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The influence of metal coordination compounds on the ionic conductivity of bilayer lipid membranes

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ABSTRACT. **The results of studies on the problem of the effect of metal coordination compounds on biological and bilayer lipid membranes are surveyed. The authors' later data, defining the mechanism of the action of metal complexes which are significant in the development of specific syntheses of physiologically active substances used in medicine and agriculture, are presented.**

The bibliography includes 50 references.

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I. Introduction

One of the most important problems in modern chemistry is that of the creation of the foundation for the specific synthesis of compounds with a specified biological activity. Its solution can be achieved only by establishing the relation between the physiological activity, on the one hand, and the molecular structure and properties of substances on the other. Despite certain advances in this field, there is at present no scientificially-based theory which would make it possible to construct effective biologically active compounds.

One of the causes of this is that the manifestation of activity is frequently inferred from the final results of the activity of substances manifested in the alteration of some kind of properties and features of the living organism as a whole (at least the living cell). The deep-seated processes at a molecular level, which are in fact the cause of the change in the normal functioning of the organism, are hardly investigated under these conditions.

The problem of creating the foundations for a specific synthesis of biologically active compounds must be solved by imparting to them properties which would enable them to act on the most important molecular mechanism among the enormous number of mechanisms of the normal vital activity of the living cell.

Among such mechanisms, it is essential to differentiate those which are linked directly to the functioning of cell membranes. Lipids, among which phospholipids predominate, constitute the structural basis of the membranes of living organisms. Bilayer lipid membranes (BLM), which are completely adequate models of the cell membranes of living organisms,[1] are therefore convenient objects for the study of the mechanisms of the action of compounds on biological membranes. Using this model, the mechanism of the action of various substances, including metal ions, has been investigated. On the other hand, only isolated studies have been devoted to the action of coordination compounds, including biologically active ones, on cell membranes.

A characteristic features of unmodified BLM is their extremely low permeability to the majority of inorganic ions, especially multicharged ions, and as a rule the absence of an appreciable selectivity among the ions. The low permeability of BLM to inorganic cations is due to the high activation energy for their transfer (of the order to 120 kJ mol^{-1} for singly charged cations[2]). At the same time, it is well known that biological membranes exhibit a high permeability (higher by 5–6 orders of magnitude than that of BLM) and manifest ion-selective properties.

The mechanisms of ionic conduction and selectivity of biological and artificial membranes have not been ultimately elucidated despite the large number of relevant studies. The accumulated literature and the authors' own data on the effect of metal cations on the lipid membranes have been surveyed[3] taking into account their physicochemical properties. It was indicated in the review that, when metal cations are bound to BLM and other membrane systems, it is necessary to take into account a series of factors: hydrolysis, the behaviour of metal compounds at different pH of the solution, in particular their solubility, their complex forming capacity, and other properties,

The subdivision of metal cations into classified groups taking into account their covalent characteristics makes it possible to estimate the sequence in which the metal cations are bound to membranes using models of the adsorption centre in lipid bilayers of different composition.

The available data suggest that the transport of metal cations through biological membranes is achieved with the aid of special transferring molecules. Evidently the transport of transition metal cations, which are important for the physiology and toxicology of living organisms, should involve the participation of organic compounds—bioligands capable of coordination interaction with these metal cations. In this connection, studies on the molecular mechanisms of the interaction of metal complexes with cell membranes have become particularly timely.

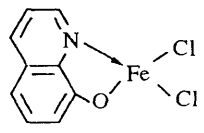
II. Interaction of metal complexes with biological membranes

Insufficient attention has been devoted hitherto to the question of coordination compounds.

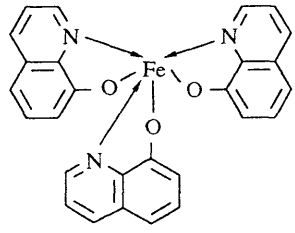
It has been assumed that the role of the metal in the complex consists in creating a suitable transport form for the ligands or, if

the metal ion itself exhibits a biological activity, then the complex is necessary for the transport of the metal through the cell membrane.[4]

Albert[5] showed that the iron(III) complex of 8-hydroxyquinoline (HQ) has the 1 : 3 composition. Neither the ligand nor the iron(III) ion possess biological activity but are very effective in suppressing pathogenic microorganisms when combined in a 1 : 1 complex.



$Fe(HQ)Cl_2$



$Fe(HQ)_3$

The author suggested that the compound containing three deprotonated 8-hydroxyquinoline molecules $Fe(HQ)_3$ is the only one among all the possible complexes which is capable of penetrating through the cell membranes of the microorganism. He believes that coordinatively unsaturated complexes are bound too strongly to membranes. After entering the cell, $Fe(HQ)_3$ decomposes with formation of 1 : 1 and 1 : 2 complexes, which exhibit a biological activity. The view that the most lipophilic complex exhibits a toxic activity on pathogenic microorganisms is supported by the fact that the introduction of hydrophobic groups into the 8-hydroxyquinoline molecule weakens the antibacterial activity of the complex.

Similar conclusions were reached in a study[6] confirming that only coordinatively saturated and neutral copper(II) complexes with low-molecular-weight lipophilic plasma components can permeate through cell membranes.

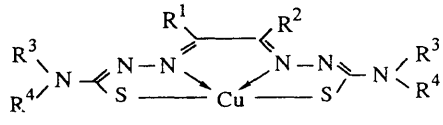
It has been shown[7] that the copper(II) complex of ceruloplasmin (CPL), which is a copper-containing serum α-2-glycoprotein, is synthesised in the liver and then enters the serum and the blood plasma. The physiological functions of ceruloplasmin involve oxidase and ferroxidase enzyme activities and participation in the iron and copper metabolisms in the organism involving transport of copper(II) ions through plasmatic membranes.

It is stated in the above monograph that bisthiosemicarbazones, which tetradentate complex-forming substances, exhibit an appreciable antitumour activity. The derivative in which R^1 = ethoxyethyl and $R^2 = R^3 = R^4 = H$ [ketoxal bisthiosemicarabazone (H_2KTS)] has been most thoroughly investigated. The effect of these compounds is due to the binding of copper(II) ions with formation of a therapeutically active complex:

$$H_2KTS + CuX_2 \longrightarrow CuKTS + H_2X.$$

This complex has been isolated and investigated in solutions and in the solid state. According to X-ray diffraction data, it has a square planar structure.

By virtue of its high stability ($\lg\beta = 18.4$), the complex is almost incapable of forming axial adducts and of entering into substitution reactions. It can therefore be regarded as coordinatively saturated.



CuKTS

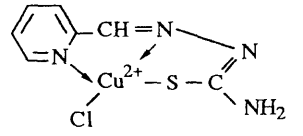
It has been demonstrated by polarographic and potentiometric methods that the compound is capable of undergoing one-electron reduction. The reaction with thiols (for example, glutathione) proceeds via a mechanism involving three stages:

$$Cu^{(II)}KTS + RSH \rightarrow Cu^{(I)}SR + {}^1/_2RSSR + H_2KTS,$$
$$CuKTS + RS^- \rightleftharpoons CuKTSRS^-,$$
$$CuKTSRS^- + RSH \rightarrow Cu^{(I)}SR + HKTS^- + RS^{\cdot},$$
$$2RS^{\cdot} \rightarrow RSSR.$$

It has been noted that the rate of oxidation of thiols is correlated with the cytotoxic activity of the complexes. It is believed that H_2KTS enters the cell only as a result of the complex formation reaction with copper(II). If the reaction does not occur, then the ligand is localised in the lipophilic part of the membrane. On reduction of the complex in the cell and the formation of $Cu^{(I)}SR$, the copper is probably distributed between the cell thiols and moves to the interior of the cell, while the H_2KTS ligand emerges from the cell. This inhibits the synthesis of DNA and the mitochondrial oxidative phosphorylation, which constitutes ultimately the cytotoxic activity of the complex.

