

Aspen Bile Acid/Cholesterol Conference 1991. Report of a Conference

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"Lipid Accumulation in Hepatic and Extrahepatic Tissues," a research seminar sponsored by the Aspen Bile Acid/Cholesterol Conference, Inc., was held at the Given Institute of the University of Colorado School of Medicine in Aspen, Colorado on August 17-20, 1991. This symposium was planned to bring together investigators who approach the general problem of tissue accumulation of lipids from different points of view. Eight presentations were devoted to the accumulation of lipids in cells of the developing atherosclerotic lesion and eight to the accumulation of lipids in liver and in peripheral tissues.

LIPID ACCUMULATION IN CELLS OF THE DEVELOPING ATHEROSCLEROTIC LESION

Chairs: Daniel Steinberg, University of California, San Diego and Scott M. Grundy, Center for Human Nutrition, Dallas

1. **Dr. Monty Krieger** (Massachusetts Institute of Technology) opened the meeting with a discussion of "The Macrophage Scavenger Receptor," the receptor that recognizes chemically acetylated LDL. That receptor, which also recognizes oxidized LDL, is believed to play a key role in the generation of macrophage foam cells in developing atherosclerotic lesions. The cDNA for the bovine receptor was first cloned in Dr. Krieger's laboratory. Two different forms, Type I (453 amino acids), and Type II (349 amino acids), were demonstrated and shown to derive by alternative splicing. Type II differs from Type I in that the cysteine-rich extracellular domain is almost totally deleted. Yet the two forms of the receptor bind and internalize acetyl LDL comparably, showing that the cysteine-rich domain is not essential for ligand binding. Under reducing conditions the receptor had an apparent molecular weight of 220,000 and on reduction it appeared to be made up of three subunits of 77,000. There was also some dimeric material apparent on immunoblots. The trimeric structure was also compatible with the presence in the

receptor of an unusual domain highly reminiscent of collagen. Recent studies indicate that disulfide cross-linkage can occur during preparation, and site-directed mutagenesis has shown that the cysteine at position 83 can be converted to glycine without affecting the efficiency of the receptor for binding and internalization of acetyl LDL.

Using transfected CHO cells it was shown that the receptor can also bind and internalize oxidized LDL, but the binding affinity is considerably lower than that of acetyl LDL. Acetyl LDL competed efficiently with oxidized LDL, but oxidized LDL competed poorly with acetylated LDL. In the discussion it was pointed out that this was the inverse of what is observed when studying degradation of acetyl LDL and oxidized LDL in mouse peritoneal macrophages.

Lipopolysaccharide (LPS) binds with reasonably high affinity to the scavenger receptor. However, other studies indicate that binding to the scavenger receptor is not the basis for LPS activation of the macrophage.

2. **Dr. George H. Rothblat** (Medical College of Pennsylvania) addressed the issue of "Intracellular Movement of Cholesterol during Foam Cell Formation." His presentation focused on the relationship between cellular cholesterol metabolism and cholesterol efflux. Using J774 mouse macrophages, he and his collaborators compared a series of reconstituted cholesterol acceptor particles containing either apoA-I, apoA-II, or apoCs to see whether they would differently affect lysosomal cholesteryl ester hydrolysis, ACAT activity, or cholesterol efflux. The cells were first loaded with cholesteryl esters (CE) in which the cholesterol moiety was labeled with tritium. Then the metabolism of the tritium-labeled free cholesterol generated in the lysosomes was compared to the metabolism of ¹⁴C-labeled free cholesterol previously exchanged into the cells (and predominantly present in cellular membranes). The studies showed that the nature of the apoprotein on the acceptor particles had no selective effect on the metabolism of lysosomal or membrane-bound cholesterol. Both cellular pools of free cholesterol were equally available for efflux and/or esterification by ACAT, regardless of

the apoprotein in the acceptor particles. However, acceptors containing apoA-I were more efficient than those containing apoA-II or apoCs. It was proposed on the basis of kinetic analyses that different apoproteins may influence the distribution of free cholesterol in different pools within plasma membranes. It was observed that as lysosomal hydrolysis of cholesteryl esters proceeded, substantial levels of free cholesterol accumulated in the J774 cells. Recent studies suggest that a significant fraction of this excess free cholesterol is trapped within the lipid-loaded lysosomes. Long-term incubation (48–72 h) of these cells results in the appearance of free cholesterol crystals within the cell.

