The effect of dietary polyenylphosphatidylcholine on microsomal delta-6-desaturase activity, fatty acid composition, and microviscosity in rat liver under oxidative stress

Pier L. Biagi, Alessandra Bordoni, Silvana Hrelia, Michele Celadon, and Edoardo Turchetto

Department of Biochemistry "G. Moruzzi," University of Bologna, Bologna, Italy

Polyenylphosphatidylcholine is a choline-glycerophospholipid containing up to 80% of total fatty acids as linoleic acid and may be an important factor in ensuring normal functioning of cell membranes. We tested the effect of a polyenylphosphatidylcholine-supplemented diet and compared it with both a trilinolein-supplemented and a laboratory chow diet on the fatty acid composition, microviscosity, and delta-6-desaturase activity of liver microsomal membranes of 12-month-old rats, in the absence or presence of oxidative stress induced by adriamycin. Polyenylphosphatidylcholine- and trilinolein-supplemented diets showed a similar increase in linoleic acid content and delta-6-desaturase activity in liver microsomes, indicating that low amounts of linoleic acid are able to partially restore the enzyme activity in old rats, independent of the source of linoleic acid. After adriamycin treatment, delta-6-desaturase activity increased in polyenylphosphatidylcholine and trilinolein groups, indicating a protective mechanism against the damage induced by polyunsaturated fatty acid peroxidation. The measurement of malondialdehyde production showed a protective effect on adriamycin-induced lipid peroxidation by polyenylphosphatidylcholine supplementation only. Microsomal membrane microviscosity did not change independent of diet and adriamycin treatment, suggesting that the response of microsomes to lipid peroxidation might be the maintenance of a given membrane order. Administration of polyenylphosphatidylcholine can prevent or minimize the liver damage induced by adriamycin treatment. (J. Nutr. Biochem. 4:690–694, 1993.)

Keywords: polyenylphosphatidylcholine; oxidative stress; delta-6-desaturase; fatty acids; microviscosity; malondialdehyde; liver microsomes

Introduction

In the naturally occurring mammalian phosphatidylcholine (PC, lecithin) the unsaturated fatty acid is esterified in the 2 position only, whereas the 1 position is esterified almost exclusively with palmitic acid.[1] Polyenylphosphatidylcholine (PPC) is a choline-glycerophospholipid containing up to 80% of fatty acids as linoleic acid (18:2 n-6, LA), localized in both the 1 and 2 positions of the glycerol backbone. Because it has been demonstrated that a large portion of orally administered PPC is absorbed,[2] and the 1 position does not undergo hydrolysis,[3] PPC is not only a good source of LA, but it also provides 1-acyl-lysophosphatidylcholine (1-lysoPC). About 50% of this 1-lysoPC is reacylated to intact PPC in the intestinal mucosa.[4] The ex novo synthesized PPC enters the liver via the lymph and the blood stream; in the liver it is partly taken up by cell membranes and subcellular membrane fractions.[4] This finding is important insofar as studies by Whyte et al.[1]

This work was supported by grants from Italian Ministery of Scientific Research (M.U.R.S.T.).
Address reprint requests to Prof. PierLuigi Biagi at the Dipartimento di Biochimica "G. Moruzzi," via Irnerio, 48, 40126 Bologna, Italy.
Received December 31, 1992; accepted June 8, 1993.

indicate that unsaturated fatty acids given orally are not effective in changing the fatty acid moieties of endogenous PC so as to produce molecules containing polyunsaturated fatty acids in the 1 and 2 positions. PPC could have many biochemical properties; as the structural and functional integrity of cell membranes depends on the presence of polyenyl phospholipids, PPC may be an important factor in ensuring normal functioning of cell membranes.[5]

The role of lipids as structural and functional components in biological membranes is well known. In old animals, changes of lipid composition and examples of age-dependent modifications of plasma membrane enzymes are documented,[6,7] and it has been demonstrated that essential fatty acid (EFA) metabolism is altered in old animals. In previous studies we demonstrated the fall-off in the activity of the delta-6-desaturase (D6D) enzyme, which converts LA to γ-linolenic acid (18:3n-6) and α-linolenic acid (18:3n-3, ALA) to stearidonic acid (18:4n-3) in aged rats.[8,9]

Appropriate dietary supplementation can modify membrane fatty acid composition and can influence D6D activity.[10] Because the importance of PPC is not only as a source of LA, but also its ability to be incorporated into both normal and damaged membranes, we tested the effects on liver microsomal membrane fatty acid composition, microviscosity, and on D6D activity in old rats of a PPC-supplemented diet and compared it with both a trilinolein-supplemented diet and a laboratory chow diet.

