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LIPID PEROXIDATION *in* BIOMEMBRANES

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PREFACE

The remarkable perfection with which the cell maintains its homeostasis and at the same time adequately responds by the changes in the functional activity, to the environmental factors and to the changes in the other cells of the same organism, is largely achieved through the strictly coordianted functioning of its membrane mechanisms: receptors, enzyme complexes, and channel-formers. In its turn, the faultless functioning of these molecular devices is possible due to the interactionof the proteins with the surrounding lipid molecules, which are not only the structural basis for the bilayer organization of biomembranes, but also act as important regulators of the activity of membrane mechanisms. This suggests the importance of metabolic processes involoving cell lipids both under normal physiological conditions and during many pathological processes.

However, strange as this may seem, the whole class of reactions of lipid metabolism, the possible existence of which was predicted by Engler and Bakh as early as at the end of the 19th and in the beginning of the 20th century,[1-2] until recently was outside the scope of attention of researchers. We mean here more specifically the peroxidation reactions resulting in the addition of an oxygen molecule to the molecule of the substrate (lipid) and the formation of the peroxide derivative (lipid peroxide).

Systematic studies on lipid peroxidation reactions in biological systems and their free-radical nature began in the 1940s and 1950s.[3-9] Over the past three decades the number of publications on this problem increased exponentially to approach nearly 1000 annually at present.10 It is clear that comprehensive analysis of all aspects of the complex problem of lipid peroxidation is a difficult task. Therefore, the author will probably disappoint those readers who are expecting to find in this book a systematic presentation and and exhaustive bibliography on the various problems of lipid peroxidation. In the author's opinion, such a task is beyond one's abilities, and moreover unnecessary. Although for the time being there is no comprehensive survey on lipid peroxidation, nevertheless many important problems have recently been examined in several remarkable monographs and reviews, notably publications on the mechanisms of initiation of lipid peroxidation and the role of the activated oxygen species and of transition metal ions,[10-13] on the regulation of the lipid peroxidation process by enzyme and nonenzyme systems,[15-16] on its involvement in various pathological states,[17-25] and on some methodological problems.[26-28] However, to the best of the author's knowledge, a number of fundamental issues have not been studied yet, among them: (1) the difficulties and the possibilities of the analysis of the endogenous products of lipid peroxidation in vivo; (2) the real scale of lipid peroxidation in vivo; (3) the possible role of lipid peroxidation in normal physiological processes; (4) the interrelation of structural and functional damage of biomembranes during in vitro lipid peroxidation and its possible occurrence and significance in vivo.

On the basis of the experience accumulated in the course of research in this field for more than 15 years, the author has attempted to offer an in-depth discussion of these problems. The materialization of this idea proved a very difficult task and it would have been impossible without the help offered by the author's colleagues in the course of joint research and discussions for many years, which resulted in the generation and formulation of many of the ideas presented in the proposed monograph.

Naturally, it is impossible to mention here the names of all colleagues with whom the author has worked on these problems, but nevertheless special thanks are due to B. N. Tarusov, E. A. Neyfakh, Y. P. Kozlov, Y. A. Vladimirov, F. Z. Meerson, V. Z. Lankin, E. B. Burlakova, L. L. Prilipko, V. B. Spirichev, O. A. Azizova, A. A. Krasnovski, V. B. Ritov, K. N. Novikov, A. A. Shvedova, Y. V. Arkhipenko, A. A. Boldyrev, and V. M. Savov, as wellas to the author's young colleagues, E. A. Serbinova, A. N. Erin, V. A. Tyurin, V. N. Orlov, D. P. Raykova, R. I. Viner, and R. A. Bakalova, because this work with them both in Moscow and in Sofia was at the same

a stimulus and a hope, and who greatly helped the author in the preparation of the monograph.

