Apolipoprotein B synthesized by Hep G2 cells undergoes fatty acid acylation

Jeffrey M. Hoeg, Martha S. Meng, Rosemary Ronan, Stephen J. Demosky, Jr., Thomas Fairwell, and H. Bryan Brewer, Jr.

Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Abstract Apolipoprotein B is the principal protein associated with cholesterol transport in the blood and has been proposed to play a central role in human atherogenesis. The unique hydrophobic nature of this large (512 kDa), glycosylated apolipoprotein differs from that of the other apolipoproteins. Since another apolipoprotein, apolipoprotein A-I, has been recently shown to have covalently bound fatty acids, potential fatty acid acylation of apolipoprotein B was investigated. The human hepatoma cell line, Hep G2, synthesizes apoB-100 and secretes the apolipoprotein into the culture medium. After a 24-hr incubation with [^{14}C]palmitate and [^{14}C]stearate, the label was incorporated into apoB-100 when assessed by a sodium dodecyl sulfate polyacrylamide gel electrophoresis, autoradiography, immunoblot analysis, and immunoprecipitation. Hydroxylamine treatment, which hydrolyzes ester and thioester bonds, removed the radiolabel. ApoB-100 isolated from Hep G2 cells by ultracentrifugation and preparative sodium dodecyl sulfate gel electrophoresis was hydrolyzed and analyzed by gas–liquid chromatography–mass spectrometry. In contrast to circulating apoB in low density lipoproteins, both palmitate and stearate were present in newly synthesized apoB-100. These results establish that newly synthesized apoB-100 undergoes covalent acylation with palmitate and stearate. The acylation of apoB may play an important role in lipoprotein particle secretion. In addition, derangements in apoB fatty acid acylation may lead to dyslipoproteinemia.—**Hoeg, J. M., M. S. Meng, R. Ronan, S. J. Demosky, Jr., T. Fairwell, and H. B. Brewer, Jr.** Apolipoprotein B synthesized by Hep G2 cells undergoes fatty acid acylation. *J. Lipid Res.* 1988. **29:** 1215–1220.

Supplementary key words low density lipoprotein • lipoprotein secretion

The human apolipoproteins have been shown to undergo several cotranslational and posttranslational modifications including proteolytic cleavage (1–4), glycosylation (5–8), and deamidation (9). These structural alterations may play physiologic as well as pathophysiologic roles. Another covalent modification of apolipoprotein structure that has been evaluated involves covalent attachment of fatty acid to the apolipoprotein. In an effort to understand the hydrophobic nature of the proteins associated with lipoproteins, Fisher and Gurin (10) reported that long chain fatty acids were associated with plasma lipoprotein that floated with a density < 1.21 g/ml. However, the identification of the amphipathic helix and its proposed role in apolipoprotein binding to the aqueous-lipid interface of lipoprotein (11, 12) focused attention upon the primary structure of the apolipoproteins. Using the human hepatoma cell line Hep G2, we recently demonstrated that newly secreted apolipoprotein A-I is covalently acylated with palmitate (13). Since apoB-100 is the principal apolipoprotein associated with circulating plasma cholesterol (14), a study of potential fatty acid acylation in newly secreted apoB-100 as well as circulating apoB-100 was undertaken.

EXPERIMENTAL PROCEDURES

Cell culture

Investigation of nascent apolipoprotein structure was conducted utilizing the hepatoma cell line Hep G2, a gift from Dr. B. Knowles and Dr. D. Aden (15). Cells were maintained in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (v/v), at 37°C in a humidified incubator in an atmosphere of 5% CO_2, 95% air. To isolate nascent apoB-100, preconfluent Hep G2 cells were incubated in a 150-cm^2 flask (Falcon) for 20–24 hr in unsupplemented, serum-free Eagle's minimum essential medium with 50 uCi of one of the following products from New England Nuclear: [U-^{14}C]palmitate (800 mCi/mmol), [1-^{14}C]stearate (60 mCi/mmol), [1-^{14}C]oleate (60 mCi/mmol), or [1-^{14}C]myristate (60 mCi/mmol). In control experiments, serum-free media aliquots were harvested after a 24-hr incubation with Hep G2 cells. These media were then incubated in the presence of 50 uCi of [U-^{14}C]palmitate for 24 hr in a 37°C incubator as previously described except that the exposure of the media to the palmitate was

Abbreviations: apoB, apolipoprotein B; $NaDodSO_4$, sodium dodecyl sulfate; LDL, low density lipoproteins; PAGE, polyacrylamide gel electrophoresis.

in the absence of cells. The apoB was immunoprecipitated from the media and the samples wre processed as outlined below.

