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The Peroxide Oxidation of Membrane Lipids and Natural Antioxidants

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The ways in which membrane lipids are oxidised are considered and the initiating and protective systems influencing the rate of this process are classified. The regulatory role of the interrelation between the composition of the membrane lipids and the degree of their oxidation, which makes it possible to regard this process as a procedure for the rapid renewal of the composition of the lipids and the alteration of the functional activity of membranes, is discussed. The kinetic characteristics of natural antioxidants as radical process inhibitors are presented and the characteristic features of their activity are deduced. They are discussed in terms of their involvement in the regulation of the peroxide oxidation of lipids.
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I. INTRODUCTION

The research into the peroxide oxidation of lipids (POL) in the USSR was initiated by studies carried out in 1954–1956 in Moscow State University and at the Institute of Chemical Physics at the USSR Academy of Sciences, which have been recognised as giving priority in this field to the Soviet Union.[1,2]

At the present time this field has been developing successfully not only in the USSR but also abroad. It is not fortuitous that so much attention has been devoted to the question of the biological significance of the oxidative processes occurring in membrane lipids. It has been established that the accumulation of the lipid oxidation products in membranes alters the structure of the latter and their functional activity. Lipid peroxides, which are the primary and labile lipid oxidation products, have attracted most attention by investigators.

The peroxide oxidation of lipids has been regarded for a fairly long time by many investigators as an undesirable process, which is actually harmful for the organism. This was promoted by the establishment of the following facts: (1) peroxides have been detected most frequently in the organs of animals following the development of various pathological conditions; (2) it has been shown in many studies that the administration of oxidised lipids to animals is toxic for the organism;[3,4] (3) the appearance of peroxy-groups in the aliphatic acid residues of membrane phospholipids alters the properties of the latter such as permeability and the stability of the lipid–protein complexes and induces the inactivation of enzymes.[5-11]

Questions associated with the role of natural antioxidants in the regulation of the peroxide oxidation of lipids are examined in this review.

II. SYSTEMS REGULATING THE RATE OF THE PEROXIDE OXIDATION OF MEMBRANE LIPIDS

The discovery in 1963[12] of two pathways leading to the peroxide oxidation of lipids, namely enzymic and non-enzymic, and the vigorous investigation of these processes in recent years have altered somewhat the assessment of the biological role of peroxides.

Many factors proved to be important for the establishment of the hypothesis of a significant role of peroxide oxidation for normal metabolism.

1. The improvement and increase of the sensitivity of methods for the determination of peroxides made it possible to detect them in many organs and tissues of intact animals.

2. Enzyme systems regulating both the rate of formation of peroxides and the rate of their utilisation have been discovered and investigated.[13-18] This means that peroxide formation and utilisation processes are characteristic of norma metabolising cells and the amount of peroxide in the membrane must be maintained at a specific stationary level.

3. Careful investigation of the toxicity of oxidised lipids showed that not so much the peroxides as the products of the more far-reaching oxidation of lipids, namely aldehydes, ketones, and acids, are toxic for the organism.[19]

4. It has been shown that lipid peroxides are intermediates employed by the organism for the synthesis of biologically active substances: prostaglandins, thromboxanes, steroid hormones, etc.[20-23] and they cannot therefore be regarded as undesirable side products of the oxidation process.

The synthesis of these substances in the organism from unsaturated aliphatic acids is achieved in several stages via the formation of short-lived labile endoperoxides under the influence of specific enzymes. For example, the cyclisation of arachidonic acid entails the formation of endoperoxides (the prostaglandins G_2 and H_2), which are transformed into the prostaglandins E_2, D_2, and $F_{2\alpha}$. When the thromboxane synthetase, localised in the membranes of thrombocyte microsomes, acts on the endoperoxides in this case, the thromboxanes A_2 and B_2—substances with pronounced adhesive properties, are formed. When the endoperoxides are acted upon by the enzymes of the microsomal fraction of the arteria vessels, the biosynthesis is switched towards the formation of prostacyclin—a substance which possesses antiadhesive and deaggregating activity, several tens of times superior to the antithrombic activity of prostaglandins.[20] Consequently, depending on which enzyme system is involved in biosynthesis, biologically active substances with different,

sometimes directly opposed, properties are formed from the same precursor—the endoperoxide of arachidonic acid. Peroxides are a universal raw material which is employed by the organism for a wide variety of purposes.

5. The peroxide oxidation of lipids is interrelated with the processes involving the renewal of the composition of the membrane phospholipids. It has been shown that an increase in the peroxide oxidation of lipids accelerates the metabolism of phospholipids, alters their relative composition, alters the lipid–protein ratios, and hence alters the structure of the membrane.[24–27]

The interrelation between the rate of the peroxide oxidation of lipids and the change in the composition of lipids is based on the acceleration of the release of phospholipids from membranes with increase of the rate of peroxide oxidation. In model experiments with isolated membranes a decrease of the phospholipid/protein ratio was observed after the induction of peroxide oxidation and a decrease of the relative amount of the readily oxidisable phospholipids such as phosphatidylethanolamine, phosphatidylserine, cardiolipin, and phosphatidylinositol. Acceleration of the "flip-flop" and the passive transfer of lipids has been demonstrated.[28,29] On the other hand, the data concerning active transport with the aid of lipid-transferring proteins are extremely contradictory. Thus it has been observed[29] that the induction of peroxide oxidation does not influence or even suppresses the transfer of phosphatidylcholine between membranes by the lipid-transferring protein.

Data concerning the role of the fluidity of lipid membranes in the activity of lipid-transferring proteins have been published.[30,31] It has been shown that a rigid state of the lipid-donor membrane is the most favourable for the operation of the lipid-transferring proteins. Since lipid peroxides increase the microviscosity of the lipid double layer in membranes,[32] they can thus increase the activity of the lipid-transferring proteins and the rate of release of lipids from the membrane.

One should bear in mind also the acceleration of the cleavage of the oxidised phospholipids with the aid of phospholipases compared with the unoxidised phospholipids.[33] Experiments *in vivo* showed in all the cases investigated that an increase in the peroxide oxidation enriches the lipid membranes in fractions more resistant to oxidation (phosphatidylcholine, sphinogomyelin, and cholesterol).[34–36] The results, which are treated from the standpoint of the alteration of the rate of catabolism of the phospholipids, have been confirmed by the alteration of the turnover times of the membrane lipids. This has been effectively demonstrated for irradiated plant cells.[36,37]

Calculations have shown that the turnover times for readily oxidisable phospholipids are satisfactorily consistent with the rate of their peroxide oxidation, which makes it possible to regard the peroxide oxidation of lipids as one of the important factors responsible for the renewal of the membrane lipids.