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REFERENCES

1. **Enlger, C. and Wild, W.,** Uber die sogenannte "Aktivierung" des Sauerstoffs und uber Superooxydbildung, *Ber. Dt. Chem. Ges.,* 30, 1669, 1897.
2. **Bakh, A. N.,** On the role of peroxide in processes of slow oxidation, *J. Russ. Phys. Chem.Soc.* ,29, 373, 1897.
3. **Kohn, H. J. and Liversadge, M.,** On a new aerobic metabolit whose production by brain is inhibited by apomorphine, emetine, ergotamine, epinephrine and menadione, *J. Pharmacol.,* 82, 292, 1944.
4. **Tarusov, B. N.,** *The Fundamental s of Biological Effects of X-Irradiation*, Medizina,Moscow, 1954, 240.
5. **Holman, R. T.,** Autoxidation of fats and related substances, in *Progressive Chemistry of Fats and Other Lipids,* Vol. 2, Pergamon, New York, 1954, 51.
6. **Horgan, V. J., Philpot, J., Porter, B. W., and Roodyn, D. B.,** Toxicity of autoxidized squalene and lineolic acid and of simpler peroxides in relation to toxicity of radiation,*Biochem. J.,* 67, 551, 1957.
7. **Emanuel, N. M. and Lyaskovskaya, Y. P.,** *The Inhibition of Fat Oxidation Processes,* Pergamon, Oxford, 1967, 240.
8. **Tappel, A. L. and Zalkin, H.,** Inhibition of lipid peroxidation in mitochondria by vitamin E, *Arch. Biochem. Biophys.,* 80, 333, 1956.
9. **Esterbauer, H.,** Lipid peroxidation products: formation, chemical properties and biological activities, in *Free Radicals in Liver Injury,* Poli, G., et al., Eds., IRL Press, Oxford, 1985, 29.
10. **Halliwell, B. and Gutteridge, J. M. C.,** *Free Radicals in Biology and Medicine,* Clarendon Press, Oxford, 1985, 346.
11. **Pryor, W. A., Ed.,** *Free Radicals in Biology,* Vol. 5, Academic Press, Orlando, Fla., 1982, 320.
12. **Pryor, W. A., Ed.,** *Free Radicals in Biology,* Vol. 5, Academic Press, Orlando, Fla., 1982, 304.
13. **Bors, W., Saran, M., and Tait, D., Eds.,** *Oxygen Radicals in Chemistry and Biology,* Walter de Gruyter, Berlin, 1984, 1025.
14. **Pryor, W. A., Ed.,** *Free Radicals in Biology,* Vol. 4, Academic Press, Orlando, Fla., 1982, 480.
15. **Cahnce, B., Sies, H., and Boveris, A.,** Hydroperoxide metabolism in mammalian organs, *Physiol. Rev.,* 59, 527, 1979.
16. **Rotilio, G., Ed.,** *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier, Amsterdam, 1986, 688.
17. **Poli, G., et al., Eds.,** *Free Radicals in Liver Injury,* IRL Press, Oxford, 1985, 229.
18. **Rice-Evans, C., Ed.,** *Free Radicals, Cell Damage and Disease,* Richelieu Press, London, 1986, 312.
19. **Halliwell, B. and Gutteridge, J. M. C.,** The importance of free radicals and catalytic metal ions in human disease, *Mol. Asp. Med.,* 8, 89, 1985.
20. **McBrien, D. C. H. and Slater, T. F., Eds.,** *Free Radicals, Lipid Peroxidation and Cancer*, Academic Press, New York, 1982, 386.
21. **Johnson, J. E., et al., Eds.,** *Modern Aging Research,* Vol. 8, Alan R. Liss, New York, 1986, 1.
22. **Yagi, K. Ed.,** *Lipid Peroxide in Biology and Medicine*, Academic Press, New York, 1982, 320.
23. **Slater, T. F.,** Free radical mechanisms in tissue injury, *Biochem. J.,* 222, 1, 1984.
24. **Demopoulos, H. B., et al.,** *Pathology of Oxygen,* Autor, A. P., Ed., Academic Press, New York, 1982,

25. **Diguiseppi, J. and Fridovich, I.,** *CRC Crit. Rev. Toxicol.,* 12, 315, 1984.
26. **Greenwald, R. A., Ed.,** *Methods of Oxygen Radical Research,* CRC Press, Boca Raton , Fl. 1985, 1.
27. **Kagan, V. E., Orlov, O. N., and Prilipko, L. L.,** *Prog. Sci. Tech. Ser. Biophys.,* Vol. 18, Boldyrev, A. A., Ed.,USSR Acad. Sci., Moscow, 1986, 136.
28. **Packer, L., Ed.,** *Methods in Enzymology,* Vol. 105, Academic Press, Orlando, Fl., 1984, 600.

THE EDITOR

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I. INTRODUCTION

The colossal potential of life to cope with different living environments is possible owing to the exceptionally well-developed mechanisms of adaptation to environmental conditions.

There is an innumerable variety of concrete mechanisms which make it possible for living creatures to adapt to different and changing environmental conditions. Nevertheless, all this variety is the manifestation of the three strategic lines of the adaptation process,[1,2] namely: (1) evolutional or genotypic adaptation; (2) phentotypic adaptation; (3) rapid (instantaneous) adaptation.

The first of these processes requires changes over many generations and depends on mastering new genetic information. The contemporary animal and plant species have been formed as a result of this process based on hereditary changes, mutations, and natural selection in the course of evolution. This type of adaptation is the starting point for the phenotypic adaptation taking place in the lifetime of the concrete individual and it takes place in the course of the interaction of the organism with the environment. Conditioning the emergency of changes which are not inherited but interact with the hereditary characteristics, phenotypic adaptation is responsible for the formation of the individual features of the organism. Phenotypic adaptation may be considered as a process taking place in the course of the individual's life, as a result of which the organism acquires the previously lacking resistance to a concrete factor of the environment. Thus it becomes possible for it to live under conditions hitherto incompatible with life and to solve hitherto unsolvable tasks.[2] The completion of the process of phenotypic adaptation requires between several hours and several months. In the cases when the adaptation to the environmental changes should be almost instantaneous, the organism is left with the only possibility of resorting to fast adaptation reactions, using — as a rule — already formed mechanisms.

Naturally, these three basic principles of adaptation involve, to one degree or another, all levels of the organization of living matter. It is usually assumed that at the level of cell macromolecules these strategic lines of the adaptation process occur by means of three basic mechanisms:[1]

1. Changes in the *type* of macromolecules in one or another system of the organism
2. Changes in the number or *concentration* of macromolecules
3. Adaptative regulation of the function of macromolecules

Probably it would not be very difficult to find the correspondence between the three mentioned strategic lines of adaptation, considered in the temporal scale (genotypic, phenotypic, and rapid adaptation), and these three mechanisms of adaptive changes of the macromolecules.

