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Detection of Lipid Radicals by Electron Paramagnetic Resonance Spin Trapping Using Intact Cells Enriched with Polyunsaturated Fatty Acid*

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Electron paramagnetic resonance (EPR) spin trapping was used to detect lipid-derived free radicals generated by iron-induced oxidative stress in intact cells. Using the spin trap α-(4-pyridyl 1-oxide)-*N-tert*-butylnitrone (POBN), carbon-centered radical adducts were detected. These lipid-derived free radicals were formed during incubation of ferrous iron with U937 cells that were enriched with docosahexaenoic acid (22:6*n*-3). The EPR spectra exhibited apparent hyperfine splittings characteristic of a POBN/alkyl radical, $a^N = 15.63 \pm 0.06$ G and $a^H = 2.66 \pm 0.03$ G, generated as a result of β-scission of alkoxyl radicals. Spin adduct formation depended on the $FeSO_4$ content of the incubation medium and the number of 22:6-enriched cells present; when the cells were enriched with oleic acid (18:1*n*-9), spin adducts were not detected. This is the first direct demonstration, using EPR, of a lipid-derived radical formed in intact cells in response to oxidant stress.

The iron-mediated peroxidation of lipids is believed to be a key factor causing cell injury. Lipid peroxidation has three components: an initiation step, propagation of the radical chain reactions, and termination (1-3). Generally, the initiation of lipid peroxidation requires iron or other catalytic metals (4). The free radicals generated, such as L$^{\cdot}$,[1] LOO$^{\cdot}$, and LO$^{\cdot}$, propagate the chain reactions in the lipid peroxidation process (1). Termination occurs due to breakdown of the radicals into non-radical short chain hydrocarbons. Polyunsaturated fatty acyl groups located in cell membranes are the principal targets for this peroxidation.

The double bond systems of polyunsaturated fatty acids (PUFA) contain methylene carbon bridges having hydrogens that are susceptible to abstraction by the initiation and chain propagation radical reactions. Through bond rearrangements, lipid-derived radicals can break down forming a variety of products (1, 2). Alkoxyl radicals formed during the peroxidation process undergo β-scission to produce alkyl radicals, which can be detected through electron paramagnetic resonance (EPR) spin trapping (5, 6). A PUFA such as docosahexaenoic acid (DHA, 22:6n-3), which contains six double bonds with five methylene bridges, is especially susceptible to peroxidation.

Although many aspects of the competing mechanisms involved in lipid peroxidation are known from investigations with chemical systems, liposomes, and subcellular fractions, few studies have examined the generation of lipid radicals in the intact cell. Using a human monocyte cell line enriched with DHA, we report here the successful use of EPR spin trapping to detect lipid-derived, carbon-centered free radicals generated in intact cells.

EXPERIMENTAL PROCEDURES

Materials—5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO), α-(4-pyridyl 1-oxide)-*N-tert*-butylnitrone (POBN), and chelating resin (C-7901) were purchased from Sigma. DHA and oleic acid (18:1*n*-9) were obtained from Nu Chek Prep (Elysian, MN). DMPO was purified with charcoal and stored frozen as a 1.0 M aqueous solution. A 0.25 M POBN aqueous stock solution was prepared immediately before use.

Cell Culture—The human U937 monocytic leukemia cell line was cultured at 37 °C under a humidified atmosphere of 95% air:5% CO_2 in RPMI 1640 medium containing 5% fetal bovine serum, 15 mM HEPES, and 2 mM L-glutamine. The cells were maintained as an exponentially growing culture by passage every other day. The cell lipids were modified by the addition of 10 μM DHA or oleic acid to the growth medium. After 2 days, the cells were washed twice with a phosphate-buffered salt solution containing 2.68 mM KCl, 1.47 mM KH_2PO_4, 136.8 mM NaCl, 8.06 mM Na_2HPO_4, pH 7.4 (PBS) and then resuspended in PBS at a concentration of about 8×10^6 cells/ml. PBS was treated with chelating resin to remove metals from solution using the batch method (7); absence of catalytic metals was verified with ascorbate (8).

Fatty acid composition was determined by gas-liquid chromatography (9). Briefly, washed cells were extracted with $CHCl_3$:CH_3OH (2:1, v/v) (10) and the lipids saponified for 60 min at 56 °C in 33% KOH:95% ethanol (1.2:20, v/v) (11). The fatty acids were then methylated for 30 min at 95 °C with 14% BF_3 in CH_3OH (12) and separated by gas-liquid chromatography using a 1.8-m column packed with 10% SP2330 on 100/120 mesh Gaschrome WAW. The resultant peaks were identified by comparing the retention times with those of known fatty acid methyl ester standards. Quantification was accomplished by integration of the peak areas. Statistical analysis was done using the *t* test.

