Polyamines as Modulators of Lipoperoxidation

D. D. PAVLOVIC[2], P. UZUNOVA[1], T. GALABOVA[1], V. PENEVA[1], Z. SOKOLOVA[1], G. BJELAKOVIC[2] and S. RIBAROV[1]

1 *Department of Biophysics, Med.- Biol. Institute, 1431 Sofia, Bulgaria*
2 *Institute of Biochemistry, Faculty of Medicine, 18000 Nis, Yugoslavia*

Abstract. The polyamines spermine and spermidine and the diamine putrescine inhibit lipid peroxidation in phospholipid liposome suspensions and rat liver homogenates. Using the chemiluminescence technique the antioxidant activity of polyamines was found to be due to reactions with the free radical intermediates of lipid peroxidation and/or superoxide radicals. Also, the antioxidant action of polyamines correlated with the amount of their amino groups: the antioxidant activity increases from putrescine to spermine.

Key words: Polyamines — Free radicals — Lipid peroxidation — Chemiluminescence

Introduction

The polyamines spermine and spermidine and the diamine putrescine, which at physiological pH are polycationic, are widely distributed in biological material (Bachrach and Plesser 1986). The intracellular concentrations of these compounds are high (up to 1 mmol/l) and fluctuate according to the stage of the cell cycle (Raina 1963; Bachrach 1970; Tabor and Tabor 1976). Their role in the regulation of cell growth, cell differentiation, nucleic acid and protein synthesis and membrane metabolism (Tabor and Tabor 1972; Janne et al. 1978; Seiler and Lamberty 1973; Schuber 1989) have attracted considerable attention during the last few decades.

Numerous publications have indicated that polyamines influence the cell membrane properties and functions (Johnson and Nordiie 1980; Kossorotow et al. 1974; Heinrich-Hirsch et al. 1977; Fukuyama and Yamashita 1976). The exact mechanisms of this action, however, is not clear as yet.

Correspondence: Stefan R. Ribarov, Medico-Biological Institute, Department of Biophysics, 1431 Sofia, Bulgaria.

Some authors suggested that the di- and polyamines have stabilizing properties which may be due to their interactions with the negative charged residues of the membrane-bound proteins (Johnson and Nordiie 1980; Kossorotow et al. 1974; Heinrich-Hirsch et al. 1977) or with the acid phospholipids resulting in relocation of the surface charge (Bachrach 1970). The polyamines-induced change of the surface charge obviously may influence some of the membrane-bound enzymes (Wojtczak and Nalecz 1979), as well as the biosynthesis of membrane lipids (Fukuyama and Yamashita 1976; Moller and Hough 1982) and glycoproteins (Navaratnam et al. 1986), etc.

Lipid peroxidation in tissues and in tissue fractions represents a degradation process which is the consequence of initiation and propagation of free radical reactions primarily involving membrane polyunsaturated fatty acids (Poli et al. 1987). It is interesting to note that there are some data in the literature (Kitada et al. 1979; 1980; 1981; Tadolini et al. 1985; Tadolini 1988) indicating that the membrane stabilizing effect of di- and polyamines may be due to their antioxidant properties. On the other hand, the initiation of lipid peroxidation usually needs generation by some mechanism of activated oxygen species such as superoxide radical (O_2^-), H_2O_2, hydroxyl radical ($\cdot$ OH), singlet oxygen, etc.

Therefore, it seemed stimulating to carry out an extended investigation concerning the ability of di- and polyamines to scavenge oxygen radicals and/or lipid radicals, i.e. their ability to inhibit lipid peroxidation, thus stabilizing the biomembranes against oxidative destruction.

The present work is an attempt to shed some light on this problem. In particular, we studied the ability of the diamine putrescine and the polyamines spermine and spermidine to react with superoxide radical and lipid radical intermediates (mainly peroxy radical – $LO_2\cdot$) and to inhibit lipid peroxidation in different lipid-containing systems.

Materials and Methods

Spermine tetrahydrochloride, spermidine trihydrochloride and putrescine dihydrochloride were obtained from Calbiochem (La Jolla, California), xanthine and xanthine oxidase from Sigma Chemical Company. The other reagents used were of finest grade purchased mainly from Fluka Chemical Company. The water employed was glass bidistilled.

