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Cellular oxidative modification of low density lipoprotein does not require lipoxygenases

(atherosclerosis/macrophage/endothelial cell/cholesterol)

CARL P. SPARROW* AND JOANNE OLSZEWSKI

Department of Atherosclerosis Research, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, 126 East Lincoln Avenue, Rahway, NJ 07065

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ABSTRACT **The oxidative modification of low density lipoprotein (LDL) may play an important role in the pathogenesis of atherosclerosis. LDL can be oxidatively modified *in vitro* by endothelial cells, mouse peritoneal macrophages, or copper ions. Studies using lipoxygenase inhibitors have suggested that lipoxygenase(s) is required for the cellular modification of LDL [Rankin, S. M., Parthasarathy, S. & Steinberg, D. (1991) *J. Lipid Res.* 32, 449–456]. We have reexamined the effect of lipoxygenase inhibitors on cellular modification and found that (*i*) inhibitors specific for 5-lipoxygenase do not block LDL modification; (*ii*) inhibitors that block lipoxygenase by donating one electron to the enzyme (reductive inactivation) prevent LDL modification by cells and also modification mediated by copper ions, implying that they act as general antioxidants; (*iii*) the lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid blocks 15-lipoxygenase activity in intact macrophages at concentrations 100 times less than those required to block LDL modification by macrophages; and (*iv*) 5,8,11,14-eicosatetraynoic acid is cytotoxic at concentrations about twice those required to prevent modification. Furthermore, macrophages and the RECB4 line of endothelial cells modify LDL with similar efficiencies despite dramatic differences in 15-lipoxygenase activity. Thus we conclude that neither 5-lipoxygenase nor 15-lipoxygenase is required for modification of LDL by cultured cells.**

There is growing evidence that an important part of the pathogenesis of atherosclerosis is the oxidative modification of low density lipoprotein (LDL) (1–4). LDL can be oxidatively modified *in vitro* by copper ions and by various cultured cells (reviewed in ref. 1). LDL can also be modified by the combined action of two purified enzymes: soybean lipoxygenase and phospholipase A_2 (5). This observation suggested the possibility that cellular modification of LDL may involve lipoxygenase(s), and it has been shown that lipoxygenase inhibitors block modification by endothelial cells (6) and macrophages (7). It has been suggested, therefore, that lipoxygenase inhibitors might prevent or reverse atherosclerosis (4). Particular attention has been focused on 15-lipoxygenase (4, 7).

Many commonly used lipoxygenase inhibitors such as nordihydroguaiaretic acid (NDGA) (8) and quercetin (9) are irreversible inhibitors that "reductively inactivate" lipoxygenase by donating one electron to the enzyme (8). In contrast, the lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) is a mechanism-based inactivator (or suicide substrate) of lipoxygenase (10). Both types of lipoxygenase inhibitors have been shown to block cellular modification of LDL (6, 7). We now report that reductive inactivators of lipoxygenase block LDL modification mediated by copper ions, implying that they act as general antioxidants and not as specific lipoxygenase inhibitors. Reductive inactivators of lipoxygenase have previously been shown to be general antioxidants (11). We also report that ETYA blocks 15-lipoxygenase at concentrations 100 times less than those required to block LDL modification. Furthermore, ETYA is cytotoxic at concentrations about twice those required to block modification. Our results cast doubt on the conclusions drawn in previous studies using lipoxygenase inhibitors to block the cellular modification of LDL.

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MATERIALS AND METHODS

Cells. The cell line RECB4, derived from rabbit aortic endothelial cells (12), was obtained from D. Steinberg (University of California, San Diego) and grown as described (13). Resident mouse peritoneal macrophages were obtained from female Swiss Webster mice by peritoneal lavage with phosphate-buffered saline. Macrophages were plated in 6-well cell culture plates at 3 million cells per well (for modification) or in 24-well plates at 0.6 million cells per well (for uptake assays) in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Macrophages were used 16–20 hr after plating.

Materials. Tissue culture supplies were from GIBCO, except fetal bovine serum, which was from HyClone. ETYA was obtained from Cayman Chemicals (Ann Arbor, MI). L-number compounds, ICI211965, and A-64077 were synthesized at Merck Sharp & Dohme Research Labs or at The Merck Frosst Center for Therapeutic Research (Kirkland, PQ, Canada).