The example analysed above describes the mechanism of the action of the coordinatively saturated complex on the cell membrane, complex formation occurring on the surface of the membrane with participation of the metal ion located within the components of the tumour cell. Only as a result of this process is the physiologically active complex transported through the biological membrane.

The case of a coordinatively unsaturated complex occurs when 2-formylpyridine thiosemicarbazone (Hα) reacts with copper(II). In contrast to CuKTS, the complex $[Cu\alpha Cl]^0$, where α is the tridentate ligand



enters readily into adduct formation reactions, for example, with ethylenediamine. The stability of the complex [CuαenCl] is indicatated by $\lg\beta = 5.53$. The complex [CuαCl] is reduced on interaction with thiols within the cell, but does not decompose, in contrast to CuKTS, and affords an adduct.

The effect of platinum and platinum group metal complexes, which exhibit an appreciable antitumour activity, on biological membranes is of special interest. It was noted in 1969 that certain platinum complexes exhibit an anticarcinogenic activity. The influence of the charge, geometrical structure, stability, etc. of the complexes on their antitumour activity was investigated. It was noted that the absence of antitumour activity in charged platinum complexes is associated with their inability to permeate through cell membranes. The interaction of *cis*-dichlorodiamineplatinum(II) with a phosphate transferring agent in mitochrondia has been investigated.[8] It was suggested that the fully or partly hydrolysed complex interacts in mitochondria with a phosphate transferring agent and modifies the SH group in the latter. According to the authors, such interaction can partly account for the antitumour activity of the complex.

Interesting data have been obtained by Stetsenko et al.[9] in a study of the electrical conductivity of a NaCl solution ($c = 1 \times 10^{-2}-0.15$ M) on treatment with $[Pt(NH_3)_2Cl_2]$ isomers ($c = 3 \times 10^{-3}$ M). The reason why *cis*-$[Pt(NH_3)_2Cl_2]$ influences the electrical

conductivity of the solution is the associative interaction of the ion–dipole type between the Cl^- ions and the polar molecules (of the *cis*-isomer). The existence of a direct relation between the antitumour activity and the ability of *cis*-$[Pt(NH_3)_2Cl_2]$ to reduce the electrical conductivity of the electrolytes suggests that the association of the biologically active *cis*-isomer promotes its retention within the extracellular liquid, where the concentration of chloride ions is high, and its supply in an unchanged form through the cell membrane to the site of the reaction with the DNA of the tumour cell.[9]

Bearing in mind the size of the complex molecule (the distance between the chlorine atoms is 340 pm) and the pore radius in the cell membrane (400 pm), through which the solutes pass in either direction relative to the concentration gradient, it has been suggested[7] that *cis*-$[Pt(NH_3)_2Cl_2]$ permeates the cell as a result of passive diffusion. This mechanism has been confirmed by experiments which make it possible to distinguish passive diffusion from active transport. The rate of accumulation of *cis*-$[Pt(NH_3)_2Cl_2]$ in the cells of the Ehrlich ascites tumour has been determined and the data were analysed on the basis of the kinetic Michaelis–Menten equatiion. The hypothesis that *cis*-$[Pt(NH_3)_2Cl_2]$ passes through membranes by means of diffusion is supported by the finding that the rate of accumulation of the preparation in the cells is not affected by substances promoting membrane permeability.[7]

The biological activity of compounds is in many respects determined also by their ability to alter the functional activity of membrane structures. The complexes inhibit the activity of membrane enzymes, interacting with the surface or deep-seated SH groups of ATPase. Thus in the anionic Pt^{2+} and Pd^{2+} complexes having the composition $K_2[MX_4]$, where X = NO_2^-, Cl^-, Br^-, I^-, or SCN^-, the inhibiting properties vary in different ways with increase in the ability of the ligands to redistribute the electron density from the complex-forming ion in the above series of ligands X. For palladium(II), the inhibiting activity of the complexes increases, while for platinum(II) it diminishes. This indicates different mechanisms of the activity of the complexes. The Pt^{2+} compounds are reduced to zerovalent platinum on contact with the enzyme, forming "couplings". On the other hand, the Pd^{2+} compounds act as electron acceptors, forming complexes with the electron-donating groups of the enzyme.[10]

It has been noted that in the series of compounds $Q_2[PdBr_4]$, where Q = K^+, $(CH_3)_3NH^+$, or $N(C_2H_5)_4^+$, the inhibiting activity of the membrane ATPase diminishes with increase in the size and hydrophobicity of the cation. The large dimensions of the cations and ionic associated species probably hinder the approach through complexes to the active centre of the enzyme, as a result of which $[N(C_2H_5)_4]_2[PdBr_4]$ does not have an inhibiting activity. The same behaviour has been observed for copper(II) complexes—$[(CH_3)_3NH]_2[CuCl_4]$ and other compounds.

The intensification of studies on substances the mechanism of the biological activity of which is associated with their interaction with biological membranes has been promoted by the studies of Mueller et al.[1] They were able to develop a method for the formation of bilayer lipid membranes constituting an adequate model of the lipid matrix of the membranes in any living organisms.

A number of investigations of the effects of various reagents on BLM have been carried out using this model.[11] However, only isolated communications have been devoted to studies on coordination compounds.

Thus it has been shown[12] that, in the presence of Cu^{2+}, Zn^{2+}, Ni^{2+}, and Hg^{2+} ions as well as tetrachlorotrifluoromethylbenzimidazole (TTFB), the bilayer lipid membranes comprising brain lipids have an N-shaped voltage–current characteristic (VCC). The decrease in the conductivity of the BLM with increase in voltage has been explained by the formation of coordination compounds of the type $[M(TTFB)_n]^{2+}$ It has been suggested[13] that the agent effecting transfer through the membrane is in this case the complex of the metal cations with TTFB and the reason for the appearance of the descending section on the voltage–current characteristic is the decomposition of the complex under the influence of the potential difference between the lipid phase of the membrane and the aqueous solution.

There have also been a few studies on the transport of metal cations through the lipid bilayers of liposomes which takes place with participation of organic ligands. For example, free aliphatic acids increase the permeability of liposomes to Yb^{3+}, Tb^{3+}, Fe^{3+}, Fe^{2+}, and Co^{2+}.[14]

It has been shown[15] that hypoglycemic sulphonylureas (glibenclamide and gliclazide) increase the permeability of sonicated dipalmitoylphosphatidylcholine vesicles to Pr^{3+} and Ca^{2+}.

In view of the inadequacy of the published factual data and the lack of systematic studies of the membrane activity of coordination compounds of metals, which constitute one of the most important preconditions for the creation of a theory of the specific synthesis of biologically active substances, we undertook a study of the molecular mechanisms of the interaction of coordination compounds of metals and planar BLM to which a number of studies have been devoted.[16–30] In each specific case, the principal problem which had to be solved was that of the composition of the membrane-active form of the complex.

In order to investigate the nature of the absorbing and permeating species, use was made of the methods for the generation of a transmembrane electrical potential differences (EPD) for an asymmetric addition of the reagents to the BLM,[31] the method based on the VCC for BLM which also exist under asymmetric conditions as regards the concentration of the reagents, as well as the method involving the measurement of the intramembrane electrical potential difference based on the conpensation for the second harmonic of the alternating current.[32]

In many instances the central ion in the complex can be coordinatively unsaturated in relation to the ligand. It is then possible to postulate the formation of a membrane-transport form having the compositions central ion–organic ligand–electrolyte anion. When this is taken into account, the mechanisms of the effect of coordinatively saturated and unsaturated compounds on BLM should differ significantly. We carried out an experimental test of these hypotheses in chloride solutions and investigated the mechanisms of the transport of chloride anions by coordinatively unsaturated metal-containing compounds.

It must be emphasised that substances which function as transferring agents for chloride anions through a membrane should undoubtedly exhibit an appreciable biological activity. This is indicated by data showing the importance of maintaining a chloride homeostasis for the normal functioning of the living cell. For example, the membrane potentials of mitochondria and liver cells have a chloride basis.