Furthermore, as polyunsaturated acyl chains of membrane phospholipids are especially susceptible to free radical-initiated oxidation, which can be generated either by metabolism of xenobiotics or by normal aerobic cellular metabolism,[11,12] we evaluated the effect of an endogenous oxidative stress induced by adriamycin, an anthracycline antibiotic that enhances lipid peroxidation, on fatty acid composition, microviscosity, and D6D activity of liver microsomal membranes in the three groups of animals.

Methods and materials

Materials

[1-^{14}C] Linoleic acid (59 mCi/mmol) was purchased from the Radiochemical Center (Amersham, UK); NADH, Coenzyme A (CoA), ATP, and trilinolein were purchased from Sigma Chemical Co. (St. Louis, MO USA). Adriamycin was from Farmitalia (Milan, Italy). All unlabeled fatty acids were obtained from Nu. Check (Elysian, MN USA); all chemicals and solvents were of the highest analytical grade. PPC was a kind gift from Rhone-Poulenc Rorer GMBH (Köln, Germany).

Methods

Normal male Wistar rats, aged 11 months, were used. Rat body weight was 370 ± 9 g. Rats were divided at random into three groups, each fed ad libitum a different diet for 1 month:

Laboratory chow diet (control diet, n = 12). The laboratory chow diet was a balanced standard diet for rats (Dottori and Piccioni, Brescia, Italy); the diet was normoproteic (proteins: 21 g/100 g), normolipidic (lipids: 4.8 g/100 g), and normoglucidic (carbohydrates: 61.5 g/100 g) and contained appropriate amounts of lipid soluble vitamins.

PPC diet (n = 16). The PPC diet was prepared by adding 275 mg PPC/100 g standard diet to the laboratory chow diet derived from the same batch.

Trilinolein diet (n = 16). The trilinolein diet was prepared by adding 200 mg trilinolein/100 g standard diet, corresponding to an equimolar content of LA with respect to the PPC diet, to the laboratory chow diet derived from the same batch.

Rat body weight and the amount of food ingested were carefully recorded daily to determine the amount of PPC and trilinolein ingested (in mg). At the beginning of the study rats ingested about 110 mg PPC or about 80 mg trilinolein daily; at the end of the dietary treatment, rats ingested about 135 mg PPC or about 100 mg trilinolein daily.

The standard diet provided 3.9 Kcal/g; the amount of calories provided by PPC or trilinolein supplementation was negligible.

Vitamin E was added to each diet (200 mg/kg) to avoid unsaturated lipid peroxidation. Diets were stored at 4° C and rats were fed fresh food daily.

The animals were housed in individual cages in strictly controlled conditions of temperature (20 ± 2° C) and humidity (60–70%), with a 12-hour light-dark cycle. Water was provided ad libitum, and water soluble vitamins and minerals were provided dissolved in water.[10]

After 30 days of dietary treatment, no differences were detected in rat body weight among the three groups (control group 452 ± 13 g, PPC group 446 ± 16 g, trilinolein group 453 ± 20 g), indicating that animals on PPC and trilinolein diets did grow as well as the controls.

Forty-eight hours before sacrifice, six rats from the control group, eight rats from the PPC group, and eight rats from the trilinolein group received 100 mg/kg bw adriamycin intraperitoneally.

Rats were sacrificed by decapitation at the same time early in the morning. Livers were quickly excised, washed, and minced with scissors.

