalysts with activities and selectivities similar to those of enzyme catalysts. The solution of this problem, which involves in essence the creation of synthetic analogues of enzymes rather than models, is associated with the creation and employment of polymers having a flexible structural organisation, like proteins. Naturally, this is not the only possible way of achieving a solution. It may be that the procedure involving the combination of polymers of the above kind, linked to active groups, with systems of molecular sieves of the type of zeolites will lead to success. It is also possible that an unpredictable solution will be found. This does not imply that the study of low-molecular-weight models of enzymes has lost its value. Their investigation will lead to the formulation of new hypotheses and concepts useful for the solution of practical problems.

However, one should not overestimate the role of studies on the modelling of enzymes as a method for the investigation of the latter. In this sense the modelling method is simply one of the trends in the overall research programme, including the study of the sequence of aminoacid residues in enzymes, the X-ray diffraction analysis of proteins, the determination of the nature of coenzymes, the study of the kinetics and mechanism of enzymic reactions, the identification of intermediate compounds, the investigation of the conformational states of the enzyme protein and the dynamics of the changes in these states, etc.

This field of research has already yielded promising practical results associated with the synthesis of highly active non-protein catalysts.

REFERENCES

1. W.Langenbeck, "Die organischen Katalysatoren und ihre Beziehungen zu den Fermenten" (Translated into Russian), Inostr.Lit., Moscow, 1961.
2. W.Frankenburger, "Katalytische Umsetzungen", Akad.Verl.M.B.H., Leipzig, 1937.
3. G.-M.Schwab, "Katalyse vom Standpuhlst der chemische Kinetik" (Catalysis from the Standpoint of Chemical Kinetics), (Translated into Russian), ONTI-Goskhimtekhizdat, Leningrad, 1934.
4. A.Mittasch and F.Theis, Ver.Chem., 1932, 278.
5. E.Müller, Elektrochem.und angew.phys.Chem., 1923, **29**, 345.
6. E.Müller, Z.phys.Chem., 1923, **107**, 347.
7. G.Bredig and F.Sommer, Z.phys.Chem., 1909, **70**, 34.
8. S.Hennichs, Chem.Ber., 1926, **59**, 218.
9. A.Antropoff, Z.phys.Chem., 1908, **62**, 513.
10. A.Lettermoser and W.Eichler, Z.Elektrochem.und angew.phys.Chem., 1929, **35**, 610.
11. E.Wiegel, Z.phys.Chem., 1929, **143**, 81.
12. W.Hückel, "Katalyse mit kolloiden Metallen", Akad. Verlag.M.B.H., Leipzig, 1927, 86.
13. C.Paal and C.Amberger, Chem.Ber., 1904, **37**, 124.
14. A.Skita and W.Meger, Chem.Ber., 1912, **45**, 3579.
15. R.Kuhn and L.Brann, Chem.Ber., 1926, **59**, 2370.
16. L.A.Nikolaev, "Biokatalizatory i Ikh Modeli" (Biocatalysts and Their Models), Izd.Vys.Shkola, Moscow, 1968, 196 pp.
17. O.Warburg, Biochem.Z., 1920, **119**, 134.
18. E.A.Shilov, Dokl.Akad.Nauk SSSR, 1938, **18**, 643.
19. E.A.Shilov, and A.A.Yasnikov, Ukrain.Khim.Zhur., 1961, **27**, 639.
20. C.Swain and I.Brown, J.Amer.Chem.Soc., 1952, **74**, 2538.
21. N.I.Kobozev and L.A.Nikolaev, Zhur.Fiz.Khim., 1945, **19**, 323.
22. L.A.Nikolaev, in "Vozniknovenie Zhizni na Zemle" (The Origin of Life on Earth), Izd.Akad.Nauk SSSR, Moscow, 1957, p.156.
23. L.A.Nikolaev, Uspekhi Khim., 1961, **30**(1), 3 [Russ. Chem.Rev., 1961(1)].
24. A.E.Martell, R.L.Gustafson, and S.Chaberek, in "Kataliz. Trudy I Mezhdunarodnogo Kongressa" (Catalysis. Proceedings of the First International Congress), (Translated into Russian), Inostr.Lit., Moscow, 1960, p.364.