Male albino Wister rats (180–200 g) were used. The animals were killed by decapitation and their livers were quickly removed, washed in cold 0.9% NaCl, weighed and homogenized in 0.15 mol/l NaCl (10% w/v) in Potter-Elvehjem type glass homogenizer with a Teflon pestle at 4 °C.

Free radical production and lipid peroxidation *in vivo* in rat liver is difficult to measure (Poli et al. 1987). Therefore, incubation studies were carried out at prooxidant conditions to determine the capacity of the tissue homogenates (or liposome suspension) to generate additional peroxides. For this purpose, to 1 ml homogenate and 0.8 ml of 50 mmol/l phosphate buffer, pH 7.4 (or 1.8 ml liposome suspension with a concentration of

5 mg total egg yolk phospholipides/ml phosphate buffer), 0.2 ml of 1 mmol/l $FeCl_2$ was added and the mixture was incubated at 37 °C for 30 min. The extent of lipid peroxidation was estimated by the malondialdehyde (MDA)-like products generated in the mixture, by the method of Asakawa and Matsushita (1980). In brief, 1 ml of 30% trichloroacetic acid was added to the reaction mixture, followed by centrifugation for 15 min at $10,000 \times g$. One ml of 0.67% thiobarbituric acid was added to 1 ml of the supernatant and the mixture was boiled at 97 °C for 20 min. After cooling, 2 ml chloroform was added, the mixture was vigorously mixed and centrifuged, and the optical absorbance was measured at 532 nm.

The capacity of the liver homogenates (or phospholipid liposomes) to undergo peroxidation in the presence of di- and polyamines was determined by recording the Fe^{2+}-induced chemiluminescence (Vladimirov et al. 1980). For this purpose, to 1 ml homogenate and 0.8 ml of 50 mmol/l phosphate buffer (or 1.8 ml liposome suspension) containing di- and polyamines to concentrations as indicated in the Figures, 0.2 ml of 1 mmol/l $FeCl_2$ was added and the chemiluminescence kinetic curve was recorded. The integral chemiluminescence for 10 min after addition of Fe^{2+} to the reaction mixture was used as a measure of lipid peroxidation.

The effect of polyamines on the luminol-dependent chemiluminescence was tested in a system consisting of 0.1 mmol/l luminol, 0.1 mmol/l xanthine, polyamines in final concentrations as indicated in the Figure legends, and 10^{-4} IU/ml xanthine oxidase, in 50 mmol/l phosphate buffer, pH 7.4.

Results and Discussion

The initial objective of the present study was to determine the capacities of the diamine putrescine and the polyamines spermine and spermidine to influence Fe^{2+}-catalyzed peroxidation of phospholipid liposomes. The main results obtained are shown in Fig 1. As it is well seen all these compounds have antioxidant activity, which however depends on the compound concentration. The antioxidant activities of the polyamines tested at 1.5 mmol/l did not differ significantly from each other, the inhibition being approximately 20–30%. At lower concentrations, spermine and spermidine have more expressed inhibitory effects on the lipid peroxidation in comparison with putrescine. These data show that at least in *in vitro* experiments the compounds tested are not very effective inhibitors of lipid peroxidation. Their moderate antioxidant activity increases from putrescine to spermine. On the other hand, the process of lipid peroxidation is rather complicated. Its characterization by measuring only the terminal malondialdehyde-like products seems inadequate. As it is well known (Vladimirov et al. 1980; Cadenas et al. 1981) lipid peroxidation is accompanied by light emission. The light emission, chemiluminescence, is mainly due to disproportionation of the peroxy radicals – $LO_2\cdot$, which are responsible for a prolongation of the oxidation chains. Therefore, the measurement of chemiluminescence represents a very convenient approach to the estimation of the level of lipid peroxidation, as well as to registration of peroxidation kinetics. However, the count rates for spontaneous chemiluminescence are extremely low, making its char-