Lipid components also take part in the process of adaptation, in addition to the protein (macromolecular) components, at the level of the membrane apparatus of the cell. In this case the change in the type of molecules (lipid molecular species) can easily take place, not only in the course of prolonged evolutional adaptation, but also in the course of phenotypic and probably of fast adaptation, because the reactions of lipid synthesis and degradation in biomembranes can take place sufficiently quickly.[3,4] Thus, unlike the adaptational changes of macromolecules, the adaptational mechanisms in lipids function basically at the expense of the changes in their molecular species, which may take place in the membrane structures of the cell as a result of both genotypic changes.

From this point of view it is probably interesting to consider light-absorbing membranes containing pigments rhodopsins, with retinal as their chromophore, which occur both in prokaryotes and in eukaryotes. In the course of the evolutional adaptation to the action of light fluxes having different intensity, nature has created molecular membrane systems consisting of similar molecular blocks, but being capable of performing totally different

functions: highly sensitive perception of weak light signals in the visual photoreceptors of different animals (photosensors) or transformation of the solar energy into energy of macroergic bonds in ATP molecules under intense light fluxes, acting as a kind of solar battery (*Holobacterium halobium*).

Irrespective of a certain structural similarity in the general pattern of these membrane phototransducers, they are essentially different both in the primary structure of the protein components[5-8] and in the composition of membrane lipids.[9-11] It may be assumed that the differences in the protein components reflect the results of the ancient genotypic adaptation to the action of light with different intensity and to the performance of different functions. The differences in the lipid composition are probably the evolutionally consolidated adaptational response to the action of some other factor, insofar as both these membrane proteins can be reconstituted in a phospholipid matrix of the same composition, with preservation of the functional activity.[12-14] The lipid component of the prokaryotic cells of *H. halobium* is represented almost exclusively by saturated molecular types of phospholipids,[11] whereas the phospholipids of the photoreceptor membranes of the retina of different animals contain 70 to 80% polyenoic fatty acid residues.[9,10] These differences are believed to result from the adaptation to various temperature conditions: the need to maintain a sufficient rigidity of the membranes of *H. halobium* in heated water reservoirs (50 to 70°C), as well as the need to maintain sufficient fluidity of the photoreceptor membranes (for guaranteeing the mobility of the proteins participating in the visual transduction), especially in cold-blooded animals.[15] Without going into detail concerning the participation of the mechanisms of genetic or phenotypic adaptation in the existence of such sharp differences in the fatty-acid composition of phototransducing membranes containing bacterial or visual rhodopsins, we shall only point out that these differences are characteristic of the membranes of pro- and eukaryotic cells in general.[16] What is very important in the context of the present monograph is that the presence of a considerable amount of polyunsaturated lipids in the plasmic and especially in the intracellular membranes is the most characteristic feature of their organization, and guarantees the normal functioning of membrane proteins.[17] The change in the saturation of the lipid components of the membranes, as a means of compensating temperature changes, is one of the most important mechanisms for maintaining the homeostasis in ectothermal animals.[18] For greater details on this problem see the monographs.[1,19]

Molecular oxygen is one of the environmental factors to which the organisms are compelled to adapt. Naturally, we are used to the thought that life on earth is unthinkable without oxygen. However, this was not always so, and probably the transition from anaerobic to aerobic life, which took place on earth 1.5 to 2 billion years ago, required the intense work of all mechanisms of adaptation, especially of genotypic adapation.[20] Strange as it seems, this was so because molecular oxygen is chemically inert.

Looking at the scheme illustrating the electron structure of the oxygen molecule, we shall see that in its ground-state molecular oxygen is biradical (Figure 1).[21-23] In the oxygen molecules, two electrons occupy the antibonding orbitals. In the ground state ($^3\Sigma_g$) of oxygen, in accordance with Hund's principle, these electrons are sited with parallel spins on the orbitals with equal energies, π_x^* and π_z^*. Oxygen has a σ-π-π-bonding structure and additionally, two electrons on the antibonding molecular orbitals with parallel spins. Such an electron configuration is the reason for the extremely low reactivity of molecular oxygen with respect to stable organic compounds having paired electrons in their orbitals. The direct penetration of electron pairs of the organic substrate S ($\downarrow\uparrow$) into the half-filled orbitals of molecular oxygen O_2 ($\uparrow + \uparrow$) should lead to the appearance of two parallel spins on one orbital.

$$O_2(\uparrow + \uparrow) + S(\downarrow\uparrow) \rightarrow SO_2(\downarrow\uparrow + \uparrow\uparrow)$$