In another series of studies, J774 cells containing cholesteryl esters stored in cytoplasmic inclusions were incubated with HDL. This did not result in a net removal of cellular cholesteryl esters. In contrast, similar incubations of mouse peritoneal macrophages produced very significant reductions in cellular cholesteryl ester. The failure of HDL to clear cholesteryl esters from J774 cells was not associated with HDL concentration or any particular properties of the HDL. Further studies show that exposure of cholesteryl ester-loaded J774 cells to cyclic AMP results in a stimulation of the clearance of cholesteryl esters when the cells are incubated with HDL. Cyclic AMP has been shown previously to increase the activity of neutral cholesteryl ester hydrolase. Preliminary data in Dr. Rothblat's laboratory suggest that the cyclic AMP may also stimulate the efflux of the free cholesterol produced on hydrolysis of the cytoplasmic cholesteryl ester, i.e., have an effect on transport of free cholesterol as well.

3. **Dr. Ta-Yuan Chang** (Dartmouth Medical School) described "Mutant Cell Models for the Study of Cholesterol Accumulation." Dr. Chang has previously cloned a mutant CHO cell line (M_1) that is defective in its ability to enhance sterol biosynthesis and expression of the LDL receptor when sterols are removed from the growth medium. The availability of this mutant offers an opportunity to identify the gene(s) responsible for up-regulation of these pathways in mammalian cells. Using recombinant DNA library construction and screening, several λ -phage clones containing various parts of the human M_1 gene have been isolated.

A similar approach has been taken using a mutant CHO cell clone (AC29) that lacks acyl CoA:cholesterol acyltransferase (ACAT). Primary and secondary transfectants have been isolated containing and expressing the human ACAT gene. Through recombinant DNA library construction and screening, genomic DNAs and cDNAs have been isolated that are components of the human ACAT gene. This approach is a promising one that should lead to the identification of the genes relevant to these critical pathways in sterol metabolism.

4. **Dr. Ira A. Tabas** (Columbia University) and **Dr. Thomas L. Innerarity** (Gladstone Foundation

Laboratories, University of California, San Francisco) presented studies of the "Endocytic Pathways of β -VLDL in Macrophages." Previous studies have established that the LDL receptor is responsible for the uptake of both LDL and β -VLDL, although the latter has a higher affinity for the receptor. However, even when comparisons are made at equal rates of delivery of lipoprotein cholesterol, β -VLDL is much more potent in stimulating ACAT activity than is LDL. This led to the hypothesis that LDL and β -VLDL are targeted to different sites within the macrophage and that different intracellular pathways influence the ability of the lipoproteins, respectively, to stimulate ACAT activity. Previously published work has shown that fluorescence-labeled LDL and β -VLDL do indeed have different endocytotic pathways in the macrophages. LDL is targeted primarily to perinuclear lysosomes whereas β -VLDL is at least partially targeted to unique, widely distributed vesicles. Further work disclosed that the heterogeneity of the β -VLDL endocytotic pattern could be partially explained by heterogeneity in the β -VLDL particles themselves. Thus, large, intestinally derived β -VLDL (which are apoE-rich) were targeted peripherally, whereas smaller, hepatically derived β -VLDL were targeted centrally, like LDL. Consistent with this hypothesis, the large β -VLDL showed a higher potential for stimulating ACAT than the smaller β -VLDL. The basis for this difference in intracellular targeting may relate to differences in the apoE content which determines the affinity of these particles for the receptor. This hypothesis was supported by studies in which some of the apoE on the large β -VLDL was "inactivated" by treating with an antibody against apoE. This caused the fate of these particles to be more like that of the smaller β -VLDL particles. Current studies are directed at isolating endosomes containing β -VLDL from macrophages and examining β -VLDL metabolism in intact cells using fluorescent microscopy techniques. The goal is to elucidate the possible functional significance of the unique endocytotic pathway for β -VLDL, with particular emphasis on the activation of ACAT.

5. **Dr. John S. O'Brien** (University of California, San Diego) spoke on "Saposin Proteins and Lipid Degradation." The saposins are a family of heat-stable, low molecular weight glycoproteins that activate lysosomal hydrolases. A common precursor, prosaposin, contains within its sequence four saposin domains and it is proteolytically cleaved to generate saposins A, B, C, and D. Each saposin is made up of about 80 amino acid residues and contains six cysteine residues; the glycosylation sites, proline residues, and hydrophobic residues are well conserved.