25. J.H.Wang, J.Amer.Chem.Soc., 1955, **66**, 4715.
26. J.H.Wang and R.C.Jarnagin, J.Amer.Chem.Soc., 1958, **80**, 786.
27. T.P.Vorob'eva, V.G.Isak, A.P.Purmal', and A.Ya.Sychev, Zhur.Fiz.Khim., 1974, **48**(6), 1439 [Russ.J.Phys.Chem., 1974(6)].
28. M.A.Lunina and T.A.Sharai, Zhur.Fiz.Khim., 1962, **36**(7), 1570 [Russ.J.Phys.Chem., 1962(7)].

29. A.E.Martel and M.Calvin, "Chemistry of the Metal Chelate Compounds", Prentice Hall, Englewood Cliffs, New York, 1959.
30. W.Langenbeck, Chem.Ber., 1927, **60**, 930.
31. A.E.Braunshtein and M.M.Shemyakin, Biokhimiya, 1953, **18**, 393.
32. D.E.Metzler and E.E.Snell, J.Biol.Chem., 1952, **198**, 353.
33. D.E.Metzler, J.Longenecker, and E.E.Snell, J.Amer. Chem.Soc., 1954, **72**, 639.
34. D.E.Metzler, M.Ikawa, and E.E.Snell, J.Amer.Chem. Soc., 1954, **76**, 648.
35. E.L.Smith, J.R.Kimmel, and A.Light, in "Molekulyarnye Osnovy Deistviya i Tormozheniya Fermentov. V Mezhdunarodnoi Biokhimicheskii Kongress" (Molecular Principles of the Action and Inhibition of Enzymes. The Vth International Biochemical Congress) (Translated into Russian), Izd.Akad.Nauk SSSR, Moscow, 1961, p.9.
36. R.Frater, A.Light, and E.Smith, J.Biol.Chem., 1965, **240**, 253.
37. A.P.Purmal', Doctoral Thesis in Chemical Sciences, Institute of Chemical Physics, USSR Academy of Sciences, Moscow, 1971.
38. A.Ya.Sychev, "Okislitel'no-Vosstanovitel'nyi Kataliz Kompleksami Metallov" (Oxidation-Reduction Catalysis by Metal Complexes), Izd.Shtiintsa, Kishinev, 1976, p.191.
39. V.M.Berdnikov, Yu.M.Kozlov, and A.P.Purmal', Khim.Vys.Energii, 1969, **3**, 321.
40. L.I.Shabarchina, V.M.Berdnikov, and A.P.Purmal', Zhur.Fiz.Khim., 1970, **44**(1), 265 [Russ.J.Phys. Chem., 1970(1)].
41. V.M.Berdnikov, Yu.N.Kozlov, and A.P.Purmal', Khim.Vys.Energii, 1969, **3**, 370.
42. T.P.Vorob'eva, V.M.Berdnikov, and A.P.Purmal', Kinetika i Kataliz, 1970, **11**, 100.
43. Yu.N.Kozlov, A.D.Nadezhdin, and A.P.Purmal', Kinetika i Kataliz, 1973, **14**, 141.
44. T.P.Vorob'eva, Yu.N.Kozlov, and A.P.Purmal', Zhur.Fiz.Khim., 1974, **48**(6), 1444 [Russ.J.Phys. Chem., 1974(6)].
45. T.P.Vorob'eva and A.P.Purmal', Zhur.Fiz.Khim., 1982, **56**(5), 1148 [Russ.J.Phys.Chem., 1982(5)].
46. Zh.P.Kachanova, E.L.Kudryavtseva, and A.P.Purmal', Zhur.Fiz.Khim., 1974, **48**(6), 1449 [Russ.J.Phys.Chem., 1974(6)].
