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Review

Influence of amphiphilic compounds on membranes★✪

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On the basis of Gortel & Grendel (*J. Exp. Med.*, 1925, 41, 439–494) discovery, the importance of the lipid bilayer as an integral and indispensible component of the cell membrane is discussed. In particular, attention focuses on the interaction between membranes and amphiphilic substances. The effect on membranes of quaternary ammonium salts, both in the form of pesticides and oxidants as well as organic compounds of tin and lead are discussed in greater detail.

LIPID LAYERS, MEMBRANES AND AMPHIPHILIC SUBSTANCES

The Gorter & Grendel experiment (1925) consisted of the formation of a monomolecular layer of lipids extracted from erythrocyte ghosts on a water surface. In such a layer, the polar parts of lipid molecules remain in water whereas the hydrocarbon chains locate in the nonpolar phase (Fig. 1A). Since the monolayer surface proved twice as large as the red cell surface, the authors concluded that the cell membrane constitutes a bimolecular lipid layer (Fig. 1B). Disregarding the mistakes they made (which, luckily, compensate each other (Bar *et al.*, 1966), and the fact that the bilayer itself does not constitute a biological membrane, the significance of that discovery cannot be overestimated. It was the first physically documented statement in

★75th Anniversary of Membrane Lipid Bilayer Concept.

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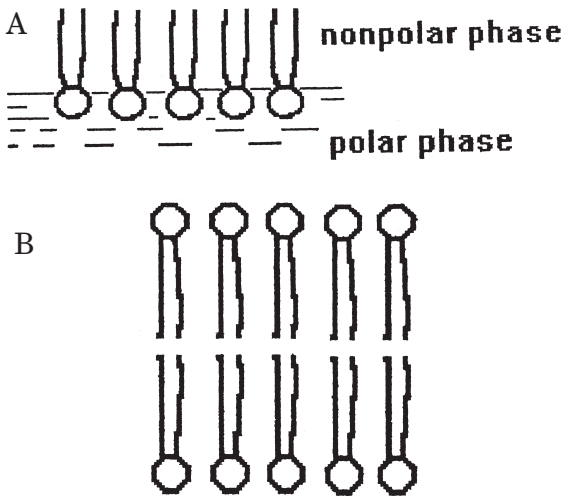
Abbreviations: QUATS, quaternary ammonium salt; BLM, black lipid membranes; CC, critical concentration.

membranology that cell lipids are arranged in a bilayer. The ensuing development of studies on biological membranes to a large degree correlated with the development of physics and its methods (e.g. electron microscopy, freeze fracture, isotopic tracer, EPR, NMR and fluorescence spectroscopy) resulting in the creation of the present, much more complex model of the membrane. Nevertheless, the lipid bilayer remained as the basic element of each new consecutive model of the biological membrane, especially of the fluid mosaic model and its modifications. Moreover, until the present time, the lipid bilayer has been used as a simple model of the biological membrane in the form of planar (bilayer or "black") lipid membranes (BLM) or liposomes.

Lipids are able to form spontaneous, entropy favoured, very diverse, liotropic, fluid crystalline structures. The type of structure formed depends to a large degree on the shape of the lipid molecules (the concept of molecular shape). A useful measure that allows the distinction of lipid molecules, from this point of view, is the dimensionless shape factor (or packing parameter), which is defined as the ratio of the cross-section area H of the head group to that of the chain C, i.e. H/C (De Kruijff et *al.*, 1985). The shape factor is defined more precisely by Izraelachvili (1994). Large values of the factor (H/C > 1) correspond to molecules of large head group section area and small chain section. For instance, the lysolecithin molecule is represented schematically as an inverted cone. The opposite situation corresponds to small H and large C value (H/C < 1). The cholesterol molecule is an example here; this molecule can be approximated by a cone. If H/C < 1 and both sections are close (this usually being the case with lecithin and other lipid molecules with two alkyl chains), the molecule can be represented as a cylinder. Schemes that correspond to the three cases (there can be more of them, in general) are presented in Fig. 1C.

