ROLE OF MEMBRANE LIPID OXIDATION IN CONTROL OF ENZYMATIC ACTIVITY IN NORMAL AND CANCER CELLS

E. B. BURLAKOVA, E. M. MOLOCHKINA and N. P. PAL'MINA

Institute of Chemical Physics, USSR Academy of Sciences, Moscow, USSR

INTRODUCTION

At present regulation of enzymatic activity by the membranes receives wide attention. The membrane is a cooperative system and its structural rearrangements represent a trigger mechanism underlying transition of the cell from one into another metabolic state. An important role in the regulation of enzymatic activity by membranes is played by lipids. Lipids may influence the activity of enzymes in different ways. In model experiments on proteoliposomes it has been shown that the enzymatic activity is affected by changes in the following characteristics:

1. ratio of lipid weight to protein weight;
2. relative amount of phospholipids;
3. composition of fatty acids; in particular, degree of non-saturation of the lipids;
4. viscosity of the membrane lipid component;
5. degree of oxidation of membrane lipids.

Now it is difficult to state the leading role of any of these alterations or to elucidate a strict correlation between the changes of certain characteristics of lipid metabolism and influence onto a certain step of enzymatic catalysis. In *in vitro* experiments it is difficult, too, to induce changes in a single property of the lipids without affecting other characteristics. For example, conclusion concerning the important role of the total amount of phospholipids for the enzyme activity was drawn based on the data of delipidization. However, this kind of treatment results not only in the shift of the ratio lipid/protein but in the alteration of the relative composition of lipids, too. For instance, delipidization of microsomal membranes from the Harding-Passey melanoma cells with water-acetone mixture was accompanied by predominant elimination of phosphatidyl ethanolamine and phosphatidyl choline whereas the relative amount of the minor phospholipid fraction, i.e., phosphatidyl serine and phosphatidyl inositol, was increased (1).

The same phenomenon is observed in studies of the role of oxidative reactions in membrane lipids. It has been shown (2) that the degree of the oxidative degradation of different mitochondrial phospholipids under conditions of ascorbic-dependent peroxidation may vary, the increase in the oxidation time resulting in predominant elimination of cardiolipine.

It has been found also (3) that in the course of the microsome peroxidation the percentage of phosphatidyl ethanolamine content decreases by approximately 10 times, whereas the relative content of phosphatidyl choline is about the same.

Other investigators have shown (4) that the incubation of microsomes with NADP-H_2/NADP-H_2-dependent peroxidation also results in considerable changes in the composition of endogenous phospholipids; in this case alterations were marked both for phosphatidyl ethanolamine and phosphatidyl choline.

Changes in the lipid composition of the membrane due to peroxidation may be regarded as a general rule for different types of membranes. It is natural to expect that the viscosity of the membrane lipid component changes at the same time.

It is even more difficult to establish which pathways of regulation of enzymatic activity occur *in vivo*.

The purpose of the papers (5–8) was to elucidate whether *in vivo* the pathways of modification of enzymatic activity with lipids are interconnected. The changes in the membrane lipid composition were studied under conditions of directed alteration of the rate of oxidative reactions in lipids by using spin probes located predominantly in lipids; microviscosity and conditions of structural transitions were investigated in membranes as well.

Different kinds of experiments have shown that changes in the lipid composition and rate of oxidative reactions in lipids are interconnected. Increase of the antioxidative activity (AOA) and decrease of the rate of oxidative reactions result in such a change of the lipid composition that these lipids are more easily oxidizable. In its turn this induces accelerated consumption of antioxidants (AO), gradual decrease of the AOA and restoration of the latter to the normal values.

On the contrary, decrease of the AOA and increase of the rate of oxidative reactions in lipids induce such a change in the lipid composition of the membrane that these lipids become more oxidizable and the rate of AO consumption by lipids is decreased whereas AOA gradually increases and returns to normal level. Such interrelations between changes of AOA and lipid composition, on the one hand, may be regarded as a physico-chemical control system preserving the constant level of the oxidative reactions in lipids (5) and, on the other hand, as a system of the turnover of phospholipids.