Lipoproteins. Plasma was obtained from fasted normal volunteers, and LDL was isolated by standard procedures (14). Concentrations of LDL are expressed on the basis of protein, measured using the micro BCA (bicinchoninic acid) protein reagent (Pierce). LDL was iodinated with ^{125}I by using the "trapped label" tyramine cellobiose (15). No significant differences were found in preliminary experiments comparing the oxidative modification of LDL labeled with ^{125}I-tyramine cellobiose and LDL labeled conventionally (13) (data not shown).

Assays of Modification of LDL. Cells were washed three times with Ham's F-10 medium and then incubated with F-10 medium containing LDL (0.10 mg/ml) with or without inhibitors. Most experiments used radioiodinated LDL (20–50 dpm/ng). In experiments involving incubation of RECB4 cells with ETYA, bovine serum albumin was added to 0.25 mg/ml (6). Controls for modification assays were performed by incubating LDL in F-10 in the absence of cells or in the presence of 10 μM $CuSO_4$. After 24 hr, media were harvested and adjusted to 20 μM butylated hydroxytoluene. Modifica-

Abbreviations: ETYA, 5,8,11,14-eicosatetraynoic acid; HODE, hydroxyoctadecadienoic acid; LDL, low density lipoprotein; NDGA, nordihydroguaiaretic acid; TBARS, thiobarbituric acid-reactive substances.

*To whom reprint requests should be addressed.

tion of LDL was assayed by measurement of thiobarbituric acid-reactive substances (TBARS) (5) and by macrophage uptake. Uptake was measured by incubating macrophages with 10 μg/ml of radiolabeled LDL for 5 hours, then quantitating cell-associated ^{125}I as previously described (13).

Inhibitors were tested at various concentrations, and the concentration required for 50% inhibition (EC_{50}) was estimated from the dose–response curve. In most experiments, both TBARS and macrophage uptake assays were performed. In general, the EC_{50} determined from the TBARS assay of a single experiment was the same or slightly higher than the EC_{50} determined from the uptake assay of the same experiment. However, day-to-day variability was significant, so when data from different experiments are averaged to obtain mean EC_{50} values, the standard deviation is usually large.

Assay of 15-Lipoxygenase. 15-Lipoxygenase is so named because it converts arachidonic acid to 15-hydroperoxyeicosatetraenoic acid (16). We used linoleic acid as the substrate for 15-lipoxygenase because (*i*) 15-lipoxygenase prefers linoleic acid over arachidonic acid as substrate (17, 18) and (*ii*) linoleic acid is not a substrate for 5-lipoxygenase or cyclooxygenase and thus fewer side products are formed. 15-Lipoxygenase converts linoleate to 13-hydroperoxyoctadecadienoic acid (16). In intact cells, this initial product is reduced to 13-hydroxyoctadecadienoic acid (13-HODE) and then released from the cells (19). To assay 15-lipoxygenase in intact cells, the cells were washed three times with F-10 and then incubated with F-10 containing 10 μM [1-^{14}C]linoleate (50 Ci/mol; 1 Ci = 37 GBq). After 15–30 min the medium was removed and 1 ml was extracted with chloroform (20) in the presence of 100 μmol of sodium citrate buffer (pH 2.8), 50 nmol of unlabeled linoleate, and 50 nmol of butylated hydroxytoluene. The chloroform phase was dried under argon, redissolved in chloroform/methanol, 1:1 (vol/vol), and subjected to thin-layer chromatography on silica-gel G plates in diethyl ether/petroleum ether/acetic acid, 50:50:1. [^{14}C]13-HODE was visualized by autoradiography and quantitated by scraping and liquid scintillation spectrometry. Macrophages released essentially no 9-HODE or hydroperoxy derivatives of linoleate, as judged by migration of standards. Macrophage production of 13-HODE was sensitive to NDGA (EC_{50} about 500 nM) and ETYA (see Fig. 1). The amounts of lipoxygenase-derived products detected within the macrophages were very low (data not shown).

RESULTS

Inhibitors of 5-Lipoxygenase Do Not Block Modification of LDL. Compounds that specifically inhibit 5-lipoxygenase did not block the oxidative modification of LDL by endothelial cells or by macrophages (Table 1). This was true for direct inhibitors of the 5-lipoxygenase enzyme as well as compounds that block 5-lipoxygenase by binding to the 5-lipoxygenase-activating protein (23). These results show that 5-lipoxygenase is not required for cellular modification of LDL.