III. The mechanism of the influence of coordinatively unsaturated compounds on the ionic conductivity of bilayer lipid membranes

Complexes with various biological properties were chosen as objects for the study of the activity of coordinatively unsaturated compounds. Thus the copper(II) complex of ε-caprolactam (CL)—the preparation phyton (kartocid)—exhibits a high fungicidal activity against the agent responsible for false mildew.[33]

Phyton L (a laboratory preparation) was obtained by refluxing copper(II) chloride and ε-caprolactam in molar proportions of 1 : 3 in dry alcohol over a period of 1 h. According to X-ray diffraction data, a single crystal of phyton L has an ionic structure.[34] The molecule consists of the cations $[Cu(CL)_6]^{2+}$, having a crystallographic symmetry such that the Cu^{2+} cation is a centre of symmetry, and of the anions $[Cu(CL)Cl_3]_2^-$. Thus the formula of phyton L is $[Cu(CL)_6][Cu(CL)Cl_3]_2$. ε-Caprolactam is a monodentate ligand and is coordinated to Cu^{2+} via the oxygen atom.

Phyton T (technical preparation) is dichloroaquotricaprolactamcopper(II) $Cu(CL)_3Cl_2H_2O$. Phyton T is synthesised by fusing together the initial components.

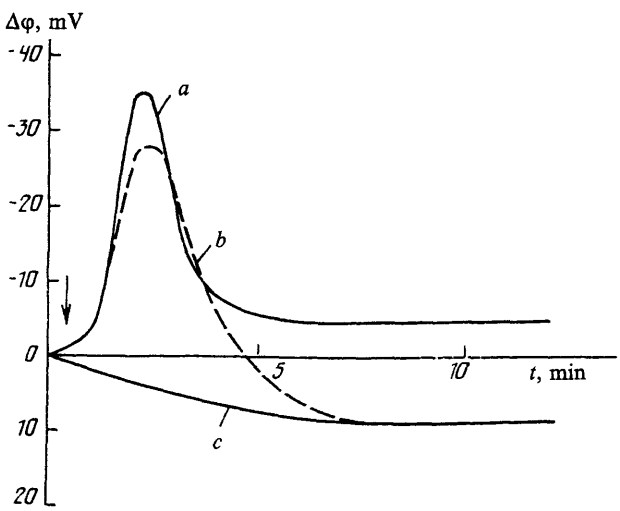


Figure 1. Typical curves for the generation of the transmembrane electrical potential difference following the introduction of reagents on one side of a brain lipid BLM; the arrow indicates the instant of the introduction of the reagents: *a*, $CuCl_2$; *b* and *c*, phyton; electrolyte—0.01 M KCl.

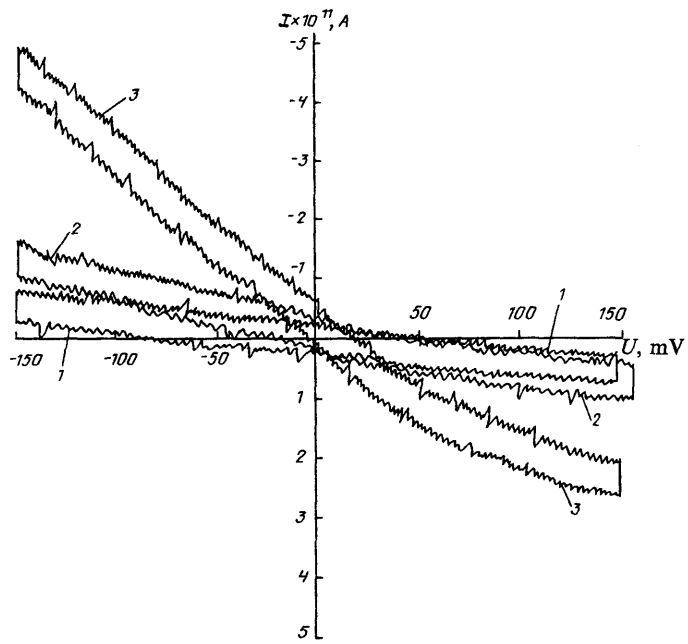


Figure 2. The voltage—current cyclic characteristics of brain lipid BLM in the presence of various concentrations of phyton on one side: *1*, in the absence of phyton; *2*, 1.7 × 10^{-4} M; *3*, 5.4 × 10^{-4} M; rate of scanning of the potential 1 mV s^{-1}; electrolyte—0.01 M KCl.

Fig. 1 presents typical curves for the generation of the transmembrane electrical potential difference when $CuCl_2$ and phyton are introduced on one side of the BLM. The introduction of ε-caprolactam did not generate a potential difference, which indicates its non-ionic nature. The nature of the transmembrane potential difference, generated on the BLM after the introduction of membrane-active agents on one side, has been studied in detail in relation to ionogenic detergents.[31,35] It was shown that the form of the potential growth—potential decrease curve depends significantly on the magnitude and sign of the charge on the adsorbing species and on the surface area of the BLM and is determined by two processes—the adsorption on the BLM of ions of membrane-active substances as well as the transmembrane transfer of these ions and the ions contained in the electrolyte solutions. The stationary potential is diffusional in nature and arises as a consequence of the transmembrane gradient of the adsorbing ions. The rate of generation of the potential is determined by the rate of adsorption of Cu^{2+} on the surface of the BLM, while the rate of dissipation of the charge is determined by the rate of the transmembrane transport (i.e. by the resistance of the membrane). The maximum on the potential growth—potential decrease curve shows that the transfer of ions through the membrane is slower than their adsorption. The existence of an excess negative charge on the BLM comprising cattle brain lipids[36] apparently determines the specific adsorption of the ions on the membrane surface.

For the asymmetric addition of dimethylformamide solutions of phytons L and T to the electrolyte surrounding the BLM, a positive charge arose also in the compartment with the phyton (Fig. 1*b*). The positive sign of the stationary potential difference indicated the preferential passage through the BLM of the negatively charged (anionic) form of phyton. Such a species was likely to have been $[Cu(CL)Cl_3]^-$. Sometimes a negative potential was recorded in the compartment with phyton and was then reversed in the course of 5—10 min (Fig. 1*c*). In this case, the curve representing the

variation of the membrane potential had a maximum, which is apparently associated with the adsorption of the coordinatively saturated complex cations $[Cu(CL)_6]^{2+}$.

The anionic nature of the membrane-transport form of phyton is indicated also by the symmetrical form of the voltage–current characteristics recorded in the presence of phyton (Fig. 2). Evidently, in the absence of the modifying agent, the voltage–current characteristic for the BLM is linear in the potential range ± 150 mV. In the presence of phyton on one side, the BLM acquired rectifying properties. For the negative polarisation of the compartment with phyton, the current was greater than for the positive polarisation, which indicated the appearance of preferential anionic conductivity in the BLM.

The addition of phyton affected also the electrical conductivity of the BLM. The unmodified BLM has a low conductivity of the order of $10^{-9}-10^{-8}$ S cm^{-2}. The introduction of $CuCl_2$ diminished slightly the electrical conductivity while the addition of ε-caprolactam did not affect the conductivity of the BLM. Under the influence of dimethylformamide solutions of phytons L and T, the electrical conductivity of the BLM increased approximately by a factor of 100 (Fig. 3). The non-linearity of the dependence of the electrical conductivity of the BLM on the concentrations of the reagents is noteworthy. At a phyton concentrate close to 10^{-2} M, the structure of the BLM was affected and became unstable.

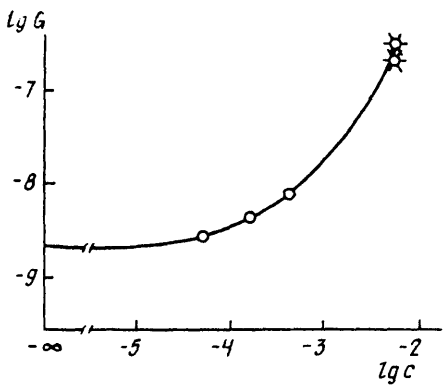


Figure 3. Dependence of the conductivity (G, S cm^{-2}) of the BLM on the phyton concentration (c, M) on one side (the points represented by stars denote the conductivity of BLM measured 20 min after the introduction of the reagent).