Microsomes were isolated as previously described.[13] All microsomal preparations were done at 0–4° C and frozen at −80° C until use. Microsomal purity was assessed, and the absence of mitochondrial membranes verified.[8] Microsomal protein was measured according to Lowry et al.[14]

The delta-6-desaturase (acyl-CoA, hydrogendonor : oxidoreductase, E.C. 1.14.99.5) (D6D) enzyme assay was carried out as previously described.[8,10] The reaction mixtures, containing 5 mM $MgCl_2$, 50 μM CoA, 2 mM ATP, 1 mM NADH, 50 mM phosphate buffer pH 7.4, 40 μM [1-^{14}C] linoleate, and approximately 2.5 mg microsomal protein, in a total volume of 1 mL, were incubated in a shaking water-bath at 37° C for 20 min. The reactions were stopped by adding 4 mL of chloroform/methanol (1:1 vol/vol) and, after the addition of 2 mL of chloroform, lipids extracted according to Folch et al.,[15] and methyl esterified with methanol / hydrochloric acid (5% by vol) as reported by Stoffel et al.[16] Fatty acid methyl esters were separated on thin layer chromatography plates, coated with silica gel G, impregnated with 10% (wt/vol) $AgNO_3$, as previously reported.[8,10,13] Enzyme activity is expressed as pmol of the radioactive fatty acid converted into the product per min per mg microsomal protein.

The fatty acid composition of microsomal total lipids was

Table 1 Fatty acid composition (mol/100 mol) of liver microsomal membranes of rats fed the experimental diets

Fatty acid	Control (n = 6)	PPC (n = 8)	Trilinolein (n = 8)
16:0	22.36 ± 0.40	22.32 ± 2.00	23.87 ± 1.39
16:1	0.95 ± 0.02	0.69 ± 0.35	1.67 ± 0.50*
17:0	0.73 ± 0.13	0.79 ± 0.11	0.92 ± 0.19
18:0	21.10 ± 0.12	20.10 ± 0.66	18.76 ± 1.05
18:1	7.81 ± 0.61	7.53 ± 1.36	8.99 ± 0.91
18:2(n-6)	13.98 ± 0.56	18.25 ± 1.53*	15.83 ± 0.72
18:3(n-6)	0.16 ± 0.02	0.12 ± 0.02*	0.17 ± 0.04
18:3(n-3)	0.17 ± 0.05	0.40 ± 0.07	0.26 ± 0.02
20:3(n-9)	0.32 ± 0.03	0.52 ± 0.09*	0.37 ± 0.10
20:3(n-6)	0.52 ± 0.12	0.35 ± 0.18	0.34 ± 0.12
20:3(n-3)	1.33 ± 0.08	0.76 ± 0.12	0.80 ± 0.22
20:4(n-6)	22.21 ± 0.76	19.92 ± 2.99	20.93 ± 1.94
20:5(n-3)	0.75 ± 0.03	0.59 ± 0.14*	0.68 ± 0.12
22:4(n-6)	0.41 ± 0.02	0.40 ± 0.12	0.94 ± 0.47*
22:5(n-6)	0.41 ± 0.06	0.42 ± 0.18	0.27 ± 0.15
22:5(n-3)	1.56 ± 0.16	1.13 ± 0.07	0.95 ± 0.30
22:6(n-3)	5.17 ± 0.04	5.48 ± 0.66	4.04 ± 0.79*
n-6:n-3	4.20 ± 0.10	4.79 ± 0.66*	5.83 ± 0.94

The fatty acid analysis was performed as reported in Methods and materials. The number of animals examined in each group is given in brackets. Data are means ± SD. Statistical analysis was performed using the one-way analysis of variance, comparing PPC supplemented and trilinolein-supplemented diet to control diet
*P < 0.05
**P < 0.01.
P < 0.001

determined by gas chromatography (Carlo Erba mod. 4160) using a capillary column (SP 2340, 0.10-0.15 μm i.d.) at a programmed temperature (160–210° C, with an 8° C/min gradient), as previously described.[10]

Microsomal membrane fluidity was estimated by means of the hydrophobic probe 1,6-diphenylhexa-2,3,5-triene (DPH, Aldrich Chemical Co., Milwaukee, WI USA).[10]

Peroxidation of microsomal phospholipid polyunsaturated fatty acids was determined measuring malondialdehyde (MDA) production by the thiobarbituric acid method.[11]

All results are expressed as means ± SD. Statistical differences were assessed by analysis of variance.

Results

Liver microsomal membrane fatty acid composition of rats fed the three different diets is reported in *Table 1*.