Lipids of the first kind (inverted cone) prefer to aggregate as micelles in the aqueous environment, lipids of the second kind (conical) are able to form a special hexagonal phase and lipids of the third kind (cylindrical) form lamellar structures. Lamellar structures are lipid bilayers separated by water layers. Such structures are formed spontaneously in the water phase by phosphatidylcholine molecules, which commonly occur in lipid bilayers of biological membranes. This observation allows the speculation that in the early stage of evolution, the spontaneous formation of lipid bilayers gave rise to the development of biological membranes (Tien & Ottova-Leitmannova, 2000)

Biological membranes are the subject of interest of many different natural sciences. Basic research has been performed by biologists, biochemists and biophysicists; more applica-



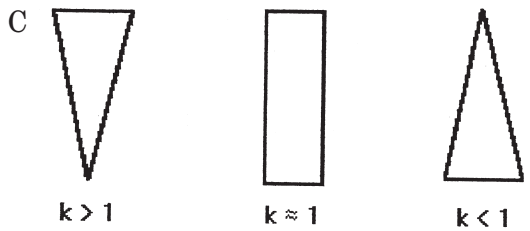


Figure 1. A. Monomolecular layer of amphiphile molecules at the interphase between polar and nonpolar phases. B. Bilayer of amphiphile molecules in a polar phase. C. The scheme presents three simple approximations of amphiphile molecules; k = H/C, H — cross-section area of the head group, C — cross-section of chains.

tion oriented research is conducted by people in the medical and pharmaceutical sciences, agriculture, biotechnology, environment protection and others. Of special interest are studies on the structure and function of membrane proteins that perform various active life functions, such as the bioconduction of chemical reactions and many biophysical processes. In turn, the lipid phase is often treated as a passive, structural element of the membrane that supports the proteins. Such a view is incorrect. Membrane lipids not only play a structural role but also constitute an element of metabolic processes. There is a close correlation between the functioning of proteins and the state of lipid phase. For example, the physical state of the lipid phase (degree of fluidity, domain and microdomain occurrence, and other features) determines the efficiency of (at least some) membrane-bound enzymes.

Protective and transport functions belong to the most important membrane functions. The membrane protects the cell, among others, against various adverse external factors, in particular against toxic chemical compounds and xenobiotics in a more general sense. This function is closely connected with the transport function that consists in controlling substance permeation across membranes. In the physiological state, the membrane allows the passage of substances that the cell needs and inhibits the transport of those that are unwanted. If the concentration of toxic substances exceeds a certain limit, the protective and transport mechanisms are disturbed, disorganisation of the cell membrane ensues and changes in the membrane structure result in disturbances in the functioning of the entire cell.

All substances can be roughly classified into three groups: those that are very soluble in water (hydrophilic), those that are not, or poorly soluble in water (hydrophobic), and substances with dual properties, containing hydrophobic and hydrophilic parts, known as amphiphilic (amphipathic) substances. The third group is well represented by lipid molecules, since they contain both a polar head group, which is always hydrophilic, and an unpolar part in the form of hydrophobic chain.

Each molecule that enters a living organism has always contact with its cell membranes. That contact may result in two events: 1) a change in the membrane itself, e.g. in the case of some toxins the membrane may suffer damage or even destruction, the latter resulting in death of the cell; 2) the substance crosses the membrane and exerts its action inside the cell.

Hydrophobic molecules in an aqueous environment practically do not interact with the cell membrane because they aggregate in water. Hydrophilic molecules, e.g. inorganic ions, cannot, in principle, permeate across undamaged lipid bilayers due to its hydrophobic interior. Under normal conditions, the transport of such molecules goes on in principle through proteinacious channels and transporters. Amphiphilic molecules, which exhibit a larger or lesser tendency both to water and unpolar phases, have the special ability to dwell in the water medium of the living organism and to interact with the lipid layer of cell membranes of the organism; that is why such substances are called membrane-active substances. It can simply be imagined that the hydrophobic part of a molecule anchors in the lipid phase of the membrane, and the polar part remains in the aqueous phase or in the membrane polar region. Such admixtures in the form of amphiphilic molecules can bring about various effects depending on, e.g., the molecular shape.