Saposins C and A both activate glucocerebrosidase by binding to the enzyme, increasing its V_{max} many-fold. Saposin D stimulates acidic sphingomyelinase. Saposin B acts in a detergent-like manner to activate a number of enzymes, notably arylsulfatase A. Some of the human

lipidoses arise from abnormalities in one or more of these saposins. The structures of the saposins, the molecular cloning of prosaposin cDNA, the nature of the activating peptide sequence of saposin C, and several specific mutations leading to saposin B deficiency were discussed.

6. **Dr. Joseph L. Witztum** (University of California, San Diego) discussed "The Role of Oxidatively Modified LDL in Atherogenesis." It has been proposed that the atherogenicity of plasma LDL can be enhanced by a number of modifications. The best studied of these is oxidative modification, which converts LDL to a form recognized by the "scavenger receptor(s)." Thus uptake of oxidatively modified LDL (Ox-LDL) can lead to the formation of foam cells, the dominant cells in the earliest atherosclerotic lesion (the fatty streak), whereas the uptake of LDL cannot. Ox-LDL has a number of additional properties that make it potentially more atherogenic than native LDL. 1) It is chemotactic for monocytes and thus could recruit cells into the developing lesions. 2) It inhibits the motility of tissue macrophages, a property that might "trap" them in the vessel wall. 3) It can stimulate the rate of release of monocyte chemoattractant protein and of macrophage colony-stimulating factor from endothelial cells. 4) It is cytotoxic, at least to cells in culture. 5) It is antigenic and gives rise to autoantibodies. 6) It stimulates the release of interleukin-1, a chemoattractant and a growth factor for smooth muscle cells.

A number of lines of evidence indicate that oxidative modification of LDL does in fact occur *in vivo*, both in rabbits and in humans but that alone does not implicate it in atherogenesis. The most direct evidence that oxidative modification is relevant comes from studies in which antioxidants have been shown to inhibit the rate of genesis of early atherosclerotic lesions. However, these studies have thus far been conducted almost exclusively in rabbits and with either probucol or butylated hydroxytoluene, an analog of probucol. Additional studies using other antioxidants and using other animal species are needed before the oxidative modification hypothesis can be accepted as proved.

7. **Dr. Sampath Parthasarathy** (University of California, San Diego) discussed "Mechanisms Involved in Oxidative Modification of LDL." LDL exposed to prooxidant conditions undergoes a bewildering array of changes, changes that involve all the major lipid classes of the particle and also the apoprotein. Some of these changes are evident very quickly—within an hour or two of incubation—while others are not evident until oxidation has been extensive. For example, the conversion of lecithin to lysolecithin begins almost immediately, as does the appearance of conjugated dienes in the polyunsaturated fatty acids. On the other hand, conversion to the form recognized by the scavenger receptors may not be evident for 6–24 h. Thus it is important for investigators to better define what they mean by "oxidized LDL" and

specify explicitly the methods used to prepare it.

The mechanisms by which cells oxidize LDL are several. In cultured smooth muscle cells, generation of superoxide anion appears to be of major importance; in endothelial cells and macrophages, on the other hand, the role of superoxide anion seems to be minor. In these cells, lipoxygenases (particularly 15-lipoxygenase) appear to play a major role. Recent *in situ* hybridization studies show that 15-lipoxygenase is strongly expressed in macrophage-rich atherosclerotic lesions but not in normal artery. Expression of the enzyme has also been demonstrated in foam cells harvested from the aortas of cholesterol-fed rabbits (10–12 weeks after balloon injury to the endothelium). While these studies are suggestive, firm conclusions about the relative roles of various oxidative mechanisms must await further studies *in vivo*.