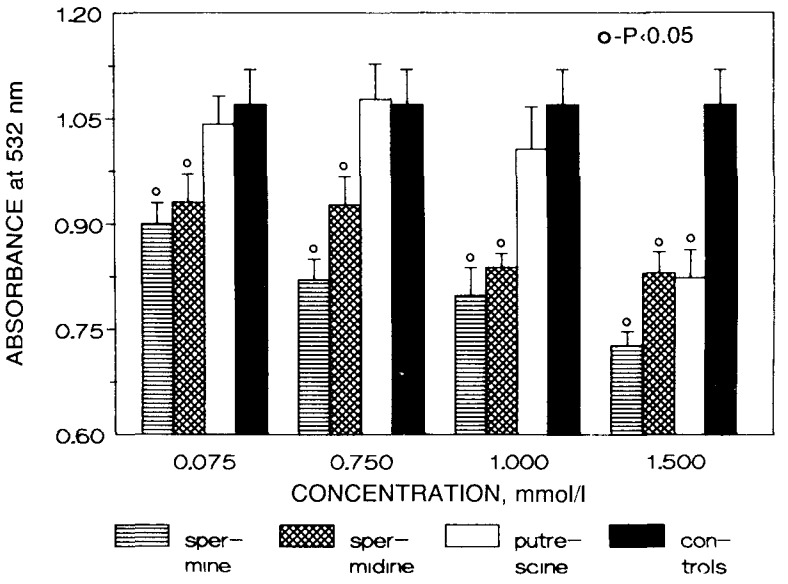

Figure 1. Inhibition by polyamines of the Fe^{2+}- induced generation of MDA in a phospholipid liposome suspension. Suspensions of phospholipid liposomes (5 mg lipids/ml) in 50 mmol/l phosphate buffer, pH 7.4, containing the tested polyamines (in concentrations as indicated in the Figure) were exposed to lipid peroxidation by addition of Fe^{2+} to a concentration of 0.1 mmol/l. The MDA content was measured after 30 min of incubation of the mixture at 37 °C.

acterization difficult (Cadenas et al. 1981). Therefore, efforts are usually made to enhance this spontaneous chemiluminescence by creating peroxidative conditions such as by adding Fe^{2+} to reaction mixture. In this context, the capacity of phospholipid liposomes to undergo peroxidation in the presence of spermine, spermidine and putrescine was also determined by recording the Fe^{2+}-induced chemiluminescence. The integral chemiluminescence for 10 min after addition of Fe^{2+} to the reaction mixture was used as a measure of lipid peroxidation. The results obtained are shown in Fig 2. All the compounds tested were found to inhibit the Fe^{2+}-induced chemiluminescence. The inhibitory effect of spermine at 1.5 mmol/l is significantly stronger than those of spermidine and putrescine (74, 35 and 27% respectively). All the compounds tested at concentration of 3 mmol/l reduced the chemiluminescence by 80-90%.

In other experiments the ability of spermine, spermidine and putrescine to inhibit Fe^{2+}-induced chemiluminescence of liver homogenates was checked. Fig. 3*A* presents the data obtained for polyamine concentrations of 0.1 mmol/l. It is evident that in such a system spermine is also a more potent inhibitor of the Fe^{2+}-induced

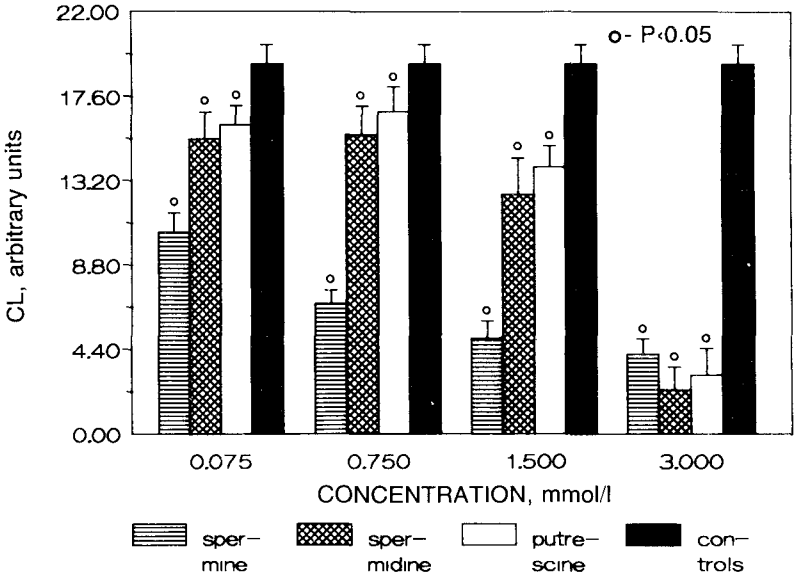

Figure 2. Inhibition by polyamines of the Fe^{2+}-induced chemiluminescence in a phospholipid suspension. To suspension of phospholipid liposomes (5 mg lipids/ml) in 50 mmol/l phosphate buffer, pH 7.4, containing the respective polyamine (in concentrations as indicated in the Figure), was added Fe^{2+} to a concentration of 0.1 mmol/l and the chemiluminescence response was recorded. The integrated chemiluminescence for 10 min after the Fe^{2+} addition was plotted on the ordinate.

chemiluminescence than the other amines tested. As is seen from the panel in Fig. 3*B*, spermine at 0.5 mmol/l reduced the chemiluminescence by more than 30%.