Inhibition of LDL Modification by Reductive Inactivators of Lipoxygenases. The ability to block LDL modification was determined for a set of compounds including lipoxygenase inhibitors known to reductively inactivate the enzyme (8) (Table 2). The compounds blocked LDL modification by cells, but they also blocked modification by copper ions (Table 2). For each compound, the EC_{50} against copper ions was slightly higher than the EC_{50} against endothelial cells. This pattern was also found for butylated hydroxytoluene, which is a general antioxidant. We conclude that members of this class of lipoxygenase inhibitors block LDL modification by acting as general antioxidants, not by acting as specific lipoxygenase inhibitors.

Table 1. Inhibitors of 5-lipoxygenase do not inhibit LDL modification by cells

Compound*	EC_{50} against leukotriene biosynthesis	Effect on LDL modification	
		Endothelial cells	Macrophages
ICI211965	10 nM	Inactive at 5 μM	Inactive at 5 μM
MK-886	3 nM	Inactive at 2 μM	Inactive at 2 μM

ICI211965, a direct inhibitor of 5-lipoxygenase (21), and MK-886, an inhibitor of the 5-lipoxygenase-activating protein (22, 23), were tested for their ability to block oxidative modification of LDL by RECB4 endothelial cells and by macrophages. Neither of the compounds had an effect on LDL modification at the highest concentrations tested. The compounds' effectiveness against 5-lipoxygenase was determined previously by other workers (21, 22) by measuring production of leukotrienes by whole cells. 5-Lipoxygenase catalyzes the first committed enzymatic step in leukotriene biosynthesis (24).

*ICI211965 is 1-[3-(naphth-2-ylmethoxy)phenyl]-1-(thiazol-2-yl)-propyl methyl ether (21). MK-886 (also known as L-663,536) is 3-[1-(4-chlorobenzyl)-3-*t*-butylthio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid (22).

Comparison of ETYA Inhibition of 15-Lipoxygenase and LDL Modification. It has been proposed that 15-lipoxygenase is required for LDL modification (4, 28), based partly on the observation that ETYA inhibits oxidative modification by cells but not by copper ions (6, 7). We assayed 15-lipoxygenase in intact macrophages using [^{14}C]linoleate as substrate. We found that the apparent EC_{50} of ETYA against cellular 15-lipoxygenase activity (60 nM) was at least 100 times less than the apparent EC_{50} of ETYA against cellular modification (10 μM) (Fig. 1). These values agree relatively well with previously reported EC_{50} values against cellular 15-lipoxygenase (29–31) and cellular modification (7).

Different time periods were used to assay LDL modification (24 hr) and 15-lipoxygenase activity (15–30 min). This raised the possibility that ETYA was less effective in assays of LDL modification because the ETYA was inactivated during the 24 hr. To address this possibility, we sequentially assayed LDL modification and 15-lipoxygenase in the same cells. Specifically, macrophages were incubated with LDL in the presence of various concentrations of ETYA. At various times, the media were harvested and TBARS was assayed; then the cells were washed and assayed for 15-lipoxygenase

Table 2. EC_{50} values of various compounds against oxidative modification of LDL by cells or copper ions

Inhibitor*	EC_{50} value, μM		
	Endothelial cells	Macrophages	$CuSO_4$
BHT	4.7 ± 3.5	3.5	8.8 ± 4.2
NDGA	0.74 ± 0.6	1.3	0.92 ± 0.5
L-670,630	0.44 ± 0.3	0.6	1.2 ± 0.6
Quercetin	0.7	NT	3.7 ± 2.0
L-651,896	0.2	NT	0.7
A-64077	NT	0.1	0.5

Compounds were tested for the ability to block oxidative modification of LDL mediated by cells or by copper ions. EC_{50} values were determined from the TBARS assay; similar results were obtained from the macrophage uptake assay. Error estimates are standard deviations of the means (see *Materials and Methods*). NT, not tested.

*BHT, butylated hydroxytoluene; NDGA (8); L-670,630, 2-phenethyl-5-hydroxy-6-(3-phenoxypropyl)-2,3-dihydrobenzofuran (25); quercetin (9); L-651,896, 2,3-dihydro-6-[3-(2-hydroxymethyl)-phenyl-2-propenyl]-5-benzofuranol (26); A-64077 (also known as zileuton), *N*-(1-benzo[*b*]thien-2-ylethyl)-*N*-hydroxyurea (27).