8. **Dr. Robert E. Pitas** (Gladstone Foundation Laboratories, University of California, San Francisco) discussed "Accumulation of Cholesterol in Arterial Smooth Muscle Cells." While most of the cholesterol-loaded foam cells in early lesions of atherosclerosis derive from circulating monocytes, some cholesterol-loaded cells of smooth muscle origin are found also. The generation of foam cells from macrophages is presumed to be due to the acetyl LDL receptor and other scavenger receptors but no such receptor expression has been reported previously for smooth muscle cells. Studies in Dr. Pitas' laboratory have shown that treatment of cultured rabbit and canine smooth muscle cells (SMC) with phorbol myristic acetate (PMA) markedly enhances their rate of uptake of chemically modified LDL (acetoacetylated LDL). PMA stimulation increased the degradation of chemically modified LDL and of oxidized LDL to a similar extent, suggesting that both ligands bind to the same SMC receptor. Competition studies were compatible with this conclusion. Expression of the scavenger receptor was also enhanced by prior incubation with platelet secretion products. Finally, fibroblasts isolated from carrageenan-induced granulomas of rabbits also expressed scavenger receptors, suggesting that the lipid accumulation in these cells may have been due to uptake of modified forms of LDL.

LIPID ACCUMULATION IN LIVER AND PERIPHERAL TISSUES

Chairs: Roger Davis, University of Colorado and Donald M. Small, Boston University

1. **Dr. Steven H. Zeisel** (University of North Carolina) discussed "Fatty Liver, Choline and Diacylglycerol." The point of departure for these studies is the fact that choline deficiency, by making labile methyl groups unavailable, is associated with a greatly increased incidence of hepatocarcinoma in experimental animals. This

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dietary deficiency is also associated with the development of a fatty liver, because choline is needed for lipoprotein secretion. It was postulated that the fatty liver might be associated with the accumulation of 1,2-*sn*-diacylglycerol, which is known to be an activator of protein kinase C. Several lines of evidence implicate protein kinase C-mediated signal transduction. For example, rats fed a choline-deficient diet for 6 or 27 weeks did show increased concentrations of diacylglycerol in the liver and increased protein kinase C activity. It is suggested that choline deficiency, by perturbing protein kinase C-mediated transmembrane signaling, induces a series of events eventually leading to the development of hepatic cancer.

2. **Dr. H. Bryan Brewer, Jr.** (National Heart, Lung, and Blood Institute) discussed "Cholesteryl Ester Storage in Tangier Disease." Most of the cholesteryl ester storage in this disease is found in the reticuloendothelial system (tonsils, spleen, liver, omentum) but some is found also in other tissues (nerve, smooth muscle cells). These patients have extremely low plasma levels of HDL and of apoproteins A-I and A-II. Yet the net flux in plasma is not markedly reduced (because the fractional catabolic rate is extremely high). ApoA-I is secreted in a pro form, but in normal individuals at steady state only 4% of the apoA-I in the plasma is still in the pro form. In contrast, as much as 70% of A-I in patients with Tangier disease is present as the pro form. The catabolic rate of the pro form is an order of magnitude higher than that of the mature form. Which tissues are involved in the degradation of HDL in Tangier patients is not known. It has been suggested that the rate of degradation in macrophages is markedly increased, but studies in Dr. Brewer's laboratory have not been able to confirm that finding.

It has been postulated that the "protective effect" of HDL against atherosclerosis and its complications is related to the role of HDL in reverse cholesterol transport. Why then, with so little HDL, do Tangier patients show little if any increase in atherosclerosis and its complications? One possibility is that they have a low LDL level in addition to a low HDL level. Another possibility is that the flux of HDL apoprotein is almost normal despite the low steady state concentration and so reverse cholesterol transport may be proceeding at a normal rate. Finally, apoprotein A-IV, which is present in relatively normal amounts in patients with Tangier disease, may be the major transport form for reverse cholesterol transport.

3. **Dr. Laura Liscum** (Tufts University) discussed "Defects in Intracellular Cholesterol Transport and Esterification." Studies in her laboratory are directed at identifying cellular factors that facilitate cholesterol movement in vivo. Previous studies showed that the pharmacological agent, U18666A (3- β [2-(diethylamino)ethoxy]androst-5-en-17-one), inhibits the intracellular transport of cholesterol derived from LDL in Chinese hamster ovary cells. This causes accumulation of LDL-derived cholesterol in

the lysosomes, limiting the movement of cholesterol to other cell membranes and delaying LDL-mediated regulation of cellular cholesterol metabolism. One explanation for these findings is that U18666A inhibits the activity or synthesis of a cellular component that is required for cholesterol movement. As a result of impaired LDL cholesterol transport, LDL-dependent growth of CHO cells is also inhibited by U18666A. By selecting for cell growth in the presence of inhibitory concentrations of U18666A, she has identified a CHO cell line, designated U18R, that is resistant to U18666A-inhibition of LDL-cholesterol trafficking. When compared to parental CHO cells, U18R cells are 100-fold more resistant to U18666A inhibition of LDL stimulation of cholesterol esterification. U18R cells are 10-fold more resistant to other aspects of LDL-mediated regulation of cellular cholesterol metabolism. In cell fusion experiments, the U18666A resistance observed in U18R cells displays a dominant phenotype. Perhaps U18666A resistance is due to overexpression of the cellular target of U18666A. Identification of the U18666A-resistance factor may provide important insights regarding intracellular LDL cholesterol regulation and trafficking.