47. Yu.M.Azizov, G.I.Likhtenshtein, and A.P.Purmal', Biokhimiya, 1972, **37**, 620.
48. Y.Felsenfeld and M.P.Printz, J.Amer.Chem.Soc., 1959, **81**, 6259.
49. K.S.Stern, Z.physiol.Chem., 1932, **209**, 176.
50. T.Samejima and K.Shibata, Arch.Biochem.Biophys., 1961, **93**, 407.
51. A.P.Purmal', in "Mekhanizm Dykhaniya, Fotosinteza i Fiksatsii Azota" (The Mechanisms of Respiration, Photosynthesis, and Nitrogen Fixation), Izd.Nauka, Moscow, 1967, p.179.
52. Zh.P.Kachanova, Yu.M.Azizov, and A.P.Purmal', in "Problemy Kinetiki i Kataliza. VIII. Kompleksoobrazovanie v Katalize" (Problems of Kinetics and Catalysis. VIII. Complex Formation in Catalysis), Izd.Nauka, Moscow, 1968, p.121.
53. L.N.Arzamaskova and A.P.Purmal', Zhur.Fiz.Khim., 1964, **38**(5), 1321 [Russ.J.Phys.Chem., 1964(5)].
54. L.N.Arzamaskova and A.P.Purmal', Zhur.Fiz.Khim., 1970, **44**(7), 1829 [Russ.J.Phys.Chem., 1970(7)].
55. M.E.Vol'pin and V.B.Shur, Dokl.Akad.Nauk SSSR, 1964, **156**, 1102.
56. M.E.Vol'pin and V.B.Shur, Zhur.Vses.Khim.Obshch. im.Mendeleeva, 1967, **12**, 31.
57. M.E.Vol'pin, M.A.Platovskaya, L.V.Kosyakova, and V.V.Shur, Dokl.Akad.Nauk SSSR, 1968, **180**, 103.
58. M.E.Volpin and V.B.Shur, "Organometallic Reactions", Wiley, New York, 1970, Vol.1, p.55.
59. M.E.Vol'pin, Zhur.Vses.Khim.Obshch.im.Mendeleeva, 1972, **17**, 396.
60. A.D.Allen and C.V.Zenoff, Chem.Comm., 1965, **2**, 121.
61. Yu.G.Borod'ko, A.K.Shilova, and A.E.Shilov, Dokl.Akad.Nauk SSSR, 1967, **176**, 1297.
62. Yu.G.Borod'ko, A.K.Shilova, and A.E.Shilov, Zhur.Fiz.Khim., 1970, **44**(3), 627 [Russ.J.Phys. Chem., 1970(3)].
63. A.E.Shilov and A.K.Shilova, Zhur.Fiz.Khim., 1970, **44**(2), 288 [Russ.J.Phys.Chem., 1970(2)].
64. A.P.Khrushch and A.E.Shilov, Kinetika i Kataliz, 1970, **11**, 86.
65. N.T.Denisov, E.I.Rudshtein, and N.I.Shuvalova, Dokl.Akad.Nauk SSSR, 1972, **202**, 623.
66. G.I.Likhtenshtein, "Metod Spinovykh Metok v Molekulyarnoi Biologii" (The Method of Spin Labels in Molecular Biology), Izd.Nauka, Moscow, 1974, 256 pp.