All these data may suggest that di- and polyamines have antioxidant activity which is due, at least in part, to their interaction with the radical intermediates of lipid peroxidation. Nevertheless we wish to turn attention to the fact that at equal conditions spermine (and the other amines tested) inhibited the Fe^{2+}-induced chemiluminescence significantly more effectively than they did with lipid peroxidation as estimated by the MDA products generated. This effect may indicate that some part of the chemiluminescence inhibition was due to interaction of the polyamines with activated oxygen species (mainly superoxide radicals (O_2^-)), produced in every lipid system undergoing peroxidation. This assumption was checked using the traditional xanthine-xanthine oxidase system as the source of superoxide radicals. The O_2^- concentration was controlled by recording luminol-dependent chemiluminescence. As it is well seen from the results presented in Fig. 4*A*, the polyamines spermine and spermidine at 1 mmol/l reduced the luminol-dependent

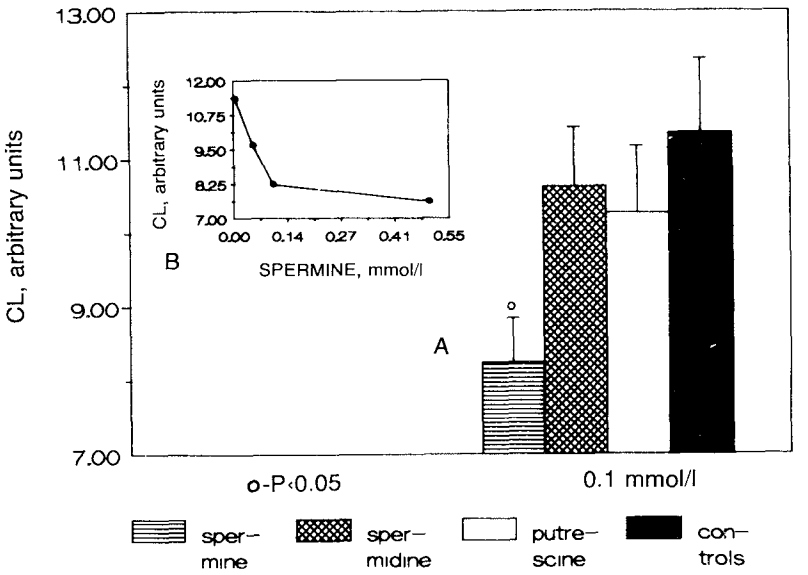

Figure 3. Inhibition by polyamines of the Fe^{2+}-induced chemiluminescence in rat liver homogenates. *A* – Comparison of the inhibitory effects of spermine, spermidine and putrescine at 0.1 mmol/l, on the Fe^{2+}-induced chemiluminescence. The respective polyamine was added to mixture of 1 ml homogenate and 0.8 ml of 50 mmol/l phosphate buffer to a concentration of 0.1 mmol/l. The chemiluminescence was initiated by addition 0.2 ml of 1 mmol/l $FeCl_2$. As a measure of lipid peroxidation, the integral chemiluminescence for 10 min after addition of Fe^{2+} to the reaction mixture was used. *B* – Inhibition of the chemiluminescence by different concentrations of spermine. The experimental conditions, except the concentration of spermine, were the same as those described in legend to Fig. 3*A*.

chemiluminescence, whereas putrescine did not. The O_2^--scavenging property of spermine was significantly stronger than that of spermidine and depended on its concentration (Fig. 4*B*). Spermine at 1 mmol/l caused 50% inhibition of the chemiluminescence. Since the luminol-dependent chemiluminescence is indicative of O_2^- concentration in the xanthine-xanthine oxidase system it seems likely that polyamines are able to scavenge O_2^-. This is in good agreement with the results reported by Drolet et al. (1986). Using xanthine-xanthine oxidase system as the source of O_2^-, these authors showed polyamines to inhibit the reduction of both nitroblue tetrazolium and cytochrome c; i.e. that polyamines have superoxide radical scavenging property.