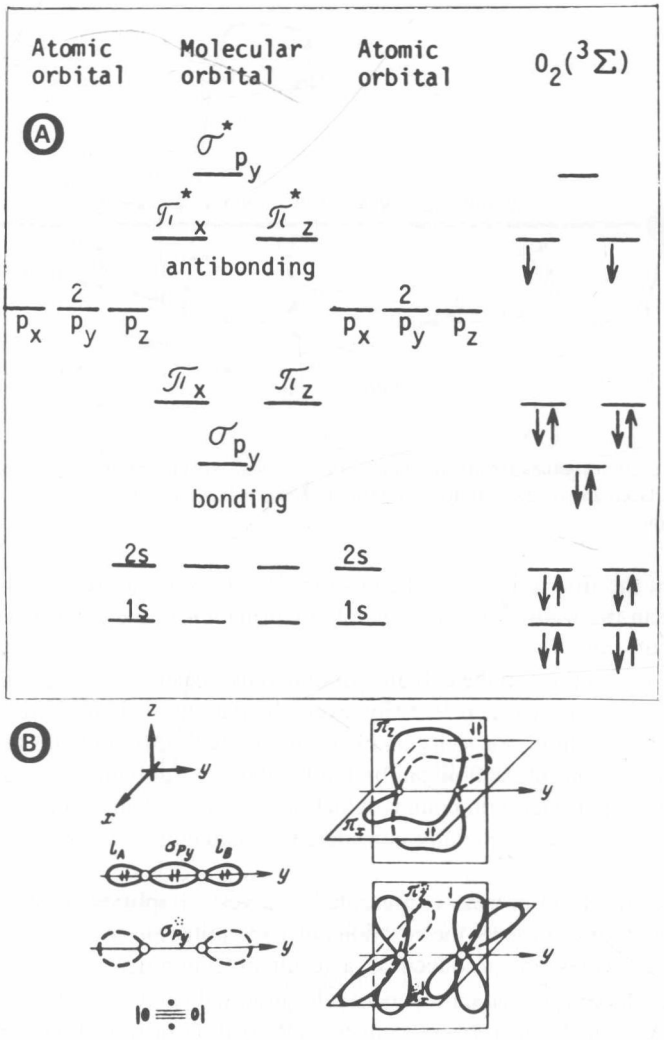

FIGURE 1. (A) Schematic presentation of the orbitals of the oxygen molecules. (B) spatial arrangement and population of the molecular orbitals of the molecule in ground state.

This is forbidden by the quantum mechanical rules of selection. The result of these quantum mechanical restrictions is the very low rate of interaction of O_2 with compounds containing paired electrons. Hence, it is clear that a direct reaction of molecular oxygen with organic compounds is practically impossible under physiological conditions.

There exist several ways of overcoming the above-mentioned spin restrictions. The following among them are important for the reaction of O_2 with biological substrates, namely:

1. Consecutive one-electron reduction of O_2 with the formation of superoxide-anion ($O\dot{-}_2M$), H_2O_2, and hydroxyl radical
2. Formation of singlet oxygen in excited state ($^1\Delta$ g)
3. Complexing of oxygen with transition metals
4. Transformation of the stable oxidizable molecules of the substrate into unstable radical intermediates with unpaired electrons

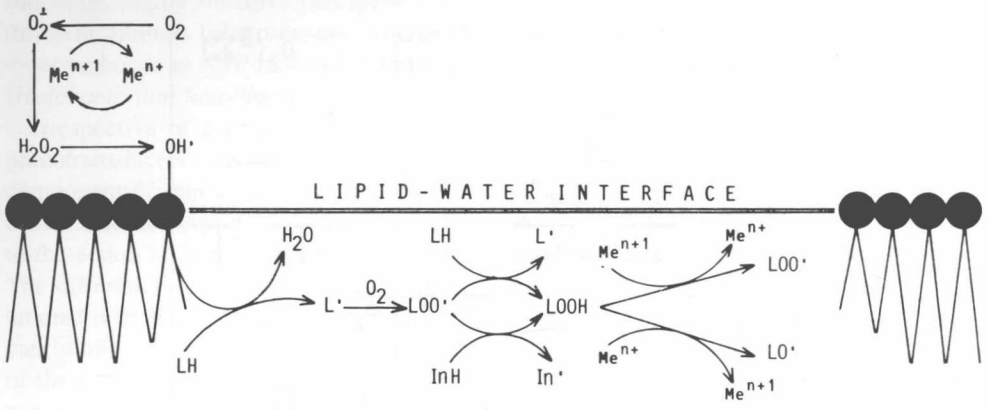

FIGURE 2. Scheme of the initiation and propagation of free-radical oxidation reactions in the phospholipid bilayer Me^{n+}, Me^{n+1} — reduced and oxidized forms of transition metals; LH — oxidation substrates; InH — inhibitor of free-radical oxidation.

It is clear that in the first three cases the electron structure of the oxygen molecule itself is involved, while in the fourth case there is a transformation in the electron configuration of the oxidizable substrate.

Many oxidation reactions in the cell are possible only because of the specific mechanisms of activation of molecular oxygen.[21-23] However, the paradox is that the resulting activated oxygen species have a high reactivity and can interact with different substrates in the cell. The substrate of such an interaction can be lipids, above all polyunsaturated lipids, leading to the formation of peroxide compounds which are capable of initiating reactions of free-radical oxidation. These are generally considered as damaging factors for biological membranes.[23-25]