What is the molecular mechanism of the interrelations between these characteristics? It has been suggested that the formation of the oxidized lipids

in vivo facilitates their release from membranes as has been found in *in vitro* experiments. Then an increase of AOA should result in a decrease of lipid release, an increase of the total lipid amount in the membrane and enrichment of the membrane lipids with the most easily oxidizable fractions actively consuming antioxidants. On the contrary, a decrease of AOA should result in the enrichment with the most stable fractions. The investigation of the oxidation process of different phospholipid fractions isolated from mouse liver has shown that the fractions of phosphatidyl ethanolamine and phosphatidyl serine containing large amounts of unsaturated fatty acids are most easily oxidizable. Lipids containing saturated fatty acids, i.e., sphingomyelin and lecithin, were found to be more stable (6). An increase of AOA did actually result in an increase of the total amount of phospholipids and relative quantities of the easily oxidizable phospholipids (phosphatidyl ethanolamine and phosphatidyl serine) whereas a reversed alteration induced an increase in the relative amounts of sphingomyelin and lecithin. Acceleration of the release of oxidized lipids from membranes may be due to increased rate of their degradation and enhancement of the metabolism by the lipid-exchange proteins.

Previously we have shown in *in vitro* experiments that the antioxidants do actually change the rate of the lipid metabolism of membranes in the presence of a supernatant containing transferring proteins. However, the mechanism of their influence has not been elucidated.

A change in the membrane lipid composition results, in its turn, in an alteration of the microviscosity of the lipid component, lipid-protein interactions and conditions for structural transitions in membranes. In the cases under study (influence of an inhibitory antioxidant, effect of irradiation, etc.) it has been found that an increase of AOA results in a transition of the membrane lipids to a more "liquid" state whereas a decrease of AOA makes the lipid phase more rigid (8). The viscosity is changed both for lipid and protein components; alterations are marked also for temperatures of the structural transitions within the membrane and values of efficient activation energy. An increase of AOA results in the appearance of structural transitions at a lower temperature; a decrease of AOA manifests itself in a higher temperature of these transitions.

Therefore, changes in the rate of oxidative reactions in lipids are interconnected with alterations of the relative lipid composition, viscosity of lipid and protein components, conditions of structural rearrangements of the membrane. The whole complex of these changes results in an alteration of the rate of reaction involving membrane-bound enzymes.

In a series of publications (5, 9–13) we have demonstrated that AOA of lipids of the cellular membranes is changed regularly, in correspondence with a stage of tumor development that is characterized by specific intensity and peculiarities of cellular proliferation and cell metabolism.

MATERIALS AND METHODS

Mitochondria and microsomes were isolated from mouse and rat liver by a differential centrifugation technique. Nuclei from the cells of tumor and liver of the cancerous animal were isolated by centrifugation in sucrose gradient. The purity of the fractions was checked by electron-microscopic observation and by measuring DNA/protein ratio (for nuclei) (14).

Lipid extraction was carried out by the technique of Bligh and Dyer modified by Kates (15) for subcellular fractions.

Lipid composition was determined by the technique of one-dimensional and two-dimensional chromatography on silica gel (14).

The intensity of free-radical oxidation of membrane lipids was evaluated from their antioxidative activity (AOA) determined by using the methyloleate model (5).

Membrane microviscosity was studied by applying spin probes localizing predominantly in the lipid layer (probe I) or in the layer adjacent to the proteins (probe II). The role of paramagnetic probes was played by iminoxyl radicals of 2,2,6,6-tetramethyl-4-capryloyl-hydroxypiperidine-1-oxyl (I) and 5,6-benzo-2,2,6,6-tetramethyl-1,2,3,4-tetrahydro-N-carbolin-3-oxyl (II). Liver tumors were induced in rats (inbred males) by feeding with 2-acetylaminofluorene (AAF) in a special diet providing the shortest possible time of tumor appearance and by feeding with diethylnitrosamine (DENA) in the normal diet.

In the first case the studies were carried out in 4, 7, and 9 weeks after the beginning of the carcinogen treatment, i.e., in rather early periods when liver did not show any macroscopic signs of tumor formation. The first tumors were seen 12 weeks after the beginning of the experiment. In the second case the studies were performed 5, 10, 16 and 27 weeks after the onset of the carcinogenic treatment. The first tumors were observed at the 26th week of treatment.

In AAF experiments functional activity of the microsomal membrane was evaluated from the state of the system of oxidases with mixed function. The measure of its state is the amount of the cytochrome P-450 (mM/mg of microsomal protein) which has been determined spectrophotometrically. In DENA experiments, two indices of the functional activity of the microsomes were tested: the amount of the cytochrome P-450 and the activity of glucose-6-phosphatase (EC 3.1.3.9.). The technique of Swanson was used to measure the enzymatic activity (16).

RESULTS AND DISCUSSION

Effect of lipids on the activity of enzymes (normal cells). In *in vitro* experiments, it has been shown that glucose-6-phosphatase, i.e., one of the key

enzymes of gluconeogenesis, is a lipid-dependent enzyme (17-18). The data of the same approach suggest that to be active glucose-6-phosphatase needs phosphatidyl ethanolamine. It is, however, unclear what is the mechanism of the action of this lipid on the enzyme either *in vivo* or *in vitro*.