Reaction Conditions—Incubation mixtures consisted of cells resuspended to 8×10^6 cells/ml, unless otherwise noted, in 0.5 ml of PBS in new disposable glass culture tubes. Just prior to placement in the EPR spectrometer, 10 mM (final concentration) POBN was added to the cell suspension. Ferrous iron from a stock solution of 5 mM $FeSO_4 \cdot 7H_2O$ in 10 ml of H_2O, containing 4 drops/10 ml of concentrated H_2SO_4 to maintain the iron as Fe^{2+}, was then added. The contents of the tube were gently mixed by vortexing and then transferred to an EPR quartz flat cell.

EPR Measurement—EPR spectra were obtained using a Bruker ESP 300 spectrometer operating at 9.79 GHz with 100-kHz modulation frequency. Each sample contained in a quartz flat cell was centered in a TM_{110} cavity. All EPR measurements were made using air-saturated solutions at room temperature. The EPR spectrometer settings were: microwave power, 40 milliwatts; modulation amplitude,

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[1] The abbreviations used are: L$^{\cdot}$, lipid carbon-centered radical; LOO$^{\cdot}$, lipid peroxyl radical; LO$^{\cdot}$, lipid alkoxyl radical; HO$^{\cdot}$, hydroxyl radical; PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; POBN, α-(4-pyridyl 1-oxide)-*N-tert*-butylnitrone; PBS, phosphate-buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

1.05 G; time constant, 1.3 s; scan rate, 60 G/335 s; receiver gain, 1×10^6.

RESULTS

Spin Trapping of Lipid-derived Radicals—When intact U937 cells enriched with DHA were incubated with 80 μM $FeSO_4$ in the presence of the spin trap POBN, a carbon-centered spin adduct was observed (Fig. 1*D*, $a^N = 15.63 \pm 0.06$ G and $a^H = 2.66 \pm 0.03$ G). This adduct was not detected when unmodified cells or cells enriched with oleic acid were incubated with POBN and ferrous iron (Fig. 1, *C* and *B*). No spin adduct was detected in incubations of DHA-enriched cells in the absence of ferrous iron (Fig. 1*A*). Based on work using cell-free incubations with purified PUFA and lipoxygenases (5, 6), we have assigned the spectra we observed to an alkyl, such as ethyl, pentyl, or pentenyl, adduct of POBN.

Iron alone produced an EPR signal at the noise level under our experimental conditions, but this signal appears to have different hyperfine splitting constants ($a^N \approx 15.1$ G, $a^H \approx 1.8$ G) than the spin adduct observed in the presence of DHA-enriched cells (Fig. 2*A*). When iron was added to 1×10^6 DHA-enriched cells, a very weak spectrum was obtained (Fig. 2*B*). As the number of DHA-enriched cells was increased further, a corresponding increase in the spin adduct EPR signal intensity was observed (Fig. 2, *C–E*). Although the strongest POBN/alkyl radical signal was obtained with 8×10^6 DHA-enriched cells, it was difficult technically to obtain such large numbers of cells for repeated experiments. Therefore, 4×10^6 DHA-enriched cells were used for the remainder of the experiments. This provided adequate sensitivity to clearly detect the radical adduct under a variety of conditions.

At a constant cell density and POBN concentration, the POBN/spin adduct signal intensity increased as the $FeSO_4$ concentration was raised (Fig. 3). Apparent saturation of the process was observed when the $FeSO_4$ concentration exceeded 140 μM. In a second experiment, a similar iron-dependent increase was observed, but the saturation effect began at 120 μM $FeSO_4$ (data not shown).

Production of Oxygen Radicals in the System—In order to demonstrate that $FeSO_4$ generates strong oxidants in this system that can initiate lipid peroxidation, we introduced 160 μM $FeSO_4$ to a 50 mM DMPO solution. DMPO is a spin trap that forms relatively stable adducts with oxygen-centered radicals. When $FeSO_4$ was added, a spectrum was obtained consistent with the spin trapping of $HO^\cdot$ by DMPO ($a^N = 14.90$ and $a^H = 14.90$, Fig. 4*B*) (13). Addition of $FeSO_4$ to a mixture of DMPO and unmodified U937 cells resulted in less DMPO/$^\cdot$OH signal (Fig. 4, *C* and *D*).