Numerous studies demonstrating the lipid-dependence of the membrane proteins, namely enzymes, receptors, channel-forming agents, translocators, etc., have been published recently. The change in the composition of lipids induced by the change in the rate of peroxide oxidation therefore alters also the activity of these membrane proteins.

6. It has been shown that the lipid peroxides themselves can both activate and inhibit the activity of enzymes[38–40] and can also play the role of allosteric effectors. The interactions of lipid peroxides with the catalytic and allosteric centres of proteins justify the differentiation from the lipid-dependent enzymes of a separate group of peroxylipid-dependent enzymes.[38,41,42]

An important factor in the action of lipid peroxides on enzymes is the possibility that they may transform the enzyme activity.[41]

Thus all these data support the view that the peroxide oxidation of lipids should be regarded as a physiological process and the lipid peroxides as the products of the metabolism of the normal metabolising cells. The low level of lipid peroxides characteristic of normal tissues by no means implies that peroxide oxidation does not occur in such tissues and can be explained by the fact that the processes involving the formation and consumption of peroxides are well balanced and that lipids are oxidised at a specific stationary level.[43]

On development of pathological states, such balance can be disturbed, the peroxides formed accumulate in the tissues and lead to serious damage to the bio-membranes.

We shall consider how a stationary lipid oxidation level is maintained in membranes. The oxidation of membrane lipids is a complex multistage process with its own characteristic features determined primarily by the involvement in the oxidation reaction of a number of enzymes, the structural organisation of the lipid double layer in the membranes, the high degree of unsaturation of the aliphatic acids forming part of the composition of the phospholipids, the multicomponent composition of the lipids, and the presence in the latter of substances capable of intensifying or weakening the effect of natural antioxidants and initiating oxidation.

It is noteworthy that only the enzymic pathway to the peroxide oxidation of lipids is at present recognised as a normal physiological process. The non-enzymic pathway to the autoxidation of lipids is still regarded by many investigators as an uncontrolled process, which is therefore abnormal. We believe that this view is incorrect.

The autoxidation of lipids is a special case of the liquid-phase oxidation of hydrocarbons whose theoretical principles have been examined in detail by Emanuel's school[44–49] and which constitutes a typical chain process with degenerate branching. A distinctive feature of such processes is an extremely high sensitivity to very slight changes in the composition and proportions of the components of the oxidation reaction and in the concentrations of various additives which can play the role of oxidation inhibitors or activators. These properties are in fact important for processes involved in the regulation of the functional activity of membranes. In contrast to oxidation *in vitro*, the rate of which can be varied appreciably within wide limits by altering the temperature, composition, and the component ratios, in membranes such changes are possible only within very restricted limits. Only processes whose rate depend significantly on slight changes in the composition of the components can therefore fulfill a regulatory function. Such requirements are met most fully by degenerate-branched chain oxidation processes. The non-enzymic oxidation of lipids can therefore hardly be regarded as an avalanche-like uncontrollable process. The question whether non-enzymic oxidation can take place in the lipid double layer in membranes in which the aliphatic acid residues of the phospholipids have a structural organisation is still controversial. However, the recognition of a mosaic structure of membranes with "lipid seas" permits the assumption that all the conditions necessary for the homogeneous liquid-phase oxidation are fulfilled in the oxidation of lipids.

The properties of lipids as oxidation substrates to some extent resemble those of polymers by virtue of the high degree of structural organisation in the lipid double layer in membranes. It has been shown that the characteristics deduced for the liquid-phase oxidation of organic substances can be legitimately applied to the low-temperature oxidation of polymers.[50] On this basis, one can assume that all the

features of the oxidation of lipids established in model systems will be manifested also in the oxidation of membrane lipids, although undoubtedly the structure of biological membranes should exert a significant influence on the intensity of this process.

Thus one may postulate that the oxidation of lipids in membranes can also take place via a non-enzymic mechanism. The question arises whether it is necessary to draw a sharp boundary between these two lipid oxidation pathways. It is noteworthy that the two lipid oxidation pathways have much in common, although they differ as regards the mode of initiation and the set of intermediate and final oxidation products.

In both cases the oxidation of lipids proceeds via a stage involving the formation of lipid free radicals which are fairly active and long-lived. The rate of oxidation depends significantly on the composition of the membrane phospholipids, especially on the number of double bonds in the unsaturated aliphatic acids (UAA), on the degree of structure formation in the lipid phase of the membrane, and on the presence of inhibitors, activators, and other substances which are highly effective even at low concentrations. In all probability the oxidation pathways at different stages can merge. Thus the active forms of oxygen produced enzymically can initiate the non-enzymic oxidation.[51-53] On the other hand, glutathione peroxidase decomposes lipid peroxides regardless of the pathway (enzymic or otherwise) which led to their formation.[54-57]

Regardless of whether the lipids are oxidised non-enzymically or with participation of enzymes, the following principal elementary stages in the development of this process must be considered: initiation, propagation, branching, and termination of the oxidation chains (Scheme 1):[44-49]

Scheme 1

$$\left.\begin{aligned} RH &\xrightarrow{k_1} R^{\cdot} \\ R^{\cdot} + O_2 &\xrightarrow{k_2} RO_2^{\cdot} \end{aligned}\right\} \text{chain initiation;} \quad (0), (1)$$

$$RO_2^{\cdot} + RH \xrightarrow{k_3} ROOH + R^{\cdot} \quad \text{chain propagation;} \quad (2)$$

$$ROOH \xrightarrow{k_4} RO^{\cdot} + HO^{\cdot} \quad \text{chain branching} \quad (3)$$

$$\left.\begin{aligned} R^{\cdot} + R &\xrightarrow{k_5} \text{molecular products} \\ RO_2^{\cdot} + R^{\cdot} &\xrightarrow{k_6} \text{molecular products} \\ RO_2^{\cdot} + RO_2^{\cdot} &\xrightarrow{k_7} \text{molecular products} \end{aligned}\right\} \text{chain termination;} \quad (4), (5), (6)$$

RH = oxidation substrate.

The overall rate of oxidation of membrane lipids is determined by the ratios of the rates of each of these stages. A change in the rate of any oxidation stage leads to the breakdown of the steady-state nature of the process, to a change in the overall rate of oxidation, and to the accumulation of lipid peroxides in membranes, which can in its turn entail the appearance of states which are not characteristic of the normal cell. We believe that there exist several initiating and protective systems in the cell which alter the rate of oxidation of lipids and which can be regarded as regulatory. These systems can be divided into four groups.[43]

System I is responsible for a rigorously defined structural organisation of lipids and thus influences the rate of the chain initiation, propagation, and termination reactions.

System II comprises enzymes responsible for the formation and destruction of the active forms of oxygen and free radicals initiating the oxidation process and the enzymes participating in the decomposition of peroxides without the formation of free radicals from the latter.