4. **Dr. James Cali** (Southwestern Medical Center, University of Texas) discussed "Sterol 27-Hydroxylase in Relation to Cerebrotendinous Xanthomatosis." Sterol 27-hydroxylase is a mitochondrial cytochrome P-450 enzyme involved in side-chain oxidation of sterols in bile acid biosynthesis. In addition to introducing a hydroxyl group at carbon 27 of several bile acid intermediates, it catalyzes their further oxidation to C₂₇ carboxylic acids. Unlike most enzymes of the bile acid pathways, which are found only in the liver, this enzyme is expressed in all tissues examined. Its extrahepatic function(s) is yet to be determined.

Several lines of evidence indicate that 27-hydroxylase is defective in the sterol storage disorder known as cerebrotendinous xanthomatosis (CTX). Recent studies by Dr. Cali in collaboration with Dr. Chih-Lin Hsieh, Dr. Uta Francke, and Dr. David W. Russell, have identified point mutations in the 27-hydroxylase (CYP27) genes of two CTX patients and the demonstration that the changes significantly impair enzyme function. In keeping with the autosomal recessive inheritance pattern of CTX, the CYP27 gene maps to the q33-qter interval of human chromosome 2.

5. **Drs. Hugo W. and Ann Moser** (Johns Hopkins) discussed "Peroxisomal Diseases." They pointed out that a total of 14 genetically determined peroxisomal disorders have now been identified and their combined incidence is estimated to be greater than 1:25,000. The disorders can be subdivided into two major groups.

In the first group, generalized disorders of peroxisome biogenesis such as the Zellweger Syndrome, the organelle fails to form normally, probably due to defects in the

mechanisms that control the targeting and import of matrix enzymes into it. There are at least six separate complementation groups, suggesting that there are at least six genetic defects that can impair import. Multiple peroxisomal functions are deficient, including an impaired capacity to degrade very long chain fatty acids, pipercolic acid, and phytanic acid and an impaired capacity to synthesize plasmalogens, bile acids, and cholesterol.

The second group comprises seven disorders in which only a single specific peroxisomal enzyme is defective. The most common of these disorders is X-linked adrenoleukodystrophy (ALD), in which there is accumulation of saturated very long chain fatty acids (VLCFA) mainly in the nervous system white matter, the adrenal cortex, and in the testicular Leydig cell. The basic defect appears to involve the enzyme lignoceroyl-CoA ligase. The disorder has been mapped to Xq 28. It is possible to normalize the levels of saturated VLCFA in plasma with a dietary regimen that involves restriction of the intake of VLCFA combined with the administration of glyceryl trioleate and glyceryl trierucate. The chemical effects of this regimen are now being tested in an international trial that involves more than 200 patients.

6. **Dr. Paul M. Coates** (Children's Hospital of Philadelphia) discussed the "Molecular Basis of Mitochondrial Fatty Acid Oxidation Defects." He pointed out that inherited disorders of mitochondrial fatty acid β -oxidation have emerged as commonly recognized causes of episodic diseases resembling Reye Syndrome in early life. Morbidity and mortality associated with these disorders can be high in patients who are undiagnosed or untreated; some patients have died suddenly and unexpectedly in the first two years of life, prompting the diagnosis of sudden infant death syndrome (SIDS). By contrast, patients in whom a defect has been identified and appropriately managed can appear perfectly normal. Of the 20 or so steps known (or suspected) to be involved in fatty acid oxidation, genetic defects have been identified in half. The most common defect, medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, has now been identified in more than 200 patients, almost all of whom are Caucasians of Northern European origin. A single common mutation, $^{985}\text{A} \rightarrow \text{G}$, causes a lys \rightarrow glu substitution at residue 304 of the mature MCAD subunit. Metabolic labeling with [^{35}S]methionine reveals that early post-translational processing and mitochondrial uptake of precursor subunits, and their proteolytic cleavage to mature intramitochondrial subunits, are all normal. However, these mutant subunits are apparently unstable in the mitochondrial matrix; there is no immunoreactive MCAD in mutant cells examined by Western blot analysis. A striking feature of this disorder is the very high frequency of this single mutation (90%) among MCAD-deficient patients. The molecular survey of this mutation may be of great benefit in planning screening strategies for this eminently

treatable disease; it may also provide intriguing insights into the evolution of the mutation.