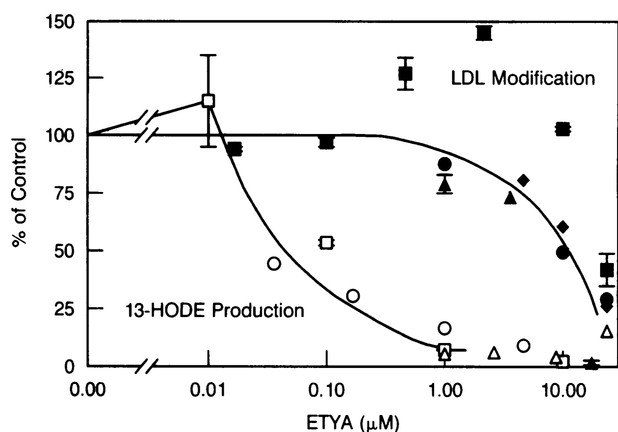

FIG. 1. ETYA inhibits LDL modification and production of 13-HODE in intact macrophages. LDL modification was assayed by TBARS, and 15-lipoxygenase activity was quantitated by measuring 13-HODE in the medium. All data are presented as percent of control. Error bars indicate the range of duplicates; data points without error bars were not done in duplicate. Filled symbols, LDL modification; open symbols, 15-lipoxygenase activity. Different symbol types represent independent experiments. The curves were drawn by eye.

activity, in the absence of LDL and ETYA. The ETYA present during the first incubation should inactivate cellular 15-lipoxygenase (10), and this inactivation should be measurable during the second incubation. The results (Fig. 2) show that macrophages exposed to ETYA (2.5 μM) and LDL (0.1 mg/ml) for 12 hr had less than 20% of control 15-lipoxygenase activity, but these cells modified LDL to the same extent as control cells.

Comparison of 15-Lipoxygenase Activity in Endothelial Cells and Macrophages. The RECB4 line of endothelial cells released no detectable lipoxygenase products when incubated with [^{14}C]linoleate. The endothelial cells were assayed in parallel with macrophages, and it would have been possible to detect 5% of the activity displayed by macrophages. Saponification of lipid extracts of endothelial cells labeled with [^{14}C]linoleate showed no 15-lipoxygenase products in cellular lipids (data not shown). This contrasts with the fact that the two cell types modify LDL with comparable efficiencies (data not shown; also see ref. 1).

ETYA Is Toxic to Macrophages and Endothelial Cells. In the standard assay of LDL modification, the cells were discarded after the 24-hr incubation. In some experiments we analyzed the cells to assess cytotoxicity of ETYA. ETYA was toxic to macrophages as judged by loss of adherent cell protein: in four independent experiments, macrophage wells given 20 μM ETYA had 55%, 53%, 39%, and 33% of the cell protein found in control wells. ETYA was toxic to endothelial cells as judged by a decrease in cellular uptake of [^{3}H]leucine: in four independent experiments, ETYA inhibited LDL modification with EC_{50} values of 24 ± 5 μM (macrophage uptake assay) and 29 ± 9 μM (TBARS); simultaneously, ETYA blocked accumulation of [^{3}H]leucine by the endothelial cells with an EC_{50} of 48 ± 14 μM. It could be argued that ETYA was toxic because it is a fatty acid and therefore has detergent properties; however, arachidonic acid, which is very similar in structure to ETYA, did not show toxicity to either cell type, nor did it block oxidative modification. It is possible that the inhibition by ETYA of cellular modification is related to its cytotoxicity.