Harvested media were chilled to 4°C and the following enzyme inhibitors were added to the medium (final concentration): Nα-*p*-tosyl-L-lysine methylester (30 mg/l), phenylmethylsulfonyl fluoride (30 mg/l), metrizimide (30 mg/l), 2-nitro-4-carboxyphenyl N, N,-diphenylcarbamate (30 mg/l), *p*-aminobenzyl-1-thio-2-acetamido-2-deoxy 1-D-glucopyranoside (30 mg/l), 1-10-phenanthroline (15 mg/l), amino-n-caproic acid (15 mg/l), benzamidine (15 mg/l), and dimethylsulfoxide (0.5%; v/v).

Apolipoprotein isolation

The harvested media were adjusted to a density of 1.25 g/ml with potassium bromide and the lipoproteins were separated from other media proteins by ultracentrifugation for 48 hr (4°C) at 100,000 *g*. LDL was isolated from plasma between d 1.030 and 1.050 g/ml by preparative ultracentrifugation (16). Lipoproteins were dialyzed against at least 400 volumes of distilled water. After lyophilization, the lipoproteins were delipidated with five separate extractions of chloroform–methanol 2:1 and apoB-100 was isolated by preparative $NaDodSO_4$-polyacrylamide gel electrophoresis.

Characterization of apoB acylation

Delipidated samples were solubilized using 1% $NaDodSO_4$, 0.1 M Tris-HCl (pH 6.8), 10% sucrose, followed by incubation of the sample for 3 min at 100° C. Samples were applied to a 5% $NaDodSO_4$ polyacrylamide gel and electrophoresis was performed as previously described (17, 18). Standards for apoB-100 and apoB-48 were obtained from a patient with Type III hyperlipoproteinemia (18). ApoB-100 and apoB-48 standards for autoradiography were iodinated using the iodine monochloride method as adapted for apolipoproteins (19). After electrophoresis, gels were either stained with Coomassie brilliant blue R-250 or the protein was transferred to modified nylon paper (Nytran: Schleicher and Schuell, Keene, NH). The paper was applied to Kodak XAR-5 film and fluorography was performed from 6 to 10 days at −70°C. Following fluorography, the nylon paper containing the radiolabeled sample was subjected to immunoblot analysis as outlined by Towbin, Staehlin, and Gordon (20) utilizing 15 μl of polyclonal rabbit-antihuman apoB serum in 30 ml of incubation buffer. This was followed by a 5-hr, 25°C incubation with ^{125}I-labeled protein A (1 μl:500 μl) (100 μCi/μg; New England Nuclear). The radiolabeled nylon paper was then reapplied to Kodak XAR-5 film at room temperature for 2 hr prior to development. ApoB-100 radiolabeled with fatty acids was deacylated after $NaDodSO_4$-polyacrylamide gel electrophoresis by hydroxylamine treatment based upon the method of Omary and Trowbridge (21).

Immunoprecipitation of apoB from the lyophilized [^{14}C]palmitate- and [^{14}C]stearate-labeled Hep G2 lipoproteins was performed after dissolving the samples in 80 μl of an incubation buffer containing 0.3% Nonidet P-40 (Sigma), 0.1% $NaDodSO_4$, 0.3% sodium deoxycholate, 0.15 M NaCl, and 0.01 M Tris-HCl (pH 7.4). Rabbit anti-human apoB antisera (25 μl) and 1 μg of human LDL apoB standard which served as carrier protein were both added to the 80 μl containing the solubilized sample. After an initial 2-hr, 37°C incubation, the samples were incubated for an additional 12 hr at 4°C. The samples were then centrifuged at 2,000 *g* for 10 min at 4°C. After removing the supernatant, the pellets were suspended in 100 μl of the incubation buffer and the sample was recentrifuged at 2,000 *g* for 10 min at 4°C. The washed protein pellets were analyzed by 5% $NaDodSO_4$ gel electrophoresis and fluorography as outlined above.