Both PPC and trilinolein supplementation determined significant modifications in microsome fatty acid composition; particularly, an increase in LA relative molar content was clearly detectable. Furthermore, there was an increase in α-linolenic acid content, concomitant with a decrease in the polyunsaturated n-3 fatty acid content. As a consequence, the n-6:n-3 ratio increased in both PPC and trilinolein group with respect to controls.

Figure 1 shows the modifications in liver microsomal membrane fatty acid composition of rats treated with adriamycin, compared with animals fed the same diet and not treated with adriamycin. Adriamycin treatment determined a decrease in palmitic and an increase in stearic acid relative molar content in all the dietary groups; furthermore, in the control and trilinolein groups an increase in LA relative molar content was detected. Adriamycin treatment did not affect the n-6/n-3 fatty acid ratio (data not shown), apart from an increase in the trilinolein group (untreated: 5.83 ± 0.94, treated: 7.64 ± 0.61, $P < 0.05$).

In *Table 2* the D6D activity of liver microsomes from rats fed the three different experimental diets, treated or not treated with adriamycin, is reported. Both the PPC and the trilinolein dietary supplementation showed an increase in the D6D activity. An even higher increase

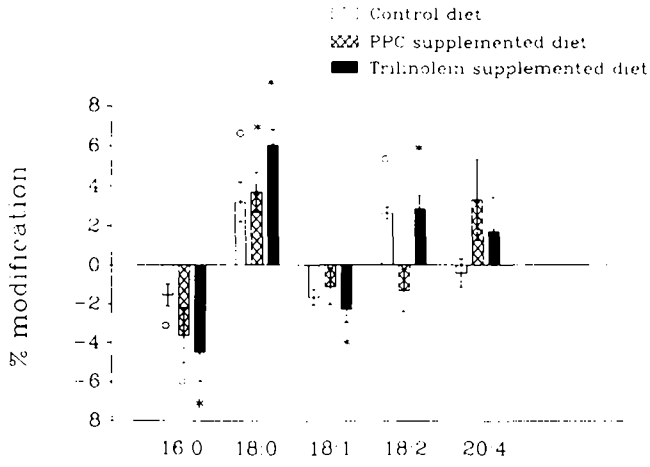

Figure 1 Differences in the relative molar content (%) of the main fatty acids of liver microsome total lipids between rats untreated and treated with adriamycin. The differences in the relative molar content of the main fatty acids were calculated by subtracting the adriamycin-untreated level from the adriamycin-treated level for each fatty acid for each dietary treatment. Data are means ± SD. Statistical analysis was by one-way analysis of variance, comparing untreated and treated animals fed the same diet *P < 0.05; **P < 0.01.

Table 2 Delta-6-desaturase activity (pmol × min^{-1} × mg protein^{-1}) in liver microsomes of rats fed the three different diets, treated or not with adriamycin

Diet	Adriamycin injection: Without	Adriamycin injection: With
Control	30.1 ± 9.2 (n = 6)	52.0 ± 12.6 (n = 6)
PPC	49.1 ± 9.6 (n = 8)	124.0 ± 4.3 (n = 8)
Trilinolein	59.8 ± 12.8 (n = 8)	130.1 ± 3.5 (n = 8)

The delta-6-desaturase assay was performed as reported in Methods and materials. The number of animals examined is given in brackets. Data are means ± SD. Statistical analysis: the two-way analysis of variance revealed a significant effect of the dietary treatment ($P < 0.001$), a significant effect of the adriamycin treatment ($P < 0.001$), and a significant interaction between the dietary and the adriamycin treatments ($p < 0.05$). The one-way analysis of variance was then performed comparing the effect of PPC- and trilinolein-supplemented diet versus control diet in both the animals not treated with adriamycin (controls versus PPC-fed: $P < 0.01$, controls versus trilinolein-fed: $P < 0.001$) and treated with adriamycin (controls versus PPC-fed: $P < 0.001$; controls versus trilinolein fed: $P < 0.001$), and the effect of adriamycin injection in the animals fed the same diet (controls: $P < 0.01$; PPC-fed $P < 0.001$; trilinolein fed: $P < 0.001$)

in enzyme activity was determined by the adriamycin treatment in all dietary groups.

Microsomal membrane microviscosity was not influenced by either the dietary supplementation or the adriamycin treatment (the mean value of fluorescence polarization was 0.180 ± 0.009 in all conditions).

