This conclusion is proved by our observations confirming that frequency potentiation is uncharacteristic for the exciting responses to acetylcholine, applied with the aid of microionophoresis on the external surface of snail neurons (unpublished data). The question as to what features of the molecules of the substance excreted from the micropipette are responsible for this microelectrode factor remains open.

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# PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

# Thermo-Induced Structural Rearrangements of Platelet Membranes by the Action of an Inductor and Inhibitors of Aggregation.

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**Key Words**: Platelets; inductors and inhibitors of aggregation; structural rearrangements of membrane.

An universal property of biological membranes is their capacity for structural rearrangements [2]. The structural rearrangements occurring in the plasma membrane reflect the intracellular metabolic processes. In studies of the effect of inhibitors and inductors of aggregation on the spectrum parameter S it was found by several authors that ADP and aspirin decrease, while α-tocopherol (vitamin E) increases the value of this parameter [6,8]. The variations under constant temperature reveal no distinctions between inductor and inhibitor actions on the membrane.

In this study an attempt was undertaken to demonstrate the generalities as well as the features inherent in the action of these compounds.

## MATERIAL AND METHODS

In the present study acetylsalicylic acid (aspirin), α-tocopherol (vitamin E), and ADP (Sigma) were used.

As a hydrophobic spin probe for platelet membrane testing the stearic acid derivative 5-doxyl stearate was used at the final concentration $10^{-4}$M. EPR spectra were recorded with a Varian E-104 radiospectrometer equipped with a variable temperature accessory at 10 mW microwave power, 1,6 G modulation amplitude and 100 G/8 min scan time. The temperature of the sample was controlled by a thermocouple within 0,5 °C.

Figure 1 shows a typical spectrum from this probe in the platelet membrane. This spectrum is characteristic of the anisotropic motion of the spin probe incorporated into the lipid bilayer of the platelet membrane. To

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estimate quantitatively the structure of the spin probe, a spectral parameter of the order of S and $2A^{1}_{11}$ was used.

Blood for the experiments was obtained from rabbit ear marginal vein. 3.8% citrate Na solution at volume ratio 1:9 to blood was added as an anticoagulant. Platelet-rich plasma (PRP) was obtained by centrifugation at 120 g for 12 min. Platelets for EPR spectroscopy study were sedimented from PRP by centrifugation at 640 g for 15-20 min. Anticoagulant "ACD" (2% glucose, 0.085 M citrate Na, 0.005 M citric acid) was added to PRP at ratio 1:10 before centrifugation. After sedimentation, the platelets were washed one time with solution: 0.154 M NaCl, 0.154 M TRIS HCl, 0.077 M EDTA (at volume ratio 90:8:2) and suspended in Tyrode's solution without $CaCl_2$ and albumin (pH 7.4).

Cell functional capacity was tested as the capacity for ADP- and thrombin-induced aggregation. It was shown that after separation, the platelets suspended in citrate-containing plasma retained completely their ability to aggregate under the influence of ADP and thrombin. The cell suspension in Tyrode's solution revealed aggregation ability with ADP addition only in the presence of calcium ions, in conformity to published data [4,7]. Thrombin-induced aggregation did not require the presence of $Ca^{+2}$.

## RESULTS

As established earlier [6], aspirin (5 mg/ml) as well as ADP (12 mg/ml) results in a substantial decrease of the order S parameter from 0.708 to 0.648 for aspirin and from 0.708 to 0.672 for ADP. At the same time α-tocopherol (5 mg/ml) causes a decrease of the flexibility of the fatty acid chains in the phospholipid bilayer and, respectively, an increase of parameter S from 0.706 to 0.736. This fact is in accordance with an investigation [8] which showed by a fluorescence polarization study that α-tocopherol increases the 1,6-diphenyl-1,3,5- hexatriene fluorescence anisotropy value in platelets and the platelet membrane at a temperature higher than 27°C.

When studying the temperature dependence of parameter $2A^{1}_{11}$ ,which is proportional to parameter S, it was found that in the case of intact platelets the curve on the Arrhenius coordinates has a break point at 17 °C (Fig.2).For the addition of the inductor of platelet aggregation - ADP - the temperature curve is unaffected but is shifted to the low temperature region with a break point at 13 °C. This is one more piece of evidence that fatty acid chain flexibility is increased by the action of ADP, which in turn results in a reduction of microviscosity.

In the presence of an inhibitor of aggregation, aspirin, a shift of the low-temperature break point from 17°C to 12°C as well as the appearance of a new one at 20°C are observed. Another inhibitor, α-tocopherol, also shifts the low-temperature break point to the low temperature region (to 15°C) and causes the appearance of a new break point at 28°C (Fig.3).