The solubility of the permeating anion in lipids may be imparted to it by the presence in its composition of a ε-caprolactam molecule, which exhibits definite lipophilic properties. The logarithm of the partition coefficient of ε-caprolactam in the octanol–water system, which is usually adopted as a measure of the lipophilicity, is $\lg K_{o/w} = 1.03$.[37]

The set of data obtained suggested that the coordinatively unsaturated complex between $CuCl_2$ and ε-caprolactam should be capable of combining with chloride ions and of transporting them across the membrane, whereas the coordinatively saturated complex of the above substances does not possess this property.

By altering the conditions in the synthesis, the coordinatively unsaturated compound $[Cu(CL)Cl_2(H_2O)]$ (yellow colour, lamellar form of the crystals) was isolated from the aqueous or aqueous ethanol solution with the ratio $CuCl_2$:CL = 1:3. The coordinatively saturated compounds $[Cu(CL)_6Cl_2](CL)_2$ (green colour, acicular form) was obtained from benzene solutions after adding to $CuCl_2$ an excess (more than 3 mol per mole of $CuCl_2$) of ε-caprolactam.[21] The study of the membrane activity of these compounds showed that $[Cu(CL)Cl_2(H_2O)]$ does indeed possess membrane activity similar to that of phyton T, i.e. it forms an ionic membrane-transport form having apparently the composition $[Cu(CL)Cl_3]^-$. On the other hand, the effect of $[Cu(CL)_6Cl_2](CL)$ reduced to the adsorption of the cationic complex on the surface of the BLM and did not differ qualitatively from the effect of the simple Cu^{2+} salt.

Another important biologically active compound is the zinc complex of ethylenethiourea. Ethylenethiourea (ETU) is the main product of the metabolism of metal-containing zineb and maneb,[38] which exhibit an appreciable fungicidal activity.[39] It has been suggested that, having entered the interior of the cell, zineb and maneb undergo acid hydrolysis with formation of ethylenethiourea, which exhibits a toxic activity.[40] The absence of fungicidal activity in ethylenethiourea itself can be explained by its poor capacity for permeation through cell walls. Zineb and maneb are regarded as a kind of depot of ethylenethiourea. On the other hand, no account has been taken whatsoever of the role in transport through the cell membrane of the metal cations in the $ZnCl_2$–ethylenethiourea complex.

Up to a concentration of 2 mM, ethylenethiourea does not alter the conductivity and capacity of the BLM. It has been shown by the method of trial hydrophobic ions[41] that ethylenethiourea molecules are incorporated in the solution/BLM boundary.[25]. Under these conditions, the ethylenethiourea dipoles are oriented parallel to the dipoles of the phospholipid molecules, which increases the dipole-induced jump in the BLM potential.

The study of the complex formation reaction between $ZnCl_2$ and ethylenethiourea in aqueous solutions by spectrophotometric and conductimetric methods showed[26] that, at an ethylenethiourea concentration below 10^{-4} M, the complex is not formed even in the presence of a 60-fold excess of zinc ions. At $ZnCl_2$ and ethylenethiourea concentrations of 10^{-3} and 10^{-4} M respectively, the tetrahedral complex $[Zn(ETU)Cl_2]$, in which ethylenethiourea is a bidentate ligand, is formed. At higher ethylenethiourea concentrations, a second ethylenethiourea molecule adds to the zineb ion with formation of the tetrahedral complex $[Zn(ETU)_2Cl_2]$. Under these conditions, ETU behaves as a monodentate ligand.

The study of the effect of the complex on the BLM established[27] that it induces an appreciable growth of membrane conductivity (Fig. 4). This occurs at a concentration of the complex close to 10^{-3} M, so that, in combination with data obtained in the study of the complex formation reaction with ethylenethiourea in aqueous solutions, this shows that at a concentration less than 10^{-3} M the complex decomposes in the solution into its component parts and that the increase in the conductivity of the BLM is induced by the effect of the complex species. The threshold character of the dependence of the electrical conductivity of the BLM on the concentration of the complex suggested the possibility, in principle, that a membrane-active complex is formed from $ZnCl_2$ and ethylenethiourea directly in the electrolyte solution. The results for the influence of $ZnCl_2$ on the properties of the BLM formed in the presence of ethylenethiourea are presented in Fig. 4. Evidently, as in

the case where the complex is catalytically active, here too the conductivity of the BLM increases after the attainment of a $ZnCl_2$ concentration of about 10^{-3} M. This indicates the formation of permeating complex ions directly from simpler species either in the bulk of the electrolyte or at the membrane/solution interface.

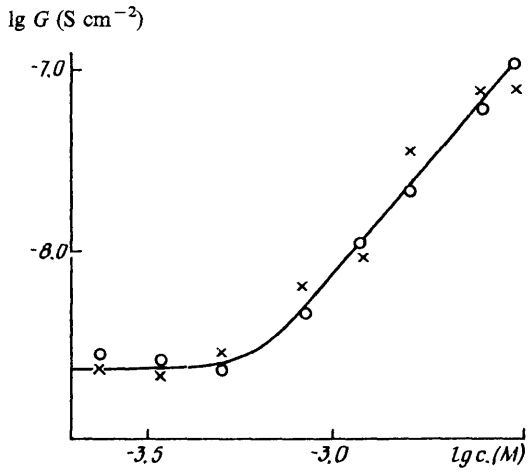


Figure 4. Dependence of the conductivity of the BLM on the concentration of the complex (circles) and $ZnCl_2$ in the presence of 2.4×10^{-3} M ethylenethiourea (crosses).

In order to determine the sign of the charge of the permeating species, a study was made of the voltage–current characteristics of BLM under asymmetric conditions. The BLM was then formed in the presence of ethylenethiourea on either side of the membrane, while $ZnCl_2$ was added on one side. Under these conditions, the voltage–current characteristics had an asymmetric form, the current being higher at a negative potential in the $ZnCl_2$ compartment. This showed that the permeating species are anions.

Measurement of the intramembrane potential difference with the aid of the second harmonic of the alternating current made it possible to determine the sign of the charge of the adsorbing species. It is seen from Fig. 5 that the position of the current I minimum (II) does not change under the influence of ethylenethiourea. This showed that ethylenethiourea does not by itself alter the intramembrane potential difference and confirmed its non-ionic nature. When $ZnCl_2$ is introduced at a concentration of 6.8×10^{-4} M in the cell compartment where ethylenethiourea had been added previously, a difference between the boundary potentials amounting to +14 mV is generated. This indicates a change in the surface charge on the *cis*-side of the BLM in the positive direction as a result of the adsorption of the Zn^{2+} cations. On subsequent increase of the $ZnCl_2$ concentration to 7.5 mM, the sign of the intramembrane potential difference is reversed, which indicates a change in the sign of the charge and hence in the nature of the species adsorbed on the membrane surface.