67. G.I.Likhtenshtein, R.I.Gvozdev, L.A.Levchenko, and L.A.Syrtsova, Izv.Akad.Nauk SSSR, Ser.Biol., 1978, **2**, 165.
68. A.V.Kulikov and G.I.Likhtenshtein, Biofizika, 1974, **19**, 420.
69. G.I.Likhtenshtein, Uspekhi Biol.Khim., 1971, **12**, 3.
70. G.I.Likhtenshtein and A.E.Shilov, Zhur.Fiz.Khim., 1970, **44**(4), 849 [Russ.J.Phys.Chem., 1970(4)].
71. L.A.Nikonova, A.G.Ovcharenko, O.N.Efimov, V.A.Avilov, and A.E.Shilov, Kinetika i Kataliz, 1972, **13**, 1602.
72. S.P.Kramer, K.O.Hodgson, W.O.Gillum, and L.E.Mortenson, J.Amer.Chem.Soc., 1978, **100**, 3398.
73. G.J.Leigh and C.J.Pickett, J.Chem.Soc., Dalton Trans., 1977, **18**, 1797.
74. J.Chatt, A.J.Pearman, and R.L.Richards, J.Chem. Soc., Dalton Trans., 1977, **19**, 1852.
75. M.W.Bishop, J.Chatt, and J.R.Dilworth, J.Less Common Metals, 1977, **54**, 487.
76. G.J.Leigh and C.J.Pickett, J.Chem.Soc., Dalton Trans., 1977, **18**, 1797.
77. L.I.Didenko, A.K.Shilova, and A.E.Shilov, Dokl. Akad.Nauk SSSR, 1980, **254**, 643.
78. N.N.Semenov, A.E.Shilov, and G.I.Likhtenshtein, Dokl.Akad.Nauk SSSR, 1975, **226**, 1374.
79. G.I.Likhtenshtein, Kinetika i Kataliz, 1977, **18**, 878.
80. I.V.Aleksandrov, Teor.Eksper.Khim., 1976, **12**, 299.
81. S.Udenfriend, C.T.Clark, C.Axelrod, and B.B.Brodie, J.Biol.Chem., 1954, **208**, 731.
82. A.P.Purmal', Yu.I.Skurlatov, and S.O.Travin, Izv. Akad.Nauk SSSR, Ser.Biol., 1980, 492.
83. E.V.Shtamm, A.P.Purmal', and Yu.I.Skurlatov, Zhur.Fiz.Khim., 1974, **48**(9), 2229 [Russ.J.Phys. Chem., 1974(9)].
84. E.V.Shtamm, A.P.Purmal', and Y.I.Scurlatov, Intern.J.Chem.Kinet., 1979, **11**, 461.
85. S.O.Travin, Yu.I.Skurlatov, N.V.Gorbunova, and A.P.Purmal', Izv.Akad.Nauk SSSR, Ser.Biol., 1979, 1480.
86. E.V.Shtamm, A.P.Purmal', and Yu.I.Skurlatov, Zhur.Fiz.Khim., 1974, **48**(9), 2229 [Russ.J.Phys. Chem., 1974(9)].

87. N.V.Gorbunova, A.P.Purmal', Yu.I.Scurlatov, and V.A.Kabanov, Intern.J.Chem.Kinet., 1977, **9**, 983.
88. Yu.I.Scurlatov, V.Y.Kovner, and S.O.Travin, Europ.Polymer J., 1979, 15, 811.
89. R.J.P.Williams, in "Molekulyarnye Osnovy Deistviya i Tormozheniya Fermentov" (Molecular Principles of the Action and Inhibition of Enzymes), Izd.Akad. Nauk SSSR, Moscow, 1962, p.160.
90. R.J.P.Williams, "Les Metaux de la vie "La Recherche"", 1973, p.529.
91. R.J.P.Williams, Inorganica Chemica Acta (Rev.), 1971, **5**, 137.
92. F.H.Westheimer, in "Molekulyarnye Osnovy Deistviya i Tormozheniya Fermentov" (Molecular Principles of the Action and Inhibition of Enzymes), Izd.Akad. Nauk SSSR, Moscow, 1962, p.9.
93. A.A.Yasnikov, "Organicheskie Katalizatory, Kofermenty i Fermenty" (Organic Catalysts, Coenzymes, and Enzymes), Izd.Naukova Dumka, Kiev, 1982, 272 pp.
94. L.V.Gorbunova, M.L.Khidekel', and G.A.Razuvaev, Dokl.Akad.Nauk SSSR, 1962, **147**, 368.
95. L.V.Gorbunova, O.N.Efimov, V.V.Karpov, and M.L.Khidekel', in "Mekhanizm i Kinetika Fermentativnogo Kataliza" (The Mechanism and Kinetics of Enzyme Catalysis), Izd.Nauka, Moscow, 1964, p.227.
