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The Role of Tocopherol, Cholesterol, and Oleic Acid in Lipid Peroxidation

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THE ROLE OF α-TOCOPHEROL, CHOLESTEROL, AND OLEIC ACID IN LIPID PEROXIDATION

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ABSTRACT

THE ROLE OF α-TOCOPHEROL, CHOLESTEROL, AND OLEIC ACID IN LIPID PEROXIDATION

The mechanism whereby α-tocopherol inhibits lipid peroxidation and the effect of cholesterol and oleic acid upon that inhibition are examined using phosphatidylcholine liposomes. A novel method for purifying soy-phosphatidylcholine (soy-pc) from lecithin tablets via flash column chromatography is introduced and confirmed to be reliable. The soy-pc is then utilized in producing five different types of liposomes: 1) large multilamellar vesicles (LMV's) produced by sonication, 2) small unilamellar vesicles (SUV's) produced by extrusion, 3) SUV's with incorporated α-tocopherol, 4) SUV's with incorporated α-tocopherol and cholesterol, and 5) SUV's with incorporated α-tocopherol and oleic acid. Each type of liposome is subjected to oxidation measurements under conditions of varying concentrations of α-tocopherol, cholesterol, and oleic acid. The first two are examined in conjunction with each other to determine how organization of the membrane model affects inhibition of lipid peroxidation by α-tocopherol. The third, fourth, and fifth are likewise compared for the purpose of determining the effect of membrane stiffness on inhibition of lipid peroxidation by α-tocopherol.

Results indicate that the first two membrane models are kinetically similar; however, LMV's appear to oxidize faster without initiator. All models tested demonstrate concentration-dependent responses to α-tocopherol. At the concentrations of soy-pc that inhibit oxygen consumption, the reactivity data are not consistent with reaction of peroxyl radicals with the tocopherol. Results of the latter three experiments indicate that the effectiveness of α-tocopherol is not remarkable in the systems observed. Cholesterol appears to have little effect on lipid peroxidation or α-tocopherol activity. Oleic acid, however, does increase lipid peroxidation and α-tocopherol antioxidant activity in homogeneous solutions.

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And finally, I would like to thank my family for their understanding. I promise to come home in a few more days.

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INTRODUCTION

In the course of the past decade, much attention has been focused on lipid peroxidation, its causes and consequences, and chemicals which possess the potential to inhibit it. Lipid peroxidation involves the destruction of membrane lipids which, in turn, leads to the destruction of the cell. Lipid peroxidation may be caused by a myriad of factors including ozone toxicity, smoke, surgery, and drugs. It is representative of certain pathogenic disease states including ischemia, neural tissue damage, and cancer. However, it is not known whether lipid peroxidation is the cause or the consequence of these disease states. Several agents have been known to inhibit lipid peroxidation. The study of these is hoped to lead to a better understanding of the mechanism of lipid peroxidation and how to prevent it.

THE MECHANISM OF LIPID PEROXIDATION

Lipid peroxidation involves the destruction of polyunsaturated lipids by a free radical chain mechanism as shown below:

initiation	$2RH + O2 \rightarrow 2R\cdot + 2\cdot OH$	(1)
propagation	$R\cdot + O2 \rightarrow ROO\cdot$	(2)
	$ROO\cdot + RH \rightarrow ROOH + R\cdot$	(3)
termination	$R\cdot + R\cdot \rightarrow RR$	(4)
	$R\cdot + ROO\cdot \rightarrow ROOR$	(5)
	$ROO\cdot + ROO\cdot \dashrightarrow ROOR + O2$	(6)

Lipid peroxidation can also be initiated by the hydroxyl radical, iron and copper atoms, and the superoxide radical. Of these, the most reactive and deleterious is the hydroxyl radical.

Ti(III), Cu(I), Fe(II), Co(II), Ni(II), and other transition metals may react with hydrogen peroxide to produce the hydroxyl radical according to the formula

$$Mn^{+} + H_2O_2 \rightarrow M^{(n+1)+} + \cdot OH + OH^{-} \qquad (7).$$

Particular attention has been focused on the role of iron in this general reaction. The Fenton reaction proceeds precisely as predicted in reaction (7):

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + \cdot OH + OH^{-} \qquad (8).$$

Ferrous complexes react more quickly than ferric complexes. The iron-catalyzed Haber-Weiss reaction is as follows:

$$O_2^{\cdot -} + H_2O_2 \xrightarrow{Fe} \cdot OH + OH^- + O_2 \qquad (9)$$

Normally, iron in the tissues is sequestered and regulated tightly as part of the natural antioxidant defense mechanism. The iron used in these reactions comes from oxidative stress itself. The superoxide radical mobilizes iron from ferritin while hydrogen peroxide mobilizes iron by degrading heme proteins (reviewed in reference 1).

CONSEQUENCES OF LIPID PEROXIDATION

The chain mechanism may result in damage to many lipid molecules before termination. In addition, the free radicals and singlet molecular oxygen produced in this reaction are capable of denaturing proteins, damaging nucleic acids, and saturating the double bonds of fatty acid molecules (2).

Lipid peroxidation results in damaged lipids as well as a complex mixture of isomeric products. In many cases it causes the release of free fatty acids which alter the properties of the membrane and integral proteins (Schaeffer). Lipid peroxides that come in contact with iron or copper ions or are subjected to high temperatures decompose into "noxious" products such as epoxides, ketones, acids, and aldehydes. Two of the major aldehyde products, malondialdehyde and 4-hydroxynonenal, attack proteins, especially at thiol and amino groups (2).

Hydrogen peroxide, hydroperoxides, hydroxyl radical, superoxide anion, and singlet molecular oxygen have been implicated as important intermediates in secondary reactions caused by ozone (3).

THE ROLE OF ANTIOXIDANTS

Peroxidized lipids assume an abnormal configuration in the cell membrane in order to signal for their own repair. The same abnormal configuration, however, alters the membrane structure to such a degree that it may create pores through which cell constituents may leak. When lipid peroxidation occurs to a large extent, repair of the peroxidized lipids is insufficient to prevent leakage and the cell dies.

Antioxidants terminate the free radical chain mechanism of lipid peroxidation. They are important in the cell's natural defense against oxidation and increasing interest has been generated to their use as preventive and curative agents. Natural defense mechanisms include mineral dependent enzymes such as glutathione peroxidase, a free radical scavenger, water-soluble antioxidants such as ascorbic acid, glutathione, and uric acid, and lipid-soluble antioxidants such as the carotenoids and a-tocopherol (4). Superoxide dismutase and catalase also protect by removing singlet oxygen and hydrogen peroxide from the body (1).