The scheme of the LPO process is presented in a very simplified form in Figure 2. The upper part of this figure presents the reactions of LPO initiation in the course of which the activated-oxygen species are produced as a result of consequent one-electron reduction. These species are the oxygen radicals: superoxide anion-radical ($O_2^{\cdot}$) and the hydroxyl radical ($HO^{\cdot}$). These oxyradicals are formed with the participation of transition metal ions, which often takes place outside the hydrophobic zone of the membrane — on its surface or in aqueous phase. The superoxide anion-radicals have a relatively low reactivity, as a result of which their interaction with the membrane phospholipids is not very important for the initiation of LPO reactions.[23-26] Conversely, the interaction of highly reactive $HO^{\cdot}$ radicals with membrane lipids (LH) results in the formation of intermediate free-radical products of a lipid nature (see the lower part of the figure): alkyl ($L^{\cdot}$), alkoxyl ($LO^{\cdot}$), and peroxyalkyl ($LO_2^{\cdot}$) radicals. This stage of the LPO process already takes place in the hydrophobic zone of the membrane. The maintenance and development of the LPO process, as well as the involvement in it of newer lipid substrates (from among membrane phospholipids), are guaranteed by the constant "regeneration" of $L^{\cdot}$-, $LO^{\cdot}$- and $LO_2^{\cdot}$- radicals, and by their interaction with membrane phospholipids. An important source of such lipid radicals are the primary molecular LPO products, hydroperoxides, which are decomposed into alkoxyl or peroxyalkyl radicals in the presence of transition metals.

With the exception of the initiation stage, in the course of which the primary oxygen and lipid radicals are formed, the most difficult and limiting reaction of the LPO process is the interaction of lipids with peroxide radicals, resulting in the formation of hydroperoxides. The rate constant of this reaction sharply increases when the number of double bonds in the oxidized molecule is increased.[27,28] This is the reason for the preferable oxidation of un-

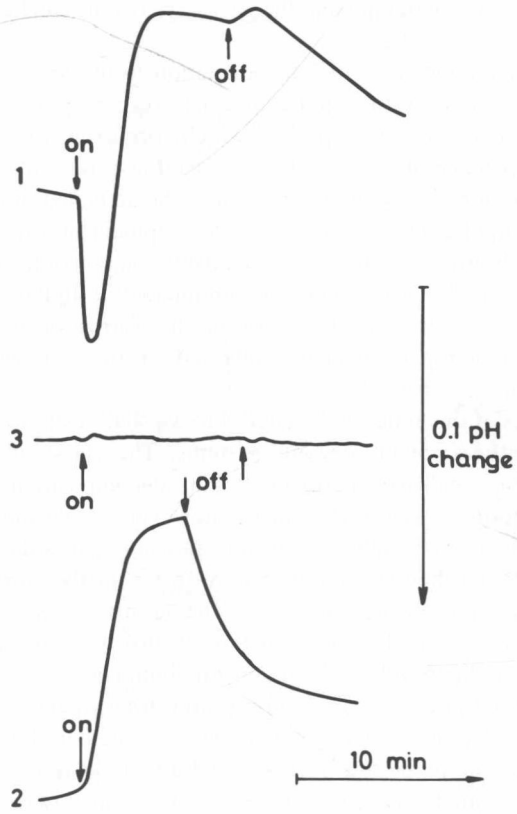

FIGURE 3. Typical photoresponses (registered by the change in pH) of *H. halobium* cells. Arrows indicate the moments when the light was switched on and off. Curves before and after the action of light with high intensity (O.2 W/cm$_2$, 30 min) in the presence of exogenous all-*trans* retinal (4×10^{-5} *M*) were practically indistinguishable. Cells in 4 *M* NaCl solution have such suspension density which gives optical density 0.4 at 570 nm in 1 cm cuvette; (1) anaerobic conditions (in N_2 atmosphere); (2) aerobic conditions (the cuvette is open); and (3) after addition of 0.1% Triton X-100 for damage of the cell membrane.

saturated lipids in the course of the LPO process in biomembranes. As a result of the described sequence of the reactions, a considerable amount of membrane polyenoic phospholipids can be involved in the LPO process.

Going back to the above-mentioned example of light-absorbing membranes in the visual cells of the retina and the purple bacteria *H. halobium*, which differ sharply in fatty acid composition, it is necessary to adduce evidence that membranes containing mainly saturated hydrocarbon chains, and therefore rather unsusceptible to the process of free-radical oxidation, prove to be rather resistant to the action of LPO inducers.[29] Thus, it is not possible to induce their photodamage under the action of high light intensity on *H. halobium* cells, even in the presence of the exogenous generator of singlet oxygen, all-*trans* retinal (Figure 3). Parallel with this, a marked photodamage manifested in suppression and disappearance of the electrical activity of the retina is observed in the photoreceptors of the retina, containing considerable amounts of polyenoic fatty acid residues, under the effect of high light intensity both in the presence of exogenous retinal and when free retinal is formed as a result of rhodopsin photolysis (for greater details see Chapter 3 and Figures 5, 6, and 8).