MDA production in liver microsomes was not influenced by dietary treatment *(Table 3)*, while adriamycin treatment induced a significant increase in MDA microsomal content in control and trilinolein-fed rats, without affecting the MDA production in the PPC group.

Discussion

In this study we evaluated the effect of two different diets providing the same amount of LA. This fatty acid was available in two different forms: esterified in the 1 and 2 position of PPC, or as trilinolein. It is important to note that PPC supplementation can determine the incorporation into cellular and subcellular membranes of a phospholipid presenting polyunsaturated fatty acids esterified in both the 1- and 2-positions. Our aim was to determine whether the importance of a PPC dietary supplementation resides only in being a source of LA, or as a source of PPC itself.

As expected, both PPC- and trilinolein-supplemented diets showed an increase in LA relative molar content in liver microsomes. This increase was higher in the PPC group, probably because LA was present in both the 1 and 2 positions of microsomal membrane phospholipids. In both groups, this increase in LA content was not associated with an increase in arachidonic acid (AA) content, although a significant increase in the D6D activity was detected in comparison with the control diet *(Table 2)*. Witters et al.[18] reported a suppressing effect on D6D activity with linoleic acid dietary supplementation. There could be several reasons for the discrepancy between our data and data previously reported: (1) they supplemented LA with a fat-free diet, while we used a normolipidic, balanced diet; (2) our LA supplementation was low, representing less than the 7% of the daily lipid intake; and (3) we examined old rats, in which D6D activity is reduced. Low amounts of LA supplementation of a balanced diet are probably able to partially restore D6D activity on n-6 fatty acids in old rats, independent of the form in which LA is available in the diet. On the other hand, although we have not directly measured the D6D activity on α-linolenic acid (ALA), the decrement in the relative content of polyunsaturated n-3 fatty acids, observed in liver microsomes of PPC- and trilinolein-fed rats *(Table 1)* indicates a decreased D6D activity on ALA. Therefore, the increased activity of the enzyme observed on LA could be determined by a shift of the enzyme activity on ALA as a substrate to LA as a substrate, and not by a "net" increase of the enzyme activity itself.

Table 3 Malondialdheyde production (nmol/mg protein) in liver microsomes of rats fed the three different diets, treated or not with adriamycin

	Adriamycin injection	
	Without	With
Control	4.45 ± 0.14 (n = 6)	5.46 ± 0.15 (n = 6)
PPC	4.82 ± 0.18 (n = 8)	5.16 ± 0.21 (n = 8)
Trilinolein	4.91 ± 0.13 (n = 8)	5.94 ± 0.13 (n = 8)

The determination of the malondialdheyde production was performed as reported in Methods and materials. The number of animals examined is given in brackets. Data are means ± SD. Statistical analysis: the two-way analysis of variance revealed a significant effect of the dietary treatment ($P < 0.001$), a significant effect of the adriamycin treatment ($P < 0.001$), and a significant interaction between the dietary and the adriamycin treatments ($P < 0.001$). The one-way analysis of variance was then performed comparing the effect of PPC and trilinolein supplemented diet versus control diet in both the animals not treated with adriamycin (controls versus PPC-fed: NS; controls versus trilinolein fed: NS) and treated with adriamycin (controls versus PPC-fed: $P < 0.01$; controls versus trilinolein-fed: $P < 0.001$), and the effect of adriamycin injection in the animals fed the same diet (controls: $P < 0.001$; PPC-fed: NS; trilinolein fed: $P < 0.001$).

Many agents known to be toxic to cells are associated with the generation of free radicals.[19] In turn, the radicals may lead to the peroxidation of polyunsaturated fatty acids in cell membranes with the formation of a wide range of toxic metabolites. The main problem in free radical damage caused by oxygen or exogenous peroxidative stress could be the loss of membrane polyunsaturated fatty acids rather than the accumulation of toxic materials, as postulated by Horrobin.[20] According to this hypothesis, some of the damage induced by free radicals may indeed be related not only to the presence of free radicals themselves and of lipid peroxides and their metabolites, but also to the absence of the damaged polyunsaturated fatty acids, which is most harmful and produces much of the pathology.[21] In this study, adriamycin treatment resulted in an increase of D6D activity on LA in all dietary groups. The peroxidation of polyunsaturated fatty acids induced by adriamycin and the consequent decreased polyunsaturated fatty acid membrane concentration may lead to an enhanced D6D activity to restore the loss due to free radical damage. This effect is much more evident in the PPC- and trilinolein-fed rats than in controls, probably as a consequence of the higher availability of the D6D substrate, LA.