We have studied changes in antioxidative lipid activity, lipid composition of liver microsomes and glucose-6-phosphatase activity under different conditions of the body. The dependence has been investigated of these characteristics on the time of day, treatment with antioxidant or carcinogens or with a combination of both drugs (7). A valid correlation was found in all the cases between a change in the glucose-6-phosphatase activity and of phosphatidyl ethanolamine concentration (Fig. 1). We tried to evaluate the degree of cooperativity in the action of phosphatidyl ethanolamine with the phosphatase activity of glucose-6-phosphatase in model experiments and in *in vivo* measurements. We found that Hill's coefficient n_H for phosphatidyl ethanolamine both in *in vivo* and *in vitro* experiments did exceed 1 (1.2-1.8), suggesting an allosteric mechanism. Similar data concerning cooperativity of action with the pyrophosphatase activity of glucose-6-phosphatase were obtained for phosphatidyl serine (19). A change in the phosphatidyl serine ratio in membrane lipids results in an alteration of the rates of glucose-6-phosphatase reactions in different directions.

From the point of view of lipid dependence it is of interest to examine the tyrosinase (EC 1.14.18.1) which has been shown to increase its activity and to be solubilized upon the action of agents inducing labilization of the biological membrane (20).

An attempt to find interrelations between a change in the tyrosinase activity due to delipidization of microsomes, on the one hand, and an alteration in the

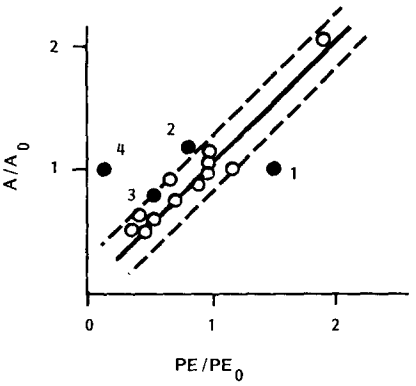


FIG. 1. Interconnections between the changes in the relative amounts of phosphatidyl ethanolamine (PEA) in microsomes of mouse liver and glucose-6-phosphatase activity (A) in normal state (0) and in chemical hepatocarcinogenesis 1, 2, 3, 4–5, 10, 16, 27 weeks of feeding rats with diethylnitrosamine (23).

total amount of lipids, phospholipids or any fraction of phospholipids per mg of protein, on the other hand, was unsuccessful (1). It has been marked that an increase in the tyrosinase activity is observed upon an increase of phosphatidyl choline removable during delipidization and a decrease of phosphatidyl ethanolamine (Fig. 2). It has been suggested that the former plays an inhibitory role, whereas the latter represents a tyrosine activator. A satisfactory correlation is actually observed between the rise of the tyrosinase activity and PEA/PC ratio in lipids of the melanoma microsomes (Fig. 2). Such a correlation may be observed, however, also in a case when the activity of tyrosinase does not depend on the amount of a certain effector lipid but does depend on the viscosity of the lipid component. It has been described that the activity of H-ATPase (EC 3.6.1.3) is controlled by changes in viscosity of the membrane lipid component. It was shown (21) that an alteration in ATP synthesis correlated with a change in fluidity of lipids of the inner mitochondrial membrane.

The data mentioned above concern the interrelations between the changes in enzymatic activity and alterations in the amount of the effector phospholipid.

It is interesting whether a correlation exists between a change in the amount of the enzyme and that in the quantity of its effector and if so, what is the nature of this correlation. From this point of view, let us consider changes in the amount of cytochrome P-450 and its effector lipid in the reaction of phosphatidyl choline hydroxylation.

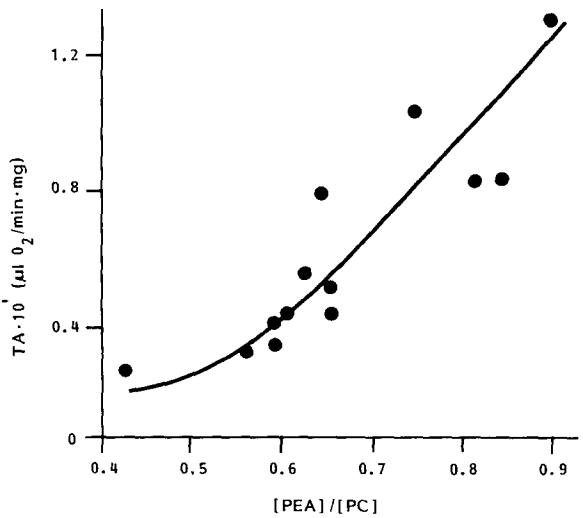


FIG. 2. Dependence of the DOPA-oxidase activity of tyrosinase (TA) on the ratio PE/PC in lipids of native and partially delipidized microsomes from the Harding-Passey melanoma at different steps of tumor growth (1).