Naturally, the scheme in Figure 2 gives only a most general idea about the initiation and development of LPO processes. A more detailed study of these problems goes beyond the

framework of the present monograph and the necessary details can be found in the following surveys.[30-32]

Thus, prolonged genotypic (evolutional) adaptation to the two most important environmental factors, namely temperature and molecular oxygen, gave rise to polyunsaturated phospholipids and activated-oxygen species, which, occurring together in biomembranes, pose a threat to the existence of the cell. This makes it necessary to create new mechanisms of adaptation, capable of resisting the interaction of the activated molecular-oxygen species with polyunsaturated lipids, which leads to the development of a radical oxidation reaction of membrane phospholipids. One of these mechanisms consists of the formation of activated-oxygen species directly in the place where oxygen interacts with the oxidized substrate, i.e., in the catalytic site of the enzyme. This prevents the formation of free activated-oxygen species capable of interacting with other compounds in the cell, which are not substrates for the concrete enzyme reaction.[33,34]

Numerous examples of this type can be cited, but we shall restrict ourselves to mentioning only the very frequently occurring enzyme systems. The classic example is the catalase-catalyzed destruction of hydrogen peroxide.[35] This decomposition of hydrogen peroxide occurs without formation of hydroxyl radicals; however, stable radicals are generated on the heme moiety.[36] A closely related family of enzymes, peroxidases, also use hydrogen peroxide as an oxidant but then generate substrate-free radicals (aromatic amines, phenols) which may have physiological significance.[37] The family of cytochromes P-450, which generate ''peroxide'' by a two-electron reduction of dioxygen, function in a manner analogous to the catalases and peroxidases, and generate both haem and substrate radicals under carefully controlled conditions.[38,39] The same is true for cytochrome oxidases.[40] The key reaction that nature has learned to achieve and control is the hetrolytic cleavage of the O–O bond of the coordinated peroxide to give water and a two-electron oxidation product of the resting enzymes, controlled by steric and electronic constraints imposed by the proteins.[38-40]

Nevertheless, the efficiency which is estimated by the oxygen uptake for the oxidation of the reaction substrate is practically never 100%. This means that part of the oxygen radicals can ''escape'' from the active site and can interact with other targets in the cell.[38-44] Moreover, there are a considerable number of nonenzyme systems capable of generating activated-oxygen species (e.g., reducers of the type of ascorbic acid, cysteine, glutathione in the presence of bound or free ions of transition metals, and photosensitizers capable of generating singlet oxygen).[45-46]

In order to prevent the interaction of the activated-oxygen species with the nonspecific substrates, special enzyme systems are functioning in the cell, the so-called enzymes for antioxidant defense: catalase, superoxide dismutase, and glutathione peroxidase, which catalyze the transformation of the products of the consecutive one-electron reduction of dioxygen into stable molecular products.[47-50] The above-mentioned defense systems are oriented toward the activated-oxygen species participating in the initiation of the reactions of free-radical lipid oxidation in the cell. They also comprise the acceptors of the activated-oxygen species of nonenzyme nature: singlet-oxygen quenchers and free-radical scavengers.[51-53] For polyunsaturated phospholipids from which the lipid radicals participating in the development of the free-radical oxidation in biomembranes are formed, there is a separate adaptational defense mechanism which includes the fatty-soluble (lipid) antioxidants (tocopherols, ubiquinols, etc.) and the systems of their regeneration.[52-55]

Thus, in the course of the adaptation of the organisms to aerobic metabolism under conditions of relatively low temperatures of the environment, it becomes necessary to include the polyunsaturated phospholipids into the membranes, as well as to generate activated-oxygen species. This, in its turn, requires the emergence of new adaptational systems controlling the level of the interaction of the oxygen radicals with the membrane-polyenoic

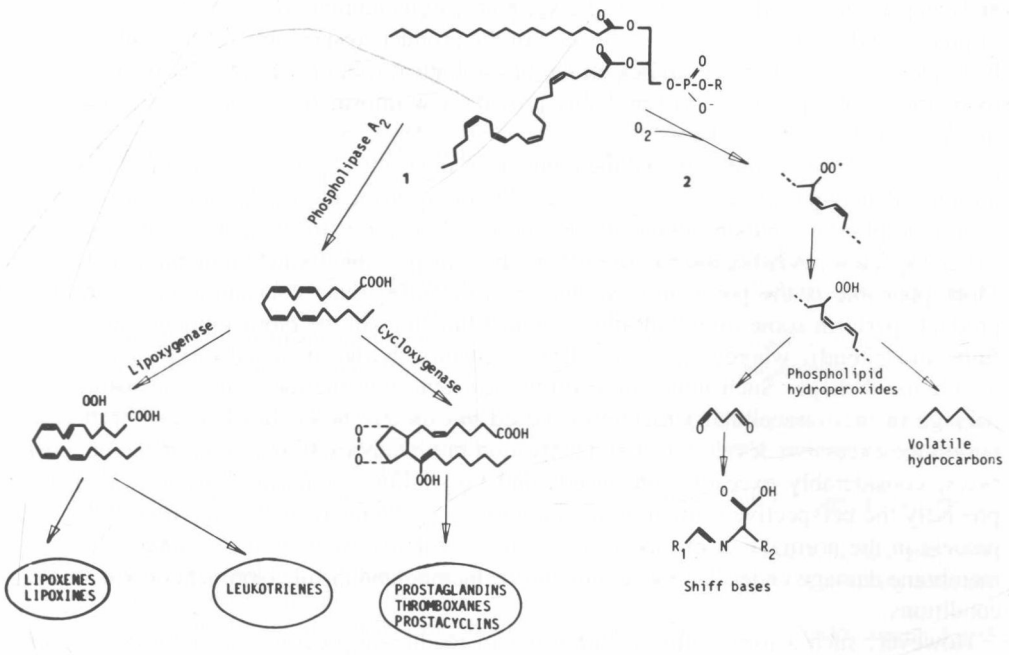

FIGURE 4. Scheme of the alternative peroxidation of polyenoic acyls of membrane phospholipids. (1) After preliminary hydrolysis with phospholipase A_2; (2) directly in the phospholipid molecule.

phospholipids, preventing the development of the free-radical oxidation reactions in the lipid phase of the membranes and their damage. The starting point in this logical sequence is the assumption that the formation of LPO products in the biomembranes inevitably leads to their damage as a result of the nonspecific involvement of phospholipids in the LPO process. However, to what extent are these notions correct? Is it possible to claim today that the LPO process is only a nonspecific process of oxidative modification of lipids?