In this case, the anionic transport complex is apparently formed as a result of the addition of chloride anions to the coordinatively unsaturated complex $[Zn(ETU)Cl_2]$ with formation of the anion $[Zn(ETU)Cl_3]^-$. The most characteristic coordination number for Zn^{2+} cations is 4. The coordinative unsaturation of $[Zn(ETU)Cl_2]$ is associated with the impossibility of altering the denticity of the ligand. Thus one can postulate that the permeating charged species is the anion $[Zn(ETU)Cl_3]^-$. The presence of ethylenethiourea in the complex imparts a certain lipophilicity to the latter (for ethylenethiourea, $\lg K_{o/w} = 0.35$ [37]), whilst chloride anions impart to the complex a negative charge, which is more favourable for transport across the BLM. It is well known that lipophilic anions have a much greater permeating capacity than cations, because the hydrophobic region of the BLM is subject to the influence of a positive electric potential.[42]

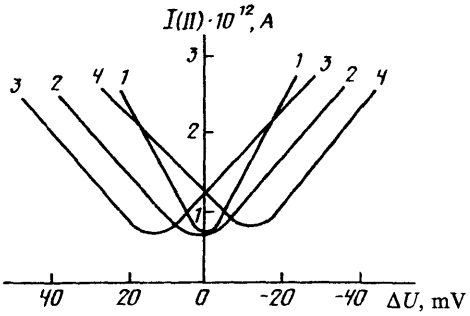


Figure 5. Dependence of the current of the second harmonic and the applied potential in the presence of 1.7×10^{-3} M ethylenethiourea on the *cis*-side: *1*, BLM in the absence of modifying agents; in the presence of modifying agents on one side: *2*, 1.7×10^{-3} M ethylenethiourea; *3*, 6.8×10^{-4} M $ZnCl_2$; *4*, 7.5×10^{-3} M $ZnCl_2$; brain lipid BLM; electrolyte—0.01 M KCl + 0.002 M acetate buffer.

Coordination compounds of cobalt(II) with *o*-phenanthroline (phen) have aroused much interest among the series of new biologically active substances.[43,44] Their appreciable catalytic activity in the oxidation reactions of biological substrates and their influence on the respiratory electron transport chain in mitochondria and on the photosynthetic apparatus of chloroplasts have been observed. Growth regulating and bactericidal activities have been found for a number of complexes. However, the membrane activity of these compounds has not been studied. At the same time, *o*-phenanthroline is appreciably lipophilic ($\lg K_{o/w} = 1.83$ [37]). This suggests a definite possibility of transmembrane transport of cobalt ions as part of coordination compounds with *o*-phenanthroline.

We investigated the effect of *o*-phenanthroline and of its coordinatively unsaturated compounds $[Co(phen)(H_2O)_2](NO_3)_2$ and $[Co(phen)Cl_2].HCl$ on BLM made up of ox brain lipids.[23,29] It was shown that, in the presence of simple salt $CoCl_2$, at a concentration of 10^{-3} M, the electrical conductivity of the BLM is reduced by 20%. On the other hand, the effect of *o*-phenanthroline or the coordination compounds $[Co(phen)(H_2O)_2](NO_3)_2$ and $[Cl(phen)Cl_2].HCl$ on BLM led to an increase in the electrical conductivity of the latter. The kinetics of this change are illustrated in Fig. 6. The effect of *o*-phenanthroline is apparently a consequence of its

lipophilic properties. In order to determine the site of location of *o*-phenanthroline in the BLM structure, the method of trial hydrophobic ions was used. It demonstrated, that, in constrast to ethylenethiourea, *o*-phenanthroline molecules do not have a particular location relative to the normal to the BLM plane. This is probably associated with the more pronounced lipophilic properties of *o*-phenanthroline compared with ethylenethiourea. The protonated form of *o*-phenanthroline, formed in the weakly acid electrolyte (pH 4.5) surrounding the membrane, is apparently the carrier of electricity through the BLM. *o*-Phenanthroline plays the role of the protonophor in this system.

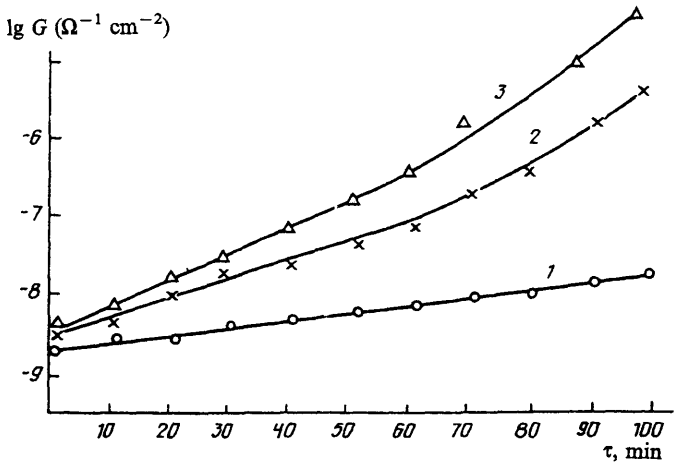


Figure 6. Kinetics of the variation of the conductivity of the BLM after the introduction of 2×10^{-4} M reagents on one side of the membrane: *1*, *o*-phenanthroline; *2*, $[Co(phen)(H_2O)_2](NO_3)$; *3*, $[Co(phen)Cl_2]HCl_2.HCl$; electrolyte—0.01 M KCl + 0.002 M acetate buffer.

The increase in the electrical conductivity of the BLM in the presence of $[Co(phen)Cl_2].HCl$ and $[Co(phen)(H_2O)_2](NO_3)_2$ is apparently induced by the passage through the BLM of lipid-soluble membrane-transport forms comprising coordination compounds.

In order to establish the sign of the charge of the permeating species, a study was made of the voltage–current characteristics of the BLM under asymmetric conditions. Fig. 7 shows that the voltage–current characteristics are asymmetric and that for $[Co(phen)Cl_2].HCl$ the current is greater at a negative potential in the cell compartment where the reagent had been added (*cis*-compartment), while in the case of $[Co(phen)(H_2O)_2](NO_3)_2$ the current is greater at a positive potential in the cell compartment. This shows that anions and cations are the permeating species in the former and latter cases respectively.

As stated above, the complexes investigated are coordinatively unsaturated. The maximum coordination number of cobalt(II) is 6, while in the given complexes it is 4, since *o*-phenanthroline is a bidentate ligand. There is therefore a possibility of a further addition to the central ion of chloride ions, a large number of which are present in the electrolyte. However, here it is necessary to bear in mind that the permeating capacity of the ions through the BLM diminishes greatly with increase in their charge, i.e. the transport of a singly-charged ion is preferred to that of a doubly-charged ion. In the case of $[Co(phen)Cl_2].HCl$, the permeating species are probably the anions $[Co(phen)Cl_3]^-$, while in the case of $[Co(phen)(H_2O)_2].(NO_3)_2$ they are the cations $[Co(phen)(H_2O)_2Cl]^+$. In the latter case, the coordination of a single chloride anion diminishes the positive charge of the ion transported. Such forms of the complex ions probably exhibit an enhanced stability in the membrane phase, i.e. in a medium with a low dielectric constant, whereas in the bulk of the electrolyte they are less stable and dissociate with elimination of chloride anions. Thus, under the influence of the electric field gradient there may be induced transport of chloride anions from one side of the membrane to the other. The greater electrical conductivity of BLM in the presence of $[Co(phen)Cl_2].HCl$ compared with $[Co(phen)(H_2O)_2](NO_3)_2$ is apparently associated with the difference between the charges of the permeating ions.[42]

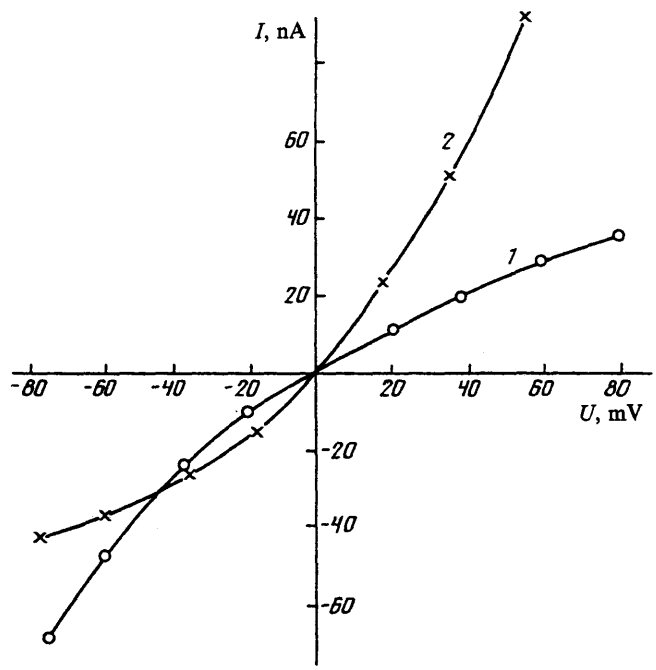


Figure 7. The voltage–current characteristics of a BLM in the presence of $[Co(phen)Cl_2].HCl$ (curve *1*) and $[Co(phen)(H_2O)_2].(NO_3)_2$ (curve *2*) on one side; the sign of the potential is indicated relative to the cell compartment to which the reagent has been added; electrolyte—0.01 M KCl + 0.002 M acetate buffer.