It should be noted that a multitude of various amphiphilic substances exist that can be beneficial or harmful to living cells. Amphiphilic properties exhibit food particles and most drugs. Numerous toxic substances are also amphiphilic (poisons, pesticides and detergents). All of them come easily into contact with the lipid phase of the membrane. In what

follows, we shall discuss the interaction of selected amphiphilic substances with membranes.

QUATERNARY AMMONIUM SALTS

Quaternary ammonium salts (QUATS) exist as amphiphilic cations in water solution. Many compounds of this group are used as pesticides, in particular as fungi-, algae-, and bactericides. The role of the membrane as the first and main target for QUATS was first pointed out by Hotchkis (1946). Since then, a vast literature has appeared on the subject. A comprehensive literature review until the beginning of 1990s is given in the work by Przestalski & Kuczera (1992).

Our own investigations were then conducted with the use of newly synthesised compounds (Witek *et al.*, 1978a; 1978b), which were known to have fungi-, algae- and insecticidal properties. The structures of some of these compounds are shown in Fig. 2. Our studies have shown that the compounds are membrane active. A question arose, what is the molecular mechanism that could explain the action of the investigated QUATS on membranes? Understanding the mechanism, being important in itself, may facilitate the synthesis of new, more effective pesticides; and it may also suggest how to introduce simpler methods for monitoring environmental pollution by those compounds. For that purpose we conducted studies that showed that the membrane activity of the compounds depends both on the character of the polar heads (size, electric charge distribution) and hydrocarbon chains (length, saturation, multiple chains). In order to investigate the effect of polar heads on membranes, we compared (Fig. 2) two groups of compounds: I (IA, IB, IC) and IV (IVA, IVB, IVC) which differed in the sizes of their polar heads and the distribution of their electric charges. We found, for instance, (comparing the action of group I compounds with that of group IV) that molecules with larger head groups and more concentrated charges induce greater disorganisation of membranes (the chains being the same).

Amphiphiles that belong to a series in which particular compounds differ in the length of their hydrophobic parts usually exhibit a non-linear dependence of their activity on hydrophobicity. The dependence is quasi-parabolic, which means that the efficiency of the interaction of such compounds with both biological and model membranes grows with an increase in their hydrophobic parts up to a certain length and then begins to diminish. In

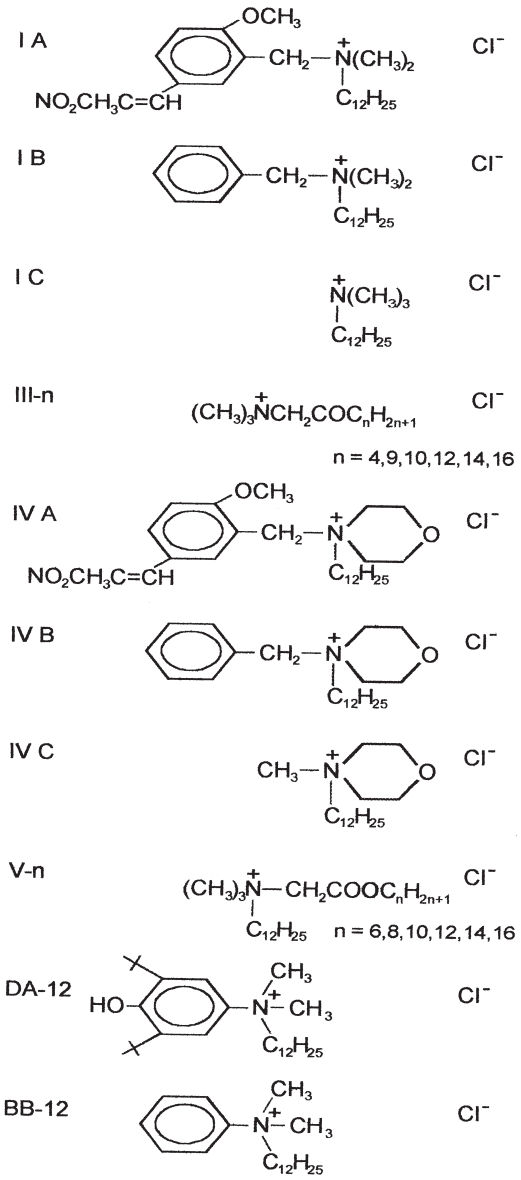


Figure 2. Structure of some quaternary ammonium salts.

