Dietary oxidized oil enhances the activity of (Na^+K^+) ATPase and acetylcholinesterase and lowers the fluidity of rat erythrocyte membrane

Israela Hayam, Uri Cogan, and Shoshana Mokady

Department of Food Engineering and Biotechnology, Technion-Israel Institute of Technology, Haifa, Israel

The effect of dietary oxidized lipids on the fluidity and function of red blood cell (RBC) membranes was studied. Male growing rats were fed diets containing 10% fresh (control) or oxidized (experimental) soybean oil for 8 weeks. Ingestion of the oxidized oil resulted in the accumulation of high levels of fluorescent peroxidation products in the RBC of the experimental animals. Membrane fluidity was studied by fluorescence polarization, using 1,6-diphenyl-1,3,5 hexatriene (DPH) as a probe. The fluidity of RBC membranes isolated from the animals of the experimental group was significantly lower than that of the membranes derived from the control following 3 and 7 weeks of feeding. This lower fluidity was accompanied by a decrease in the content of membrane polyunsaturated fatty acids and an increase in the activity of the membrane-bound enzymes acetylcholinesterase and (Na^+K^+)ATPase. Despite the excessive oxidative state of the oil used, the changes observed in membrane composition, dynamics, and function emphasize the potential risk of dietary oxidized lipids. (J. Nutr. Biochem. **4**:563–568, 1993.)

Keywords: dietary oxidized lipids; erythrocytes; rats; membrane fluidity; enzyme activity

Introduction

The composition and organization of membrane lipids play a major role in defining the activity of membrane-bound functional proteins.[1] The phospholipid bilayer of membranes is composed largely of polyunsaturated fatty acids. Consequently, membrane lipids are highly susceptible to peroxidation processes. Free radical reactions in lipid domains result in damage to membrane proteins, thereby leading to alteration and impairment of membrane dynamics and function.[2] In vitro peroxidation of membranes was shown to result in reduction in the degree of unsaturation of membrane lipids,[3] in a decrease in membrane fluidity,[4] and in modified enzyme activity.[3]

Ingestion of oxidized oil appears to promote in vivo lipid peroxidation. Thus, dietary oxidized lipids were shown to induce peroxidation of liver,[5] kidney, heart,[6] and plasma.[7] Nonetheless, limited information is available in the literature with regard to membrane damage caused by dietary oxidized lipids. The present investigation was undertaken to study the effect of dietary oxidized oil on membrane composition and function. The red blood cell (RBC) membrane was chosen as a model, membrane dynamics were assessed in terms of membrane fluidity, and the activity of the intrinsic enzymes acetylcholinesterase and (Na^+K^+)ATPase was used to evaluate membrane function.

Address reprint requests to Dr. Shoshana Mokady at the Department of Food Engineering and Biotechnology, Technion-Israel Institute of Technology, Haifa 32000, Israel.
Received August 12, 1992; accepted April 29, 1993.

Methods and materials

Preparation of the oxidized oil

Oxidized oil was prepared by bubbling air through fresh soybean oil at 80° C for 5 days in the presence of 2.3 mg/L each of $CuSO_4$ and $FeSO_4$. The extent of peroxidation was determined by assaying the peroxide number[8] and the content of thiobarbituric reactive substances (TBARS)[9] in the oil.

Animals and diets

Male rats of the Charles River CD strain (animal colony, Department of Food Engineering & Biotechnology, Tech-

nion, Haifa, Israel) weighing 60 ± 6 g were used. The rats were divided into two groups (12 animals each) and fed diets containing 18% protein and 10% of either fresh or oxidized oil *(Table 1)* for 8 weeks. The animals were housed in wire cages maintained at 24° C, with a light-dark cycle of 12 hr. Food and water were supplied ad libitum. The diets were stored at −18° C and weekly portions were kept at 4° C. Under these conditions the tocopherol content of both diets was essentially unchanged. The diets were provided daily and the residue of previous ration was discarded. The facilities met the requirements of the Institutional Animal Care and Use Committee.

Following 3 and 7 weeks feeding, blood was collected from the tail vein and erythrocyte membranes were isolated as described before[10] for determination of fluidity and enzyme activities. At the end of the 8-week feeding period the animals were killed by CO_2 asphyxiation, and blood was collected over EDTA by heart puncture and centrifuged at 2000*g* for RBC separation. Erythrocyte membranes were isolated according to Schwoch and Passow[11] and stored at −18° C for fatty acid analysis.