In the paper (22) a certain correlation has been established between changes in the phosphatidyl choline concentration and that of cytochrome P-450 in rat liver microsomes after phenobarbital treatment. Similar dependence has been found by us both in experiments when rats were fed with a certain diet and in those when rats were treated with phenobarbital (23). Fig. 3 demonstrates that in line with an increase of the cytochrome P-450 amount a rise of the phosphatidyl choline quantity is observed.

Simultaneous changes in the quantity of the enzyme itself and that of its effector lipid are possibly a mechanism of "alarm" change of the activity of membrane-bound enzymes.

It is noteworthy that phosphatidyl choline is an activator of P-450 cytochrome in hydroxylation reactions whereas in reactions of peroxide formation this compound behaves as an inhibitor (24). Therefore, an increase of the concentration of phosphatidyl choline results in simultaneous acceleration of the hydroxylation reaction and inhibition of peroxide formation.

The data presented show that the composition of the membrane lipid component as well as its degree of oxidation and viscosity are the characteristics that cannot change independently of each other. It follows that coordinated changes in lipids result in a coordinated alteration of the activity of lipid-dependent membrane-bound enzymes. It should be noted that the composition and fluidity of membrane lipids play an important role in the processes of binding of hormones, growth factors and other signal substances with membrane receptors as well as upon interaction between an antigen and an antibody, in the course of transmission of a nerve impulse, upon contact inhibition. It has been established that a change in the composition of the membrane lipids results in changes of the basal and induced activity of cyclic AMP (25, 26).

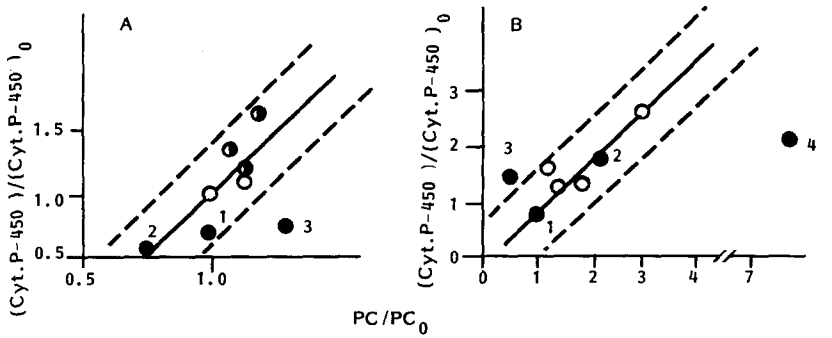


FIG. 3. Changes in the relative amounts of PC and quantity of cytochrome P-450 in microsomes of rat liver during hepatic carcinogenesis induced by acetylaminofluorene (A) and diethylnitrosamine (B). A: 1 - healthy rats fed with a special diet; 2 - healthy rats treated with phenobarbital to induce cytochrome P-450 system; 3 - rats treated with the carcinogen.

Lipids do influence the activity of DNA and RNA polymerases (27, 28). The data mentioned above being taken into consideration, the importance is clear of study of the regulatory chain "lipid oxidation–lipid composition–viscosity–activity of membrane-bound enzymes"upon such a pathological process as tumor growth.

Disturbances in the chain "lipid oxidation–lipid composition–enzyme activity" during tumor growth (chemical carcinogenesis). Tumor cells are known to be characterized by disturbances of the control of cellular metabolism. One of the tasks of this paper is the elucidation of disturbances which take place during chemical carcinogenesis in the correlation beween the changes in the intensity of oxidative reactions in lipids, lipid composition and amount of lipid-dependent enzymes typical of the normal cells; here glucose-6-phosphatase and cytochrome P-450 were chosen as models.

Figure 4 demonstrates changes in AOA of lipids and phospholipid composition of rat liver microsomes in the course of the feeding with a diet devoid of (4a) or containing (4b) the carcinogen. It can be seen (4a) that an increase in AOA is accompanied by a drop in PC content, a rise in the amount of easily oxidizable PEA and phosphatidyl serine (PS), whereas a decrease in AOA is paralleled by an increase in PC content and a decrease of PEA and PS. The data obtained are similar to findings described for the changes of the lipid AOA in the liver of intact mice. Figure 4b demonstrates that during the carcinogenesis at all the periods tested the correlation between AOA of microsomal lipids and phospholipid composition typical of animals not treated with the carcinogen is completely absent. For example, a drop in AOA is accompanied by a decrease of PC content and increase of the amount of PEA.