the case of biological objects, the effect is known as the cut-off effect and it is commonly observed (Witek *et al.*, 1978a; 1978b; Rucka *et al.*, 1981; 1983; Devinsky *et al.*, 1990; Balgavy & Devinsky, 1996).

Examples of the described behaviour were observed in experiments with liposomes. Namely, it was found that the permeabilities of sulphate and phosphate ions through liposomal membranes, and also through membranes of red blood cells, were dependent on the length of the alkyl chain of the amphiphilic substances incorporated in the membranes (Kleszczyńska *et al.*, 1981; Przestalski & Kuczera, 1992). The measured ion permeabilities reached maximum values for compounds containing 12–14 carbon atoms in their hydrophobic chain and then decreased. Also, the stability of planar lipid membranes depended on the length of the hydrophobic chain of the compound used and reached a minimum for hydrocarbon chains containing 16 carbon atoms (Sarapuk *et al.*, 1992). In both cases, the amphiphilic compounds used were two different homologous series of QUATS of the general formulae $((CH_3)_3N + CH_2OC_nH_{2n+1}$ (III n), where n = 4, 10, 12, 14, 16 and 18, and $(CH_3)_3N + CH_2COOC_nH_{2n+1}$ (V n), where n = 8, 10, 12, 14 and 16). Note that the hydrocarbon chains of the studied compounds are separated from the polar heads by different spacer groups that change the effective hydrophobic part of the compound. The differences in localization of extreme values of the measured parameters in particular experiments were probably due to the difference in the lipid composition of the model membranes used and different spacer groups inbuilt into both homologous series of compounds. It was found earlier that the hydrophobicity of the spacer oxymethylene group is equivalent to almost two methylene groups and its introduction into a hydrocarbon chain of QUATS increased the total hydrophobicity of a compound (Sarapuk *et al.*, 1992). In contrast, the $-CH_2COO-$ spacer group, present in the hydrocarbon chain of compounds of the second homologous series, decreased the total hydrophobicity of the compounds studied because carbonyl group exhibit some polarity and do not allow the same deep incorporation of the surfactant molecule into model lipid membranes, compared with a compound containing an oxymethylene spacer.

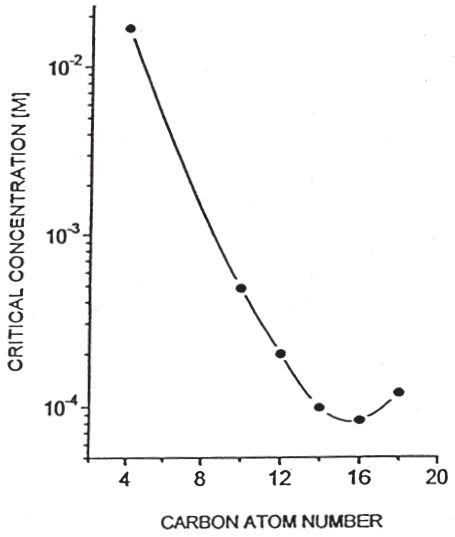


Figure 3. Dependence of the critical concentration of the III-a series on the number of carbon atoms in the alkyl chains.

A maximum of the interaction of the first studied homologous series with BLM is shown in Fig. 3; the dependence of the so-called critical concentration (CC) on the length of the hydrophobic part of the compounds is presented. The CC was defined as the concentration of a compound added to the bath solution that caused destruction of model membranes in a time shorter than 3 min.