Oil digestibility

This was expressed by the following ratio:

$$\frac{(\text{oil consumed (g)} - \text{lipid extract from feces (g)}) \times 100}{\text{oil consumed(g)}}$$

This balance was carried out over a 72-hr period.

Fluorescence measurement

A suspension of the ghosts (65 mg protein/L) was used to measure erythrocyte membrane fluidity by steady state fluorescence polarization, using 1,6-diphenyl-1-3-5 hexatriene (DPH) as a probe.[12] The polarization of fluorescence was expressed as the fluorescence anisotropy (r) and the anisotropy parameter, $[(r_0/r) - 1]^{-1}$, was calculated with the limiting anisotropy of DPH value of $r_0 = 0.362$. An inverse relationship exists between the membrane lipid fluidity and the anisotropy parameter.[13]

Lipid peroxidation

This was determined in the erythrocytes by measuring the level of polar fluorescent, lipid peroxidation products.[10,14] The method consists of extracting the tissue with a 2:1 chloroform-methanol mixture followed by water addition and phase separation. Previous studies indicated that the concentration of the water-soluble fluorescent peroxidation products was much higher than that of the nonpolar products. Thus, only the level of the polar components was determined. The fluorescence was measured using a self-constructed fluorometer that consists of a 200-watt mercury light source and a high intensity monochromator (Bausch & Lomb, Rochester, NY USA), and a Model R562 Hamamatsu photomultiplier (Middlesex, NJ USA) used as a detector. The excitation wavelength was 365 nm and the emitted light was passed through a cut-off filter No 3-74 (Corning Glass Works, Corning NY USA). 0.2 mg/L of quinine sulfate in 0.001 mol H_2SO_4 served as a standard.

TBARS in plasma were determined according to Buege and Aust.[15] The TBARS method gave barely detectable values when employed for studying peroxidation of RBC and therefore could not be used to that end. On the other hand, the fluorescent peroxidation products that represent complex, cross-linked molecules, accumulating as end products of peroxidation, could be detected with high sensitivity.

Table 1 Composition of the basal diet

	g/kg diet
soy protein*	300
soybean oil†	100
mineral mixture‡	40
vitamin mixture‡	10
choline chloride	2
methionine	3
fiber§	20
corn starch†	525

*60% protein (N × 6.25).
†Commercial product, "Shemen" Israel. Fresh and oxidized oils used for the control and experimental diets, respectively.
‡AIN-76.
§Alpha cellulose fiber, Sigma Chemical Co., St. Louis, MO USA.

Lipid analysis

Lipids were extracted from erythrocyte ghosts by the procedure of Folch et al.,[16] and cholesterol[17] and phospholipids[18] were determined. For fatty acid analysis the lipid extract was subjected to thin layer chromatography on silica gel G plates using petroleum ether:diethyl ether:glacial acetic acid (80:20:1.5) as a solvent mixture. Phospholipids were recovered from the plates for fatty acid analysis. The fatty acid profiles of the membrane lipids and those of the dietary oils were determined after methylation with H_2SO_4 in methanol[19] using a model 5890 Hewlett Packard gas chromatograph (Arondale, PA USA) equipped with a flame ionization detector. The methyl esters were resolved on a wide bore fused silica column, Supelcowax 10 (Supelco, Bellefonte, PA USA). The flow rate of the carrier gas (nitrogen) was 27 mL/min. Oven temperature was maintained at 160° C for 16 min and then raised to 180° C at a rate of 5° C/min. The injector and detector port temperatures were 230° C and 250° C, respectively.

Enzyme activity

Activity of acetylcholinesterase (AChE; EC 3.1.1.7) in the ghosts was assayed by a spectrophotometric method[20] using 15–30 μg protein per reaction mixture. The activity of (Na^+K^+)ATPase (EC 3.6.1.4) was measured by determining the inorganic phosphorus released in the presence and in the absence of ouabain according to Bordoni et al.[21]

Statistical methods

Most data were statistically analyzed using Student's *t* test. The fluorescence measurement data *(Table 2)* were analyzed by one-way analysis of variance followed by Newman-Keuls multiple comparison test.[22] Differences between groups were considered significant at $P < 0.05$.