In the absence of exogenous oxidative stress, the administration of PPC- and trilinolein-supplemented diets did not result in any modification of MDA production in liver microsomes with respect to the control diet.

The measurement of MDA after adriamycin treatment revealed a significant increase in MDA microsomal content in the control and trilinolein groups, while MDA content did not change in the PPC group, with respect to animals fed the same diet but not treated with adriamycin. These data indicate that PPC has a protective effect on adriamycin-induced lipid peroxidation. Previous studies indicate that PPC administration prevents liver membrane damage due to the formation of radicals and hydroperoxides consequent to carbon tetrachloride[22] or tetracycline poisoning[23] and to ionizing radiation.[24] Although it has not been clearly established how PPC exerts its protective effect, it is believed that

it is based on PPC's ability to be incorporated into normal and damaged liver membranes.

Microsomal membrane microviscosity did not change irrespective of diet and adriamycin treatment. Although lipid peroxidation initiated by adriamycin in vitro in rat liver microsomes is accompanied by an increase of the apparent microviscosity,[25] the presence of an inverse correlation between MDA production and cholesterol concentration in microsomes has been demonstrated.[26–28] Then, the response of microsomes in vivo after an increase of lipid peroxidation might be the maintenance of a given membrane order by mobilizing part of its cholesterol content.

In conclusion, our data indicate that the prophylactic administration of PPC can prevent or minimize the liver damage induced by adriamycin treatment. Administration of PPC may represent an example of a drug treatment based on the use of a naturally occurring compound, with the aim of enhancing the effectiveness of physiological processes.

On the basis of the evidence at hand, PPC appears worthy of consideration for further experimentation, either by itself, or in association with other dietary regimens.

Acknowledgments

The authors thank Rhone-Poulenc Rorer GMBH (Köln, Germany) for the kind gift of polyenylphosphatidylcholine (PPC) and Miss M. Zini for her technical assistance.

References

1 Whyte, M., Goodman, D.S., and Karmen, A. (1965). Fatty acid esterification and chylomicron formation during fat absorption in rat: III. Positional relation in triglycerides and lecithin. *J. Lipid Res.* **6,** 233–240

2 Blomstrand, R. (1955). The intestinal absorption of phospholipids in the rat. *Acta Physiol. Scand.* **34,** 147–157

3 Fox, J.M., Betzing, H., and Lekim, D. (1979). Pharmacokinetics of orally ingested phosphatidylcholine. In *Nutrition and the Brain* Vol. 5, (A. Barbeau, J.H. Growdon, and R.J. Wurtman, eds.) p. 95–108, Raven Press, New York, NY USA

4 LeKim, D. and Graf, E. (1976). Tierexperimentelle studien zur pharmakokinetik der "essentiellen" phospholipide (EPL). *Drug Res.* **26,** 1772–1782

5 Samochowiec, L. and Wojcioki, J. (1970). Effects of essential phospholipids (EPL) on the lipid content of the serum and liver in white rats after a single dose of ethyl alcohol. *J. Med.* **1,** 299–303

6 Rubin, M.S., Swislocki, N.J., and Sonenberg, M. (1973). Changes in rat liver plasma membrane phospholipids during aging. *Proc. Soc. Exp. Biol. Med.* **143,** 1008–1013

7 Hegner, D. and Platt, D. (1975). Effect of essential phospholipids on the properties of ATPases of isolated rat liver plasma membranes of young and old animals. *Mech. Ageing Dev.* **4,** 191–200

8 Hrelia, S., Bordoni, A., Celadon, M., Turchetto, E., Biagi, P.L., and Rossi, C.A. (1989). Age-related changes in linoleate and α-linolenate desaturation by rat liver microsomes. *Biochem. Biophys. Res. Commun.* **163,** 348–355