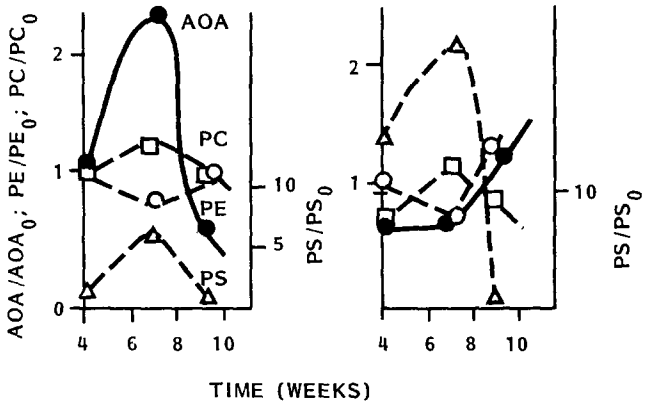


FIG. 4. Changes in AOA of lipids in rat liver microsomes and alterations in phospholipid composition during hepatic carcinogenesis induced with 2-acetylaminofluorene. a — rats were fed with a diet devoid of the carcinogen; b — rats were fed with the diet; 1 — AOA, 2 — PC, 3 — PE, 4 — PS.

Figure 5 shows what is seen during feeding of rats with the carcinogenic DENA: changes in AOA of rat liver microsomes are accompanied by changes in content of major phospholipids, i.e., PC and PEA. Comparison of Figs. 5a and 5b (intact rats and carcinogenesis, respectively) demonstrates that the response of the phospholipid content of microsomes to a change in the intensity of lipid oxidation differs from the normal pattern in this case of carcinogenesis as well.

Let us consider now whether any disturbances occur in correlation between the amount of effector phospholipids and functional activity of liver microsomes in the course of chemical carcinogenesis. Figure 1 visualizes the *in vivo* dependence of the activity of glucose-6-phosphatase on the PEA amount inherent to healthy animals. Points 1, 2, 3, 4 reflect the case when animals were fed with DENA. It is evident that the regularity inherent in the normal conditions is absent even at rather early periods of treatment.

Figure 3 demonstrates interrelations between the microsomal PC content and the amount of cytochrome P-450 in the absence of pathological states as well as disturbance of this correlation during chemical carcinogenesis. It can be readily seen that in experiments both wth AAF and DENA deviations from the correlation between the changes in PC content and the alterations in P-450 cytochrome amount typical of healthy animals take place at periods close to or coinciding with the time of liver tumor appearance.

Hence the results presented here allow us to state that in the process of liver tumor growth induced by the action of chemical carcinogens disturbances appear in the regulatory interrelations between oxidative reactions in membrane lipids, phospholipid content and functional state of the microsomal membrane. Changes in the link "lipid AOA–phospholipid composition" appear at early periods of carcinogen treatment. The interconnection between the effector amount (PEA) and activity of the microsomal glucose-6-phosphatase is disturbed early, too. As to the

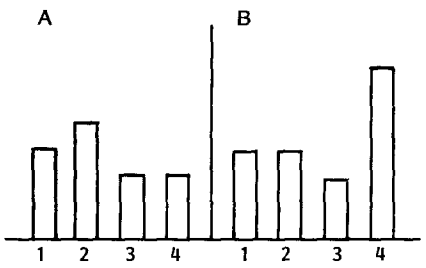


FIG 5. Changes in AOA lipids of rat liver microsomes and alterations in content of PC and PEA during hepatic carcinogenesis induced with DENA. a — normal conditions, b — carcinogenesis. 1 — AOA, 2 — amount of PE, 3 — amount of PC, 4 — amount of PS, at the 10th week of the experiment.

correlation between the PC content and the amount of cytochrome P-450 which reflects the activity of the system of microsomal hydroxylases, this characteristic is disturbed at the time slightly preceding appearance of the tumor.

Changes in the regulatory chain observed in tumor cell. The model of the Ehrlich ascites carcinoma (ACE) was used to study the character of interrelations between the changes in AOA, lipid content, lipid oxidizability and membrane structure in nuclei of tumor cells and liver of a sick animal.

To answer the question whether the regulatory system "AOA–lipid content–lipid oxidizability–structure of nuclear membrane" is restored or altered, it was necessary to compare changes in each of these links with each other.