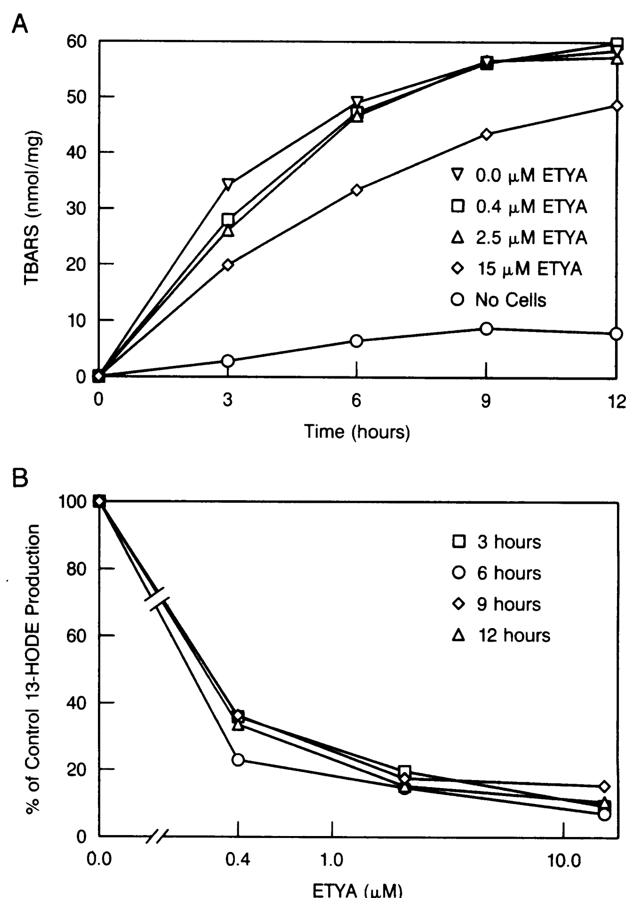

FIG. 2. Sequential assays of LDL modification and 15-lipoxygenase activity in macrophages treated with ETYA for various times. Mouse peritoneal macrophages were incubated with LDL in the presence of various concentrations of ETYA. At various times, media were removed and assayed for TBARS. The cells were washed and then incubated with 10 μM [^{14}C]linoleate for 30 min to assay cellular 15-lipoxygenase activity. (*A*) Time course of modification. Each curve represents one concentration of ETYA. No cells, LDL incubated in the absence of cells. (*B*) Dose–response of cellular 15-lipoxygenase to ETYA. Each curve shows the 15-lipoxygenase activity remaining in intact macrophages after a particular time of incubation with LDL.

DISCUSSION

It has been hypothesized that cellular oxidative modification of LDL requires lipoxygenase activity (4, 28), based on recent observations that lipoxygenase inhibitors block cellular modification of LDL (6, 7). We believe this hypothesis may not be true, for the reasons discussed below.

(*i*) Compounds that directly and specifically block 5-lipoxygenase action did not prevent cellular modification of LDL (Table 1). Although the specific 5-lipoxygenase inhibitor piriprost (32) was reported to block LDL modification by macrophages (7), it was used at a very high concentration and no assessment of possible cytotoxicity was reported (7).

(*ii*) Some of the lipoxygenase inhibitors previously used to block LDL modification inhibit lipoxygenase by reductive inactivation; i.e., they donate one electron to the enzyme (8). Compounds capable of donating one electron are usually general antioxidants (11). We now report that reductive inactivators of lipoxygenase block LDL modification mediated by cells or mediated by copper ions, implying that the compounds act as general antioxidants, not as specific lipoxygenase inhibitors (Table 2).

(*iii*) ETYA is a lipoxygenase inhibitor that blocks cellular modification of LDL but not copper-mediated modification (6, 7). We now show that ETYA blocks 15-lipoxygenase activity at concentrations 100 times lower than the concentrations required to block LDL modification (Fig. 1). Furthermore, macrophages treated with ETYA such that they

had less than 20% of control 15-lipoxygenase activity were still able to modify LDL to the same extent as control cells (Fig. 2). We also found ETYA to be cytotoxic at concentrations about twice those required to block modification. Currently, there is disagreement in the literature over whether 20 μM ETYA is toxic to macrophages (7, 33).

(*iv*) Mouse peritoneal macrophages displayed at least 20 times as much 15-lipoxygenase activity as did the RECB4 line of endothelial cells. However, these two cell types are comparably efficient at LDL modification.

(*v*) The origin of the "lipoxygenase hypothesis" was the observation that the introduction of lipid hydroperoxides into LDL by soybean lipoxygenase initiated LDL oxidation (5). However, the presence of peroxidases in cells causes lipoxygenase products to be released from cells as alcohols, not as hydroperoxides (19, 34). Alcohols would not be expected to initiate oxidation of LDL.