In order to explain the observed effect, various factors should be taken into account. These are: change in hydrophobicity or lipophilicity of compounds following a change in their hydrophobic length, change in partition coefficient with the length of hydrophobic part of compound and the mismatch between alkyl chains of lipids and the studied compounds, with all the following consequence. Since the biological activity of QUATS is connected with their interaction with the lipid

phase of biological membranes, the explanation is also valid for their pesticidal activity.

It is rather easy to explain the low biological activity of compounds of short alkyl chains. The mismatch between their chains and those of lipids is rather large. There appears a so-called free volume beneath the terminal methyl group of an amphiphile compound incorporated into the lipid bilayer. Such a free volume can be treated as a kind of defect. It enables lipid alkyl chains to achieve greater mobility or to be more fluid. The hydrophobic interaction of an incorporated molecule with neighbouring lipid molecules is weak. Both the factors and the interaction between polar heads of lipids and a compound tend to destabilize the lipid bilayer. However, because of the small value of the partition coefficient (Balgavy & Devinsky, 1996), a high concentration of short alkyl chain amphiphile is needed to destroy model and/or biological membranes. Of course, the biological applicability of such compounds is somewhat doubtful. An increase of the alkyl chain length of an amphiphilic compound is followed by an increase in the hydrophobic interaction, which in turn „sucks" the amphiphilic molecule further into the membrane. The free volume gradually vanishes as the fluidity of that space of the membrane and the partition coefficient increase. The combined result of these changes is the growing destabilization of the membrane, whose measure can be a decrease in the amphiphile concentration that destroys the membrane or the biological activity of the amphiphile compound that grows with the alkyl chain elongation.

Such a simple picture cannot explain the decrease of activity when alkyl chains reach a certain length. It seems possible that, in such a case, the terminal methyl group of a compound may localize in the area of the terminal groups of the hydrocarbon chains of lipids that form the opposite monolayer, where QUATS are not incorporated. Some theoretical considerations confirm the possibility of the occurence of such a "sewing" of both monolayers (Sarapuk & Kubica, 1998) and the resulting structure may exhibit an increased stability and diminished potential biological activity of an amphiphile. This phenomenon is commonly called interdigitation and has been observed for some amphiphilic compounds, alcohols, acetone, drugs, anesthetics and other substances (Lobbecke & Cevc, 1995).

The interdigitation effect can be used to explain the appearence of the maximal permeability of ions and dyes through liposomal and erythrocyte membranes (Kleszczyńska *et al.*, 1981; Przestalski & Kuczera, 1992; Komatsu & Okada, 1997). The proposed model of the interaction between long-chain amphiphiles and lipid molecules can also be applied to the description of the interaction of these compounds with biological membranes. In such a case, it can explain the cut-off effects in biological activity observed for compounds with long enough hydrophobic parts of a homologous series of some pesticides.

It should be emphasized that the extensive use of lipid model membranes (liposomes and planar lipid membranes) for testing the potential biological activity of compounds is well justified, as it has been shown that there is a very good agreement between the results obtained in model experiments and those obtained from tests *in vivo* (Rucka *et al.*, 1981; 1983; Witek *et al.*, 1978a; 1978b). In particular concerning the comparison of parameters describing the total break of erythrocyte membranes, i.e., the concentration of QUATS inducing 100% hemolysis of erythrocytes (C_{100}), and the mentioned critical concentration (CC) found for planar membranes. It must be noted that the hemolysis phenomenon is commonly regarded as connected with the lipid phase of the erythrocyte membrane. The agreement found between the results of both sets of studies is the evidence that QUATS act directly on the lipid phase. However, one cannot exclude their action, directly or *via* the lipid phase, on membrane proteins (Trela *et al.*, 1997a).

A good agreement between C_{100} and CC parameters was also commonly found in studies

on the interaction of a series of other various amphiphilic compounds with model membranes, thus proving the correctness of the chosen approach to the problem. Examples are studies of bifunctional surfactants that can be used, depending on the demand, as common pesticides or as antioxidants. The current studies concern another group of potential pesticides, namely some organophosphorous compounds and the results so far obtained seem to qualify some of those compounds as good pesticides.