System III regulates the metabolism of the membrane phospholipids and influences the rate of oxidation by altering the composition of the phospholipid UAA, the ratios lipid/protein, phospholipids/cholesterol, etc.

System IV comprises low-molecular-weight substances which fulfill the role of initiators, catalysts, inhibitors, quenching agents, synergistic agents, etc. and which influence the chain branching and termination stages.

It is already possible to state that the operations of these systems are inter-related and that the principal role cannot be attributed to any of them: each system operates within rigorously specified limits and in different cases the dominant and rate-limiting reactions change. It is therefore undesirable to speak of the first or second line of defence, as has been done in Fridovich's studies.[14,17,18]

We shall consider briefly the operation of these systems in different stages of the oxidation process.

The Chain Initiation and Propagation Stage. The steady-state concentration of lipid peroxy-radicals is determined by the composition of the lipid UAA (the strength of the double bonds), the concentrations of substances capable of rupturing the double bonds, and the rate of destruction of lipid peroxy-radicals. It is now known that several types of free radicals, differing both in the way they are formed and in their activity, exist in the cell. We shall not consider the primary mechanism of the generation of free radicals in membranes, because a whole series of reviews have been devoted to these questions.[18,51,57,58] We shall only note that, regardless of the nature of the free radicals formed, on the basis of the principle of the indestructibility of the free valence, they are exchanged for the lipid peroxy-radicals and the rate of this exchange is fairly high. An increase in the concentration of the lipid peroxy-radicals in membranes leads to an increase of the overall rate of oxidation, which is proportional to their concentration. It must be specially emphasised that we are considering only short-lived lipid peroxy-radicals. A change in the concentration of lipid peroxy-radicals, their additional formation or destruction caused by a change in the rate of operation of particular systems, leads to a change in the steady-state nature of the process.

Systems influencing the conditions governing the formation of active radicals in membrane lipids will therefore alter also the overall rate of oxidation. In all probability, the structural organisation of membrane lipids (system I), which influences the accessibility of the membrane phospholipid UAA to the action of oxygen, plays a decisive role in this instance. The closer the packing of the UAA in the membrane phospholipids, the smaller their accessibility to O_2 or singlet oxygen (O_2^*), and the lower the rate of initiation of free radicals. Any agents which interfere with the packing of the UAA (for example, chaotropic agents) accelerate the oxidation of lipids. Factors which maintain the membrane lipid structure inhibit oxidation.

Substances capable of deactivating O_2^* (singlet oxygen quenching agents) also alter the rate of initiation and inhibit the overall rate of oxidation (System IV).

A change in the activity of the enzymes involved in the formation or destruction of free radicals and the active forms of oxygen (system II) also exerts a major influence on the overall rate of oxidation of lipids. The activity of such enzymes depends on the degree of structural organisation in the lipid double layer in the membranes.

Indeed, it has been shown, for example, that the activity of the enzymes responsible for the formation of peroxy-radicals requires the lipids be in a liquid state, an increase in viscosity resulting in the inhibition of these enzymes.[56,59]

At the same time, in the non-enzymic generation of free radicals, the rate of initiation is also greatly influenced by a change in the viscosity of the lipid component. The liberation of radicals from the cage is significantly inhibited with increase of the viscosity.[60,61]

Attention should be drawn to the fact that in a normal metabolising cell, whose membranes have not been significantly changed, the degree of structural organisation in the lipids in all probability changes within restricted limits. When the peroxide oxidation of lipids in membranes is modelled by *in vitro* experiments, the structural organisation in the lipid double layer is disturbed. Therefore, the quantitative characteristics obtained in such model systems can be transferred, but with some caution, to the *in vivo* oxidation of lipids, the degree of structural organisation of which has a decisive influence on the rate of their oxidation.

Certain vitamins with long-chain side groups (system IV) are able to stabilise membrane lipids by improving the structural organisation of the lipid double layer. Recently the hypothesis of a structural interaction of tocopherols with components of biological membranes has gained wide currency.[62-64] According to the concept of these authors, the tocopherol molecule stabilises the membrane as a result of the specific interaction between its phytyl side group and the aliphatic acid chains of the membrane phospholipids. This model presupposes the interaction between the methyl groups of the side chains in tocopherols and the *cis*-double bonds of the UAA in such a way that the CH_3 group at the C(4) and C(8) atoms of the tocopherol side chain enters the "pockets" formed by the double bonds of the UAA residues of the phospholipids. The interaction takes place via van der Waals forces and increases the packing density of the membrane phospholipids, which hinders the penetration of oxygen to the UAA double bonds and the formation of lipid peroxy-radicals and entails a decrease of the overall rate of oxidation.

Vitamins with a different structure, which give rise to intermediate radical species in the oxidation reactions (vitamins A, C, D, and F) at certain concentrations, can play the role of initiators and can accelerate the oxidation of lipids by increasing the rate of formation of free radicals.[13,65-68] Variable valence metal ions, particularly the Fe^{2+} and Cu^{2+} ions[7,9,69] (system IV) can play a similar role of initiators. The rate of propagation of the oxidation chain depends significantly on the composition of the UAA of the phospholipids and on the degree of their saturation. For this reason, the changes in the metabolic rate of phospholipids and in the rate of their emergence from the membranes alter also the overall rate of oxidation (system III).

Thus the overall rate of oxidation of lipids at the chain initiation and propagation stages depends on all four systems: on the change in the structural organisation of the membrane lipids (system I and IV); on the activity of the enzyme system involved in the formation and destruction of free radicals and active forms of oxygen (system II); on the presence of singlet oxygen quenching agents (system IV); on the composition of the phospholipid UAA (system III); on the presence in the lipid of substances playing the role of oxidation initiators (system IV).

The Chain Branching Stage. The primary products of the oxidation of lipids are hydroperoxides, which are fairly labile and are capable of spontaneous decomposition into free radicals, which makes it possible to regard the oxidation of lipids as a degenerate-branched chain process. The rate of the chain branching reaction is determined both by the stability of the hydroperoxides formed and their amount. The stability of the hydroperoxides depends in its turn primarily on the structure of the UAA. The factors which alter the aliphatic acid composition of the membrane phospholipids (system III) therefore alter not only the susceptibility of these lipids to oxidation but also the stability of the hydroperoxides formed. The steady-state amount of peroxides in the lipid double layer depends both on the rate of their formation and on the rate of their release from the membranes.