The ability of polyamines to neutralize O_2^- is very interesting. As mentioned above, O_2^- is generated in any lipid system subjected to peroxidation. On the other

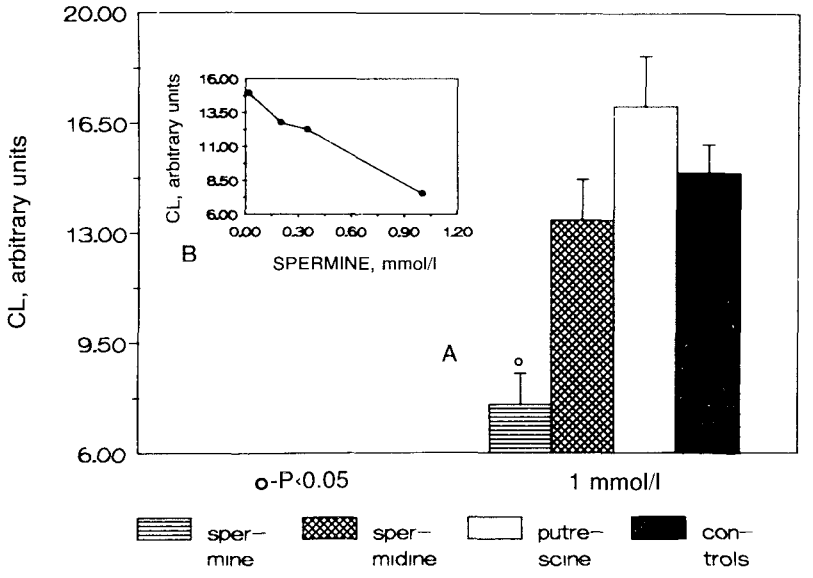

Figure 4. Inhibition of the luminol-dependent chemiluminescence in xanthine-xanthine oxidase system by polyamines. *A* – Comparison of the inhibitory effects of spermine, spermidine and putrescine at 1 mmol/l on the luminol-dependent chemiluminescence. The respective polyamine was added to mixture of 0.1 mmol/l luminol and 0.1 mmol/l xanthine in 50 mmol/l phosphate buffer, pH 7.4, to a concentration of 1 mmol/l. Chemiluminescence was iduced by addition of xanthine oxidase to a concentration of 10^{-4} IU/ml. *B* – Inhibition of the luminol-dependent chemiluminescence by different concentrations of spermine. The experimental conditions, except the concentration of spermine, were the same as those described in legend to Fig. 4*A*.

hand, O_2^- may take part in some reactions in which other reactive oxygen species such as hydroxyl radicals (·OH) and/ or singlet oxygen ($^1O_2$) are produced. Both ·OH and $^1O_2$ are powerful triggers of lipid peroxidation. Therefore, O_2^- scavenging by polyamines in the lipid containing systems will result in decreased initiation of new oxidation chains.

Taking into account all these findings it may be concluded that spermine, spermidine and putrescine at concentrations similar to those found in some biological structures have some antioxidant activities. The antioxidant action of polyamines seems to be related to the number of amino groups on their molecules. Putrescine, spermidine and spermine have two, three and four amino groups, respectively. Therefore the antioxidant activity of spermine is the highest. On the other hand, in phospholipid liposome suspensions the polyamines inhibited Fe^{2+}-induced chemiluminescence significantly stronglier than they did with Fe^{2+}-catalyzed production

of MDA products. This difference may suggest that the inhibition of Fe^{2+}-induced chemiluminescence is not due to reaction between the polyamine molecule and the lipid peroxy radical only: reaction between the polyamine and the superoxide radical generated during Fe^{2+}-catalyzed lipid peroxidation seems to be involved as well. Therefore, it may be speculated that the antioxidant action of the polyamines is realized at the levels of initiation and propagation of the lipid peroxidation chains. The antioxidant action of polyamines at the level of initiation is due to their ability to scavenge O_2^-. At the level of propagation of the oxidation chains the antioxidant action of polyamines is obviously due to their reactions with the lipid peroxy radicals. It is difficult to say which of the two-level antioxidant action of the polyamines is more important for biological structures. Possibly, it is different in different biomembranes and depends on the kind and location of the free radical generators within the cells. This problem is important from biological point of view and needs therefore further experimental investigations.