Figure 6 demonstrates curves of changes of AOA and different lipid

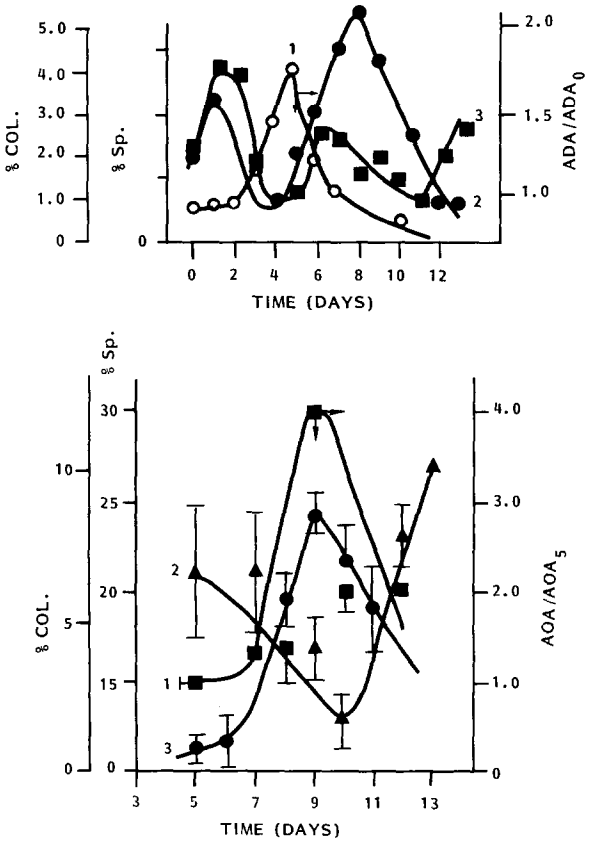


FIG. 6. Changes of AOA (1), percentage of cholesterol (col) (2) and sphingomyelin (Sp) (3) expressed as a fraction of the total lipids during ACE development. a — in nuclei of liver cells of cancerous animals; b — in nuclei of tumor cells.

fractions (cholesterol and sphingomyelin) in nuclei of liver cells during development of ACE.

Under normal conditions, changes of these fractions are most tightly bound with AOA changes; to be exact, they are of antagonistic character.

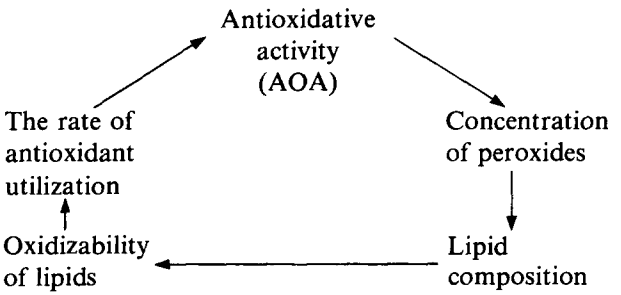
In nuclei of liver cells from a sick animal, counterphase changes of AOA and cholesterol content take place that are inherent in a healthy cell. The percentage of sphingomyelin content in nuclei of liver cells from a sick animal is changed stepwise, the lowest value, however, being parallel to the highest change in AOA. Hence, study of this link of the regulatory chain did not reveal any differences between healthy and sick animals.

At the same time tumor growth is accompanied by considerable (about 3-fold) extremal accumulation of sphingomyelin and drop of cholesterol content in the ACE cell nuclei accompanied with an increase of AOA with a distinct peak on the 9th day of tumor growth (Fig. 6b).

It is noteworthy that the accumulation of sphingomyelin is a typical feature of both tumor tissue and cellular organelles isolated from such tissue for other tumor strains as well (29–33).

Comparison of the data obtained makes it possible to say that contrary to the normal cell, nuclei of tumor cells show parallel changes of AOA and content of a heavily oxidizable phospholipid, i.e. sphingomyelin, that testifies to changes in correlation between AOA and lipid composition.

According to the scheme of regulation of cellular metabolism with oxidative reactions in membrane lipids (cf. scheme), AOA and lipid composition are related to the oxidizability of lipids (S). On the basis of the data concerning different rates of peroxide formation in separate lipid fractions, the value of S was estimated as a ratio of the sum of easily oxidizable lipids (phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol) to the sum of the heavily oxidizable lipids (phosphatidyl choline, sphingomyelin, cholesterol). In calculations a coefficient has been introduced reflecting the ability of each phospholipid for peroxide formation.



The scheme of the connection between the lipid oxidation and membrane lipid composition.

