2. A. P. Elkin, Biofizika, No. 3, 444 (1977).
3. G. Zontag and K. Shtrenge, Coagulation and Stability of Dispersed Systems [in Russian], Leningrad (1973), p. 51.
4. B. I. Kuznik, Usp. Sovrem. Biol., 75, No. 1, 61 (1973).
5. L. D. Landau and V. V. Deryagin, Eksp. Teor. Fiz., No. 11, 802 (1941).
6. P. E. Lukomskii, "Myocardial infarction," Dissertation Lecture [in Russian], Moscow (1971).
7. A. E. Lukomskii and V. A. Lyusov, in: Preventive Cardiology [in Russian], Moscow (1977), p. 322.
8. M. S. Machabeli, Coagulopathic Syndromes [in Russian], Moscow (1970), p. 64.
9. M. P. Popov, Lab. Delo, No. 9, 511 (1976).
10. S. S. Kharamonenko and A. A. Rakityanskaya, Electrophoresis of Blood Cells Under Normal and Pathological Conditions [in Russian], Minsk (1974), p. 65.
11. A. L. Chizhaevskii, Electrical and Magnetic Properties of Erythrocytes [in Russian], Kiev (1973), p. 41.
12. M. P. Popov, E. G. Tseitlin, I. D. Boenko, et al., Probl. Gematol., No. 6, 43 (1977).
13. Yu. E. Shevchenko, Biofizika, No. 3, 534 (1977).
14. V. N. Zakharcehenko, Colloid Chemistry [in Russian], Moscow (1974), p. 109.
15. D. A. Haydon and J. L. Taylor, Nature, 217, 739 (1968).

ROLE OF LIPID PEROXIDATION AND α-TOCOPHEROL IN CONDUCTIVITY OF ARTIFICIAL MEMBRANES MADE FROM LIVER PHOSPHOLIPIDS OF RATS WITH BURNS

M. I. Agadzhanov, S. A. Badzhinyan, and V. G. Mkhitaryan

UDC 617-001.17-092.9-07:616.36-008.6-07

The state of membrane permeability for potassium and calcium ions was studied in bilayer phospholipid membranes (BPM) from the liver of rats with burns. A sharp increase in the conductivity of BPM was found under these circumstances. This process was accompanied by an increase in lipid peroxidation. Administration of α-tocopherol (1 mg/kg body weight) restored these indices to normal. Model experiments with methyl oleate and cumyl hydroperoxide confirm the peroxide mechanism of injury to membrane formations.

KEY WORDS: burns; conductivity of membranes; lipid peroxides; α-tocopherol.

Although burns are serious conditions, there is as yet no general agreement regarding the pathogenesis of the disturbances they cause [4]. Considerable attention has been paid to the neurogenic theory [5, 7]; in recent years the theory of an autoimmune mechanism of the pathological changes in burns has been researched intensively [10].

Burns cause serious disturbances in the whole of the body, which are based on a disturbance of homeostasis. Displacement of sodium, water, and protein from the blood stream, hypovolemia, an increase in the blood concentrations of metabolic and breakdown products, hypoxemia, tissue hypoxia, and other disturbances are observed. All these disturbances may be largely attributed to pathology of membranes, for which there is much indirect evidence [8, 9].

It was shown previously that in various stress states there is intensification of free-radical processes [3], accompanied by elevation of the level of lipid peroxidation. Burn trauma is known to be a powerful stressor, leading to a considerable increase in the lipid peroxide level [6]. This increase, in turn, increases the permeability of cell membranes [2].

The object of this investigation was to study permeability of membranes in burns at the molecular level in model experiments.

Department of Biochemistry, Erevan Medical Institute. Department of Biophysics, Central Research Laboratory, Erevan Postgraduate Medical Institute, Ministry of Health of the USSR.
(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 87, No. 5, pp. 422-425, May, 1979. Original article submitted December 28, 1977.