To confirm that $FeSO_4$ mediated the production of an oxidizing radical such as $HO^\cdot$ in the absence of cells, 200 mM

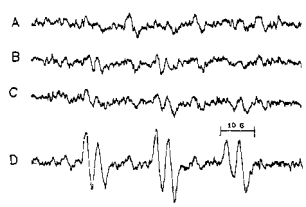

FIG. 1. **EPR spectra observed during incubation of U937 cells with POBN.** The incubations were done at room temperature in air-saturated PBS solutions containing 10 mM POBN and 4×10^6 cells in a total volume of 0.5 ml. Within 1–2 min after introduction of 80 μM $FeSO_4$, EPR scans were initiated. *A*, DHA-enriched cells without added $FeSO_4$; *B*, unmodified cells plus $FeSO_4$; *C*, oleic acid-enriched cells plus $FeSO_4$; *D*, DHA-enriched cells plus $FeSO_4$. The EPR spectrometer conditions are those listed under "Experimental Procedures."

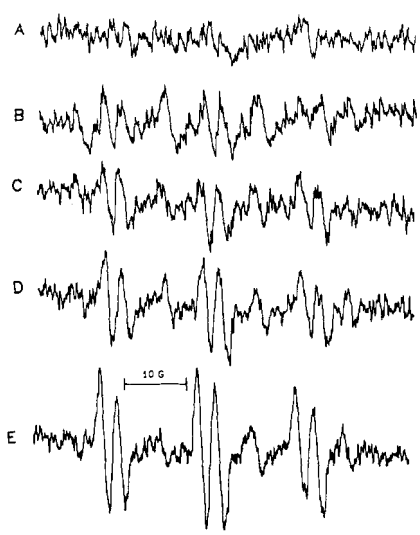

FIG. 2. **Effect of cell density on POBN-spin adduct signal intensity.** DHA-enriched U937 cells were incubated with 10 mM POBN and 100 μM $FeSO_4$. *A*, cell-free control; *B*, 1×10^6 cells; *C*, 2×10^6 cells; *D*, 4×10^6 cells; *E*, 8×10^6 cells. The total volume of the incubation was 0.5 ml.

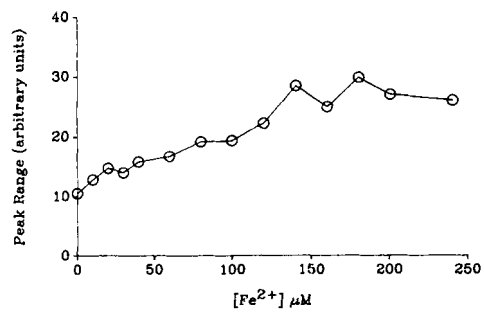

FIG. 3. **Effect of $FeSO_4$ concentration on the relative peak height of POBN-spin adduct spectral intensities.** The incubations contained 4×10^6 DHA-enriched U937 cells and 10 mM POBN in a total volume of 0.5 ml. When no $FeSO_4$ was added, the peak height was 10 or less arbitrary units; this is the noise level at the instrument settings used. Peak heights were determined by adding the absolute values of the maximum and minimum deflections, which included the low-field peak of the first doublet and the high-field trough of the second doublet. Similar results were obtained in two separate experiments; only one is presented in this figure.

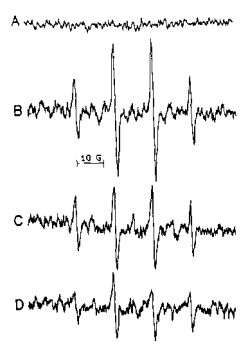

FIG. 4. **DMPO-spin adducts formed during incubation with $FeSO_4$.** The incubations contained 50 mM DMPO and, where present, 160 μM $FeSO_4$ in air-saturated PBS solutions at room temperature. The total volume of the incubation contents was 0.5 ml. *A*, PBS buffer control; *B*, $FeSO_4$ in PBS; *C*, $FeSO_4$ plus 2×10^6 U937 cells; *D*, $FeSO_4$ plus 8×10^6 U937 cells. Spectrometer conditions were those listed under "Experimental Procedures": power, 20 milliwatts; scan rate, 80 G/335 s.

dimethyl sulfoxide was added to a 50 mM DMPO solution. Without added $FeSO_4$, no spectrum was observed. When 160 μM $FeSO_4$ was added, however, the anticipated decrease of DMPO/$^{\cdot}$OH and appearance of DMPO/$^{\cdot}CH_3$ (14) was observed (data not shown). These results are consistent with the formation of $HO^{\cdot}$, which when formed in the presence of cells could initiate lipid peroxidation.