96. V.V.Karpov and M.L.Khidekel', Zhur.Org.Khim., 1968, **4**, 861.
97. V.V.Karpov and M.L.Khidekel', Zhur.Org.Khim., 1967, **3**, 1669.
98. M.L.Khidekel', in "Nauchnye Osnovy Podbora Katalizatorov" (Scientific Principles of the Selection of Catalysts), Izd.Nauka, Moscow, 1966, p.207.
99. M.L.Khidekel', in "Kompleksoobrazovanie v Katalize. Problemy Kinetiki i Kataliza" (Complex Formation in Catalysis. Problems of Kinetics and Catalysis), Izd.Nauka, Moscow, 1968, No.13, p.126.
100. E.G.Chepaikin and N.L.Knidekel, J.Mol.Catal., 1978, **4**, 103.
101. R.B.Ivanova and M.L.Khidekel', Zhur.Org.Khim., 1969, **39**, 2393.
102. V.V.Abalyaeva, A.S.Astakhova, E.I.Bakhanova, and M.L.Khidekel', Izv.Akad.Nauk SSSR, Ser. Biol., 1969, 89.
103. British Patent No.1 453 966, 1973.
104. Bulgarian Patent No. 29 186, 1980; Byul.Izobret. Bolgarii, 1980(10).
105. Swiss Patent No. 586 651, 1973.
106. S.D.Kushch, M.V.Klyuev, E.N.Izakovich, M.L.Khidekel', K.N.Dyumaev, V.M.Rogovin, and V.N.Alekseev, USSR Patent No. 767 089, 1980; Byul.Izobret., 1980(36), 116.
107. B.R.James, "Homogeneous Hydrogenation" (Translated into Russian), Izd.Mir, Moscow, 1976, 570 pp.
108. V.S.Psthezhetskii, A.P.Lukjanova, and V.A.Kabanov, J.Molec.Catalysis, 1977, 2, 49.
109. V.S.Pshezhetskii and A.A.Yaroslavov, Bioor.Khim., 1977, **3**, 1117.
110. V.S.Pshezhetskii, T.A.Kuznetsova, and V.A.Kabanov, Bioor.Khim., 1975, **1**, 1215.
111. V.S.Psthezhetskii, G.A.Murtazaeva, and V.A.Kabanov, Europ.Polymer J., 1974, **10**, 571.
112. N.A.Vengerova, Yu.E.Kirsh, and V.A.Kabanov, Vysokomol.Soed., 1971, **13A**, 2509.
113. A.A.Rakhnyanskaya, Yu.E.Kirsh, and V.A.Kabanov, Vysokomol.Soed., 1977, **19A**, 1770.
114. D.E.Koshland, Proc.Nat.Acad.Sci.USA, 1958, **44**, 98.
115. D.E.Koshland, J.Theor.Biol., 1962, **2**, 75.
116. S.Bauer, "Theoretical Biology" (Translated into Russian), Izd.VIEM, Moscow, 1935, 180 pp.
117. N.I.Kobozev, Zhur.Fiz.Khim., 1945, **19**, 1413.
118. N.I.Kobozev, L.A.Nikolaev, I.A.Zubovich, and Yu.M.Gol'dfel'd, Zhur.Fiz.Khim., 1945, **19**, 48.
119. N.I.Kobozev, Uch.Zap.Moskov.Gos.Univ., 1955, **174**, 125.
120. N.I.Kobozev, Zhur.Fiz.Khim., 1960, **34**(7), 1443.
121. Yu.I.Khurgin, D.S.Chernavskii, and S.E.Shnol', Mol.Biol., 1967, **1**, 419.
122. S.E.Shnol', Symposium, "Kolebatel'nye Protsessy v Biologicheskikh i Khimicheskikh Sistemakh" (Vibrational Processes in Biological and Chemical Systems), Izd.Nauka, Moscow, 1967, p.22.
123. L.A.Blyumenfel'd, "Problemy Biologicheskoi Fiziki (Fizika Zhiznennykh Protsessov)" [Problems of Biological Physics (The Physics of Vital Processes)], Izd.Nauka, Moscow, 1974, 335 pp.
124. V.I.Ivanov and M.J.Karpeisky, Adv.Enzymol., 1969, **32**, 21.

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