This reaction is desirable because the tocopherol radical is only mildly reactive. There are several possible fates of the tocopherol radical; it may react with ascorbate to form

semihydroascorbate or it may react with ubiquinol. Another plausible mechanism for the action of α-tocopherol stems from the rigidity it confers upon membranes. Such added stiffness would hypothetically decrease the range of movement of molecules within the bilayer, entrapping the radical. The purpose of these experiments is to determine which mechanism, if either, are correct.

IMPLICATIONS

Lipid peroxidation has been implicated in the pathogenesis of many disease states. It has been linked to cancer, atherosclerotic plaques, tissue damage caused by trauma or oxygen deprivation, issue damage resulting from toxins such as carbon tetrachloride, strokes, myocardial infarction, drugs, surgery, damage resultant from air polution, and the natural aging process (2).

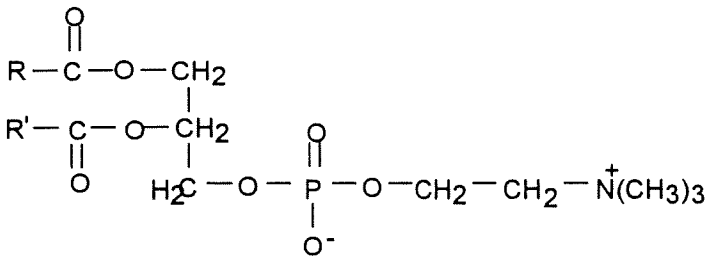
α-tocopherol as been demonstrated to possess cancer-preventing effects. Perchellet et al. have shown that α-tocopherol is capable of inhibiting DBMA-induced skin cancer in mice (5). Likewise, it is also capable of inhibiting DBMA-induced oral cavity and mammary gland cancers (6) Antioxidants, including α-tocopherol, "block the formation of chemical carcinogens in the stomach, protect DNA and lipid membranes from oxidative damage, and enhance immune function" (4).

PURIFICATION OF PHOSPHATIDYLCHOLINE

INTRODUCTION

PHOSPHATIDYLCHOLINE - GENERAL CHEMICAL INFORMATION

Phosphatidylcholine belongs to a group of compounds known as phospholipids. Phospholipids are esters of two molecules of fatty acid joined to one molecule of glycerol, the remaining hydroxyl group on glycerol being joined by a phosphate group to a nitrogen-containing molecule such as choline (2). Phospholipids vary in composition with respect to both head groups and hydrocarbon chains. The hydrocarbon chains frequently have one or more *cis* double bonds (7). Other phospholipids include phosphoserine, phosphoethanolamine, and phosphoinositol. The chemical structure of phosphatidylcholine is shown below:



There are two main portions of the molecule which contribute to its unique properties: the choline "head group" and the hydrocarbon "tail". R and R' normally represent even-numbered fatty-acid chains, most commonly of 16 and 18 carbon lengths. In addition, the *sn*-1 position usually contains a saturated fatty acid while the *sn*-2 position usually contains unsaturated fatty acids. X-ray crystallography studies show that the two hydrocarbon chains and the two carbonyls are not equivalent because of their specific sites of attachment to glycerol. Intermolecular interactions between the N-methyl hydrogens and the phosphate of a neighboring phosphatidylcholine contribute to the properties of the membrane they compose (3). Phosphatidylcholine is an ampipathic molecule and as such usually forms a bilayer sheet because of hydrophobic forces (8).

At physiological pH, phosphatidylcholine is a zwitterion, with a positive charge on the quaternary ammonium and a negative charge on the phosphate. These charges and different charges on other phospholipids confer characteristic surface properties to membranes (9). Membrane surface charges are very important as proteins will not bind to zwitterionic-only membranes (10).

FUNCTION OF PHOSPHATIDYLCHOLINE IN BIOLOGICAL MEMBRANES

Phosphatidylcholine orients perpendicular to the surface of a membrane, with its hydrophobic tail inside and hydrophilic head outside the membrane, and thereby

contributes to the formation of a semipermeable barrier to proteins and ions. Phosphatidylcholine is the most abundant phospholipid in the outer leaflet of most biological membranes. There is an "asymmetric transverse distribution" of phospholipids between both leaflets of the plasma membrane of most cells. It has been established that the aminophospholipids phosphatidylserine and phoshpatidyl-ethanolamine are localized in the cytoplasmic membrane leaflet while phospholipids containing choline head groups such as phosphatidylcholine and sphingomyelin are present in the outer monolayer. The distribution of red cell fatty acids is dependent on dietary lipid alteration (11). In addition, other cell types demonstrate different lipid distributions according to temperature (12).

Phosphatidylcholine, like many other lipids, may exist in an ordered (*trans*) or a fluid (*gauche*) state. A certain degree of fluidity is required for membrane flexibility, activity, and movement of proteins, lipids, and antioxidants (2). The Tm, transition or melting temperature, is the temperature at which the transition between the ordered and fluid states occurs. The Tm depends on the fatty acid chain length and the degree of unsaturation (8). Phospholipid behavior is anisotropic; accordingly, *trans-gauche* isomerism may lead to kinks in the chain where small molecules can fit and diffuse through (9). X-ray crystallography studies have demonstrated that "lipids generally arrange in crystals in typical bilayer structures and adopt a limited number of preferred conformations, both with respect to their hydrophobic moieties and their head groups" (13). In the ordered or "gel" state, motion of the tail is restricted but the head can still move and is somewhat independent (9).

As the major phospholipid component of biological membranes, phosphatidylcholine confers many characteristic properties to them. The ampiphilic nature of phosphatidylcholine enables the formation of the bilayer structure which is responsible for partitioning cellular constituents from the external environment. Fatty acid chain length and degree of unsaturation contribute to membrane fluidity and are essential for protein function and movement as well as diffusion of small molecules. The lack of a net charge contributes to the surface characteristics of membranes, but not its overall surface charge. Phosphatidylcholine is also involved in the second messenger system whereby cells receive instructions on their membrane surface.

Phosphatidylcholine's role as the major component of biological membranes has led to its frequent use as a model membrane for experimentation. As a liposome, it is also used to transport drugs, water-soluble and fat-soluble agents because of its amphiphilicity (14).