Results

Bubbling air through the soybean oil sample for 5 days at 80° C resulted in an oxidized oil having a peroxide value of 105 mmol O_2/kg oil and TBARS value of 4×10^{-2} mmol MDA/kg oil compared with very low levels observed for the control, which are typical of fresh,

Table 2 Fluorescence anisotropy parameter of diphenylhexatriene in erythrocyte membrane of rats fed fresh (control) and oxidized oils

Feeding duration	*n*	37° C		25° C	
		Control	Oxidized	Control	Oxidized
beginning of experiment	5	1.20 + 0.02[a]		1.84 + 0.03[a]	
3 weeks	10	1.27 + 0.02[a]	1.39 + 0.03[b]	1.90 + 0.05[a]	2.0 + 0.05[b]
7 weeks	10	1.26 + 0.04[a]	1.40 + 0.03[b]	1.90 + 0.03[a]	2.0 + 0.03[b]

Values are means ± SEM.
For each temperature, values having different superscript are significantly different from the controls at $P < 0.05$ based on Newman-Keuls multiple range test.
The fluorescence anisotropy parameter is defined as $[(r_0/r)-1]^{-1}$, where r is the fluorescence anisotropy and r_0 is the limiting anisotropy, which equals 0.362 for diphenylhexatriene.

Table 3 Analysis of fresh and oxidized oils

	Fresh oil	Oxidized oil
Peroxide value, mmol/kg oil	0.5	105
TBARS*, mmolMDA/kg oil	4.5×10^{-4}	4×10^{-2}
Fatty acid composition†, g/100g, fatty acids		
16:0	11.1 ± 0.06	12.0 ± 0.20
18:0	2.9 ± 0.03	3.5 ± 0.02*
18:1	22.2 ± 0.03	27.2 ± 1.50*
18:2	55.4 ± 0.50	50.0 ± 1.00*
18:3	7.7 ± 0.20	6.0 ± 0.50*

*TBARS, thiobarbituric acid reactive substances.
†The results are given as mean ± SEM.
*Values are significantly different from those of controls at $P < 0.05$ based on Student's *t* test, $n = 6$.

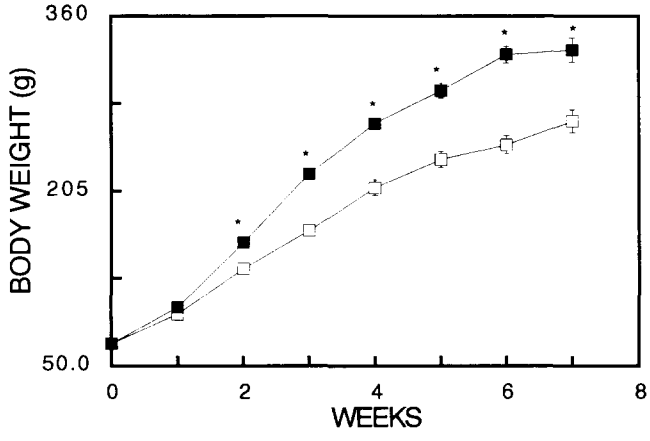

Figure 1 Growth rate of rats fed oxidized oil (□) and control (■). Values are means ± SEM. *Values are significantly different from those of controls at $P < 0.05$ based on Student's *t* test, $n = 12$.

commercial products *(Table 3)*. With regards to fatty acid composition, oxidation resulted in a lower linoleic and linolenic acid concentrations and apparently greater stearic and oleic acid concentrations *(Table 3)*.

Body weight was significantly higher in the control group compared with the experimental group as of the second week *(Figure 1)*. However, there was no difference in food intake (values not shown) and in the digestibility coefficient between the groups consuming the

Table 4 Lipid metabolism indicators in response to the dietary oils

	n	Control	Oxidized
Oil digestibility, %	5	95.6 ± 0.1	96.0 ± 0.3
Relative liver weight, g/100g body wt.	8	3.2 ± 0.2	3.8 ± 0.2*
Liver lipid content, g/100g liver	5	3.2 ± 0.1	2.8 ± 0.1*
Plasma triglyceride mmol/L	8	1.9 ± 0.08	2.0 ± 0.13*

The results are given as means ± SEM.
*Values are significantly different from those of controls at $P < 0.05$ based on Student's *t* test.

oxidized or the control oils *(Table 4)*. Relative liver weight in the experimental group was higher than that of the control rats *(Table 4)*. In addition, compared with the controls, the experimental animals showed alterations in lipid metabolism as expressed by a lower liver lipid content and a higher triglyceride level *(Table 4)*.