The chloride selectivity of BLM in the presence of the given complexes is indicated by measurements of the potential arising when a transmembrane electrolyte concentration gradient is established. For example, in the presence of 2×10^{-4} M $[Co(phen)(H_2O)_2](NO_3)_2$ with the ratio of the concentrations of the

KCl electrolyte on the two sides of the membrane amounting to 10, an ionic potential of 53 mV is developed, which corresponds to the transport number of the anions $t_{Cl^-} = 0.91$.

The influence of the coordinative saturation of the Co^{2+} ions and of the lipophilic properties of the ligands on the membrane activity of the complex compounds was investigated with the aid of the coordinatively saturated complex $[Co(phen)_2Cl_2].3.5(H_2O)$ and the coordinatively unsaturated complex [Co(dimphen)Cl_2].HCl, where dimphen is 2,9-dimethylphenanthroline. The lipophilic properties of 2,9-dimethylphenanthroline are more pronounced ($\lg K_{o/w} = 2.49$ [37]) than those of *o*-phenanthroline. It is striking that, in the presence of the coordinatively saturated complex $[Co(phen)_2Cl_2].3.5H_2O$, the electrical conductivity of the BLM does not increase. It is also seen that the more lipophilic cobalt(II) complex of the dimethyl derivative of *o*-phenanthroline is more effective than [Co(phen)Cl_2].HCl. In the presence of [Co(dimphen)Cl_2].HCl on one side of the membrane, the voltage–current characteristics of BLM are asymmetric (the membrane current at a negative potential in the *cis*-compartment is greater than at a positive potential).

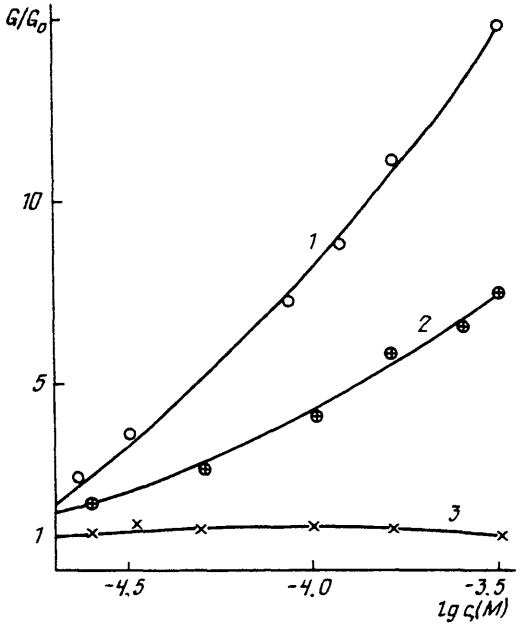


Figure 8. The influence of cobalt(II) complexes of *o*-phenanthroline and its dimethyl derivatives on the relative conductivity of the brain lipid BLM: *1*, [Co(dimphen)Cl_2].HCl; *2*, [Co(phen)Cl_2].HCl; *3* $[Co(phen)_2Cl_2].3.5H_2O$; electrolyte—0.01 M KCl + 0.002 M acetate buffer.

The study of the ionic selectivity of BLM in the presence of the given complexes by the method involving the measurement of the diffusion potential under conditions where a transmembrane chloride ion gradient has been established demonstrated the chloride selectivity of BLM in the case of coordinatively unsaturated complexes (the Cl^- transport numbers were 0.91–0.93) and the absence of ionic selectivity in the case of the coordinatively saturated complex. The results permit two conclusions: firstly, the coordinatively saturated complexes are unable to transport chloride ions through BLM and, secondly, an increase in the lipophilicity of the coordinatively unsaturated complexes improves their membrane-transport properties in relation to chloride anions.

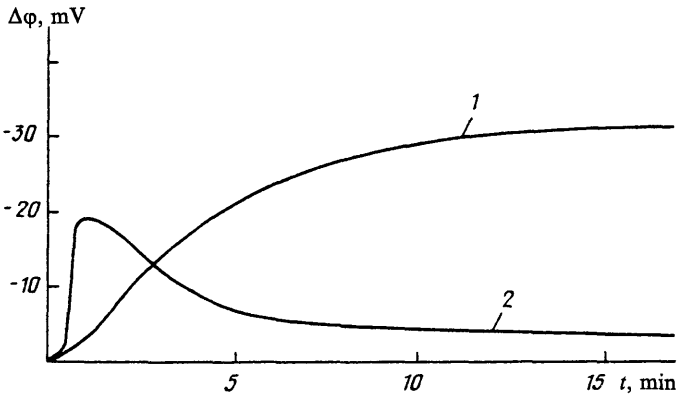
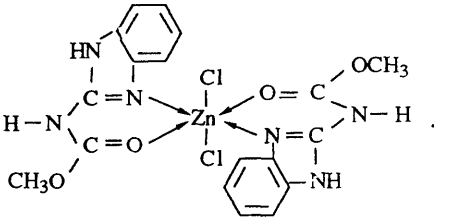


Figure 9. Generation of the transmembrane potential difference after the introduction of 2.7×10^{-6} M $[Zn(BMC)_2Cl_2]$ (curve *1*) and 2.5×10^{-4} M $ZnCl_2$ (curve *2*) on one side of the membrane.

IV. The mechanism of the influence of coordinatively saturated metal complexes on the ionic conductivity of bilayer lipid membranes

The coordinatively saturated complex of $ZnCl_2$ with *N*-methyl-(2-benzimidazolyl)carbamate (BMC) exhibits a fungicidal activity.[45] Its structure and properties have been described.[46] The complex $[Zn(BMC)_2Cl_2]$, where BMC is a bidentate ligand, has an octahedral configuration:



In order to determine the sign of the charge of the membrane-active species, a study was made of the generation of the transmembrane potential difference after the introduction of $[Zn(BMC)_2Cl_2]$ and $ZnCl_2$ on one side of the BLM (Fig. 9). When both reagents were added, the potential difference had a minus sign in the *cis*-compartment. The form of the curve in the case where $ZnCl_2$ was introduced indicated a facilitated adsorption of the cations and a negatively charged surface of the BLM made up of ox brain lipids. The absence of a maximum following the introduction of $[Zn(BMC)_2Cl_2]$ indicated the retardation of the stage involving the adsorption of ions formed on dissociation of the above complex compared with the stage involving their transport. The sign of the generated potential difference shows that the permeating ions are cations.

Additional evidence indicating that the permeating species are cations on exposure of the membrane to $[Zn(BMC)_2Cl_2]$ was provided by the voltage–current characteristics of the BLM recorded after the introduction of the reagent on one side only. They had the rectification form—the current corresponding to positive polarisation in the *cis*-compartment was higher than that corresponding to the negative polarisation. The cationic transport complex is apparently formed as a result of the hydrolysis involving the elimination of a chloride ion:

$$[Zn(BMC)_2Cl_2] + H_2O \rightleftarrows [Zn(BMC)(H_2O)Cl]^+ + Cl^- .$$

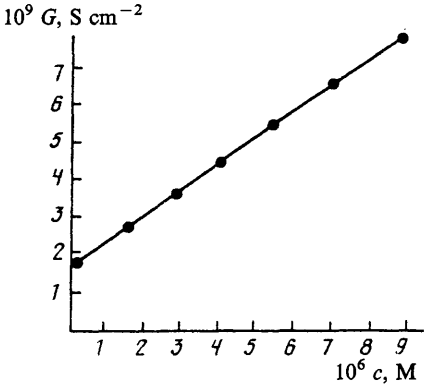
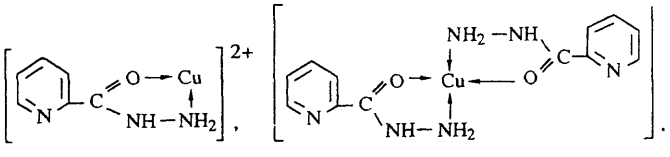


Figure 10. The influence of the $[Zn(BMC)_2Cl_2]$ concentration on the conductivity of the ox brain lipid BLM; electrolyte—0.01 M KCl + 0.002 M acetate buffer.