Figure 4 presents two alternative ways for the oxidation of the polyenoic lipids of biomembranes by means of free-radical reactions. Polyenoic fatty acyls can be subjected to free-radical oxidation both after preliminary hydrolysis of phospholipids by type A_2 phospholipases, as well as in esterified form, directly in the molecules of the membrane phospholipids. In the first case the reaction products catalyzed by cyclooxygenases or lipoxygenases are two groups of physiologically active compounds: (1) prostaglandins, thromboxanes, and prostacyclins, and (2) leukotrienes, lypoxins, and lypoxenes.[56,57] The oxidation of fatty acyls in the phospholipids can also be catalyzed by lipoxygenases (e.g., lipoxygenase from reticulocytes,[58] microvessels,[59] etc.) with the formation of stereospecific hydroperoxides. The enzyme or nonenzyme initiation of the peroxidation of the fatty acid residues of phospholipids, taking place at the expense of the interaction with the oxygen radicals, results, as a rule, in the formation not of one or several stereospecific products, but of a wide range of different compounds (for greater details see Chapter 1).

Is it possible to conlclude that the stereospecific products of the free-radical oxidation of lipids (fatty acids and phospholipids) are physiological regulators, while the "nonspecific" products of the oxidation of phospholipids are responsible for the damage of biomembranes and do not participate in the normal cell metabolism? It should be assumed that such a conclusion would be extremely precipitous. At present there is evidence that the LPO products are capable of forming single ionic channels in the lipid bilayer.[60,61] They can also determine the appearance of selective calcium conductivity of artificial and natural membranes,[62,63]

and can participate in the physiological disassembly of membrane structures[64] in the processes of phago- and pinocytosis,[65,66] etc. Several of the products responsible for these effects have been identified.[62,67] Further studies on the physiological role of the products of free-radical oxidation of phospholipids will probably provide new information about their participation in the normal cell metabolism.

However, it is very unlikely that the products of free-radical peroxidation of phospholipids are only damaging agents, something like "extra costs for the production", in which the polyenoic phospholipids necessary for the normal functioning of the cell and the activated-oxygen species inevitably interact to form products responsible for the biomembrane damage. More plausible is the point of view that the low steady-state concentrations of the LPO products perform some important physiological functions in the biomembranes (not always fully understood), whereas excessive LPO activation is the universal mechanism of biomembrane damage. Such notions are fully consistent with the idea[68] that the pathological damage to the intracellular structures is based not on any new "biochemical" processes, but on the excessive development and activation of already existing normal metabolic processes, considerably exceeding the needs and possibilities of normal physiology. This is precisely the perspective in the investigation of the problems related to the role of the LPO process in the normal cell metabolism, and to the role of excessive LPO activation for the membrane damage under the impact of extreme factors, and the development of pathological conditions.

However, such a formulation of the problem requires a precise answer to the question: what are the normal (low) steady-state concentrations of the phospholipid peroxidation products in the cell, and what should be considered to be increased concentrations of the LPO products, i.e., activation of this process? Before being able to find the answer to this question, it is necessary to study in greater detail the problem of the variety of products of free-radical lipid peroxidation.

LIST OF ABBREVIATIONS

BHA	butylated hydroxyanisole (2,3-*tert*-butyl-hydroxyanisole).
BHT	butylated hydroxytoluene (2,6 -di-*tert*-butyl-*p*-cresol).
Ca^{2+}-ATPase	Ca^{2+}-dependent adenosine 5′-triphosphatase.
CMC	critical micellization concentration.
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid).
ERG	electroretinogram.
ESR	electron spin resonance.
LPO	lipid peroxidation.
MDA	malonyl dialdehyde.
NMR	nuclear magnetic resonance.
ROS	rod outer segments.
TBA	2-thiobarbituric acid.
V_{ATP}	activity of Ca^{2+}-ATPase (rate of ATP hydrolysis).
$V^{o}_{out\ Ca^{2+}}$	rate of Ca^{2+}-outflow through the "passive" channels.
$V^{ATP}_{out\ Ca^{2+}}$	rate of Ca^{2+}-outflow through the "active" channels.