Typical Arrhenius plots *(Figure 2)*, as well as values at 25° and 37° C of the fluorescence anisotropy parameter $[(r_0/r)-1]^{-1}$ of DPH *(Table 2)* showed that the membranes isolated from the erythrocytes of the experimental group were less fluid than those derived from the controls following 3 and 7 weeks of feeding.

The temperature dependence of AChE activity is illustrated by Arrhenius plots in *Figure 3*. The activity of the enzyme in the ghost membranes derived from the experimental group was significantly higher compared with that of the respective enzyme of the control animals over the entire temperature range of 24–38° C, except for the value obtained at 29° C. The temperature of the breakpoint in the Arrhenius plots and activation energy below and above this point were found to be similar in both enzyme preparations.

An activity of 0.30 ± 0.06 mmol P/g protein released per hr, observed for (Na^+K^+)ATPase in the erythrocyte membranes derived from the experimental group, was significantly higher $P < 0.05$ than the respective value of 0.15 ± 0.03 mmol P/g protein/hr found for the control rats.

Table 5 demonstrates the effect of the dietary oxidized oil on erythrocyte and plasma lipid peroxidation. Compared with the control, the experimental group exhibited a higher content of water-soluble fluorescent

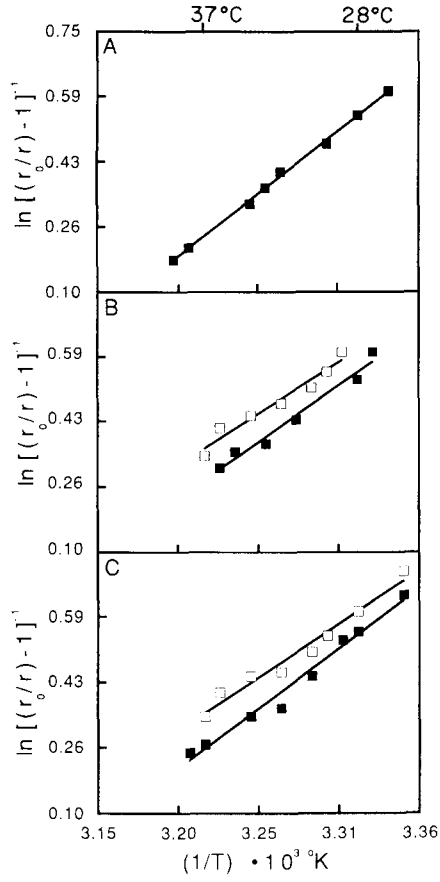

Figure 2 Typical Arrhenius plots of the temperature dependence of the diphenylhexatriene fluorescence anisotropy parameter of erythrocyte membranes derived from experimental (□) and control (■) animals. (A) At the beginning of the experiment; (B) and (C) following 3 and 7 weeks of feeding, respectively. The fluorescence anisotropy parameter is defined as $[(r_0/r) - 1]^{-1}$, where r is the fluorescence anisotropy and r_0 is the limiting anisotropy, which equals 0.362 for diphenylhexatriene.

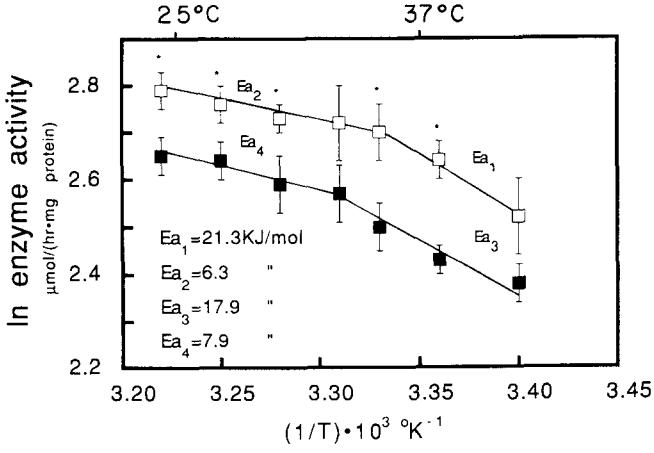

Figure 3 Arrhenius plots of the temperature dependence of acetylcholinesterase activity in erythrocyte membranes derived from experimental (□) or control (■) animals. Ea, energy of activation. Values are means ± SEM. *Values are significantly different from those of controls at $P < 0.05$ based on Student's t test, $n = 5$.

peroxidation products in the RBC. TBARS concentration in plasma was significantly higher in the experimental compared with the control group.