Enzymes utilising ROOH (system II) therefore diminish their amount and hence the rate of the chain branching reaction and the overall rate of oxidation (for example, glutathione peroxidase, catalase, peroxidase).[14-17,69,70] Mercapto-compounds, which decompose peroxides via a molecular pathway without the formation of free radicals, have the same effect on the overall rate of oxidation (system IV).[71] Variable-valence metal ions, especially Fe^{2+} ions at low concentrations, catalyse the decomposition of peroxides into free radicals and thereby increase the overall rate of oxidation.[69]

Thus the overall rate of oxidation in the chain branching stage depends directly on the operation of three systems: on the activity of the enzyme system regulating the metabolism of membrane phospholipids (system III); on the activity of the enzyme system utilising hydroperoxides without the formation of free radicals (system II); on the presence in lipids of phosphorus- and sulphur-containing substances, which decompose peroxides without the formation of free radicals, and on the concentration of variable valence metal ions (system IV).

As regards the involvement of system I in the regulation of the rate of chain branching, its influence operates via other systems (systems II and III). As already stated, the effective lipid transfer between membranes, the catabolism of the oxidised forms of the lipids, and the operation of a number of enzymes of system II depend on the viscosity of the lipid. Nor is it possible to eliminate the influence of the viscosity of the medium on the [rate?] constant for the decomposition of peroxides into radicals. However, we were unable to find in the literature any special studies devoted to these questions.

The Chain Termination Stage. This stage consists of reactions involving the quadratic termination of peroxy- or alkyl-radicals derived from lipids (their recombination or disproportionation) and linear termination reactions at the interface or in the bulk phase, for example, linear termination by the molecules of natural antioxidants.

The change in the composition of peroxy-radicals resulting from the change in the composition of phospholipids (system III) can alter the rate of their recombination, the steady-state concentration of peroxy-radicals, and ultimately also the overall rate of oxidation of lipids. A change in the concentration of substances whose molecules are responsible for linear chain termination (system IV) alters markedly the overall rate of oxidation of lipids.

It must be specially emphasised that only natural antioxidants (AO) possess the ability to react directly with lipid peroxy-radicals. If an additional amount of free radicals are formed as a result of some kind influence on membrane lipids, whose generation could not be prevented either by the structural (system I) or enzymic (system II) protective systems, then the exchange reaction of these radicals with the lipid substrate can result in their exchange with the lipid peroxy-radicals. The excess concentration of lipid peroxy-radicals can be abolished only by natural antioxidants and their influence on the overall rate of oxidation greatly exceeds the effectiveness of the action of other systems. This is the reason for the special role of natural antioxidants in the regulation of lipid peroxide oxidation processes.[5]

Thus the overall rate of oxidation of lipids in the chain termination stage depends on the change in the UAA composition in the phospholipid metabolism (system III) and on the activity of the system of natural antioxidants and their synergists (system IV).

The principal features of the operation of systems III and IV have been analysed within the framework of this review. The characteristics of the operation of systems I and II are not considered in detail because the literature contains a number of detailed reviews on this topic.[15,18,57,72,73]

III. THE COMPOSITION OF LIPIDS AND THE INTENSITY OF THEIR OXIDATION

The present stage of the investigation of the characteristic features of the peroxide oxidation of lipids is characterised by an increased interest in kinetic studies in terms of the comparative aspects of the oxidation of individual phospholipids as well as phospholipids forming part of the composition of membranes by means of a series of models of increasing complexity. The models employed permit the elucidation of the role of fluidity, change, hydrophobic properties, and other physicochemical characteristics of the lipid double layer in the kinetics of peroxide oxidation.

Table 1. The influence of the medium on the kinetic characteristics of the initiated oxidation of lipid substrates of different composition.

Oxidation substrate	Yield of radicals	$10^3 k_2/\sqrt{k_6}$, litre$^{1/2}$ mol$^{-1/2}$ s$^{1/2}$	Refs.
Methyl oleate*	0.7	4.4	[76]
Methyl linoleate*	1.2	13	[76]
Methyl linolenate*	0.83	32	[76]
Cholesterol*	—	3.2	[77]
Phosphatidylcholine*	0.66	610	[78]
Phosphatidylcholine**	0.04	17.0	[78]

*Solution in chlorobenzene.
**Aqueous suspension.

Previously it was known that phospholipids whose composition includes unsaturated aliphatic acids are most readily oxidised. Studies in recent years have shown that the oxidation of phospholipids is influenced by the structure not only of the aliphatic acid residues but also by that of other components. Thus in a study of the oxidation of phosphatidylcholine (PC) and phosphatidylethanolamine (PEA), which have identical aliphatic acid compositions, it was found that the latter lipid is oxidised faster.[75] One of the important questions concerning the kinetics of the oxidation of phospholipids involves the study of the problem of the way in which the kinetic characteristics of the oxidation process vary in liposomes or micelles, compared with the oxidation of phospholipids in a homogeneous solution in an inert solvent. Table 1 presents the kinetic characteristics of the initiated oxidation reactions of the methyl esters of oleic, linoleic, and linolenic acids as well as cholesterol in benzene and of phosphatidylcholine in chlorobenzene and in the form of micelles.[76-78] Table 1 shows that the phosopholipid is oxidised significantly faster in a homogeneous medium (by a factor of 20) than the methyl esters of aliphatic acids and cholesterol. On passing to oxidation in the form of micelles, the yield of radicals diminishes sharply and the rate constant ratio $k_2/\sqrt{k_6}$ decreases by a factor of 35. According to Ingold,[78] changes in the viscosity and polarity of the medium exert the main influence on the rate of the chain propagation reaction [reaction (2)] in Scheme 1.

The high polarity of the peroxy-radical increases the possibility of its migration to the surface of the micelle, as a result of which the probability of recombination increases and the rate of the chain propagation reaction falls.

A further increase in the complexity of the model and its closer approach to the real conditions in the peroxide oxidation of lipids in cell membranes involves the study of the characteristic features of the oxidation reaction and of its inhibition in systems for the oxidation of mixtures of hydrocarbons. In the oxidation of two or several substrates, it is essential to take into account the occurrence of the so-called chain termination and propagation cross-reactions. Data for the kinetics of the co-oxidation of the methyl esters of aliphatic acids have been published.[79]

As a result of the co-oxidation, the same substrate can intensify the oxidation of slowly oxidised substrates and can inhibit that of rapidly oxidised substrates. Studies by the authors of this review revealed that the addition of PC to the rapidly oxidised microsomal lipids inhibits their oxidation, while its addition to slowly oxidised lipids intensifies it. Analogous data have been obtained for cholesterol and PEA.[80,81]

The next stage in the increase of complexity involves the study of the characteristics of reactions during which products functioning as inhibitors or initiators of the oxidation process are formed. Such substances include, in particular, cholesterol and PC.[80] The antioxidant activity of the products of the oxidation of such lipids has been demonstrated.[80,81]

The study of peroxide oxidation processes in a mixture of lipids made it necessary for investigators to take into account not only the change in the rate of initiation and the occurrence of chain propagation and termination cross-reactions, but also the changes in the charge, viscosity, and hydrophobic properties of the mixture. A change in the latter leads in its turn to an alteration of the elementary reaction constants and also of the activity of the enzyme initiating or inhibiting the peroxide oxidation of lipids. Thus complex changes in the inter-relations of the components of the regulatory systems III and IV take place during the oxidation process. In order to understand the characteristic features of the regulation of the peroxide oxidation of lipids by antioxidants, it is necessary to consider in greater detail their kinetic characteristics.