OXIDATION OF PHOSPHATIDYLCHOLINE

One example of how abnormal phosphatidylcholine functioning affects membranes is illustrated by lipid peroxidation. The free radical chain mechanism may be initiated as a result of radiation, surgery, drugs, disease, or other factors. Lipid peroxidation results in damage to the lipid bilayer and release of toxic products. The toxic products may be used as an indicator of extent of tissue damage, but their presence does not imply whether lipid

peroxidation caused or was a consequence of that damage. Since phosphatidylcholine is such an essential part of a living cells, it and its pathogenesis has become an intriguing subject of research.

METHODS AND MATERIALS

Phosphatidylcholine was purified from Spring Valley brand Lecithin tablets obtained from Wal-Mart. The lecithin tablets were purified from soybeans. The contents of a capsule were weighed and dissolved in 10ml chloroform. This solution was then added to a silica gel flash chromatography column 42 cm in length and 1cm in diameter. The column was washed under slight pressure with two successive solvents, 300 mls of 99% ethyl acetate: 1% methanol, and 200 mls of 100% methanol, respectively. Evaporation and weight determination of each fraction demonstrated that the final fraction, that of 100% methanol, contained the major portion of soy-pc.

RESULTS

Identification of phosphatidylcholine was confirmed using fast atom bombardment mass spectroscopy. Figure 1 represents phosphatidylcholine molecules containing one C_{18} and one C_{16} chain, with varying degrees of unsaturation. Figure 2 represents phosphatidylcholine molecules containing two C_{18} chains with varying degrees of unsaturation. The peak weights of 760.7 and 784.8 amu correspond well to the expected value of approximately 780 amu.

MASS SPECTROSCOPY ANALYSIS

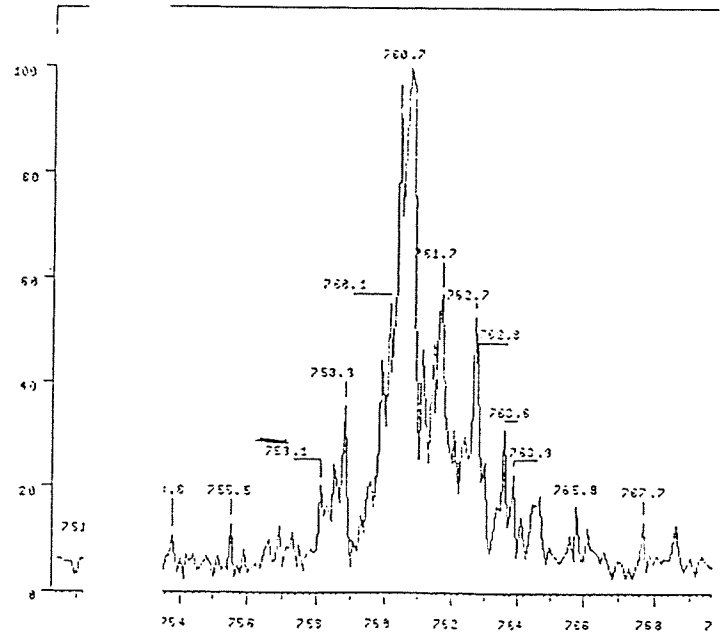


Fig.1

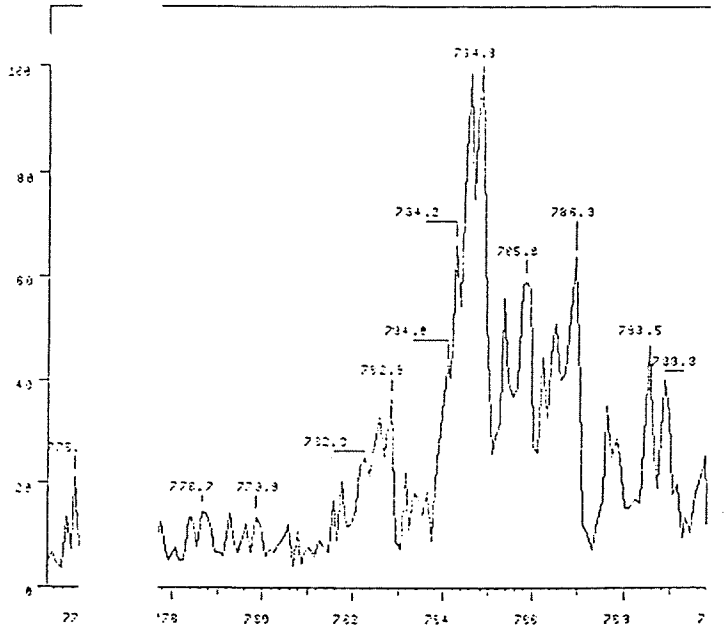


Fig.2

CONCLUSION

The FAB-mass spectroscopy results indicate that lecithin tablets provide an inexpensive and reliable source of phosphatidylcholine.

MEASUREMENT OF LIPID PEROXIDATION AND INHIBITION BY α-TOCOPHEROL: LARGE MULTILAMELLAR VESICLES AND SMALL UNILAMELLAR VESICLES

INTRODUCTION

The purpose of this study is to determine the mechanism by which α-tocopherol inhibits lipid peroxidation in the membrane bilayer. Two different types of membrane models were used: large mulitilamellar vesicles (LMV's) and small unilamellar vesicles (SUV's). The first membrane model resembles an onion skin in its organization. The second membrane model more closely approximates a true biological cell membrane, although it is somewhat smaller. LMV's generally range in size from 100-1000nm; SUV's are much smaller, ranging from 15-25nm. LMV's contain an aqueous compartment between each concentric layer. SUV's contain only one central compartment. LMV's are not well-suited for trapping large volumes of solution because the concentric bilayers take up most of the internal space. They are, however, well-suited for carrying lipophilic compounds. SUV's are also not well-suited for trapping large volumes of solution. In addition, SUV's require a high energy input for their production but they are the best characterized and most uniform (15).

LIPOSOME PREPARATION

Phospholipids automatically form bilayers in solution. The different methods of liposome preparation are aimed at producing liposomes of different sizes and structures. Mechanical dispersion consists of evaporating the lipid onto a solid support and then shaking it in an aqueous medium. This type of preparation includes shaking by hand, sonication, centrifugation, membrane extrusion, and the French Press method. Solvent dispersion involves the alignment of bilayers at the interface of organic and aqueous solvents. This is most frequently accomplished by ether injection. The final type, detergent solubilization, include bile salt preparations, different types of dialysis, and the use of solubilized Sendai virus particles (15). The methods used in the following experiments are of the mechanical dispersion type since this is the simplest method.