9 Hrelia, S., Celadon, M., Rossi, C.A., Biagi, P.L., and Bordoni, A. (1990). Delta-6-desaturation of linoleic and α-linolenic acids in aged rats: a kinetics analysis. *Biochem. Int.* **22,** 659–667

10 Biagi, P.L., Bordoni, A., Hrelia, S., Celadon, M., and Horrobin, D.F. (1991). Gamma-linolenic acid dietary supplementation can reverse the aging influence on rat liver microsome delta-6-desaturase activity. *Biochim. Biophys. Acta* **1083,** 187–192

11 Freeman, B.A. and Crapo, J.D. (1982). Biology of disease. Free radicals and tissue injury. *Lab. Invest.* **47,** 412–426

12 Slater, T.F. (1984). Free radical mechanisms in tissue injury. *Biochem. J.* **222,** 1–15

13 Bordoni, A., Biagi, P.L., Turchetto, E., and Hrelia, S. (1988). Aging influence on delta-6-desaturase activity and fatty acid composition of rat liver microsomes. *Biochem. Int.* **17,** 1001–1009

14 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

15 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.* **256,** 497–509

16 Stoffel, W., Chu, F., and Ahrens, E.H., Jr. (1959). Analysis of long chain fatty acids by gas liquid chromatography. *Anal. Chem.* **31,** 307–308

17 Buege, J.A. and Aust, S.D. (1978). Microsomal lipid peroxidation. In *Methods in Enzymology* vol. 52, (S. Fleischer, and L. Packer, eds.) p. 302–310, Academic Press, New York, NY USA

18 Witters, L.A., Kowaloff, E.M., and Diruch, J. (1979). Glucagon regulation of protein phosphorylation. *J. Biol. Chem.* **254,** 245–248

19 Halliwell, B. and Gutteridge, J.M.C. (1985). *Free Radicals in Biology and Medicine,* Oxford University Press, Oxford, UK

20 Horrobin, D.F. (1991). Is the main problem in free radical damage caused by radiation, oxygen and other toxins the loss of membrane essential fatty acids rather than the accumulation of toxic materials? *Med. Hypoth.* **35,** 23–26

21 Horrobin D.F. and Begin, M.E. (1989). Essential fatty acids: metabolism, interaction with free radicals and clinic use. In *Handbook of Free Radicals and Antioxidants in Biomedicine* Vol. II, (J. Miguel, A.T. Quintanilha, and H. Weber, eds.) p. 17–26, CRC Press, Boca Raton, FL USA

22 Bartsch, G.G. and Gerber, G.B. (1975). Changes in lipid content and synthesis after liver damage with carbontetrachloride and other agents. *Acta Hepato-Gastrenterol.* **22,** 228–236

23 Skakun, N.P. and Stepanova, N.Y. (1986). Efficacy of legalon and essential in tetracielyne affections of the liver. *Antibiot. Med. Biotekhnol.* **31,** 781–784

24 Porokhynyak, L.A., Drogovoz, S.M., and Rogozhin, B.A. (1987). Effect of hepatoprotective agents in tetracycline affection of the liver. *Antibiot. Med. Biotekhnol.* **32,** 282–285

25 Demant, E.J.F. and Jensen, P.K. (1984). Destruction of phospholipids and respiratory-chain activity in pig heart submitochondrial particles induced by an adriamycin-iron complex. *Eur. J. Biochem.* **132,** 551–556

26 Mataix, F.J., Huertas, J.R., Battino, M., Barzanti, V., Maranesi, M., Parenti-Castelli, G., Littarru, G.P., Turchetto, E., and Lenaz, G. (1992). Mitochondrial and microsomal cholesterol mobilization after oxidative stress induced by adriamycin in rats fed with dietary olive and corn oil. *Life Sci* **50,** 2111–2118

27 Chen, X., Xue, A., Morris, C., Ferrans, V.J., Herman, E.H., El-Hage, A., and Levander, O.A. (1986). Effect of selenium deficiency on the chronic toxicity of adriamycin in rats. *J. Nutr.* **116,** 2453–2465

28 Nagaoka, S., Masaki, H., Aoyama Y., and Yoshida, A. (1986). Effect of excess dietary tyrosine or certain xenobiotics on the cholesterogenesis in rats. *J. Nutr.* **116,** 726–732