Our data alone cannot exclude a role for 12-lipoxygenase in LDL modification. Mathur *et al.* (35) showed that macrophage 12-lipoxygenase activity was increased following cholesterol loading with acetylated LDL, implying that foam cells in atherosclerotic lesions may have elevated 12-lipoxygenase activity. In cultured cells, however, 12-lipoxygenase has been reported to be inhibited by ETYA at concentrations similar to those required to inhibit 15-lipoxygenase (29, 31). This implies that our conclusions about the ETYA inhibition of 15-lipoxygenase may also be valid for 12-lipoxygenase; i.e., both enzymes are inhibited by ETYA concentrations significantly lower than the concentrations required to block the oxidative modification of LDL. We believe that at this time there is no evidence supporting a role for any cellular lipoxygenases in LDL modification.

There are observations linking 15-lipoxygenase with atherosclerosis. Atherosclerotic rabbit aortic tissue contains much higher levels of 15-lipoxygenase activity than does normal tissue (36). Macrophages in rabbit and human atheroma express 15-lipoxygenase (4, 28). 15-Lipoxygenase products have also been reported to decrease platelet adherence to endothelium (37). The final understanding of the possible importance of 15-lipoxygenase in atherosclerosis awaits further study.

The mechanism by which cells modify LDL remains unknown. It has been postulated that cellular modification is dependent on release of superoxide by cells (38, 39). This model may be correct for some cell types but not for other cell types: highly purified superoxide dismutase inhibited modification of LDL mediated by copper ions or by smooth muscle cells but not modification mediated by endothelial cells (6). It is tempting to speculate that certain cell types have on their surface an enzyme that initiates oxidation of LDL, perhaps by catalyzing the oxidation of LDL phospholipids. Such an enzyme might be dependent on transition metal ions; this would explain the observation that cellular modification requires transition metal ions in the medium (40). Understanding the mechanism of cellular modification of LDL might lead to the identification of novel therapeutic agents useful for the treatment of vascular disease.