IV. NATURAL ANTIOXIDANTS

The natural antioxidants or bioantioxidants are substances of plant or animal origin which inhibit the development of oxidation processes in model reactions. It has been demonstrated for model oxidation reactions that the lipids isolated from various organs of intact animals (liver, spleen, brain, and blood) do indeed inhibit the development of oxidation processes.[5] The overall antiradical activity of the natural antioxidants contained in the lipids of the livers of intact animals, which has the high constant $k_7 = 2.0 \times 10^6$ litre mol^{-1} s^{-1}, has been determined by chemiluminescence[5] and the effective active fraction of the natural antioxidants, which amount to 0.3% of the total mass of lipids, has been calculated.

Cell bioantioxidants consist of exogenic antioxidants supplied with food and endogenic antioxidants which enter the cell by the humoral pathway or are synthesised in it.

Many experimental data show that the deficiency of particular natural antioxidants in the organism leads to the intensification of the oxidative processes in lipids and to the appearance in the latter of oxidation products in greater than normal amounts.

Various biologically active substances, namely tocopherols, ubiquinones, steroid hormones, etc., exhibit antioxidant activity in model experiments. It has been suggested that these substances fulfill the antioxidant function in the organism together with other functions specific to them.

The question of the contributions of various components of lipids to their antioxidant activity (AOA) has been widely discussed in the literature. However, there is no single point of view on this question. Some investigators attribute the antioxidant activity of lipids to the presence in them of an individual substance (for example, tocopherol or ubiquinone) others believe that the activity of lipids is determined by the set of properties of various components, a change in the composition of which is expressed by a change in the properties of lipids as a whole.[5] We believe that the AOA of lipids depends on the antioxidant properties of natural antioxidants, their amount, their possible mutual influence, and their interaction with substances which are not themselves antioxidants but increase or decrease the activity of the latter. In order to demonstrate the validity of a particular point of view, it is necessary to investigate the kinetic characteristics of natural antioxidants.

The structures of natural antioxidants have many features in common. Three groups of substances have the closest chemical structures: tocopherols, ubiquinones, and vitamins of group K.[82-87] On the basis of the similarity of the structures of these compounds and the interrelation of their metabolisms, it has been suggested that they can be interconverted in the organism. However, experiments involving the administration of 14C-labelled α-tocopheryl acetate showed that this hypothesis is incorrect.[110,119]

Each of these groups includes structurally related compounds: quinones, quinols, chromenols, and chromanols.[87] Quinols (hydroquinones) and cyclic chromenols and chromanols are reduced forms, while quinones are oxidised forms. In the course of many reactions occurring in lipids, intermediate forms such as epoxy-compounds, phenoxy-radicals, and semiquinone radical-anions can also be produced. In the organism, in the lipid double layer of the membrane, these forms can be interconverted and there exist enzymes which catalyse such interconversion. Each group of natural antioxidants is present in the lipids preferentially in one of the most stable forms for the given compound.

Thus vitamins of group K exist in blood in the form of quinones. Under the influence of dehydrogenase, the quinone gives rise to the hydroquinone form of vitamin K_1, which participates in the formation of prothrombin in the γ-glutamic acid carboxylation stage. Hydroquinone is then converted with the aid of epoxygenase into the epoxide of vitamin K_1, which is then converted by reductase into the oxidised quinone form.[88,89] The vitamin K isolated from lipids and converted into the hydroquinone form is extremely unstable, is readily oxidised in air, and loses its ability to react with peroxy-radicals.

The quinone form of ubiquinones is also the most stable. In the electron transport along the respiratory chain in the presence of reductants of the type of NAD, the quinone forms of ubiquinones are readily converted into the hydroquinone forms and conversely, with formation of semiquinone radicals. The hydroquinone form is fairly unstable and is oxidised to the quinone form by atmospheric oxygen. The cyclic forms of ubiquinones (ubichromenols) are the most stable. They are not involved in electron transfer along the respiratory chain. It has been suggested that these substances behave as antioxidants in lipids.[82,83,86]

Tocopherols are present in lipids mainly in the cyclic form as 6-hydroxychromans and this applies to both free tocopherol and its esters. In the organism the ester forms are readily hydrolysed to free tocopherol by enzymes.[90] In oxidative reactions, tocopherols, which function as antioxidants, react with peroxy-radicals to form phenoxy-radicals and these are then converted into quinones, dimers, and trimers.[91]

All the natural antioxidants belonging to the group considered have aliphatic substituents in the 2-position, which consist of several isopentane units. For ubiquinones, the number of isopentane units varies from 0 to 10 (from 0 to 50 carbon atoms), for vitamin K from 0 to 4 (up to 20 carbon atoms), and for the tocopherol homologues encountered in nature the number of units is always 3 and the phytyl side chain has 16 carbon atoms.

The side chains of natural antioxidants differ in the degree of unsaturation: there are no double bonds in the side substituents in tocopherols, in vitamins K the first isopentane unit is unsaturated, and each chain of ubiquinones contains one double bond in each isopentane unit.

The presence of long side substituents is a common property of the given group of natural antioxidants. The study of different forms of these substances has shown that they can react directly with hydrocarbon peroxy-radicals, i.e. they exhibit an antiradical activity.