Fatty acid composition of the erythrocyte membranes derived from the animals of both groups is shown in *Table 6*. A reduction in arachidonic and linoleic acids and an apparent elevation in stearic and in palmitoleic acids ($P < 0.05$) was found in the membranes isolated from the RBC of the experimental animals. These alterations were accompanied by an elevation of membrane phospholipids content yielding a lower cholesterol to phospholipid molar ratio *(Table 6)*.

Discussion

Humans are exposed constantly to oxidized dietary lipids. Although the oxidative levels of these lipids are rather low, effects over a life-time might be cumulative. Therefore, the present study, in which dietary oil of a high oxidative level was employed for a relatively short time period, might bear relevance to oxidative processes that take place in humans due to lipid consumption.

Table 5 The effect of oxidized oil on the level of fluorescent polar peroxidation products in erythrocytes, and TBARS levels in plasma

Parameters	*n*	Control	Oxidized
Erythrocytes, fluorescence units/g HB†	10	290.0 ± 10.0	446.0 ± 13.0*
plasma TBARS‡, mol MDA/L	5	2.5 ± 0.5	4.6 ± 0.3*

Values are means ± SEM.
*Values are significantly different from those of controls at $P < 0.05$ based on Student's t test.
Fluorescence of 100 units arbitrarily defined as that observed with 0.1mg/L quinine-sulfate in 0.01 mol/L H_2SO_4.
†Hb, hemoglobin.
‡TBARS, thiobarbituric acid reactive substances.

Table 6 Lipid composition of erythrocyte membranes isolated from rats fed oxidized and control oils

Parameters	Control	Oxidized
Cholesterol, mmol/g protein	0.6 ± 0.1	0.6 ± 0.1
Phospholipid, mmol/g protein	0.5 ± 0.1	0.7 ± 0.1*
Cholesterol/phospholipid molar ratio	1.2 ± 0.04	0.9 ± 0.04*
Fatty acid g/100g fatty acids		
14:0	1.9 ± 0.01	2.9 ± 0.24
16:0	31.7 ± 2.00	35.9 ± 1.60
16:1	0.4 ± 0.04	2.5 ± 0.04*
18:0	19.8 ± 0.30	25.5 ± 1.80*
18:1	8.5 ± 0.90	10.6 ± 0.90
18:2	8.9 ± 0.40	4.9 ± 0.70*
20:4	28.6 ± 1.3	18.3 ± 0.40*

Values are means ± SEM.
*Values are significantly different from those of controls at $P < 0.05$ based on Student's t test.

The incorporation of the oxidized oil into the diet did not affect food intake, yet weight gain in the experimental group was lower than that observed for the control animals. These results are in accordance with those of Andrews et al.,[23] who suggested that reduction in growth rate is directly related to the extent of peroxidation of the dietary oil. The increased levels of TBARS in the plasma and of fluorescent peroxidation products in the erythrocytes are indicative of the oxidative status of these tissues. Such oxidative processes of body tissues and the possible alteration of liver function,[7] as manifested in the reduced liver lipid accumulation and increased plasma triglyceride levels, may explain in part the reduced body weight of the animals caused by the dietary oxidized oil.

RBC are prone to peroxidative damage because they are rich in unsaturated fatty acids and are exposed to high oxygen concentration. Hemoglobin and iron in RBC are powerful catalysts of lipid peroxidation.[24] The changes in the fatty acid profile of the oxidized oil were relatively small compared with the respective alterations found in the erythrocyte membrane. It is therefore conceivable that the marked changes observed in membrane fatty acid composition of the experimental animals were primarily due to peroxidation processes induced in vivo by the ingestion of the oxidized oil. These changes might have been initiated already at the plasma fatty acids level, as evidenced by the high plasma TBARS content.