Fatty Acid Modification of U937 Cell Lipids—As shown in Table I, the percentage of 22:6 contained in the cell lipids was substantially greater when the U937 cells were grown in a medium supplemented with 10 μM DHA, as compared with cells grown in medium containing either supplemental oleic acid or no fatty acid supplement. Significant differences were also observed in the percentage of palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1). The average fatty acid chain length and number of double bonds were increased in the cells supplemented with DHA. Furthermore, the total content of polyunsaturated fatty acids was nearly doubled in the DHA-supplemented cells, and the percentage of saturated and monounsaturated fatty acids was reduced substantially. By contrast, addition of 10 μM oleic acid to the growth medium produced an increase in the 18:1 content of the cell lipids. The percentage of 16:0 and 18:0 was significantly reduced in oleic acid-enriched cells as compared with unmodified cells. These differences resulted in a lower percentage of saturated fatty acids and a higher percentage of monounsaturated fatty acids in the lipids of cells enriched in oleic acid. The fatty acid changes produced by modification of the U937 cell lipids with DHA and oleic acid are similar to those previously obtained in other cultured cell lines (9, 15, 16).

TABLE I

Fatty acid composition changes in U937 cells following incubation with fatty acid supplementation

Cells were grown for 2 days with 10 μM supplemental fatty acid. Cells were washed three times and extracted with $CHCl_3/CH_3OH$ (2:1, v/v) and the lipid extracts subjected to alkaline hydrolysis. Fatty acids contained in the saponifiable fraction were methylated, and the methyl esters were separated by gas-liquid chromatography.

Fatty acid	Fatty acid composition[a]		
	Unmodified	18:1 modified	22:6 modified
	%		
14:1	0.2 ± 0.2	1.7 ± 0.5[b]	0.2 ± 0.1[c]
16:0	21.1 ± 2.3	13.4 ± 1.3[b]	19.6 ± 1.5[c]
18:0	16.1 ± 1.5	9.0 ± 0.6[b]	14.8 ± 1.5[c]
18:1	20.3 ± 4.3	33.7 ± 4.9	14.5 ± 1.9[c]
18:2	2.2 ± 0.4	1.9 ± 0.4	1.4 ± 0.3
18:3	1.3 ± 0.5	3.8 ± 0.6[b]	1.9 ± 0.2
20:3	0.4 ± 0.2	1.4 ± 0.3[b]	1.5 ± 0.5
20:4	8.5 ± 1.5	4.9 ± 0.3	5.6 ± 0.7
20:5	2.3 ± 0.9	2.4 ± 0.7	0.8 ± 0.8[c]
22:6	2.8 ± 1.2	2.2 ± 0.4	27.7 ± 2.5[b,c]
% saturated[d]	37.9 ± 2.5	25.6 ± 1.8[b]	35.9 ± 2.9[c]
% monounsaturated[d]	26.1 ± 4.6	38.7 ± 4.1	17.3 ± 2.2[c]
% polyunsaturated[d]	24.9 ± 3.5	20.9 ± 2.0	40.9 ± 2.1[b,c]
Chain length[d,e]	16.1 ± 0.4	15.2 ± 0.5	17.8 ± 0.2[b,c]
Double bonds[d,f]	1.31 ± 0.11	1.22 ± 0.08	2.31 ± 0.12[b,c]

[a] Individual fatty acid peak areas are expressed as a percent of the total fatty acid peak area. Values do not total 100% because only those fatty acids which exhibit significant changes in composition are listed. Fatty acids are designated by the number of carbons:number of double bonds that they contain; for example, arachidonic acid, 20:4, contains 20 carbons and 4 double bonds. The values represent duplicate analyses performed on at least three separate samples ± S.E.

[b] Significantly different from unmodified cells, $p < 0.05$.

[c] Significantly different from oleic acid-enriched cells, $p < 0.05$.

[d] Included in these computations are the fatty acids listed above, as well as peaks identified as 14:0, 16:1, 18:2, 18:4, 20:1, 20:4, 22:4, and 22:5. Unidentified peaks comprised 5.8–14.8% of the total fatty acids.