0007-4888/79/8705-0433$07.50

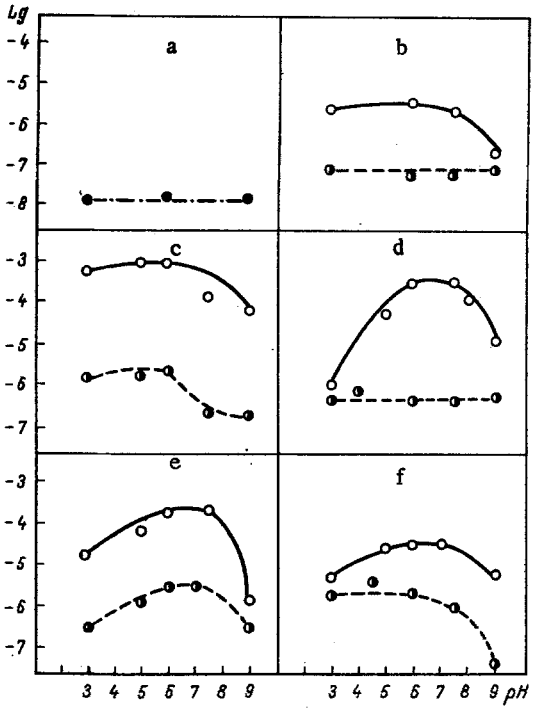

Fig. 1. Effect of α-tocopherol on conductivity of BPM from rat liver phospholipids in 0.1M KCl. Abscissa, pH values at which measurements were made; ordinate, logarithm of membrane conductivity (log G; in $\Omega^{-1}cm^{-2}$). a) Normal; b) after 1 h, c) after 1 day, d) after 3 days, e) after 7 days, f) after 15 days. Empty circles denote burns; filled circles burns + α-tocopherol.

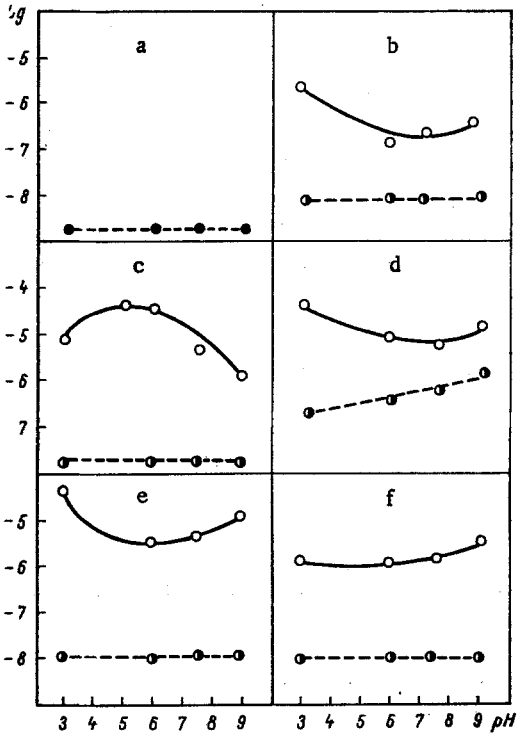

Fig. 2. Effect of α-tocopherol on conductivity of BPM from rat liver phospholipids in 0.1 M $CaCl_2$. Legend as in Fig. 1.

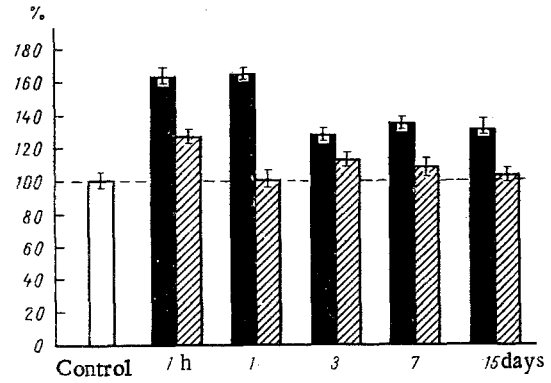

Fig. 3. Effect of α-tocopherol on level of lipid peroxidation in liver of rats after burn trauma (changes in % of control). Black columns represent burns; obliquely shaded columns burns + α-tocopherol.

EXPERIMENTAL METHOD

Male albino rats weighing 120-160 g were used. Third degree burns (12-15% of the body surface) were caused by the action of water at 80°C for 10 sec. After trauma some animals were given an intraperitoneal injection of vitamin E in a dose of 1 mg/kg immediately after burning and again after 3, 7, and 12 days.

Vitamin E was injected as α-tocopheryl acetate, which is hydrolyzed *in vivo* to α-tocopherol [12]. Tests on the liver tissue were carried out 1 h and 1, 3, 7, and 15 days after trauma.