The experimental group showed a significant decrease in erythrocyte membrane fluidity as measured by DPH fluorescence polarization. The decrease in fluidity is in line with the observation that the content of membrane polyunsaturated phospholipid fatty acyl groups was reduced in the experimental group. Furthermore, malondialdehyde, which is released in the course of lipid peroxidation processes, is a bifunctional compound that can react with free amino groups of proteins and lipids to form fluorescent cross-linked products between various membrane components,[25] thereby leading to membrane rigidification. The observed low cholesterol to phospholipids molar ratio in the erythrocyte membranes of the experimental group, which would act to raise the fluidity, points to a partial compensation process in the direction of maintenance of membrane homeoviscosity. Our results are also in agreement with those of Ohyashiki and Mohri,[26] who found using in vitro studies that peroxidation of the intestinal brush-border membrane lipid caused a decrease in membrane fluidity.

Membrane composition and organization determine the membrane fluidity, which in turn controls various membrane functions, such as the activity of membrane-bound enzymes and transport systems.[1] The activity of the two intrinsic enzymes, acetylcholinesterase and (Na^+K^+)ATPase, in the RBC membrane was higher in the experimental group compared with the control. Similar results were obtained by Bordoni et al.[21] and by Levin et al.,[10] who found that decreased membrane fluidity was associated with high activity of (Na^+K^+) ATPase or AChE, respectively. In some instances it was observed that a decrease in membrane fluidity may be accompanied by an elevation in the activity of membrane-bound enzymes. In that respect, it has been proposed that alteration in membrane fluidity can change the balance between the protein interactions with water and lipid phases and will therefore displace the protein to a new equilibrium position. When lipid fluidity is decreased, the new equilibrium position will be of an overall weaker protein-lipid interaction and greater protein-water association. Thus, the protein may be displaced toward the aqueous phase, and its active site might be more accessible to the substrate,[1] yielding a higher enzyme activity. Furthermore, AChE is known to be composed of two subunits, the interaction of which is essential for activity.[27] Thus, the reduction in fluidity is likely to enhance the protein-protein interaction between the subunits resulting in elevated activity of the enzyme.[1] Characteristic of the erythrocyte AChE is a breakpoint in the Arrhenius activity pattern in the vicinity of 28–30° C,[10,28] which suggests the existence of a local lipid phase transition in the microenvironment of this enzyme. The similarity in the breakpoint temperature and the enzyme energy of activation below and above the transition of the experimental and control membrane preparations suggests that the microenvironment of the enzyme was not affected by the peroxidation processes induced by the dietary oxidized oil. This is in agreement with the finding of Levin et al.,[10] who observed a breakpoint of erythrocyte AChE activity in the vicinity of 30° C for both control and peroxidized membranes derived from riboflavin-deficient animals.

In conclusion, this study demonstrates that feeding oxidized oil results in peroxidation of membrane components and changes in membrane fluidity and activity of membrane-bound enzymes. Despite the excessive oxidation of the oil used, the results emphasize the potential risk of dietary oxidized lipids.

Acknowledgments

The authors wish to thank Abigail Morgenstern for her skillful technical assistance.

References

1 Shinitzky, M. (1984). Membrane fluidity and cellular function. In *Physiology of Membrane Fluidity*, (M. Shinitzky, ed.), Vol. 1, p. 1–51, CRC Press, Boca Raton, FL USA

2 Sevanian, A. and Hochstein, P. (1985). Mechanisms and consequences of lipid peroxidation in biological systems. *Ann. Rev. Nutr.* **5,** 365–390

3 Hogberg, J., Bergstrand, A., and Jakobsson, S.V. (1973). Lipid peroxidation of rat liver microsomes. Its effect on the microsomal membrane and some membrane-bound microsomal enzymes. *Eur. J. Biochem.* **37,** 51–59

4 Ohyashiki, T., Ohtsuka, T., and Mohri, T. (1988). Increase of the molecular rigidity of the protein conformation in the intestinal brush-border membranes by lipid peroxidation. *Biochim. Biophys. Acta* **929,** 383–392

5 Corcos Benedetti, P., D'Aquino, M., Di Felice, M., Gentili, V., Tagliamonte, B., and Tomassi, G. (1987). Effect of a fraction of thermally oxidized soybean oil on growing rats. *Nutr. Rep. Inter.* **36,** 387–401