[e] Average number of carbons per fatty acid contained in cell lipids.

[f] Average number of double bonds per fatty acid contained in cell lipids.

DISCUSSION

Although much is known about lipid peroxidation in chemical systems (1, 2) and subcellular fractions (17), investigation of this process in intact cells has been limited. One reason is that free radicals formed during cellular peroxidations are very short-lived; therefore, the steady-state concentrations are too low to be detected directly by current EPR methods. However, EPR spin trapping has been successfully applied to detect radicals generated in cell-free systems containing PUFA and either chemical oxidants (18) or enzymes (5, 6), as well as in liver or hepatoma microsomes (19, 20). Although EPR spin trapping also has been applied to intact cells, this approach has required disruption of the cells and extraction with organic solvents in order to detect the spin adduct (21, 22). The potential problems with extraction are loss of spin adduct or the production of artifactual EPR signals. In the present study we have successfully applied EPR spin trapping to detect directly lipid-derived spin adducts originating from intact cells, without the use of organic solvent extraction.

This was achieved with a system containing undifferentiated U937 cells, a human monocytic leukemia that grows to a high cell density as a suspension. A high density of suspended cells is required in the incubation mixture to facilitate EPR detection of lipid-derived radicals in a quartz flat cell. If an adherent cell line were to be used, it would be necessary to treat the cultures with either trypsin or chelating agents to produce a cell suspension. This may introduce artifacts because trypsinization will alter the cell surface properties, and chelating agents, because of their interaction with catalytic transition metals, will affect the course of lipid peroxidation. Another potential advantage of the U937 cell line is that it can undergo differentiation to a macrophage-like phenotype when treated with phorbol esters (23). Therefore, this system also can be used to determine whether differentiation can influence lipid peroxidation. Although the U937 cells become adherent when they differentiate, it may be possible to overcome this by developing conditions that allow the differentiated cells to grow on microcarrier beads. This would provide a cell suspension of sufficient density for direct addition to the EPR quartz flat cell.

Spin adducts of lipid radicals produced during lipid peroxidation only were detected when the U937 cells were enriched with DHA, a PUFA containing 22 carbons and 6 double bonds. Lipid peroxidation is a complex free radical chain reaction that is initiated by the abstraction of an H atom from a methylene bridge of a PUFA such as DHA (2).

$$LH + X^{\cdot} \rightarrow L^{\cdot} + XH \quad (1)$$

In the presence of O_2, $L^{\cdot}$ becomes part of a chain-propagating reaction series.

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \quad (2)$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad (3)$$

If iron is present it can react with the lipid peroxide formed during the chain propagation process (Equation 3) producing lipid alkoxyl radicals.

$$LOOH + Fe^{2+} \rightarrow LO^{\cdot} + HO^{-} + Fe^{3+} \quad (4)$$

These alkoxyl radicals can undergo β-scission yielding a va-

riety of products including aldehydes and short chain alkyl radicals such as ethyl, pentyl, or pentenyl radicals.

$$LO^{\cdot} \rightarrow RCH_2^{\cdot} + RC{=}O \quad (5)$$

The resultant alkyl radical can be detected by EPR spin trapping using POBN (5, 6).

$$RCH_2^{\cdot} + POBN \rightarrow POBN/^{\cdot}CH_2R \quad (6)$$

In our spin trapping studies PUFA enrichment of cell lipids with DHA resulted in an easily detectable level of POBN/$^{\cdot}CH_2R$ when these cells were subjected to oxidative stress.

PUFA enrichment enhances the suceptibility to peroxidation of cell lipids as measured by malondialdehyde and ethane production (1, 23). When exposed to ferrous iron, we observed a 10-fold increase in malondialdehyde and ethane production with DHA-enriched U937 cells compared with unmodified or oleic acid-enriched cells,[2] indicating that DHA-enriched cells are indeed more suceptible to peroxidation. Consistent with these observations, the intensity of the POBN/lipid radical EPR signal obtained from DHA-enriched cells is substantially increased compared with the signal obtained from unmodified or oleic acid-enriched cells. Thus, PUFA enrichment of cells can be used as a tool to augment the sensitivity of EPR spin trapping for the detection of lipid peroxidation events. It remains to be determined whether this enhancement is specific for DHA or would occur if the cells are enriched with other forms of *n*-3 PUFAs or the more prevalent *n*-6 PUFAs.