Table 2. The rate constants for the interaction of peroxy-radicals with different forms of antioxidants.[6,94,98,103]

Antioxidant	k_7, litre mol^{-1} s^{-1}		
	quinone	hydroquinone	cyclic form
Tocopherols*			
α-Tocopherol	$(2.5\pm0.2)\cdot10^3$	$(4.7\pm0.5)\cdot10^6$	$(3.1\pm0.3)\cdot10^6$
β-Tocopherol	—	—	$(1.9\pm0.2)\cdot10^6$
γ-Tocopherol	—	—	$(1.4\pm0.1)\cdot10^6$
δ-Tocopherol	—	—	$(0.85\pm0.1)\cdot10^6$
Chroman-C11	—	—	$(3.2\pm0.3)\cdot10^6$
Chroman-C_6	—	—	$(2.9\pm0.1)\cdot10^6$
Chroman-C_1	—	—	$(3.2\pm0.3)\cdot10^6$
Ubiquinones**			
Ubiquinone Q-0	—	$(3.3\pm0.1)\cdot10^5$	—
Ubiquinone Q-2	—	$(3.1\pm0.1)\cdot10^5$	—
Ubiquinone Q-6	$(3.0\pm0.3)\cdot10^2$	$(3.1\pm0.2)\cdot10^5$	$(1.4\pm0.1)\cdot10^5$
Ubiquinone Q-9	$(2.8\pm0.2)\cdot10^2$	$(3.6\pm0.3)\cdot10^5$	—
Ubiquinone Q-10	—	$(3.3\pm0.1)\cdot10^5$	—
Vitamins K_1***			
Vitamin K_1 (20)******	$(2.1\pm0.2)\cdot10^2$	$(5.8\pm0.4)\cdot10^6$	—
Vitamin K_1 (15)	$(1.9\pm0.2)\cdot10^2$	—	—
Vitamin K_1 (5)	$(1.3\pm0.1)\cdot10^2$	—	—

*Measurement at 60 °C.
**Measurement at 50 °C.
***Measurement at 37 °C.
****The numeral in brackets indicates the number of carbon atoms in the side chain of the vitamin K_1 molecule.

Table 2 lists the constants for the reactions of natural antioxidants with peroxy-radicals. It is seen that only the reduced forms of the natural antioxidants, which have rate constants for their interaction with peroxy-radicals of the order of 10^5–10^6 litre mol^{-1} s^{-1}, exhibit an appreciable antiradical activity. The reduced form of vitamin K_1 has the highest constant $k_7 = (5.8 \pm 0.4) \times 10^6$ litre mol^{-1} s^{-1}. The antiradical activity of the phenolic forms of tocopherols

(homologues and analogues) is of the same order of magnitude (10^6 litre mol^{-1} s^{-1}). Ubiquinones and ubichromenols have constants k_7 amounting to 10^5 litre mol^{-1} s^{-1} and for the cyclic form, which has only one hydroxy-group, k_7 is smaller by a factor of 2 than for the hydroquinone form.[92-94]

For the quinone forms of tocopherols, ubiquinones, and vitamin K, the rate constants for this reaction are lower by two orders of magnitude. The esters (tocopheryl acetate) hardly react with free radicals.[92]

Thus the ability to inhibit the peroxide oxidation reaction of lipids is characteristic only of the reduced forms of natural antioxidants and is associated with the presence of the hydroxy-group in the molecule.

Since among all the reduced forms of the natural antioxidants tocopherols are the most stable, the antiradical activity of substances of different structures, differing in the number of substituents in the benzene ring of the chroman nucleus (homologues) and in the length of the aliphatic substituent in the 2-position (analogues), containing between 16 and 1 carbon atoms (chromans), has been investigated in relation to tocopherols. It has been established that the rate constants for the interaction of all the tocopherols investigated with peroxy-radicals are of the order of 10^6 litre mol^{-1} s^{-1} and the activation energies for this reaction are in the range 12.5–15 kJ mol^{-1}.[92]

The tocopherol homologues encountered in nature in different plants differ in the number and positions of the CH_3 groups in the benzene ring of the chroman nucleus but always have the same side substituents in the 2-position. It has been established[92] that the successive introduction of methyl groups in the 5-, 7-, and 8-positions in the tocopherol molecule increases the constant k_7. All these data agree well with experiments on the photolysis of tocopherols and with their oxidation-reduction potentials.[96,97]

Comparison of the antiradical activities of synthetic analogues of α-tocopherol, which are not encountered in nature, has shown[92,98] that the length of the phytyl side chains does not influence significantly such activity: the shortening of the side chain by each isopentane unit (5 carbon atoms) decreases the constant k_7 by 10–15% (Table 1). The introduction of one double bond into the side chain has approximately the same effect.

Analogous data have been obtained by Ingold and coworkers[99] for α-tocopherol and chroman C_1, which has a methyl group in the 2-position. The constants k_7 for the ubiquinones Q-2 and Q-10, differing by eight isopentane units, are hardly different (Table 2).[94] The values of k_7 for the synthetic analogues of vitamin K_1 change when the chain is shortened by two isopentane units by not more than 30% (Table 2). Consequently, the length and the degree of unsaturation of the side chain in the molecules of natural antioxidants do not exert an appreciable influence on the rate of their reaction with peroxy-radicals. Bearing this in mind, one may conclude, on the basis of a comparison of the constants k_7 for tocopherol and the cyclic form of ubiquinone (ubichromenol), that the replacement of the CH_3 group by the methoxy-group lowers the antiradical activity by a factor greater than 10.[93,94]

An analogous dependence has been observed also for the hydroquinone forms of vitamin K and the ubiquinol QH_2-6, whose constants differ by an order of magnitude.

Thus natural antioxidants have a labile hydroxy-group and react comparatively readily with hydrocarbon peroxy-radicals. The substituent in the *o*-position with respect to the hydroxy-group has a significant influence on the antiradical activity of natural antioxidants. Aliphatic side substituents have only a weak influence on the antiradical activity of such antioxidants.

Comparison of the overall antiradical activities of the natural antioxidants present in the lipids of the livers of intact animals and of individual antioxidants showed that they are comparable to the activities of the reduced form of vitamin K_1 and α-tocopherol.

However, the reduced form of vitamin K_1 is unstable and in the lipids which have been isolated vitamin K_1 can exist only in the form of a quinone and the activity of the total natural antioxidants can be usefully compared only with that of α-tocopherol. For lipids, this quantity proved to be 1.5 times lower than the activity of α-tocopherol, which we believe to be associated with the presence in lipids of antioxidants having a lower antiradical activity than that of tocopherol.

It is of interest to note that natural antioxidants have constants k_7 higher by 1–2 orders of magnitude than the majority of the phenols most widely used as antioxidants.[100] The activation energies for the interaction of peroxy-radicals derived from ethylbenzene with tocopherols of different structure (12–15 kJ mol^{-1})[92] and with ubiquinone QH_2-9 (17.4 ± 3.3 kJ mol^{-1} [94]) are lower than the corresponding value for the reaction with ionol (E = 20.5 kJ mol^{-1}).[101,102] On the basis of the low activation energies for the reactions involving the termination of peroxy-radicals by the molecules of natural antioxidants and the high rate constants for these reactions, it might have been expected that the above substances would inhibit the oxidation of model systems more effectively than synthetic phenols. However, in model low-temperature autoxidation systems (the most varied systems including both simple and complex systems) natural antioxidants proved to be much less active in inhibiting oxidation than synthetic antioxidants such as ionol, butoxyanisole, propyl gallate, and nordihydroguaiaretic acid, which has the constant $k_7 = 10^4$–10^5 litre mol^{-1} s^{-1}. Tests carried out by different investigators on tocopherols established that the efficiency of their antioxidant action varies in a complex manner with their concentration, the degree of unsaturation of the substrate, and the oxidation temperature.