6 Yoshida, H. and Kajimoto, G. (1989). Effect of dietary vitamin

E on the toxicity of autoxidized oil to rats. *Ann. Nutr. Metab.* **33,** 153–161
7 Corcos Benedetti, P., Di Felice, M., Gentili, V., Tagliamonte, B., and Tomassi, G. (1990). Influence of dietary thermally oxidized soybean oil on the oxidative status of rats of different ages. *Ann. Nutr. Metab.* **34,** 221–231
8 Association of Official Analytical Chemists (1980). *Official Methods of Analysis,* 13th ed, p. 440–441, AOAC, Arlington, VA USA
9 Bermbein, F., Bermbein, M.C., and Wilber, K.M. (1947). The reaction between thiobarbituric acid and the oxidation products of certain lipids. *J. Biol. Chem.* **174,** 257–260
10 Levin, G., Cogan, U., Levy, Y., and Mokady, S. (1990). Riboflavin deficiency and the function and fluidity of rat erythrocyte membranes. *J. Nutr.* **120,** 857–861
11 Schwoch, G. and Passow, H. (1973). Preparations and properties of human erythrocyte ghosts. *Mol. Cell. Biochem.* **2,** 197–218
12 Livshin, L., Mokady, S., and Cogan, U. (1987). Protein malnutrition and the function and fluidity of the intestinal microvillus membrane in growing rats. *J. Nutr.* **197,** 684–688
13 Shinitzky, M. and Barenholz, Y. (1978). Fluidity parameters of lipid regions determined by fluorescence polarization. *Biochim. Biophys. Acta* **515,** 367–394
14 Tappel, A.C. (1973). Lipid peroxidation damage to cell components. *Fed. Proc* **32,** 1870–1875
15 Buege, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods Enzymol.* **52,** 302–310
16 Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509
17 Chiamori, N. and Henry, R.J. (1959). Study of the ferric chloride method for determination of total cholesterol and cholesterol ester. *Am. J. Clin. Pathol.* **31,** 305–309
18 Rouser, G., Siakotos, A.N., and Fleischer, S. (1966). Quantitative analysis of phospholipids by thin layer chromatography and phosphorous analysis of spots. *Lipids* **1,** 85–86
19 Mehlenbacher, V.C. (1960). *The Analysis of Fats and Oils,* p. 571, The Garrard Press, Champaign, IL USA.
20 Whittaker, M. (1984). Cholinesterase. In *Methods of Enzymatic Analysis,* (H.U. Bergmeyer, J. Bergmeyer, and M. Grassl, eds), Vol. 4 p. 52–63, Verlage Chemie, Weinheim, Germany
21 Bordoni, A., Biagi, P.L., Parenti Castelli, G., Hrelia, S., Rossi, C.A., Lercker, G., Izpisua, J.C., Barber, T., Cabo, J., and Lenaz, G. (1989). Effect of a hyperlipidic diet on lipid composition, fluidity and (Na^+K^+) ATPase activity of rat erythrocyte membranes. *Membrane Biochem.* **8,** 11–18
22 Snedecor, G.W. and Cochran, W.G. (1980). *Statistical Methods,* (7th ed), Iowa State University, Ames, IA USA
23 Andrews, J., Wendell, H.G., Meal, J.F., and Stein, R. (1960). Toxicity of air-oxidized soybean oil. *J. Nutr.* **70,** 199–210
24 Chiu, D., Kuypers, F., and Lubin, B. (1989). Lipid peroxidation in human red cells. *Seminars Hemat.* **26,** 257–276
25 Lio, T. and Yoden, K. (1988). Fluorescence formation from hydroperoxide of phosphatidylcholine with amino compound. *Lipids* **23,** 65–67
26 Ohyashiki, J. and Mohri, J. (1986). A change in the lipid fluidity of the porcine intestinal brush-border membranes by lipid peroxidation. *Biochim. Biophys. Acta* **861,** 311–318
27 Beauregard, G., Potier, M., and Roufogalis, B.D. (1980). Modulation of erythrocyte acetylcholinesterase by cardiolipin: effect on subunits coupling, revealed by irradiation inactivation. *Biochem. Biophys. Res. Comm.* **96,** 1290–1295
28 Bloj, D., Morero, R.D., and Farias, R.N. (1974). Effect of essential fatty acid deficiency on the Arrhenius plots of acetylcholinesterase from rat erythrocytes. *J. Nutr.* **104,** 1265–1272