7. **Dr. Nathan M. Bass** (University of California, San Francisco) discussed "Function and Regulation of Liver Fatty Acid Binding Protein." Liver fatty acid-binding protein (L-FABP) is an abundant, cytoplasmic, 14.2 kDa protein expressed in hepatocytes and intestinal epithelial cells. It is a member of a family of FABP that differ in primary structure, ligand binding properties, and tissue distribution. Recent work from Dr. Bass' laboratory was discussed relating to 1) the evolution of FABP and 2) L-FABP regulation:

1) The primary structure of liver FABP from the nurse shark was determined and shown to bear very close sequence homology (>60%) with the FABP expressed in mammalian heart, myelin, and adipose tissue, and relatively less homology (<30%) with mammalian L-FABP. The similarity between the shark liver and mammalian adipose tissue FABP includes the presence in both of a tyrosine kinase phosphorylation consensus sequence believed to play a role in the transduction of insulin receptor-initiated signals in adipocytes.

2) In studies of L-FABP and peroxisomal fatty acyl oxidase (FACO) regulation in primary cultured hepatocytes, oleate alone induced neither L-FABP nor FACO but markedly induced the mRNA of both in the presence of an inhibitor of carnitine palmitoyl transferase I (CPT1), tetradecylglycidic acid (TDGA). Thus, oleate, in the presence of CPT1 inhibition, induces both L-FABP and FACO pretranslationally, providing support for the hypothesis that impaired or overloaded mitochondrial β -oxidation leads to an adaptive response, viz., increases in peroxisomal β -oxidation enzymes and L-FABP, mediated by long-chain fatty acids or their metabolites.

The mechanism of L-FABP and FACO induction by clofibrate, a peroxisome-proliferating hypolipidemic agent, was also studied in primary cultured hepatocytes. Evidence was presented suggesting a significant role for the products of cytochrome P450 IVA1 (dicarboxylic fatty acids) in the induction of FACO and L-FABP by peroxisome proliferators. A role for fatty acid metabolites other than long chain dicarboxylic acids (the ultimate products of P450 IVA1-mediated ω -hydroxylation) in this process of adaptation also remains a possibility.

8. **Dr. R. Andrew Zoeller** (Boston University School of Medicine) discussed "Mutant Cell Lines Defective in Plasmalogen Biosynthesis." Although plasmalogens make up a large portion of the phospholipid mass in humans, a specific function has yet to be identified for these lipids. In an effort to pinpoint roles that specific lipids serve in cellular processes, lipid-deficient mutants in established cell lines were generated and isolated. A series of plasmalogen-deficient mutants in a macrophage-like, murine cell line (RAW 264.7) were generated. Three mutants (RAW.7, RAW.12, and RAW.108) were found to be defec-

tive in the first step of plasmalogen biosynthesis, the acylation of dihydroxyacetonephosphate (DHAP acyltransferase). All of the mutant strains were different from previously reported, plasmalogen-deficient Chinese hamster ovary cell mutants in that they possessed intact, functional peroxisomes. The RAW.12 strain is unique in that it also lacks the final step in the biosynthesis of plasmenylethanolamine, the desaturation of the fatty alcohol moiety located at the *sn*-1 position (Δ^1 -desaturase). This has been determined by the following criteria: 1) RAW.12 cells

accumulated the precursor of plasmenylethanolamine, plasmanylethanolamine; 2) growth of RAW.12 cells in the presence of 20 μ M 1-hexadecylglycerol did not restore plasmenylethanolamine content; and 3) homogenates of RAW.12 were unable to desaturate plasmanylethanolamine. These cells represent new classes of mutants that will enable us to examine the function of plasmalogens in a responsive cell line without the complication of generalized peroxisomal deficiencies.