The transport of cations is ensured by the lipophilic properties of the BMC molecules ($\lg K_{o/w}$ = 0.35 [37]) and also by the low electric charge on the complex (+1) and apparently proceeds via a mechanism involving the direct passage of hydrophobic ions through the BLM. Evidence for this mechanism of the transmembrane transport of the cations $[Zn(BMC)_2(H_2O)Cl]^+$ is provided by the linear dependence of the conductivity of the BLM on the concentration of the cations (Fig. 10). Such linear dependence is characteristic of the direct passage of hydrophobic ions through the BLM.[41]

The complex of gallium(III) chloride with isonicotinic acid hydrazide (INAH), having the formula $[Ga(INAH)_3]Cl_3.H_2O$, is coordinatively saturated. Isonicotinic acid hydrazide (isoniazide) inhibits the growth of tuberculosis bacteria. The main side effect of isoniazide therapy is liver damage. On the other hand, the use of isoniazide in a complex with copper ions makes it possible to reduce greatly the negative side effect of the preparation. The copper(II) complex of INAH is an active antitumour agent. The protonated forms of the complex exhibit a biological activity:



It is believed [7] that the role of the metal reduces to creating a liposoluble compound which enhances the transport of the physiologically active ligand through the biological membrane. The use of isoniazide in a complex with the triply charged gallium cation can probably lead to new effects in therapy using this preparation.

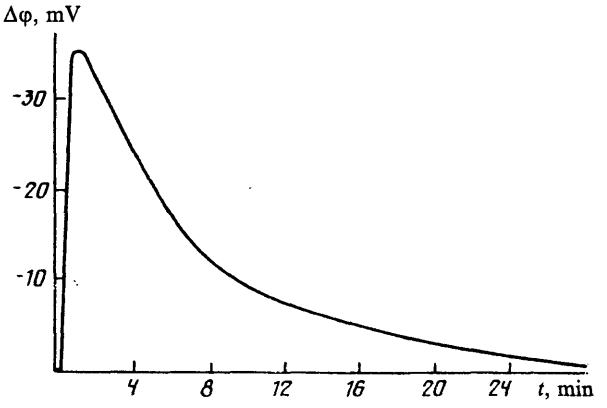


Figure 11. Generation of the transmembrane potential difference following the introduction of 1.6×10^{-6} M $[Ga(INAH)_3]Cl_3$ on one side of the membrane; the instant of the introduction of the reagent was adopted as the zero time; electrolyte—0.01 M KCl + 0.002 M acetate buffer.

We investigated the membrane activity of the $GaCl_3$ complex with isoniazide in relation to ox brain lipid BLM.[28] A typical potential difference generation curve after the introduction of the complex on one side of the BLM is illustrated in Fig.1. The minus sign of the potential difference in the *cis*-compartment of the cell indicates the adsorpton of the positively charged complex species on the BLM surface. It is seen that the residual (diffusion) potential diminishes almost to zero, which indicates the absence of the transmembrane transport of any kind of ionic form of the reagent. The maximum on the curve indicates an appreciable adsorption capacity of the complex, which is associated with the presence in its composition of the complex cation with the +3 charge.

The presence of the lipophilic isoniazide organic ligand ($\lg K_{o/w}$ = −0.5 [37]) leads to an increased adsorption capacity of the complex compared with the simple gallium salt $Ga(NO_3)_3$ (Fig. 12). When account is taken of the equality of the charges on the adsorbing species (+3), the higher value of $\Delta\varphi$ corresponds to a greater amount of cations bound to the BLM surface.

Studies on the ionic selectivity of BLM in the presence of the $GaCl_3$ complex with isoniazide demonstrated the absence of the ability of the latter to combine with and transport chloride anions to the BLM. On the other hand, comparison of the concentration variations of the relative conductivities and capacities of the BLM formed from n-octane and n-tetradecane solutions of lipids showed that, with increase in the concentration of the reagent, the properties of the BLM vary as a consequence of structural rearrangements in them (Fig. 13). It is essential to note the qualitative difference between the concentration variations of the capacities of the

membranes formed from n-octane and n-tetradecane lipid solutions, which is due to the different contents of the hydrocarbon solvent in the BLM.[22]

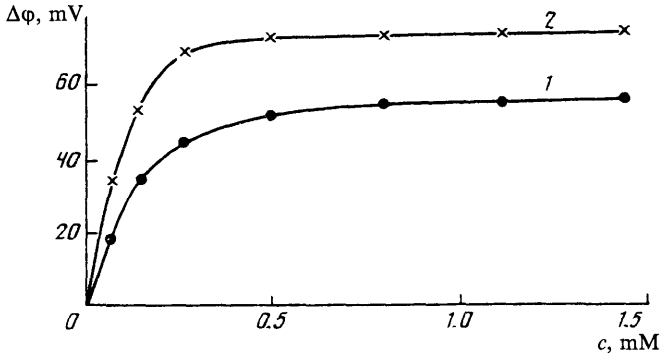


Figure 12. Dependence of the difference between the boundary potentials of the brain lipid BLM on the concentrations of $Ga(NO_3)_3$ (curve *1*) and $[Ga(INAH)_3]Cl_3$ (curve *2*); electrolyte—0.01 M KCl + 0.002 M acetate buffer.

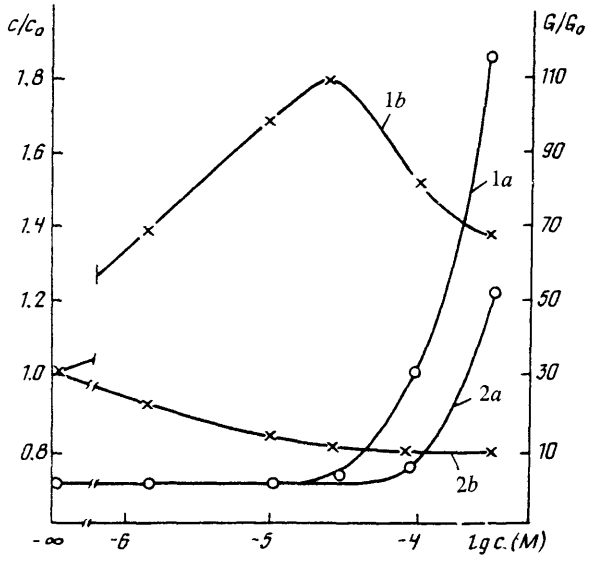


Figure 13. Dependence of the change in the relative conductivity (*a*) and relative capacitance (*b*) of the BLM in n-octane (curve *1*) and n-tetradecane (curve *2*) on the $[Ca(INAH)_3]Cl_3$ concentration; c_0 and G_0—the capacitance and electrical conductivity of the unmodified membranes.

V. Conclusion

The systematic studies which have been carried out make it possible to put forward the following considerations concerning the mechanisms of ion transport through biological membranes with participation of metal coordination compounds. There is no doubt about the validity of the hypothesis that the complex species is more favourable as a transport form for the transfer of both heavy metal ions and lipophilic ligands through the cell membrane.

However, the mechanisms of their action depend on a number of factors: coordinative unsaturation, charge, and stability of the complex, the lipophilicity of the ligands, etc. Under these conditions, the mechanism of the actual interaction of the complexes with the biological membrane, frequently accompanied by a change in its structure, is quite distinct.