Our analysis of the kinetic curves for the oxidation in the presence of tocopherols obtained in our own experiments and taken from the investigations of other workers,[92,103-106] showed that in all cases the dependence of the induction period of the oxidation reaction on the tocopherol concentration is unusual. Whereas for the majority of synthetic inhibitors the dependence of the efficiency of the inhibition on concentration is hyperbolic, for tocopherols the corresponding dependence passes through an extremum with the maximum inhibition effect at a specific concentration.[103] This maximum efficiency of the action of tocopherols is shown most markedly at low oxidation temperatures (at 20° and 40 °C). Comparison of the antioxidant activities of different tocopherol homologues showed that this type of relation with an extremum is characteristic of all the homologues, is independent of the oxidation substrate, and is most marked for α-tocopherol.[103] For the α-tocopherol analogues with short side substituents, the deviation from linearity and a decrease of the inhibiting effect with increase of their concentration have also been established,[92] i.e. the length of the side chain did not exert a significant influence on this feature of tocopherols. In all probability this unusual feature of the antioxidant activity of tocopherols is characteristic of the 6-hydroxychroman molecules themselves and is associated with the activity and stability of the radicals formed from them.

The elementary oxidation reactions involving the antioxidants and their radicals are examined in Scheme 2. The weakening of the antioxidant activity of tocopherols can be accounted for by the occurrence in the oxidation system of side reactions with participation of tocopherol or its radicals. Such reactions may be those of the inhibitor radical with a

hydrocarbon molecule [reaction (10)] and with a hydroperoxide molecule [reaction (7)] and the reaction of the inhibitor with the hydroperoxide [reaction (11)] or with oxygen [reaction (12)]. The rates of these reactions depend significantly on the structure of the antioxidant and the oxidation condition. We were interested in the properties of tocopherols under conditions close to physiological conditions: a low oxidation temperature (20–40 °C), a low degree of oxidation, and a high degree of unsaturation of the substrate.

Scheme 2

$$InH + RO_2^{\cdot} \underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} ROOH + In^{\cdot} \qquad (7), (-7)$$

$$\left.\begin{array}{l} RO_2^{\cdot} + In^{\cdot} \xrightarrow{k_8} \text{molecular products} \\ In^{\cdot} + In^{\cdot} \xrightarrow{k_9} \text{molecular products} \end{array}\right\} \text{chain termination;} \qquad \begin{array}{l}(8)\\(9)\end{array}$$

$$In^{\cdot} + RH \xrightarrow[+O_2]{k_{10}} RO_2^{\cdot} + \text{molecular products} \quad \text{chain propagation,} \qquad (10)$$

$$InH + ROOH \xrightarrow{k_{11}} \text{molecular products} + \text{free radicals;} \qquad (11)$$

$$InH + O_2 \xrightarrow{k_{12}} In^{\cdot} + HO_2^{\cdot} \qquad (12)$$

HIn = inhibitor.

Under these conditions, the oxidation reaction of tocopherol itself [reaction (12)] may be neglected, since tocopherols are stable even on heating to 100 °C.[107]

Under the conditions of a low degree of oxidation, where the concentration of hydroperoxides in the substrate is low, it is possible to neglect reactions (−7) and (11). It remains to consider the possibility of the occurrence of reaction (10).

Reaction (10) was introduced into the scheme for the inhibited oxidation of hydrocarbons in order to calculate the rate constants from chemiluminescence data.[95,108] Depending on the constants k_6, k_8, and k_9, whose ratios make it possible to estimate the limits within which the constant k_{10} varies for α-tocopherol in the initiated ethylbenzene oxidation model, k_{10} is in the range $(0.5–1.2) \times 10^{-1}$ litre mol^{-1} s^{-1}, i.e. cannot be neglected in estimating the antioxidant activity.[92]

The ratio of the rates of the reactions which decrease the efficiency of the action of antioxidants can vary with the concentration of free radicals, since an increase of the rate of initiation W_i entails a quadratic increase of the rates of reactions (8) and (9) and a linear increase of the rate of reaction (10). Thus, with increase of W_i in the system, the contribution of the chain propagation by the antioxidant radical to the overall rate of oxidation diminishes, i.e. the efficiency of the antioxidant increases. An extended kinetic scheme for the oxidation of hydrocarbons has been examined[95] and relations confirming the increase of the efficiency of the antioxidants with increasing concentration of free radicals in the system have been obtained. This explains the finding that natural antioxidants exhibit the greatest efficiency in models involving the oxidation of highly unsaturated aliphatic acids.

If this oxidation system contains substances capable of removing from the reaction sphere the radicals of natural antioxidants or substances which regenerate their oxidised forms, a synergistic effect should be observed under these conditions. The role of synergists is usually assumed by substances having a low-oxidation-reduction potential and which readily pass from the oxidised to the reduced form (for example, ascorbic and citric acids).[45,109] For tocopherols, synergism with dibasic organic acids was established as early as 1942.[111]

It has been shown recently by direct experiments[112] that ascorbic acid reacts directly with tocopherol radicals, which is accompanied by an increase of the concentration of ascorbic acid radicals, while that of the tocopherol radicals falls.

We shall consider which properties of membrane lipids and natural antioxidants ensure a high efficiency of the regulation of the peroxide oxidation of lipids. As already stated, the oxidative processes occurring in membranes have their characteristic features: a structured lipid double layer, the multicomponent composition of the lipids, a high degree of unsaturation of the aliphatic acids forming part of the composition of the membrane phospholipids, and the presence in the latter of substances capable of intensifying or weakening the action of natural antioxidants.

It has been shown in a recently published study[113] that the activity of tocopherols in structured substrates is higher by 1–2 orders of magnitude compared with oxidation in the liquid phase. The authors believe that such increase of activity is associated with the fact that the OH groups of the tocopherols in the structured substrate are directed towards the hydrophilic medium. If radical species of the type of OH^- and O_2^- approach the membrane from this medium, then their termination by the tocopherol molecules is more effective the greater the number of hydroxy-groups of the antioxidants are present on the outer surface of the membrane.