METHODS OF MEASURING LIPID PEROXIDATION

There are several widely used procedures for measuring lipid peroxidation. Most involve the measurement of a product or products of the peroxidation reaction. The thiobarbituric (TBA) reaction detects the presence of malonaldehyde, which, as already discussed, is a product of the decomposition of lipids. However, malonaldehyde is metabolized by the liver within a few hours. Therefore the absence of this product does not prove the absence of lipid peroxidation. Other useful methods quantitify the presence

of conjugated dienes by UV spectrophotometry or the presence of characteristic fluorescent chromophores which result from the decomposition products formed by malonaldehyde and certain proteins. In addition, iodimetric and ethane evolution procedures have been used (16). Takayama et al. have produced an assay for phosphatidylcholine hydroperoxide, a product of oxidative stress, using high performance liquid chromatography and UV absorption techniques. Their assay is useful in determining oxidative damage caused by surgery, drugs, and disease as well as testing the activity of antioxidants (17). More recent techniques used to measure lipid peroxidation include LC-Mass Spectrophotometry, Electron Spin Resonance (ESR), and oxygen consumption using an oxygen electrode.(18).

METHODS AND MATERIALS

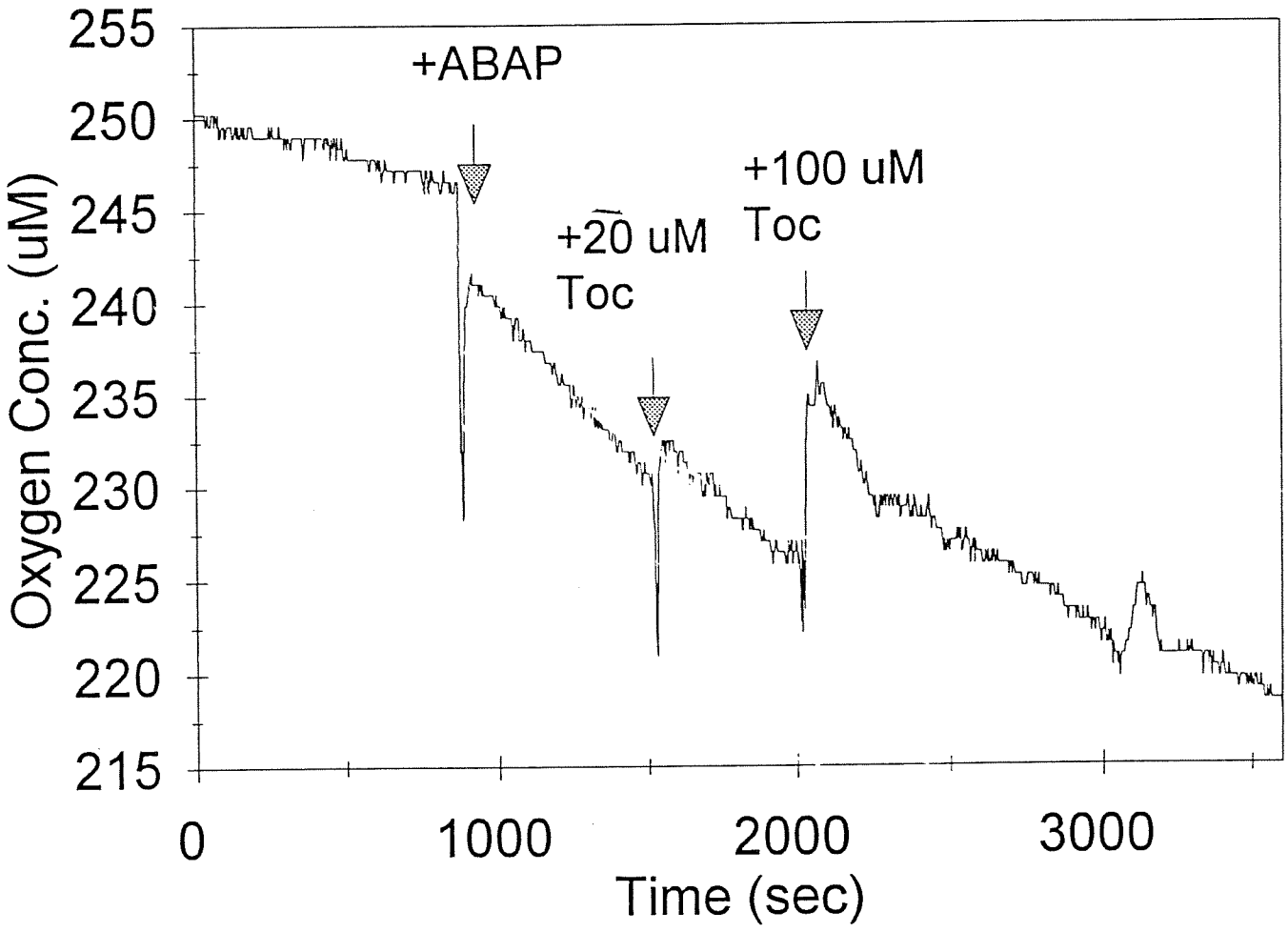
Phosphatidylcholine purified in the previous experiment was used to make membrane models. First, a stock solution of 30 mg/ml pc was prepared in phosphate buffer. Then, two different membrane models were prepared for these experiments; one model by sonication and the other by a liposome extruder from the Avestin company. The 100nm polycarbonate extruder membrane was expected to produce vesicles of roughly the same size. The first method produces large multilamellar vesicles (LMV's) and the second method produces small unilamellar vesicles (SUV's).

Oxidation consumption measurements were taken on a YSI Model 5300 Biological Oxygen Monitor. This monitor is a Clarke-type oxygen electrode, consisting of a silver anode, a platinum cathode, and KCl electrolyte. Before taking measurements, 3.5 mls of phosphate buffer (pH 7.4) and 0.5 mls of phosphatidylcholine were allowed to equilibrate in the cell at 37°C with the magnetic stirrer. Equilibration involved two steps; 15 minutes without the probe in the solution, and 15 minutes with the probe in the solution. If after these two steps the monitor did not display a nearly flat line, the solution was allowed to equilibrate further. Additions of 0.5M ABAP and varying concentrations of α-tocopherol were made using a microliter syringe. Results were visualized and slopes were calculated using the Quattro Pro for Windows program.

RESULTS

Graphs 1 and 2 depict two similar experiments using the two different types of phosphatidylcholine vesicles. The first section of figures 3 and 4 show that there is little oxygen consumption before the addition of ABAP. The second section demonstrates the response to 0.1ml of ABAP. The third and fourth sections demonstrate responses to 15μl and 75μl of α-tocopherol, respectively. Table 1 represents the corresponding numeric data taken from figures 3 and 4.