The number of moles of fatty acid per mole of apoB was determined by assessing the degree of [^{14}C]palmitate incorporation (sp act 850 mCi/mmol) into the apoB band present after electrophoresis in a 5% $NaDodSO_4$ polyacrylamide gel. After the apoB immunoprecipitate from the media was separated by electrophoresis, the gel was stained and scanned using a laser scanning densitometer (LKB Ultrascan XL, Uppsala, Sweden). The peak height and area of the densitometrograms of the samples were compared to the density of the bands of stained apoB standards that ranged in concentration from 0.5 to 4 μg and the protein concentration within the band was calculated. The stoichiometry was then determined by slicing the apoB band from the gel, quantitating the radioactivity within the band, and then calculating the moles of fatty acid as reflected in the degree of radioactivity in the band to the moles of apoB present in the band assuming a molecular mass of 512 kDa for apoB.

For mass spectrometric analysis, 100 μg of Hep G2 apoB was isolated by $NaDodSO_4$ gel electrophoresis and a methyl arachidate internal standard was added to the sample. After a 16-hr hydrolysis with 6 N HCl, the hydrolysate was extracted with n-hexane. The hexane extract was dried and esterified at 70°C for 20 min with methanolic HCl (Applied Sciences Lab, Deerfield, IL). The fatty acid methyl ester content of the extract was then evaluated on a Finnigan 4510 GLC–MS system equipped with a 30-m fused-silica (SE-54) capillary column, and the methyl arachidate peak was defined as 100% of the relative ion current (22).

RESULTS

The preconfluent Hep G2 cells secrete apoB-100 but not apoB-48 as is evident from the Coomassie blue-stained $NaDodSO_4$ gel electrophoretogram (**Fig. 1**). Therefore, like normal human liver (23), only the larger molecular weight

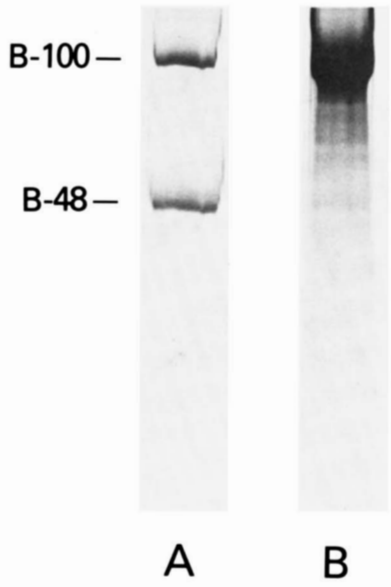

Fig. 1. The secretion of apolipoprotein B by Hep G2 cells. Hep G2 cells were incubated for 24 hr in serum-free medium. The lipoproteins secreted into the medium were isolated by preparative ultracentrifugation (d < 1.25 g/ml), and the apolipoproteins were analyzed by 5% $NaDodSO_4$-PAGE. Hep G2 cells secrete an apolipoprotein that comigrates with the apoB-100 control. No band corresponding to apoB-48 was observed.

species is present in the tissue culture media that were evaluated. Cells that were labeled for 24 hr with [^{14}C]palmitate and [^{14}C]stearate secreted apoB-100-containing lipoproteins that contained the radioactive tracers (**Fig. 2**, lanes B and C) and comigrated with isolated ^{125}I-labeled LDL (Fig. 2, lane A). When parallel unlabeled samples were analyzed by apoB antisera and protein A, Hep G2-secreted apoB (Fig. 2, lane E) comigrated with plasma LDL apoB (lane D). However, incubations with radiolabeled myristate and oleate were not incorporated into apoB. Therefore, Hep G2-secreted apoB-100 incorporated both [^{14}C]palmitate and [^{14}C]stearate labels during a 24-hr incubation and this incorporation showed some degree of specificity for the fatty acid moiety.

The studies with radiolabeled fatty acids also indicated the stoichiometry of fatty acid acylation of apoB secreted by Hep G2 cells. There were 3.2 moles of palmitate incorporated into 1 mole of apoB, assuming that all the radioactivity was in the form of palmitate and that the molecular mass of apoB is 512 kDa. However, only 1% of the radioactivity within palmitate was incorporated into apoB when the fatty acid was incubated for 24 hr at 37°C with media that had been preconditioned by Hep G2 cells compared to directly incubating the cells with the radiolabeled material. Therefore, the acylation process is discrete and is mediated by Hep G2 cells.

Hydroxylamine treatment of radiolabeled Hep G2 apoB-100 released the radioactivity from the protein (**Fig. 3**). Since alkaline hydroxylamine treatment cleaves ester and thioester bonds, it has been used to establish the presence of acyl bonds (21). Under these conditions, hydroxylamine cleaved the radiolabeled fatty acid from apoB-100 (Fig. 3, lane 2), indicating that Hep G2 cells incubated with [^{14}C]palmitate secrete an apoB-100 that is esterified with the radiolabeled fatty acid.