Phospholipids were extracted from the tissues by Folch's method [15]. Bilayer phospholipid membranes (BPM) were formed from phospholipids dissolved in heptane in a concentration of 20 mg/ml. The electrical conductivity of the BPM was measured in solutions of 0.1M KCl and 0.1M $CaCl_2$ by means of a dc electrometer [1] at pH values of 3.0 (acetate buffer), 6.0 and 7.4 (phosphate-citrate buffer), and 8.0 and 9.0 (Tris-HCl buffer). The BPM thus prepared withstood a constant voltage up to 400 mV. The experiments were carried out at 100 mV.

The level of lipid peroxidation was studied on the basis of the intensity of the color reaction with thiobarbituric acid at 535 nm [11]. To study the role of α-tocopherol in the development of membrane pathology in experiments *in vitro* α-tocopherol was added in a dose of 0.04 mmole to BPM obtained from phospholipids of animals with burns. To confirm the peroxide mechanism of the disturbance of membrane conductivity, methyl oleate and cumyl hydroperoxide, in doses of 0.05 mmole each per experiment, were added to BPM obtained from normal phospholipids.

EXPERIMENTAL RESULTS

The data on conductivity of BPM from rat liver phospholipids are given in Figs. 1 and 2. Conductivity of membranes from phospholipids of intact rats was found to average $(9.0 \pm 0.2) \cdot 10^{-8}\ \Omega^{-1}\ cm^{-2}$ for potassium ions and $(5.0 \pm 0.2) \cdot 10^{-10}\ \Omega^{-1}\ cm^{-2}$ for calcium ions and to be unaffected by changes in the pH of the medium (see Figs. 1a and 2a).

In the experiments of series I the conductivity of BPM from phospholipids obtained from the liver of burned rats was studied. In all cases changes were observed in conductivity depending on the pH of the medium (see Figs. 1b-f and 2b-f). The conductivity of the membrane for potassium ions 1 h after burning was maximal at pH values close to neutral (6.0 and 7.4) and thereafter it fell by two orders of magnitude (Fig. 1b), whereas for calcium ions at the same pH values conductivity was minimal (Fig. 2b). Maximal conductivity of BPM for calcium ions was observed at pH 3.0. The strongest effects were found after 1, 3, and 7 days (Fig. 2). The shapes of the conductivity curves for potassium and calcium 1 day after burning were similar. At pH 6.0 and 7.4 conductivity in both cases increased by two to three orders of magnitude and its minimal value occurred at pH 9.0. After 3 and 7 days, although the shapes of the curves were different for potassium and calcium ions, the effects of a marked increase in conductivity at pH 6.0 and 7.4 were still observed in all cases (see Figs. 1 and 2). As regards the 15-day burns, in this case the conductivity of BPM was much lower than at the previous times for both potassium and calcium ions; this was evidently due to restoration of the damaged structures and functions which was taking place at this time. It is interesting to note that the maximal increase in conductivity of BPM for potassium ions was observed at near-physi-

ological pH values (6.0 and 7.4). As regards calcium ions, the maximum of conductivity of BPM for this ion was observed mainly at extreme pH values (3.0 and 9.0).

In the experiments of series II the conductivity of BPM obtained from liver phospholipids of rats with burns treated with α-tocopherol was studied.

These experiments showed that α-tocopherol has a restorative action on membrane conductivity. Almost complete recovery of conductivity of the BPM, independent of the pH of the medium, was observed at all times with calcium ions (Fig. 2), and incomplete although well marked recovery was observed with potassium ions (Fig. 1).

To prove the role of α-tocopherol in the formation of the lipid layer of the membranes it was shown by experiments *in vitro* that the addition of α-tocopherol to phospholipids from the liver of rats with burns considerably restores the normal conductivity of BPM.

In the next series of experiments the intensity of lipid peroxidation was studied in the liver of rats during the same period after burn trauma. The results showed that the greatest increase in the level of lipid peroxides was observed 1 h and 1 day after burning. Later the level fell a little but still remained higher than the control. Injection of α-tocopherol almost completely restored this process to normal.

The results indicate that burn trauma leads to a marked increase in the intensity of lipid peroxidation in the liver, and this is accompanied by an increase in conductivity of BPM. Administration of α-tocopherol in accordance with the scheme mentioned above leads in general to a return to normal indices.