Allowance for these phenomena is a necessary precondition for the specific synthesis of physiologically active substances with a wide range of therapeutic activities.

Experimental data for the effects of different complexes on BLM have shown that the most important factor in the interaction is the coordinative saturation of the complexes and the lipophilic ligands. It is known that the effect of hydrophilic heavy metal salts is associated with adsorption on the surface of the biological membrane. An increasing number of data have appeared recently showing that the specific chemical properties of the metal cations as well as the structure and properties of the adsorption centres are strongly manifested in this kind of binding process. Here it must be emphasised that in the case of BLM the above interaction does not entail an increase in the conductivity of the BLM.

However, an increase in the lipophilicity of the reagent should lead to an increase in the membrane activity of the heavy metal salts. Thus on coordination of the lipophilic ε-caprolactam, the complex $[Cu(CL)Cl_2(H_2O)]^0$ causes an appreciable increase in the activity of the BLM.

The use of the lipophilic 8-hydroxyquinoline ($\lg K_{o/w} = 1.13$) as the ligand and the formation of the compound $[Cu(HQ)_2]^0$ increase the electrical conductivity of BLM approximately by an order of magnitude. The membrane activity of the trichlorophenol (TCP) complex of copper(II) $[Cu(TCP)_2]^0$, where the lipophilicity of each TCP molecule is $\lg K_{o/w} = 3.7$, increases even further.

All the complexes indicated are coordinatively unsaturated but even the coordinatively saturated compound $[Zn(BMC)_2Cl_2]^0$, in which the BMC molecule has a definite lipophilicity $\lg K_{o/w} = 0.35$, constitutes an energetically favourable transport form whereby transfer through a biological membrane is effected.

On the other hand, the complexes which do not include lipophilic molecules do not exhibit a membrane activity. Thus in the presence of $[Cu(NH_3)_4]SO_4$ there is no change in the electrical conductivity and capacity of BLM up to a concentration of 2 mM. The lipophilicity parameter for ammonia molecules is low, amounting to $\lg K_{o/w} = -1.5$. The low membrane activity of $[Cu(NH_3)_4]SO_4$ compared even with $CuSO_4$ is associated with the stability of the complex ion whose instability constant is 10^{-12}.

The effect of ligands (ethylenethiourea, BMC, 8-hydroxyquinoline, ε-caprolactam, *o*-phenanthroline, etc.) does not alter the permeability of BLM to K^+ and Cl^- ions, which is associated with their non-ionic nature and probably with the absence of a marked surface activity of such species. On the other hand, polar molecules are able to be incorporated in the BLM structure. The dipoles of such molecules are then oriented in a different manner relative to the dipoles of the lipid molecules. By becoming incorporated similarly in BLM, polar molecules alter the dipole-induced potential jump at the BLM/aqueous solution interface, which may affect the change in the permeability of membranes to metal cations.

A significant difference between the mechanisms of the action of coordinatively unsaturated and saturated complexes is that, as

observed for the first time by ourselves, the coordinatively unsaturated complexes become transferring agents for chloride anions and probably other anionic substrates via a diffusional mechanism.

The substance synthesised as the agent transferring chloride anions through membranes must possess a number of properties which make possible, firstly, the addition of chloride anions to the complex-forming ion via a coordinate bond and, secondly, the transport of the chloride ions through the internal hydrophobic region of a BLM as a part of the composition of the lipophilic transport complex. This imposes definite requirements on the central ions and ligands which are determined by calculating the energy of the transport of species through BLM. The theoretical foundations of the specific synthesis of biologically active substances, inducing the transport of chloride anions through BLM, have been described in detail.[24,30] We shall describe only the main ones.

The principal property of the ligand molecules in the process indicated is their ability to impart lipophilic properties to the transport complexes formed, which enables them to permeate through the internal hydrophobic region of the BLM. On the other hand, it has been shown[48] that the permeability of the membrane to extremely hydrophilic and extremely hydrophobic molecules is very low. The optimum partition coefficient $\lg K$ in the organic solvent/water system, adopted as a measure of the hydrophobicity of the molecule, is in the range $-1 \leqslant \lg K \leqslant 1.5$.

The coordinatively unsaturated complex species, with which the anionic substrate combines, must have a positive electrical charge or, in the absence of the latter, a dipole moment. On the basis of electrostatic considerations, a +1 charge is optimum. In the absence of electrostatic attraction of chloride anions to the coordinatively unsaturated complex, one can assume, taking into account the non-equivalence of the coordination, that combination with chloride ions does not occur. This is indicated by the results of a study[9] where it was shown that the addition of chloride anions is observed for the polar *cis*-$[Pt(NH_3)_2Cl_2]$ and is not observed for the non-polar *trans*-$[Pt(NH_3)_2Cl_2]$ if it is assumed that both compounds are coordinatively unsaturated.

One can postulate that the mechanism described plays a definite role in the transport systems of biological membranes. A complex transport form having the composition coordinated ion–lipophilic ligand–hydrophilic substrate is then created. Aminoacids, polypeptides, as well as purine and pyrimidine bases, which are components of protein molecules, can play the role of ligands.[7] By virtue of their lipophilic and polar properties, the above ligands can be incorporated in the lipid bilayer and, furthermore, are able to interact with transition metal cations. This results in the formation of a compound, coordinatively unsaturated by the ligand, which can combine with chloride anions and effect their transfer. In a real biological system, other hydrophilic substrates (dihydrogen phosphate, hydrogen carbonate, carboxylate, etc. anions) can also take the place of chloride ions.

Hydrolytic enzymes, in particular carbonic anhydrase and carboxy- peptidase, which are coordinatively unsaturated compounds of zinc and histidine or with histidine and glutamic acid, can probably serve as examples of such systems in living organisms. Proof of the direct binding of a chloride anion to zinc in carbonic anhydrase was obtained from line broadening in the ^{35}Cl NMR spectra.[49] Similar results have been obtained for other anionic substrates and in the case of carboxypeptidase.

Another mechanism of the interaction of coordination compounds with cell membranes is that involving a change in the structure of the membranes. The change in the structure of membranes, in particular BLM, is associated with the charge of the active complex species. It is noteworthy that anionic complexes do not interact with BLM because of the presence of a negative charge on its surface. In the presence of highly lipophilic ligands, the latter can only be incorporated in the lipid bilayer.

Coordination compounds with a +2 charge and above are adsorbed on the BLM surface and the adsorption is stronger the higher the charge of the adsorbing species for identical lipophilicities of the ligands in the coordination sphere of the complex.

We have shown,[22,28] in relation to the interaction of the gallium complex of isonicotinic acid hydrazide $[Ga(INAH)_3]^{3+}$ with BLM, that the properties of the bilayers change as a consequence of the compaction of their structure caused by the decrease in the electrostatic forces repelling the polar heats of the lipid molecules in the adsorption of complex cations on the BLM surface. The concluding stage of the increase in the electrical conductivity of BLM with increase in the concentration of the complex involves the phase transition of the BLM from the liquid-crystal to the gel state. The increase in the permeability of bilayers in such phase transitions has been pointed out.[50] This results in an increase in the thickness of BLM, which do not contain the solvent, this being explained by the transition from the *gauche*-conformation to the fully *trans*-conformation in the lipid molecules. The increase in the conductivity of BLM under the influence of a cationic complex in the phase transitions can be induced by the local formation of micellar and hexagonal phases, whose permeability is high, and also by the appearance of lipid domains at the boundaries of which there is a possibility of the diffusion of ions. For the BLM obtained from individual lipids, the change in conductivity during the phase transition has a discrete character. In our case, the change becomes more marked slowly and smoothly, which is a characteristic feature of the phase transitions in BLM formed from a mixture of lipids. Under these conditions, the conductivity increases significantly only in the region of high concentrations of the complex.

The data obtained for the mechanisms of the action of complexes are important for the development of the specific synthesis of biologically active substances and must be taken into account in discussing the physiological properties of such compounds.

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