REFERENCES

1. **Hochachka, P. W. and Somero, G. N.,** *Biochemical Adaptation,* Princeton University Press, Princeton, New Jersey, 1985, 561.
2. **Meerson, F. Z.,** *Adaptation, Stress and Prophylaxis,* Springer-Verlag, Berlin, 1984, 329.
3. **Kennedy, E. P.,** The biosynthesis of phospholipids, in *Lipids and Membranes: Past, Present and Future,* Jos, A. F., Op Den Kamp, Roelofsen, B., and Wirtz, K. W. A., Eds., Elsevier, Amsterdam, 1981, 171.
4. **Jobe, A., Kirkpatrick, E., and Gluck, L.,** Labeling of phospholipids in the surfactant and subcellular fractions of rabbit lung, *J. Biol. Chem.,* 253, 3810, 1978.
5. **Ovchinnikov, Yu. A., Abdulaev, N. G., Feigina, M. Yu., Artamonov, I. D., Bogachuk, A. S., Zolotarev, A. S., Eganyan, E. R., and Kostetskii, P. V.,** Visual rhodopsin. III. Total amino acid sequence and arrangement in the membrane (in Russian), *Bioorg. Khim.,* 9, 1331, 1983.
6. **Findlay, J. B. C. and Pappin, D. J. C.,** The opsin family of proteins, *Biochem. J.,* 238, 625, 1986.
7. **Nathans, J., Thomas, D., and Hogness, D. S.,** Molecular genetics of human color vision: the genes encoding blue, green, and red pigments, *Science,* 232, 193, 1986.
8. **Ovchinnikov, Yu. A., Abdulaev, N. G., Vasilov, R. G., Vturina, I. Yu., Kuryatov, A. B., and Kiselev, A. V.,** The antigenic structure and topography of bacteriorhodopsin in purple membranes as determined by interaction with monoclonal antibodies, *FEBS Lett.,* 179, 343, 1985.
9. **Bazan, N. G. and Reddy, T. S.,** Retina, in *Handbook of Neurochemistry,* Vol. 8, Lajtha, A., Ed., Plenum Press, New York, 1985, 507.
10. **Daemen, F. J. M.,** Verterbrate rod outer segment membranes, *Biochim. Biophys. Acta,* 300, 255, 1973.
11. **Plachy, W. Z., Lanyi, J. K., and Kates, M.,** Lipid interactions in membranes of extremely halophilic bacteria, *Biochemistry,* 13, 4906, 1974.
12. **Westerhoff, H. V. and Dancshazy, Zs.,** Keeping a light-driven proton pump under control, *Trends Biochem. Sci.,* 9, 112, 1984.
13. **Lozier, R. H., Niederberger, W., Bogomolni, R. A., Hwang, S. B., and Stoeckenius, W.,** Kinetics and stoichiometry of light-induced proton release and uptake from purple membrane fragments, *Halobacterium halobium* cell envelopes, and phospholipid vesicles containing oriented purple membrane, *Biochem. Biophys. Acta,* 440, 545, 1976.
14. **Kuhn, H.,** Interactions of rod cell proteins with the disk membrane: influence of light, ionic strength, and nucleotides, in *Current Topics in Membranes and Transport,* Vol. 15, Miller, W. H., Ed., Academic Press, New York, 1981, 171.
15. **Anderson, R. E. and Risk, M.,** Lipids of ocular tissues. IX. The phospholipids of frog photoreceptor membranes, *Vision Res.,* 14, 129, 1974.
16. **Stubbs, Ch. D. and Smith, A. D.,** The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function, *Biochim. Biophys. Acta,* 779, 89, 1984.
17. **Kimelberg, H. K.,** Membrane fluidity and membrane activities, in *Phys. Meth. Biol. Membr. and Model Syst.,* Proc. NATO Adv. Study Inst., New York, 1985, 277.
18. **Hazel, J. R. and Prosser, C. L.,** Molecular mechanisms of temperature compensation in poikiloterms, *Physiol. Rev.,* 54, 620, 1974.
19. **Kreps, E. M.,** *Lipids of Cell Membranes* (in Russian), Nauka Publishers, Leningrad, 1981, 338.
20. **Hochachka, P. W.,** Assessing metabolic strategies for surviving O_2 lack: role of metabolic arrest coupled with channel arrest, *Mol. Physiol.,* 8, 331, 1985.
21. **Metelitza, D. I.,** *Activation of Oxygen by Enzymic Systems* (in Russian), Nauka Publishers, Moscow, 1982, 249.
22. **Diguiseppi, J. and Fridovich, I.,** The toxicology of molecular oxygen, *CRC Crit. Rev. Toxicol.,* 12, 315, 1984.
23. **Halliwell, B. and Gutteridge, J. M. C.,** The importance of free radicals and catalytic metal ions in human diseases, *Mol. Aspects Med.,* 8, 89, 1985.
24. **Koster, J. F., Biemond, P., Montfoort, A., and Stam, H.,** The involvement of free radicals in pathological conditions, *Life Chem. Rep.,* 3, 323, 1986.
25. **Halliwell, B. and Gutteridge, J. M. C.,** Oxygen toxicity, oxygen radicals, transition metals and disease, *Biochem. J.,* 219, 1, 1984.
26. **Halliwell, B. and Gutteridge, J. M. C.,** *Free Radicals in Biology and Medicine,* Oxford University Press, Oxford, 1985, 352.
27. **Emanuel, N. M. and Lyaskovskaya, Yu, P.,** *The Inhibition of Fat Oxidation Processes,* Pergamon Press, Oxford, 1967, 240.
28. **Witting, L. A.,** Lipid peroxidation in vivo, *J. Am. Oil Chem. Soc.,* 42, 908, 1965.
29. **Szundi, I. and Kagan, V. E.,** Comparison of the effects of high-light intensity on rhodopsin-containing membranes of the retina and of *Halobacterium halobium* cells in the presence of exogenous retinal, *Photobiochem. Photobiophys.,* submitted, 1987.