There is some evidence [14] that vitamin E and phospholipids play an important role in the stabilization of cell membranes. Vitamin E is considered to stabilize the membrane structure by specific physicochemical interaction between its phytyl side chain and water-repellent residues of polyunsaturated fatty acids of the phospholipids, especially derivatives of arachidonic acid. From this point of view the data on the quantitative content of vitamin E and also of phospholipids and their individual fractions in the liver after burns are particularly interesting. The content of endogenous α-tocopherol at all periods after burning was found to be reduced in the liver, by 17, 55, 24, 33, and 38% 1 h and 1, 3, 7, and 15 days after burning, respectively. The use of therapeutic doses of vitamin E immediately after burning restored the normal level of endogenous α-tocopherol, whereas at the other times its content was somewhat higher than normal. As regards total phospholipids, their content at these times after burning was 30, 46, 45, 16, and 52%, respectively, below normal. These changes are connected mainly with shifts in the fractions of lecithins and phosphatidylethanolamines; under the influence of vitamin E these also return to normal.

The biochemical investigations showed a parallel between changes in the principal lipid components of the membranes and their functions. In experiments *in vitro* the addition of oxidized methyl oleate or cumyl hydroperoxide to normal phospholipids reduced the resistance of BPM for K^+ from $(7.0 \pm 0.2) \cdot 10^7$ to $(6.4 \pm 0.2) \cdot 10^4$ and $(2.5 \pm 0.2) \cdot 10^5$ $\Omega \cdot cm^2$ respectively. It is evident that the increased conductivity of BPM in burns is due to an excess of lipid peroxidation. Other investigations [2] have also revealed evidence of the participation of lipid peroxides in increasing membrane permeability.

The results of the present experiments suggest the following mechanism of disturbance of membrane permeability in burns. Lipid peroxides, being aggressive compounds, cause peroxidation of tissue lipids, especially unsaturated phospholipids, and inactivation of α-tocopherol. Considering the character of the bond between α-tocopherol and phospholipids [13], it is to be expected that conducting pores will be formed in areas of peroxidation in the bilayers, through which the test ions can pass. This hypothesis is supported by the results showing a decrease in the degree of unsaturation of the fatty acids in the phospholipids, mainly on account of polyenic acids, especially arachidonic. The difference between the behavior of potassium and calcium ions relative to BPM from phospholipids of animals with burns is evidently attributable to definite conformational changes arising in the structure of the membranes, including changes in the polyenic acids.

Experiments with BPM thus showed that in burns there is a disturbance of membrane permeability connected with intensification of lipid peroxidation, which leads to oxidation of the principal lipid components of the membranes. These changes contribute to the formation of conducting pores in the membranes. Administration of α-tocopherol by the scheme indicated above enables this process to be regulated and prevents the development of the profound disturbances that are characteristic of burn trauma.

LITERATURE CITED

1. S. A. Badzhinyan, Biol. Zh. Arm., No. 6, 23 (1976).
2. Yu. A. Vladimirov and A. N. Archakov, Peroxidation of Lipids in Biological Membranes [in Russian], Moscow (1972), p. 166.
3. V. P. Kaznacheev, in: Proceedings Plenary Meeting of the All-Union Scientific Medical Society of Pathophysiologists [in Russian], Erevan (1974), pp. 73-74.
4. N. I. Kochetygov, Burns [in Russian], Leningrad (1973).
5. V. B. Lemus and V. V. Davydov, Nervous Mechanisms and Corticosteroids in Burns [in Russian], Leningrad (1974).
6. V. G. Mkhitaryan, M. I. Agadzhanov, and E. A. Melik-Agayan, Zh. Éksp. Klin. Med., No. 1, 3 (1975).
7. I. R. Petrov, Khirurgiya, No. 1, 107 (1950).
8. M. I. Remizova and N. I. Kochetygov, Patol. Fiziol., No. 3, 63 (1976).
9. L. B. Rozin, A. A. Batkin, and R. N. Katrushenko, Burn Shock [in Russian], Leningrad (1975), p. 21.
10. N. A. Fedorov, in: Mechanisms of Injury, Resistance, Adaptation and Compensation (Abstracts of Proceedings of the Second All-Union Congress of Pathophysiologists) [in Russian], Vol. 1, Tashkent (1976), pp. 21-23.
11. J. G. Biery and A. A. Anderson, Arch. Biochem., 90, 105 (1960).
12. H. E. Gallo-Torres, Lipids, 5, 379 (1970).
13. J. A. Lucy, Ann. N. Y. Acad. Sci., 203, 4 (1972).
14. A. T. Diplock and J. A. Lucy, FEBS Lett., 29, 205 (1973).
15. C. B. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).