GRAPH 1. OXIDATION OF LARGE MULTILAMELLAR VESICLES



GRAPH 2. OXIDATION OF SMALL UNILAMELLAR VESICLES

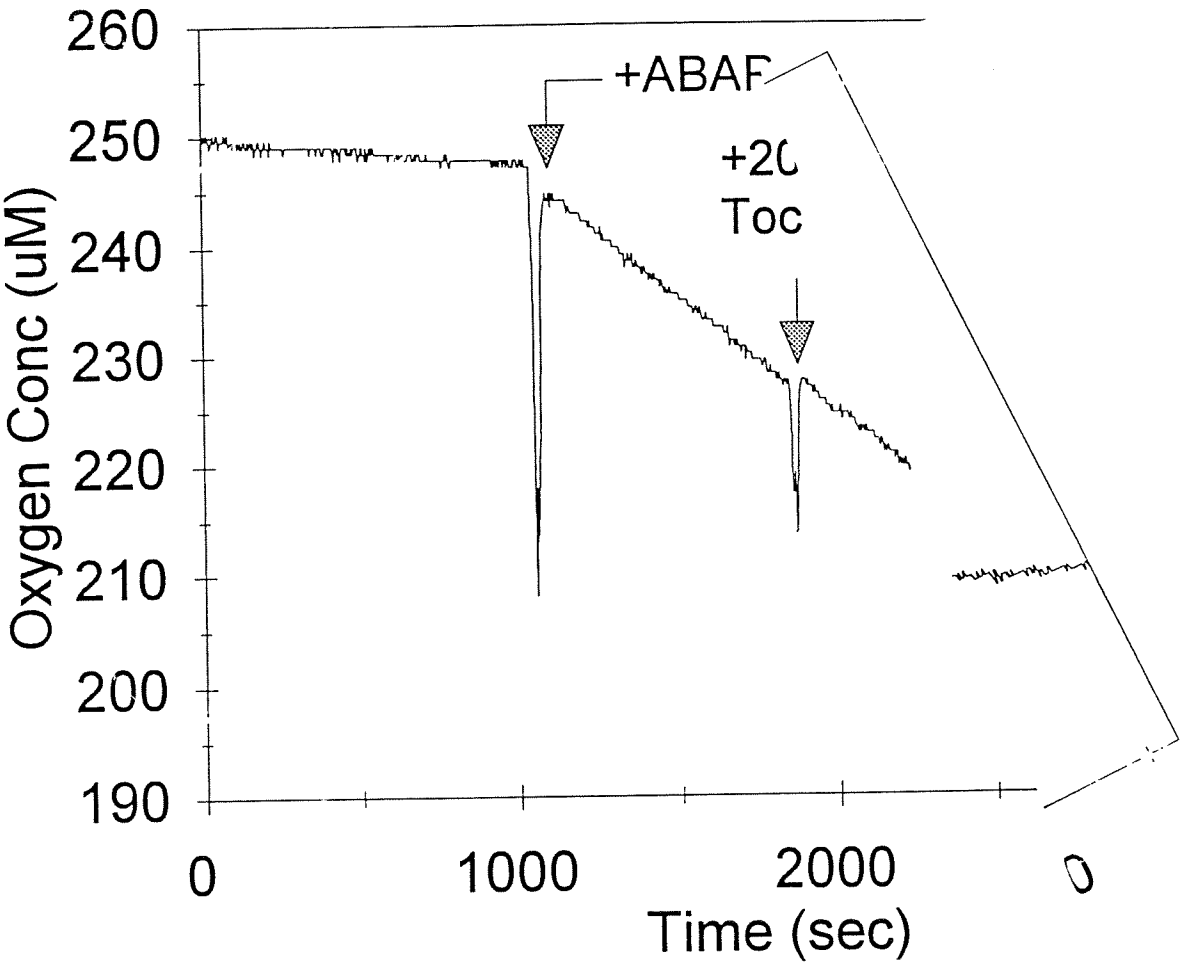


TABLE 1. RATES OF OXIDATION OF LARGE MULTILAMELLAR VESICLES AND SMALL UNILAMELLAR VESICLES

SAMPLE	LMV RATE	LMV % INHIB	SUV RATE	SUV % INHIB
Soy pc alone	-0.0041		-0.0022	
Soy pc + ABAP	-0.00186	0	-0.0237	0
+20 μM α-Toc	-0.0151	19	-0.0231	3
+100 μM α-Toc	-0.0077	58	-0.0122	49

CONCLUSION

Several observations may be made from figures 3 and 4 and table 1. While the graphs may look different at first, numerical analysis reveals that they are kinetically similar. LMV's are less structured than SUV's and therefore exhibit slightly different properties such as a faster rate of oxidation without the initiator. However, both membrane models demonstrate concentration-dependent responses to α-tocopherol and both models demonstrate that inhibition does not depend on free radical chemistry alone. Further research is needed to determine what minimum level of α-tocopherol, if any, is required to elicit some response in either membrane model. Further research is also needed to more fully comprehend the precise mechanism of lipid peroxidation inhibition by α-tocopherol.

MEMBRANE STRUCTURE AND ITS EFFECT ON α-TOCOPHEROL AS AN ANTIOXIDANT

INTRODUCTION

The purpose of this experiment is to determine what effect membrane stiffness has on the antioxidant activity of α-tocopherol. The experiment has three components: the first investigates lipid peroxidation in soy-pc liposomes with varying concentrations of α-tocopherol, the second investigates lipid peroxidation in soy-pc liposomes with α-tocopherol and varying concentrations of oleic acid, the third investigates lipid peroxidation of soy-pc liposomes with α-tocopherol and varying concentrations of cholesterol. The first part of the experiment serves to illustrate the effect of increasing concentration of α-tocopherol incorporated into the liposome on oxidation. The second and third experiments demonstrates the effects that oleic acid and cholesterol have on antioxidant activity by altering the fluidity of the membrane.