Thus, a study of the temperature dependence of parameter $2A^{1}_{11}$ in the presence of an inductor and inhibitors of aggregation reveals significant differences in their action on the platelet membrane. Whereas ADP just shifts the Arrhenius curve break point to the low-temperature region, both aspirin and α-tocopherol cause a low-temperature shift as well as a new break point appearance (at 20 °C for aspirin, 28 °C for α-tocopherol). As currently assumed, the Arrhenius curve break point at 17°C most likely is due to conformational rearrangements of the biomembrane phospholipid bilayer,

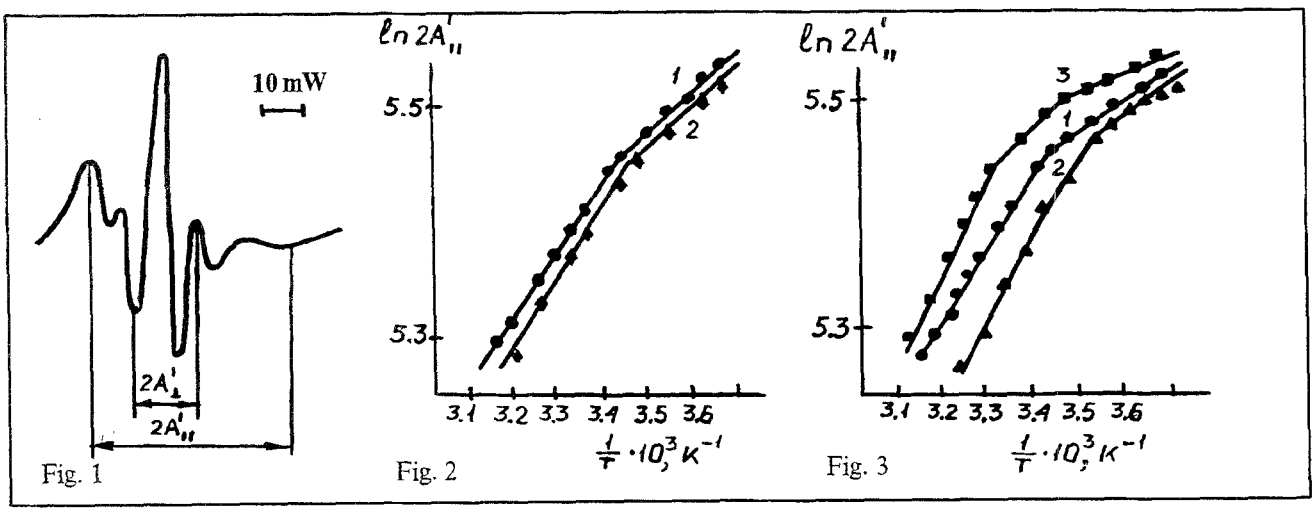


Fig.1 EPR spectrum of 5-doxyl stearic acid in platelet membrane at 37°C (recording conditions: P-10mW, c=6.3x10³, $H_M$=1.6 G, $\tau_{scan}$=8 min, τ=0.128 sec.
Fig.2 Temperature dependence of EPR spectrum parameter $2A^{1}_{11}$ of spin probe in platelets (Arrhenius coordinates). 1) control platelets, 2) platelets + ADP + $Ca^{2+}$.
Fig.3 Temperature dependence of EPR spectrum parameter $2A^{1}_{11}$ of spin probe in platelets in the presence of inhibitors of aggregation. 1) control platelets, 2) platelets + aspirin, 3) platelets + α-tocopherol.

i.e., it characterizes the changes in lipid-lipid interaction. The shift of this break point to the low-temperature region under the influence of ADP, aspirin, and α-tocopherol indicates the effect of these compounds on the platelet membrane phospholipid regions. A high-temperature break point in the presence of aspirin and α-tocopherol is likely to appear as a result of their influence on protein-lipid interactions, since a high-temperature transition is currently thought to depend upon the protein-lipid interactions, as has been determined by an erythrocyte membrane study [1].

Evidently, both inhibitors affect the cyclooxygenase pathway of arachidonic acid metabolism. Aspirin has been shown to block cyclooxygenase activity by enzyme acetylation, while α-tocopherol, due to its antioxidant properties, is able to compete with the enzyme for oxygen binding. Besides, α-tocopherol when inserted in the platelet membrane, reduces the latter structural flexibility and increases the local bilayer's microviscosity, just as cholesterol does [3].

Possibly, the metabolic processes dependent on membrane fluidity are inhibited in this case. Therefore, α-tocopherol, apart from its structural-functional role, seems to be directly involved in metabolism regulation, namely, in blocking the synthesis of endoperoxides, prostaglandins and thromboxane $A_2$ by the uptake of oxygen.

Since the data concerning with a significant drop of the malonic dialdehyde level confirm the effect of aspirin on platelet membrane metabolism, and taking into account the slight effect of α-tocopherol on malonic dialdehyde formation, it is more likely that α-tocopherol has a predominant effect on the structural properties of the platelet membrane.

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# The Effect on the Blood of Prolonged Mortal Hypoxia Endurance under the Extracorporeal Influence of a Magnetic Field

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Among the great number of studies of the influence of a constant magnetic field on the organism [1-3, 11], there are only a few concerning the antihypoxic effect of this factor [5,6]. There is no consensus in these studies, because the analyzed results were obtained under the influence of the magnetic field on the whole organism. At the same time it is known that, depending on the functional state of the organism and the preferential action of that factor on different regions (the head - the central nervous system, the adrenal sphere - the endocrine system, the main blood vessels, the parenchyma, *etc.*), the integral direction of all the physiological reactions can vary greatly: in some cases an antihypoxic effect is shown, while in others, the opposite result is achieved [7,8,10].

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