We have found that in nuclei of liver cells the value of S is highest on the 2nd and 5th days of ACE development (Fig. 7a) whereas AOA reaches its maximum on the 5th day.

In nuclei of tumor cells, S is changed stepwise but at the same time the lowest value on the 9th day of ACE development is parallel to the maximum value of AOA (34).

A direct correlation between AOA and S exists in membranes isolated from organs and tissues of healthy animals.

In nuclei of liver cells taken from a sick animal a deviation from this regularity is observed: on the 2nd day after implanting when no changes are seen for AOA an increase of S is marked. However, at later periods of tumor development these indices are altered in parallel.

In ACE nuclei, changes in the correlation between these parameters are expressed more sharply: ACE and S are in counterphase during all the time of tumor development.

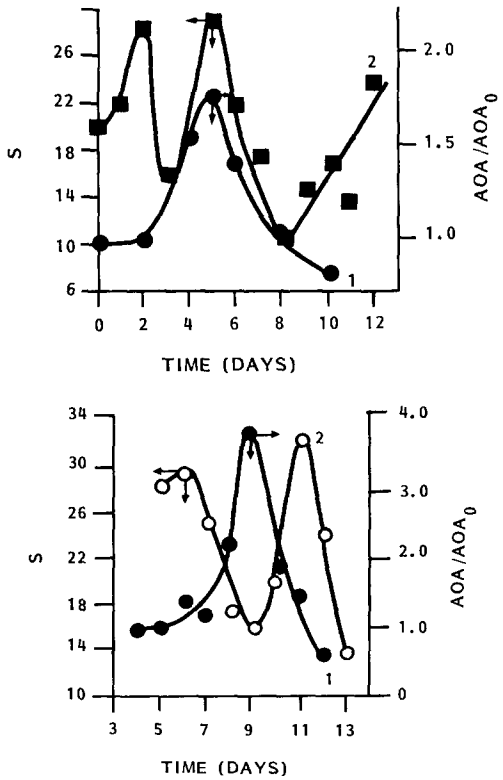


FIG. 7. Changes of AOA (1) and S – of oxidizability of lipids (2) during ACE development: A — in nuclei of liver cells of cancerous animals; B — in nuclei of tumor cells.

Changes occurring in the system "AOA–composition of lipids–oxidizability of lipids" should apparently be seen upon the study of the membrane structure as well.

For normal physiological processes when the mentioned regulatory scheme functions normally, a counterphase dependence has been established of the times of rotatory correlation of probes I and II (8).

Studies of the microviscosity of nuclear membranes of liver cells from cancerous animals have shown that in the process of ACE development an antagonistic character of changes is observed for the value $\frac{\text{exp.}}{\text{contr.}}$ for probes I and II (Fig. 8a). Similar to the normal conditions, this value changes in conterphase with respect to AOA.

The behavior of probes in the nuclei of ACE cells is distinguished from the normal one: changes in parallel of the times of rotational correlation of probes

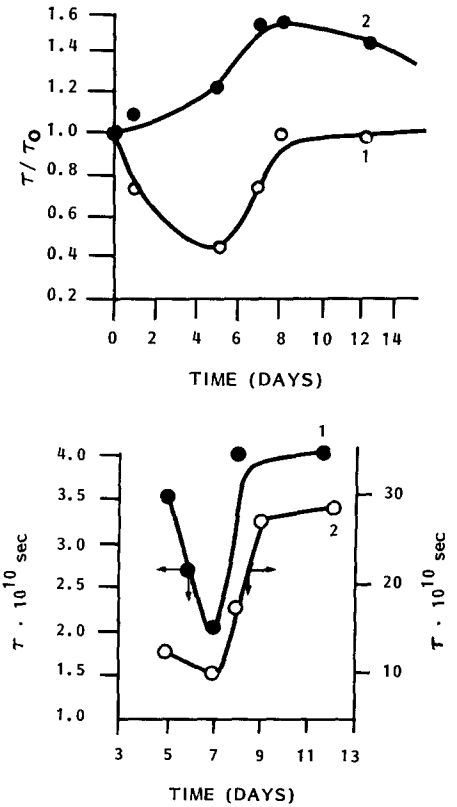


FIG. 8. Changes in the time of rotatory correlation (τ) of the probes I (1) and II (2) during ACE development: a — nuclei of liver cells of cancerous animals; b — in nuclei of tumor cells.

I and II were marked (Fig. 8b). This may testify to an alteration in the lipid-protein interactions in the membrane of tumor cells.

At the same time deviations in the regulatory chain lead to the loss of the antagonistic character of the changes of AOA and for probe I. Investigation of the temperature dependence for probes I and II has shown that in the process of malignant growth the nuclear membrane of the liver cell of a sick animal is found to be more viscous but to remain more rigid than the nuclear membrane of the tumor cells.