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1. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. & Witztum, J. L. (1989) *N. Engl. J. Med.* **320**, 915–924.
2. Yla-Herttuala, S., Palinski, W., Rosenfeld, M. E., Parthasarathy, S., Carew, T. E., Butler, S., Witztum, J. L. & Steinberg, D. (1989) *J. Clin. Invest.* **84**, 1086–1095.
3. Palinski, W., Rosenfeld, M., Yla-Herttuala, S., Gurtner, G. C., Socher, S. S., Butler, S. W., Parthasarathy, S., Carew, T. E., Steinberg, D. & Witztum, J. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1372–1376.
4. Yla-Herttuala, S., Rosenfeld, M. E., Parthasarthy, S., Glass, C. K., Sigal, E., Witztum, J. L. & Steinberg, D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6959–6963.
5. Sparrow, C. P., Parthasarthy, S. & Steinberg, D. (1988) *J. Lipid Res.* **29**, 745–753.
6. Parthasarathy, S., Wieland, E. & Steinberg, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1046–1050.
7. Rankin, S. M., Parthasarathy, S. & Steinberg, D. (1991) *J. Lipid Res.* **32**, 449–456.
8. Kemal, C., Louis-Flamberg, P., Krupinski-Olsen, R. & Shorter, A. L. (1987) *Biochemistry* **26**, 7064–7072.
9. Riendeau, D., Falgueyret, J.-P., Nathaniel, D. J., Rokach, J., Ueda, N. & Yamamoto, S. (1989) *Biochem. Pharmacol.* **38**, 2313–2321.
10. Downing, D. T., Ahern, D. G. & Bachta, M. (1970) *Biochem. Biophys. Res. Commun.* **40**, 218–223.
11. Thody, V. E., Buckle, D. R. & Foster, K. E. (1987) *Biochem. Soc. Trans.* **15**, 416–417.
12. Buonassisi, V. & Venter, J. C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1612–1616.
13. Sparrow, C. P., Parthasarthy, S. & Steinberg, D. (1989) *J. Biol. Chem.* **264**, 2599–2604.
14. Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955) *J. Clin. Invest.* **34**, 1345–1353.
15. Pittman, R. C. & Taylor, C. A., Jr. (1986) *Methods Enzymol.* **129**, 613–628.
16. Yamamoto, S. (1990) *Free Radical Biol. Med.* **10**, 149–159.
17. Soberman, R. J. (1988) *Methods Enzymol.* **163**, 344–349.
18. Kuhn, H., Sprecher, H. & Brash, A. R. (1990) *J. Biol. Chem.* **265**, 16300–16305.
19. Spector, A. A., Gordon, J. A. & Moore, S. A. (1988) *Prog. Lipid Res.* **27**, 271–323.
20. Bligh, E. G. & Dyer, J. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–918.
21. Bird, T. G. C., Bruneau, P., Crawley, G. C., Edwards, M. P., Foster, S. J., Girodeau, J.-M., Kingston, J. F. & McMillan, R. M. (1991) *J. Med. Chem.* **34**, 2176–2186.
22. Gillard, J., Ford-Hutchinson, A. W., Chan, C., Charleson, S., Denis, D., Foster, A., Fortin, R., Leger, S., McFarlane, C. S., Morton, H., Piechuta, H., Riendeau, D., Rouzer, C. A., Rokach, J., Young, R., MacIntyre, D. E., Peterson, L., Bach, T., Eiermann, G., Hopple, S., Humes, J., Hupe, L., Luell, S., Metzzger, J., Meurer, R., Miller, D. K., Opas, E. & Pacholok, S. (1988) *Can. J. Physiol. Pharmacol.* **67**, 456–464.
23. Dixon, R. A. F., Diehl, R. E., Opas, E., Rands, E., Vickers, P. J., Evans, J. F., Gillard, J. W. & Miller, D. K. (1990) *Nature (London)* **343**, 282–284.
24. Samuelsson, B. (1983) *Science* **220**, 568–575.
25. Riendeau, D., Falgueyret, J.-P., Guay, J., Ueda, N. & Yamamoto, S. (1991) *Biochem. J.* **274**, 287–292.
26. Bonney, R. J., Davies, P., Dougherty, H., Egan, R. W., Gale, P. H., Chang, M., Hammond, M., Jensen, N., MacDonald, J., Thompson, K., Zambias, R., Opas, E. E., Meurer, R., Pacholok, S. & Humes, J. L. (1987) *Biochem. Pharmacol.* **36**, 3885–3891.
27. Carter, G., Young, P. R., Albert, D. H., Bouska, J., Dyer, R., Bell, R. L., Summers, J. B. & Brooks, D. W. (1991) *J. Pharmacol. Exp. Ther.* **256**, 929–937.
28. Yla-Herttuala, S., Rosenfeld, M. E., Parthasarathy, S., Sigal, E., Sarkioja, T., Witztum, J. L. & Steinberg, D. (1991) *J. Clin. Invest.* **87**, 1146–1152.
29. Ibe, B. O., Falck, J. R., Johnson, A. R. & Campbell, W. B. (1989) *Am. J. Physiol.* **256**, C1168–C1175.
30. Sigal, E., Grunberger, D., Highland, E., Gross, C., Dixon, R. A. F. & Craik, C. S. (1990) *J. Biol. Chem.* **265**, 5113–5120.
31. Salari, H., Braquet, P. & Borgeat, P. (1984) *Prostaglandins Leukotrienes Med.* **13**, 53–60.
32. Sun, F. F. & McGuire, J. C. (1983) *Prostaglandins* **26**, 210–221.
33. Wilkins, G. M. & Leake, D. S. (1990) *Biochem. Soc. Trans.* **13**, 1170–1171.
34. Bryant, R. W., Bailey, J. M., Schewe, T. & Rapoport, M. (1982) *J. Biol. Chem.* **257**, 6050–6055.
35. Mathur, S. N., Albright, E. & Field, F. J. (1989) *Biochim. Biophys. Acta* **1001**, 50–59.
36. Simon, T. C., Makheja, A. N. & Bailey, J. M. (1989) *Atherosclerosis* **75**, 31–38.
37. Buchanan, M. R., Haas, T. A., Lagarde, M. & Guichardant, M. (1985) *J. Biol. Chem.* **260**, 16056–16059.
38. Cathcart, M. K., McNally, A. K., Morel, D. W. & Chisolm, G. M. (1989) *J. Immunol.* **142**, 1963–1969.
39. Hiramatsu, K., Rosen, H., Heinecke, J. W., Wolfbauer, G. & Chait, A. (1987) *Arteriosclerosis* **7**, 55–60.
40. Steinbrecher, U. P., Parthasarathy, S., Leake, D. S., Witztum, J. L. & Steinberg, D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3883–3887.