ANTIOXIDANTS

It is known that free-radical processes constitute an important group of biochemical processes in living organisms; an example being the respiration chain in plants and animals. Besides the normal free-radical processes that occur in the chains of biochemical reactions and ensure the proper function of organisms, there are also undesirable processes. One of them is the free-radical autooxidation of the lipid components of biological membranes. The oxidation products of free-radical reactions, such as peroxides or oxirenes, are the cause of various illnesses. Hence, among others, the interest in antioxidants as factors in the prevention of illness.

The most commonly known and used group of antioxidants are phenol derivatives substituted of the 2 and 6 positions with large-size groups that constitute a steric hindrance. They operate as free radical scavengers. The investigation of the mode of action of natural phenol antioxidants, such as those of the tocopherol group, prompted efforts in search of their natural counterparts. Our intention was to find compounds which: 1) bind to the membrane, 2) exhibit an antioxidant activity that protects membranes against peroxidation, 3) do not cause membrane destruction.

The first group of antioxidants was obtained by providing a "common" amphiphilic benzylammonium salt with two tert-butyl groups and the free radical scavenger –OH– group in the polar part of molecule (Fig. 2) (Witek *et al.*, 1994). All the other compounds synthesized up to now have the same structure of the polar antioxidative part but differed, within the homologous group, in hydrophobicity. Like the previously studied quaternary ammonium salts, they were checked for their potential biological activity and antioxidant efficiency in model membrane studies (Sarapuk *et al.*, 1993; 1998; Gabrielska *et al.*, 1995; Kleszczyńska *et al.*, 1999; 2000). The results obtained showed that all the factors already mentioned determined the functions of the studied antioxidants, i.e., polar-head stereochemistry, including net charge and its distribution, the number and alkyl chain(s) length and the presence of various spacer groups that can change the hydrophobicity of a compound. However, the potential efficiency of these bifunctional QUATS was found to be somewhat weaker than that of corresponding salts with less developed polar heads. In order to explain this finding, two compounds were chosen (DA-12 and BB-12, see Fig. 2) and some additional experiments performed. First, we applied the so-called relaxation method (Przestalski *et al.*, 1996), in which the compound was put on the water surface in a Langmuir vessel and the surface tension was measured with a Wilhelmi plate. The aim was to determine the relation between surface tension and time. It was found that, in the case of the BB-12 molecule without t-butyl and hydroxyl groups in the polar head, the surface tension decreased slower than for the DA-12 molecule. This seems to indicate that this compound is "drowning" into the water subphase more slowly or that it has a less polar character than DA-12 molecule. The results of the described experiments were confirmed by the results of calculations of the dipole moment by methods used in quantum chemistry (Stewart, 1989). It was found (assuming, for simplicity, that the alkyl chain of both molecules had only 2 car-

bon atoms) that the dipole moment of the DA-12 molecule was over 2 times greater than that of BB-12 molecule, evidencing greater polarity of DA-12 molecule. Therefore, DA-12 can not incorporate so deeply into the lipid bilayer as BB-12, and this explains why the studied antioxidants exhibited a weaker pesticide efficiency. The weaker efficiency has an important advantage because it was shown that they can protect erythrocyte membranes against oxidation at concentrations that do not cause any hemolytic effects while "common" pesticides used in the same concentrations cause 100% hemolysis.

The character of the changes in the structure of the membrane lipid phase, induced by the compounds discussed in this review, have been determined by measuring the dependence of surface pressure on mean molecular surface for monomolecular layers formed of selected DA-n and BB-n compounds and lecithin. The obtained three-dimensional plots of the relationship between the surface pressure of mixed monolayers, molecular surface and molar fraction showed that the obtained isotherms differ for the two compounds. These experiments indicate that the BB-n and DA-n compounds induce the formation of different liquid crystalline structures in the lecithin monolayer, which must be connected with different depths of incorporation of the two types of molecules in the lipid layer of membrane.

ORGANOTIN AND ORGANOLEAD COMPOUNDS

The object of our current investigations are organometallic compounds of the type R_nMeX_m, where R denotes organic radicals (alkyl or phenyl, in our case), Me – metal (here Sn or Pb), X – anionic group (here Cl^-), n and m numbers of functional groups (here m was equal to 4, 3, 2; and n was 0, 1 and 2).