To demonstrate the utility of PUFA enrichment as a tool, we initiated lipid peroxidation in our system by adding varying amounts of ferrous iron. The results with DMPO (Fig. 4) suggest that ferrous iron initiates the peroxidation process in this system through the generation of a strong oxidant that has $HO^{\cdot}$ characteristics. Thus, iron is involved in both the initiation process (Equation 1) as well as in the chain branching reaction (Equation 4) that forms alkoxyl radicals. Therefore, the degree of lipid peroxidation in a short time should increase as the amount of ferrous iron added to the incubation is increased. The results shown in Fig. 3 demonstrate that at lower iron concentrations there is indeed a direct correlation between lipid peroxidation products, monitored as POBN/$^{\cdot}CH_2R$, and the amount of ferrous iron added to the cell suspension.

Although lipid peroxidation has been extensively studied in chemical and subcellular systems, there is a wide variety of mechanistic pathways possible depending upon reaction conditions (2). Studies in intact cells should indicate which subset of these multiple pathways is important in a physiological setting. The present results suggest that in intact cells ferrous iron leads to the production of alkyl radicals through the initial formation of an $HO^{\cdot}$-type oxidant. Chemical systems have demonstrated that alkyl radicals arise from β-scission of lipid alkoxyl radicals formed from the reaction of lipid peroxides with ferrous iron (2, 5, 6). Although our experiments to date have not demonstrated the presence of all the intermediates in the pathway outlined by Equations 1, 2, and 3, our data provide the first direct EPR evidence for the formation of alkyl radicals generated through ferrous iron-mediated lipid peroxidation in an intact mammalian cell.

[2] J. A. North and A. A. Spector, unpublished observations.

REFERENCES

1. Porter, N. A. (1984) *Methods Enzymol.* **105,** 273–282
2. Gardner, H. W. (1989) *Free Radical Biol. & Med.* **7,** 65–86
3. Halliwell, B. and Gutteridge, J. M. C. (1989) *Free Radicals in Biology and Medicine*, pp. 188–276, Clarendon Press, Oxford
4. Miller, D. M., Buettner, G. R., and Aust, S. D. (1990) *Free Radical Biol. & Med.* **8,** 95–108
5. Chamulitrat, W., and Mason, R. P. (1990) *Arch. Biochem. Biophys.* **282,** 65–69
6. Iwahashi, H., Albro, P. W., McGown, S. R., Tomer, K. B., and Mason, R. P. (1991) *Arch. Biochem. Biophys.* **284,** 172–180
7. Buettner, G. R. (1990) *Methods Enzymol.* **186**, 125–127
8. Buettner, G. R. (1988) *J. Biochem. Biophys. Methods* **16,** 27–40
9. Guffy, M. M., North, J. A., and Burns, C. P. (1984) *Cancer Res.* **44,** 1863–1866
10. Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226,** 497–509
11. Burns, C. P., Luttenegger, D. G., Wei, S. L., and Spector, A. A. (1977) *Lipids* **12,** 747–752
12. Morrison, W. R., and Smith, L. M. (1964) *J. Lipid Res.* **5,** 600–608
13. Buettner, G. R. (1987) *Free Radical & Biol. Med.* **3,** 295–303
14. Buettner, G. R., and Mason, R. P. (1990) *Methods Enzymol.* **186,** 127–133
15. Burns, C. P., and North, J. A. (1986) *Biochim. Biophys. Acta* **888,** 10–17
16. Burns, C. P., and Spector, A. A. (1987) *Lipids* **22,** 178–184
17. Pederson, T. C., and Aust, S. D. (1975) *Biochim. Biophys. Acta* **385,** 232–241
18. Tien, M., Svingen, B. A., and Aust, S. D. (1981) *Fed. Proc.* **40,** 179–182
19. Rosen, G. M., and Rauckman, E. J. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 7346–7349
20. Davies, M. J. (1987) *Chem. Phys. Lipids* **44,** 149–173
21. Poli, G., Chiarpotto, E., Albano, E., Biasi, F., Cecchini, G., and Dianzani, M. U. (1986) *Front. Gastrointest. Res.* **9,** 38–49
22. Poli, G., Albano, E., Tomasi, A., Cheeseman, K. H., Chiarpotto, E., Parola, M., Biocca, M. E., Slater, T. F., and Dianzani, M. U. (1987) *Free Radical Res. Commun.* **3,** 251–255
23. Harris, P., and Ralph, P. (1985) *J. Leukocyte Biol.* **37,** 407–422