References

Asakawa T., Matsushita S. (1980): Thiobarbituric acid test for detecting lipid peroxides. Lipids **14,** 401—406

Bachrach U. (1970): Metabolism and function of spermine and related polyamines. Annu. Rev. Microbiol. **24,** 109—134

Bachrach U., Plesser Y.M. (1986): A sensitive, rapid, chemiluminescence based method for the determination of diamines and polyamines. Anal. Biochem. **152,** 423—431

Cadenas E., Wefers H., Sies H. (1981): Low-level chemiluminescence of isolated hepatocytes. Eur. J. Biochem. **119,** 531—536

Drolet G., Dumbroff E.B., Legge R.L., Thompson J.E. (1986): Radical scavenging properties of polyamines. Phytochemistry **25,** 367—371

Fukuyama H., Yamashita S. (1976): Activation of rat liver choline kinase by polyamines. FEBS Lett. **71,** 33—36

Heinrich-Hirsch B., Ahlers J., Peter H.W. (1977): Inhibition of Na,K-ATP-ase from chick brain by polyamines. Enzyme **22,** 235—241

Janne J., Poso H., Raina A. (1978): Polyamines in rapid growth and cancer. Biochim. Biophys. Acta **473,** 241—293

Johnson W.T., Nordiie R.C. (1980): Stimulation of glucose-6-phosphatase by polyamines is a membrane-mediated event. Life Sci. **26,** 297—302

Kitada M., Igarashi K., Hirose S., Kitagawa H. (1979): Inhibition by polyamines of lipid peroxide formation in rat liver microsomes. Biochem. Biophys. Res. Commun. **87,** 388—394

Kitada M., Yamaguchi N., Igarashi K., Hirose S., Kitagawa H. (1980): Effects of polyamines on ethylmorphine N-demethylation in rat liver microsomes. Jpn. J. Pharmacol. **30,** 579—586

Kitada M., Naito Y., Igarashi K., Hirose S., Kanakubo Y., Kitagawa H. (1981): Possible mechanism of inhibition by polyamines of lipid peroxidation in rat liver microsomes. Res. Commun. Chem. Pathol. and Pharm. **33,** 487—497

Kossorotow A., Wolf H.U., Seiler N. (1974): Regulatory effects of polyamines on membrane-bound acetylcholinesterase. Biochem. J. **144,** 21—27

Moller F., Hough M.R. (1982): Effect of salts on membrane binding and activity of adipocyte phosphatidate phosphohydrolase. Biochim. Biophys. Acta **711,** 521—531

Navaratnam N., Virk S.S., Ward S., Kuhn N.J. (1986): Cationic activation of galactosyltransferase from rat mammary Golgi membranes by polyamines and basic peptides and proteins. Biochem. J. **239,** 423—433

Poli G., Albano E., Dianzani M. (1987): The role of lipid peroxidation in liver damage. Chem. Phys. Lipids **45,** 117—142

Raina A. (1963): Studies on the determination of spermidine and spermine in the developing embryo. Acta Physiol. Scand. Suppl. **218,** 60, 1—81

Schuber F. (1989): Influence of polyamines on membrane function. Biochem. J. **260,** 1—10

Seiler N., Lamberty U. (1973): Interrelationships between polyamines and nucleic acids. J. Neurochem. **20,** 709—717

Tabor C.H., Tabor C.W. (1972): Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine and related amines. Adv. Enzymology **36,** 203—268

Tabor C.W., Tabor C.H. (1976): 1,4-Diaminobutane (putrescine), spermidine and spermine. Annu. Rev. Biochem. **45,** 285—307

Tadolini B. (1988): Polyamine inhibition of lipoperoxidation. The influence of polyamines on iron oxidation on the presence of mimicking phospholipid polar heads. Biochem. J. **249,** 33—36

Tadolini B., Cabrini L.,Landi L., Varani E., Pasquali P. (1985): Inhibition of lipid peroxidation by spermine bound to phospholipid vesicles. Biog. Amine **3,** 97—106

Vladimirov Yu.A., Olenev V., Suslova T., Cheremisina Z. (1980): Lipid peroxidation in mitochondrial membrane. Adv. Lipid Res. **17,** 173—249

Wojtczak L., Nalecz M.J. (1979): Surface charge of biological membranes as a possible regulator of membrane-bound enzymes. Eur. J. Biochem. **94,** 99—107

Final version accepted February 10, 1992