Cholesterol is a natural lipid component of biological molecules. Being a lipid, it is also subject to lipid peroxidation (2). Cholesterol is an ampipathic molecule which, when inserted into a lipid bilayer, has a rigid steroid nucleus and hydroxyl group oriented toward the aqueous surface and its hydrocarbon tail in the center of the bilayer. The first portion is inflexible, the second has a wide range of movement. These properties affect the fluidity, the transition temperature, and the permeability of the membrane. At high concentrations of cholesterol, the fluidity and permeability of the membrane are decreased above the transition temperature and increased below it. Such alteration can lead to elimination of the phase transition altogether. Cholesterol has been shown to increase the density of pc liposomes. It is included in membranes to reduce or completely remove the transition (fusion) of SUV's especially. It is equally distributed across the lipid bilayer and partially inhibits SUV disintegration when subjected to plasma. (15) Incorporation of cholesterol into soy PC liposomes is expected to produce less fluid membranes.

Oleic, arachidonic, and linoleic acids are other important natural components of biological membranes. Oxidative damage has been shown to release oleic and other unesterified fatty acids from phospholipids *in vivo* (19, 20). Free radicals have been demonstrated to stimulate phospholipases which cleave the fatty acids from phospholipids (21). The free fatty acids from this reaction may subsequently contribute to oxidant injury and edema (22) or modulate guanylate cyclase and adenylate cyclase activity(23, 24). Unsaturated fatty acids contribute to the fluidity of membranes and lower the melting temperature. Alterations in the fluidity of the membrane subsequently causes changes in the possible range of motion of other substituents such as proteins and allows conformational changes. Thus unsaturated fatty acids can lead to the change in activity of membrane proteins and characteristics of the membrane itself (25). Incorporation of oleic acid into PC liposomes is therefore expected to lead to the formation of more fluid membranes.

METHODS AND MATERIALS

A 20 mg/ml soy-pc/chloroform solution was prepared from lecithin tablets by flash column chromatography as previously described. A 5 mM α-tocopherol/chloroform stock solution was prepared from reagents obtained from Sigma Chemical Company. A 5 mM stock solution of cholesterol/chloroform was prepared from reagents obtained from Fisher Scientific Company and Sigma Chemical Company. A 5 mM oleic acid/chloroform stock solution was prepared from reagents obtained from Sigma Chemical Company. A phosphate buffer of pH 7.4 and high ionic strength was also prepared. All samples oxidized contained 5mg/ml of soy-pc.

The concentrations listed below refer to final concentrations in a 4 ml solution. After evaporation to dryness, 4 ml of phosphate buffer were added, swirled, and sonicated for approximately 5 minutes. Solutions were extruded through an Avestin liposome extruder and then allowed to equilibrate in the oxygen electrode cell using a magnetic stirrer for 15 minutes. Once the solutions were equilibrated measurements were taken on the oxygen electrode. 30 minutes into the measurement, 100 µl of 0.5M ABAP was added to the solution by removing the electrode and pipeting the ABAP directly into the reaction mixture. Measurement was continued for another 30 minutes. Results were visualized and slopes were calculated using the Quattro Pro for Windows program.

RESULTS

The rate of oxidation of 100µl of 0.5M ABAP in phosphate buffer was determined to be -0.01116.

TABLE 2. RATE OF INHIBITION OF OXIDATION OF SOY-PC LIPOSOMES BY α-TOCOPHEROL

FINAL [α-TOCOPHEROL]	RATE OF OXIDATION	% INHIBITION
0	-0.03357	0
10µM	-0.00833	75.2
50µM	-0.00406	87.9
100µM	-0.00810	75.9
200µM	-0.00117	96.5

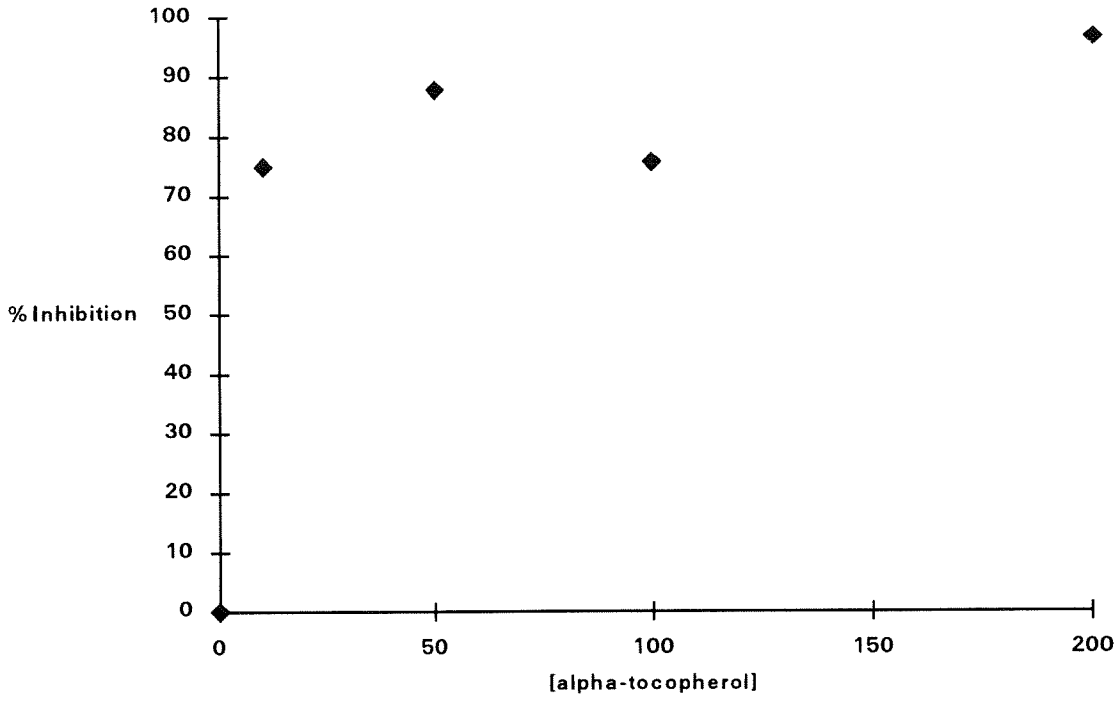
TABLE 3. RATE OF OXIDATION OF α-TOCOPHEROL AND CHOLESTEROL

FINAL [CHOLESTEROL]	FINAL [α-TOCOPHEROL]	RATE 1	% INHIBITION
1mM	0	-0.01016	0
1mM	10μM	-0.00572	43.7
1mM	50μM	-0.00357	64.9
1mM	100μM	-0.00566	44.3
1mM	200μM	0.00241	76.3
10μM	0	-0.01088	0
10μM	10μM	-0.01211	-11.3
10μM	50μM	-0.00962	11.6
10μM	100μM	-0.00250	77.0
10μM	200μM	-0.00626	42.5