The maximum inhibition of the oxidation reaction by natural antioxidants in model systems can be achieved in the oxidation of highly unsaturated substrates with a low content of peroxides and at low temperatures. Such conditions are in fact characteristic of the oxidation of lipids in biomembranes, i.e. the physiological conditions are optimal for the manifestation of the antioxidant activity of natural antioxidants. The high antiradical activities of natural antioxidants constitute the basis of the sharp change in the rate of the peroxide oxidation of lipids, which ensures a high effectiveness of regulation even when their concentration is altered only slightly.[106]

Since the reduced phenolic forms of natural antioxidants have constants k_7 higher by a factor of 100–1000 than those for quinones, the transition of natural antioxidants from one form to another, involving an appreciable decrease or increase of the antiradical activity, can also be regarded as a means for a rapid change in the intensity of the oxidative reactions occurring in lipids.[106,114]

The question arises whether ubiquinones and vitamins K_1, having unstable reduced forms which are readily converted into inactive quinones, can be regarded as natural antioxidants. In all probability they can be so regarded, because lipids always contain different reducing agents and enzymes capable of converting the quinones into the phenolic form.

Furthermore, one should bear in mind that in a lipid environment the stability of the phenolic forms of these compounds is higher. The question also arises whether the radicals formed from the antioxidant molecules can be active within the membrane. It has been established in experiments on animals that the relation between the antioxidant activity of lipids and the amount of natural antioxidants present in them passes through an extremum, as in model systems.[92] This permits the conclusion that the properties of weak antioxidants, established for them in model experiments, are characteristic of the natural antioxidants also in systems *in vivo*. This feature of the antioxidant activity of natural antioxidants can also be regarded as regulatory; in the presence of an excess of such substances in lipids, their inhibiting activity falls. We believe that the radicals formed from natural antioxidants fulfill, as it were, the role of a buffer which maintains the rate of oxidation of lipids at a specific level.[43,114]

Phenomena of this kind, namely a sharp change in efficiency following a slight increase in the concentration of the given substance up to the reversal of the effect, were first discovered and investigated in detail by Academician N.M.Emanuel' and were called the "critical phenomena" in inhibited liquid-phase oxidation reactions.[44]

In model hydrocarbon oxidation reactions, it was found that variable-valence metal ions at low concentrations activate the oxidation reaction, catalysing the decomposition of hydroperoxides, while at high concentrations they inhibit such reactions. The same type of relation has been established for Fe^{2+} ions in a study of the oxidation of lipids in membranes.[69] Retinol activates the oxidation of liposomes when present in small doses and stabilises them when present in large doses.[9,68] Such dual activity apparently constitutes a means for a rapid alteration of the rate of oxidation (regulation by the concentration).

A change in the composition of the membrane lipids, leading to a change in the rate of peroxide oxidation by altering the rate of initiation, has a significant influence on the rate of the reactions involving chain propagation by the radicals formed from natural antioxidants [reaction (10)]. Thus it has been established[95] that the contribution of the rate of reaction (10) to the antioxidant activity of natural antioxidants is less significant for higher rates of initiation and for a high degree of unsaturation of the oxidation substrate (regulation by the substrate).

Natural antioxidants participate in the regulation of the peroxide oxidation of lipids not as independent substances but as interacting components of a single system. The possibility of changing the concentration and activity of one set of natural antioxidants by administering others to animals constitutes proof of the validity of this postulate. For example, after a single administration of α-tocopheryl acetate (200 mg kg^{-1}) to intact animals, an increase of the concentration of the total antioxidants is observed in the liver lipids (by a factor of 37), while the amount of free α-tocopherol increases by a factor of only 10 during the same period.[5] An increase of the amount of a particular natural antioxidant is possible only when the product of the antiradical activity of the administered antioxidant and its concentration (k_7c) is comparable to the same product for the antioxidants present in the cell.[5]

At the same time one should bear in mind that the transformation of the relatively inactive forms of antioxidants into highly reactive forms is possible in the cell, so that, after the introduction of, for example, quinones, which can be reduced to phenols, an increase of the total amount of natural antioxidants is also observed.[115] Although ubiquinone and vitamin K_1 in the form of quinones cannot compete with tocopherols, in the reduced forms, as hydroquinones or ubichromenol, they can replace tocopherol in free-radical oxidation reactions.

After the introduction of vitamin K_1, not only an increase of the amount of natural antioxidants in lipids but also an increase of the activity of the lipids of the experimental animals compared with that of the control lipids was established, i.e. substances whose antiradical activity is greater than the total activity of the natural antioxidants and even greater than the activity of pure α-tocopherol accumulate in the lipids. Only the reduced form of vitamin K_1 exhibits such a high activity.[6]

The data presented confirm our hypothesis that tocopherols, ubiquinones, and vitamins of the K group are interrelated in the organism via their antioxidant functions. The question whether the system of natural antioxidants is linked with other systems regulating the peroxide oxidation of lipids remains open. In the first place one should note that it is difficult to draw a clear-cut boundary between these systems. Indeed, the phenolic forms of natural antioxidants are typical antioxidants, but at the same time they play the role of structural factors and on this basis can be assigned to the protective system I. On the other hand, quinones, which are potential antioxidants, can interact with $O_2^{\cdot-}$ (system II) to form semi-quinone radicals.[46,116,117] This results not only in the destruction of the possible oxidation initiation centres ($O_2^{\cdot-}$) but also in the formation of semiquinone radicals capable of terminating the oxidation chain on recombination with the lipid peroxy-radicals or on interaction with the OH group of the semiquinone. This leads to the simultaneous destruction of the oxidation initiation centres and the appearance of active free radical acceptors, which makes it possible to regard this reaction as a means for the inclusion of the oxidised forms of antioxidants in the regulation of the intensity of the peroxide oxidation of lipids in response to the increase of the rate of initiation of the oxidation process.

In all the cases considered, the interrelation of different systems for the regulation of the peroxide oxidation of lipids is based on physicochemical characteristics.

However, one must not forget also the biochemical mechanisms of such inter-actions. Thus it has been shown[24,26,35] that there exists a system for the maintenance of the steady-state in the peroxide oxidation of lipids, based on the relation between the rate of the peroxide oxidation of lipids and the rate of change of their composition. In response to the intensification of the peroxide oxidation of lipids, the composition of the lipids changes in such a way that they become more resistant to oxidation.[24] The presence of such a relation complicates the consideration of the characteristic features of the regulation of the peroxide oxidation of lipids but it enables the cell to switch the rate of the peroxide oxidation and the associated metabolism to a new level in response to the action of endogenic and exogenic factors. This has the consequence of a change in the structure and ultimately in the functional activity of the membrane.

The steady state nature of the lipid oxidation process constitutes the basis for the maintenance of a specific composition, structure, and function of membranes as highly specific, complex, and multicomponent systems. The membrane lipids fulfill a dual function: on the one hand, they maintain a rigorously specified structural organisation of the membranes, responsible for the effective fulfillment of their biological functions, and, on the other hand, they ensure the lability of their composition and the rapid response of the cell to changes in the organism.

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