The behavior of probe I in the nuclear membrane of liver cells from the cancerous animal is in its turn parallel to the change in the relative content of sphingomyelin and, in particular, of cholesterol: a drop in the viscosity and the lowest amount of these lipids is observed at the period from the 3rd to the 5th day of ACE development.

Increased viscosity of the lipids of the nuclear membrane of tumor cells may be primarily accounted for by an increased percentage of cholesterol and sphingomyelin as compared to the liver cell nuclei from the sick animal, since these lipid fractions and, in particular, cholesterol are essential for mantaining the viscosity and structural entity of membranes.

The behavior of probe I in the nuclear membrane of tumor cells does not coincide with changes of relative content of either sphingomyelin or cholesterol.

Thus, during the ACE development the interconnection of the lipid composition with the membrane structure is retained in the liver cell nuclei of the sick animal, whereas in the nuclei of tumor cells this value undergoes alterations.

Comparison of the behavior of the probe I and S value in the nuclei of liver cells of the sick animal has shown that during ACE development changes of these characteristics are actually in counterphase (Fig. 8a and 7a). Alterations of these parameters of the regulatory chain in the nuclei of tumor cells are also of antagonistic character at the 5th–9th day of ACE development whereas at other times such a character is not observed (Fig. 8b and 7b).

Therefore, the comparison of the ability of the lipid substrate for oxidation, on the one hand, and microviscosity, on the other hand, of the lipid component of the membrane in the nuclei of ACE cells makes it possible to suggest that the changes of these characteristics are most close to the normal values among all the tested parameters of the regulatory system.

On the basis of the data obtained concerning the changes in the antioxidative activity, lipid composition and behavior of the "lipid" and "protein" probes we have found considerable differences in the physico-chemical system of control of oxidative reactions in lipids of the liver cell nuclei of the sick animal and nuclei of tumor cells during the ACE development.

It follows from this comparison of the links of the regulatory chain that

during ACE development in the nuclei of liver cells of cancerous animals the system of interconnections "AOA–lipid composition–membrane structure" is retained. Despite the fact that some links are distinct from the normal pattern, the functioning of the system as a whole is unchanged.

Analysis of the corresponding parameters of the system under study in the nuclei of ACE cells has shown that in the process of the tumor growth a change takes place in the chain "AOA–lipid composition–membrane structure" as compared to the normal conditions and to the liver cells of a sick animal as well.

Primary deviation in this system evidently appears in the link "AOA–lipid composition of lipids and their oxidizability" which then induces changes in the following links of the chain. Summing of these changes results in establishing interconnections atypical of the normal conditions within the system of regulation of oxidative reactions in the lipids of the nuclear membrane.

SUMMARY

The changes in lipid fluidity, in the degree of their relative composition produce alterations of membrane-bound enzyme activities, their cooperative properties, substrate specificity, sensitivity to the action of allosteric regulation and equilibrium between free and bound forms. We have found *in vivo* regular interrelation of the rate of oxidation reactions in lipids and the changes in their composition. An increase in the rate of oxidation reactions in lipids leads to reduction in the relative amount of phospholipids, enrichment of phospholipids with oxidation-resistant lipids and more rigid lipid component of the membrane. Inhibition of oxidation reactions in lipids is interrelated with an increase in the relative amount of phospholipids, with membrane enrichment with easily oxidizable fractions, and with more liquid lipid components of the membrane. We regard such a relationship between rate of oxidation and change of lipid composition as a physico-chemical system maintaining the steady state level of oxidation reactions in lipids.

In the experiments on normal animals we have proved the interrelationship between the activity, substrate specificity and cooperative properties of glucose-6-phosphatase, cytochrome P-450 and between the composition and properties of membrane lipid phase. In carcinogenesis, there is a disturbance of the relationships in this regulatory system. A link, oxidation rate–lipid composition, was shown to be the most vulnerable.

The changes in phospholipids in response to the alteration of lipid antioxidative properties at the early stages of carcinogenesis differed from those in the normal state.

At the same time, the interrelation in the link: lipid composition–enzyme

activity, was in most cases kept much longer and a disturbance was observed only at the latest stages just before tumor formation. Similar ruptures of connections in this regulatory system were observed in tumor cells in the process of transplantable tumor growth. In the organs of the host animal the relationships between different parameters weakened, although by the type of response they were the same as in the norm. The factors that normalize the lipid membrane composition and the rate of lipid peroxidation also produced an anticarcinogenic effect.

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