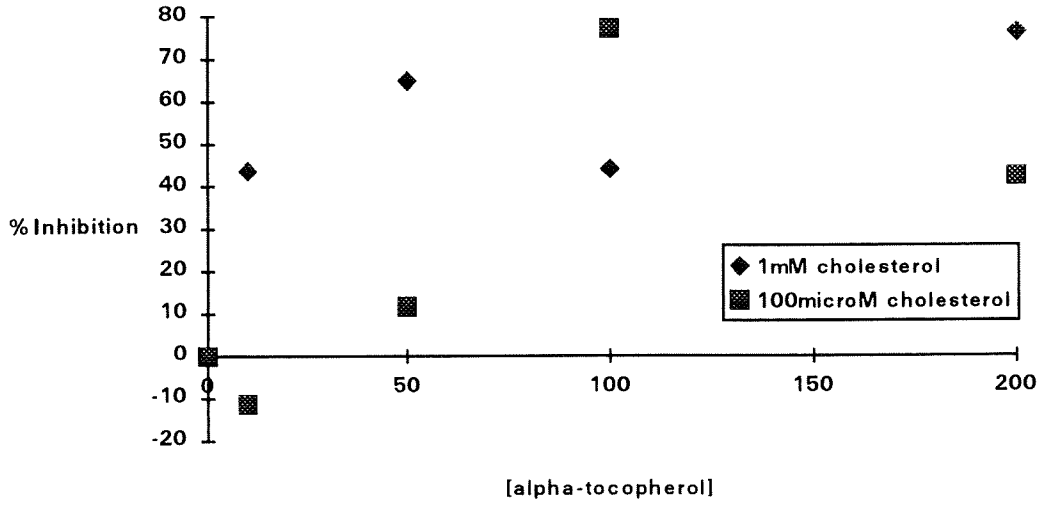
TABLE 4. RATE OF OXIDATION OF α-TOCOPHEROL AND OLEIC ACID

Final [oleic acid]	**Final [α-tocopherol]**	**Rate 1**	**% Inhibition**
10mM	0	-0.00028	0
10mM	10μM	0.00125	546.4
10mM	50μM	-0.00274	-878.6
10mM	100μM	0.00503	1896.4
10mM	200μM	-0.01572	-55142.8
100μM	0	-0.01965	0
100μM	10μM	-0.01056	46.3
100μM	50μM	-0.00655	66.7
100μM	200μM	0.00186	90.5

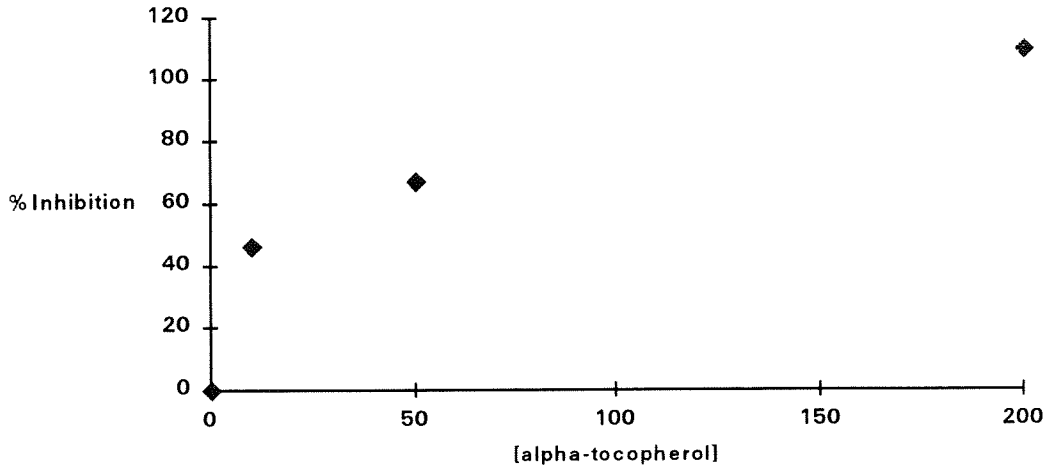
GRAPH 3. [A-TOCOPHEROL] VS. % INHIBITION



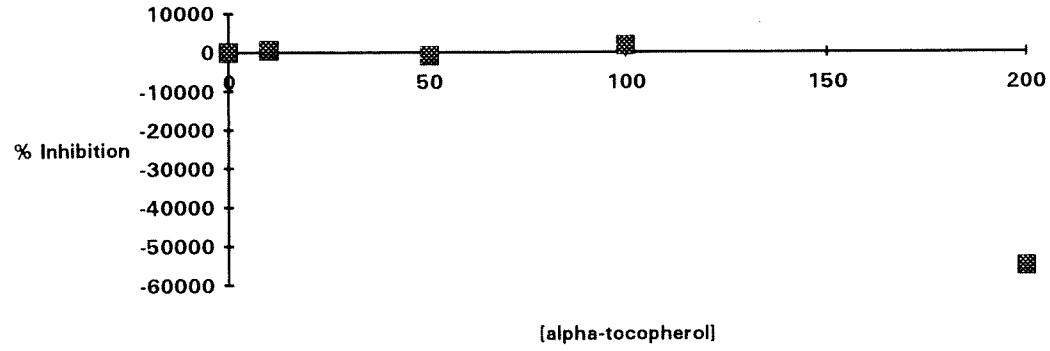
GRAPH 4. [A-TOCOPHEROL] VS. % INHIBITION WITH INCORPORATED CHOLESTEROL



GRAPH 5. [A-TOCOPHEROL] VS. % INHIBITION WITH INCORPORATED OLEIC ACID (100μM)



GRAPH 6. [A-TOCOPHEROL] VS. % INHIBITION WITH INCORPORATED OLEIC ACID (10MM)



CONCLUSION

Table 2 indicates that the data taken from this experiment are consistent with the data taken from the previous experiment. The rate of oxidation of soy-pc without α-tocopherol appears erroneous but has been tested repeatedly.

Cholesterol does not appear to have a sizable effect on either lipid peroxidation or the activity of α-tocopherol. This does not correspond well with the idea that stiffening the membrane should lead to decreased lipid peroxidation.

At high concentrations of oleic acid, the solution was not homogeneous. This is reflected in the impossible data of table 4 and the erratic appearance of graph 6. At lower

concentrations, however, oleic acid appears to increase lipid peroxidation and the effectiveness of α-tocopherol. Whether or not this is accomplished by a structural mechanism in which oleic acid acts by increasing the fluidity of the membrane remains unknown.