In water, the compounds may exist in various forms. For instance R_3MeCl, i.e., trialkyltin and trialkyllead chlorides, can give rise to the following forms (depending on pH) among others: R_3Me^+, $R_3Me(H_2O_2)^+$, $R_3Me(OH)$, Cl^-, H_3O^+.

Compounds containing a metal and organic radical are amphiphilic. They interact with living organisms and exhibit toxic action (Przestalski *et al.*, 2000). Such organic tin and lead compounds are, in general, considerably more toxic than inorganic compounds of the metals. They markedly contaminate the environment and come from many sources; in particular from the paint industry and plant protection chemicals, leaded gasoline being the main single source of lead organic compounds. The enormous literature (Antonowicz *et al.*, 1998; Brej, 1998; Strużyńska *et al.*, 1997; Wierzbicka, 1998) on the action of lead and tin refers mostly to inorganic forms and the effect on living organisms. The literature on the interaction between organic compounds of the metals and organisms is significantly less abundant. In particular, the literature is poor on the effect of organotins and organoleads on membranes, especially model lipid membranes. This is strange because their effect on membranes should be considered first in view of the amphiphilic character of the compounds. Our investigations indicated the following harmful effects of the compounds on lipid membranes: disorganisation of monomolecular lipid layers (Trela *et al.*, 1997a; 1997b) destabilisation of BLMs (Langner *et al.*, 1998; Sarapuk *et al.*, 2000), of erythrocytes (Kleszczyńska *et al.*, 1997) and of algae (Trela *et al.*, 1997b), effect on phase transitions in lipid layers (Różycka-Roszak *et al.*, 2000), effect both on lipids and membrane enzyme proteins (Przestalski *et al.*, 2000). In particular, we have found an effect of a change in membrane polarisation (electric charge) on membrane accessibility of amphiphilic cations of organic tin and lead compounds (Kuczera *et al.*, 1997; 1999; Kleszczyńska *et al.*, 1998). A decrease in the negative charge of the membrane (e.g., by incorporation of quaternary ammonium salts,

or other cations, in concentrations which do not cause membrane disturbance) results in the membrane being less accessible for electrostatic reasons to cations of the discussed