OVERALL CONCLUSION

Flash column chromatography provides an efficient, reliable and inexpensive source of soy pc. Soy pc is a common component of biological membranes and synthetic liposomes used for research or medicinal purposes. FAB Mass Spectroscopy anaylsis revealed that the product is indeed soy pc.

LMV's and SUV's are organized differently, yet contain the same components. Hence, they can be expected to behave similarly but not exactly the same. Oxidation studies revealed that this was in fact the case.

Cholesterol and oleic acid are puported to confer opposite properties to membranes; hence one might expect that they would behave oppositely when subjected to lipid peroxidation and inhibition with α-tocopherol. Cholesterol did not appreciably affect oxidation or the activity of α-tocopherol. Oleic acid, however, increased oxidation and antioxidant activity of α-tocopherol at low concentrations. This indicates that fatty acids released by lipid peroxidation compound the problem.

Data obtained from comparison of SUV's and LMV's indicate that a free radical mechanism does not completely explain the inhibition of lipid peroxidation by α-tocopherol. The cholesterol data do not appear to concur with a structural mechanism of inhibition but the oleic acid data do. Therefore, it is possible that there may be more than one or a more complex mechanism.

REFERENCES

1. Halliwell, B., Gutteridge, J.M.C., and Cross, C.E. Free radicals, antioxidants, and human disease: where are we now? *J. Lab. Clin. Med* (June 1992):119(6):598-620.

2. Halliwell, B. Lipid Peroxidation, Free Radical Reactions, and Human Disease. Current Concepts. The Upjohn Company; Kalamazoo, MI, 1991.

3. Gray, J.I. Measurement of Lipid Oxidation: A Review. *J. Am. Oil Chem. Soc.* (1978): 55(Jun): 539-546.

4. Byers, T. and G. Perry. Dietary Carotenes, Vitamin C, and Vitamin E as Protective Antioxidants in Human Cancers. *Annu. Rev. Nutr.* (1992):12:139-159.

5. Perchellet, J.P., Owen, M.D., Posey, T.D., Orten, D.K., and Schneider, B.A. Inhibitory effects of glutathione level raising agents and D-α-tocopherol on ornithine decarboxylase induction and mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. *Carcinogenesis* (1985):6:567573.

6. Shklar, G. Oral mucosal carcinogenesis in hamster: inhibition by vitamin E. *J. Natl. Cancer Inst.* (1982):68:791-797.

7. Metcalfe, J.C., Kornberg, and Phillips, eds. *Biochemistry of Cell Walls and Membranes II; International Review of Biochemistry*. Vol. 19. University Park Press; Baltimore, MD., 1978.

8. Stryer, L. *Biochemistry*, 3rd ed. W.H. Freeman and Company; New York, 1988.

9. Yeagle, P. *The Membranes of Cells*. Academic Press, Inc., Orlando, FL., 1987.

10. Bergers, J.J., Vingerhoeds, M.H., Vanbloois, L., Herron, J.N., Janssen, L.H.M., Fischer, M.J.E., and Crommelin, D.J.A. The role of protein charge in protein-lipid interactions - pH dependent changes of the electrophoretic mobility of liposomes through adsorption of water-soluble, globular proteins. *Biochemistry* (1993): 32(17): 4641-4649.

11. Dodge, J.T. and Phillips, G.B. Composition of phospholipids and of phospholipid fatty acids and aldehydes in human red cells. *J. Lipid Res.* (1967): 8: 667-675.

12. Jones, A.L., Hann, A.C., Harwood, J.L., and Lloyd, D. Temperature-induced membrane-lipid adaptation in *Acanthamoeba-Castellanii*. *Biochem. J.* (1993): 290(Feb.): 273-278.

13. Pascher, I., Lundmark, M., Nyholm, P.G., and Sundell, S. Crystal Structures of membrane lipids. *Biochim. Biophys. Acta* 1992: 1113: 339-373.

14. Blume, G. and Cevc, G. Molecular mechanism of the lipid vesicle longevity *in vivo*. *Biochim. Biophys. Acta* (1993): 1146(2): 157-168.

15. New, R.R.C., ed. *Liposomes: A Practical Approach*. Oxford University Press, New York, 1990.

16. Plaa, G.L. and Witschi, H. Chemicals, Drugs, and Lipid Peroxidation. *Ann. Rev. Pharm. and Tox.* (1976):16:125-141.

17. Takayama, F., Egashira, T., Kudo, Y., and Yamanaka, Y. Chemiluminescence-HPLC assay of phosphatidylcholine hydroperoxide generated by ischemia-reperfusion in the liver of rats. *Biochem. Pharmacol.* (1992):44(12):2412-2414.

18. Panasenko, O.M., Vol'nova, T.V., Azizova, O.A., and Vladimirov, Y.A. "Free Radical Modification of Lipoproteins and Cholesterol Accumulation in Cells Upon Atherosclerosis." *Free Rad. Biol. & Med.* (1991):10:137-148.

19. Lunt, G.G. and Rowe, C.E. "The Production of Unesterified Fatty Acid in Brain." *Biochim. Biophys. Acta* (1968):152:681-693.

20. Marion, J. and Wolfe, L.S. "Origin of the Arachidonic Acid Released Post-Mortem in Rat Forebrain." *Biochim. Biophys. Acta* (1979):574:25-32.

21. Chan, P.H. and Fishman, R.A. "Alterations of Membrane Integrity and Cellular Constituents by Arachidonic Acid in Neuroblastoma and Glioma Cells." *Brain Research* (1982);248:151-157.

22. Miquel, J., Quintanilha, A.T., and Weber, H. *CRC Handbook of Free Radicals and Antioxidants in Biomedicine*. CRC Press; Boca Raton, FL; 1989.

23. Anderson, W.B. and Jaworski, C.J. "Modulation of Adenylate Cyclase Activity of Fibroblasts by Free Fatty Acids and Phospholipids." *Arch. Biochem Biophys.* (1977):180;374-383.

24 Asakawa, T., Takenoshita, M., Uchida, S., and Tanaka, S "Activation of Guanylate Cyclase in Synaptic Plasma Membranes of Cerebral Cortex by Free Fatty Acids." *J. Neurochem.*(1978):30;161-166.

25. Schaeffer, B.E. and Zadunaisky, J.A. "Stimulation of Chloride Transport by Fatty Acids in Corneal Epithelium and Relation to Changes in Membrane Fluidity." *Biochim. Biophys. Acta*(1979): 556:131-143.