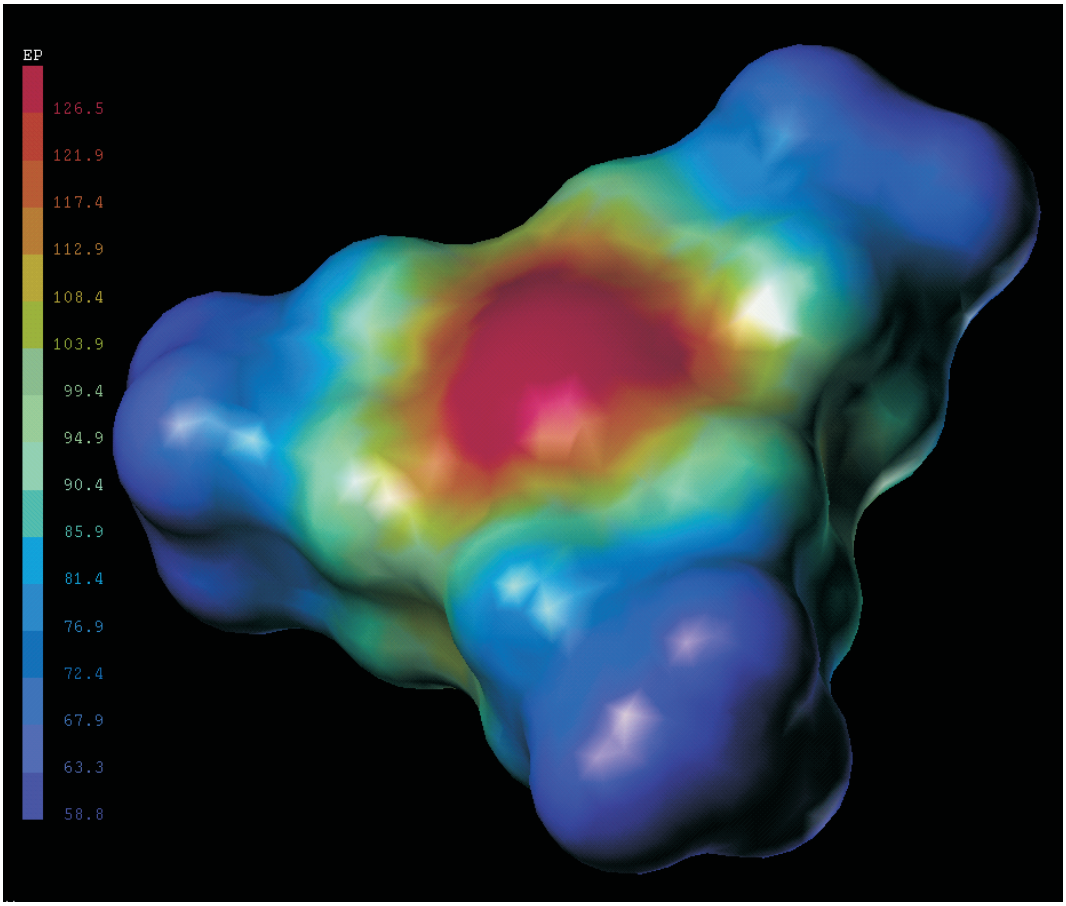


Figure 4. Electrostatic potential (EP) of tripropyltin cation mapped on a Connoly surface.

A Connoly surface was obtained by sliding a probe sphere of 1.4 019 Å on a van der Waals spheres of all atoms of the cation. The probe sphere corresponds to a solvent (water) molecule. Therefore, the Connoly surface is inpenetrable to solvent molecules and is the surface of contact with the external medium. EP is expressed in e/Å (e – elementary charge). The molecule graphics were performed in SYBYL/MOLCAD module (Tripos Inc., S. Louis 1996, SYBYL v.6.4).

organometallic compounds (organotins in particular; the example of electric potential distribution of tripropyltin chloride is shown in Fig. 4). For the same reasons, the accessibility increases when the negative charge increases (e.g., with incorporation of sodium alkylsulfonates). Both the effects, synergistic and antagonistic, should in principle be taken into account when considering the problems of environmental protection. An increase in positive charge of the membrane makes the cell membrane less accessible for cations of the metals and is thus less harmful to the cell. An increase in negative charge should result in the accumulation of the compounds in the cells of an organism, e.g., plants grown in areas contaminated with the discussed metals and their removal together with the plants. The last remark is rather speculative and requires studies with plants and economic calculations that would confirm its practical importance.

CONCLUSIONS

In this review we have indicated the importance of the lipid phase in the cell membrane;

in many phenomena, the phase is no less important than membrane proteins. In particular, the lipid phase constitutes the main target in the interaction of the cell membrane with amphiphilic substances, whose characteristic property is greater or lesser solubility in both lipids and the aqueous medium. Many food, drug and toxin compounds belong to the class of amphiphilic substances. At least part of the compounds we studied induces structural changes in the lipid phase of membrane and results in structural defects, which in turn disturb membrane function (emphasising the permanent problem of biophysics, which is the search for a relation between the physical structure of the membrane and its biological properties).

On the basis of studies of practical importance we indicated that: 1) compounds containing quaternary ammonium salt groups with pesticidal properties exhibit biological activity that is proportional to the size of their polar heads and increases with the concentration of the electric charge on them, and is related to the length of the alkyl chain (maximal effects were observed for 12–14 carbon atom alkyl chains); 2) compounds with antioxidant properties anchor well to the lipid phase of the membrane, without damaging it even at relatively high concentration; 3) the tin and lead organometallic compounds we studied are membrane-active toxins (which does not exclude other effects). By manipulating membrane polarity one can both minimise the effect of the compounds on membranes and induce synergistic effects, which may find application in plant protection treatments.

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