The location of fatty acid acylation on the amino acid backbone remains to be identified. Using purified thrombin (Dr. J. W. Fenton II, Albany, NY), the four standard thrombin fragments of apoB were identified (24). However, none of the radiolabeled palmitate remained with any of the thrombolytic fragments. The labile ester or thioester bonds may be susceptible to thrombolysis. The precise localization of the acylation will require new methods of ester bond stabilization or a form of labeling the hydrolysis product on the hydroxyl group of the amino acids, since standard methods used in hydrolyzing peptide bonds will also cleave the labeled fatty acid from the serine, threonine, or tyrosine residues that are likely to participate in fatty acid acylation.

To confirm the nature of the esterified side chain, mass spectroscopic analysis of Hep G2 apoB-100 was undertaken. Both palmitate and stearate were present in Hep G2 apoB-100 purified by $NaDodSO_4$ gel electrophoresis (**Fig. 4**, peaks 1 and 2, respectively). The other peaks on the chromatograph shown in Fig. 4 include the solvent front (peak at 3:26 min) and non-fatty acid contaminants eluted from the gels during apoB isolation (peaks at 7:10, 9:50, and 12:40 min). However, apoB-100 from plasma LDL (d < 1.050, > 1.030 g/ml) isolated by our methods including preparative $NaDodSO_4$ gel electrophoresis did not appear to be fatty acid-acylated.

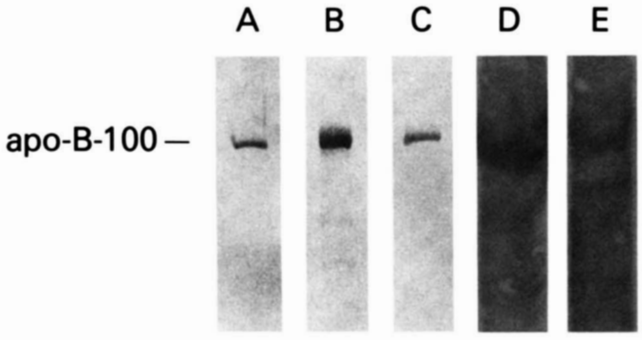

Fig. 2. The secretion of ^{14}C-labeled apoB-100 after incubation with [^{14}C]palmitate and [^{14}C]stearate. After a 24-hr incubation with radiolabeled fatty acid, media lipoproteins were isolated by ultracentrifugation and subjected to lyophilization, delipidation, and $NaDodSO_4$-PAGE as outlined in Methods. Proteins in the gel were transferred to nylon paper. Lane A illustrates an autoradiograph of ^{125}I-labeled LDL on the paper, while the lanes B and C reflect [^{14}C]palmitate- and [^{14}C]stearate-labeled apoB, respectively, from Hep G2 cells. Subsequent immunoblot analyses of unlabeled LDL and Hep G2 lipoproteins are illustrated in lanes D and E, respectively. These results indicate that the apoB-100 secreted from Hep G2 cells is radiolabeled.

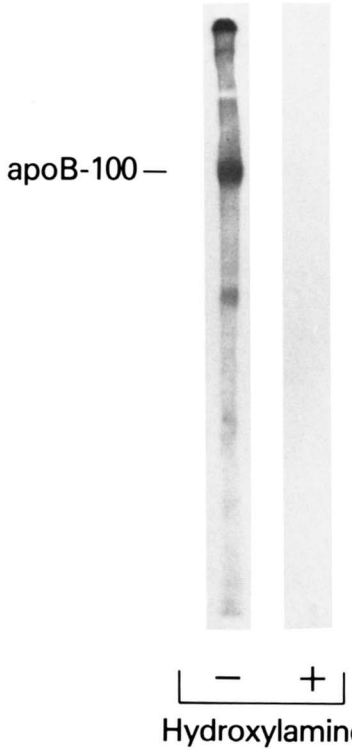

Fig. 3. The effect of hydroxylamine treatment on the labeling of ^{14}C-labeled apoB. ApoB isolated from Hep G2 cells radiolabeled with [^{14}C]palmitate underwent NaDodSO$_4$-PAGE and autoradiography either without (lane A) or with (lane B) exposure to hydroxylamine. Hydroxylamine removal of the radioactivity indicates that the interaction of the ^{14}C label with the protein was through an ester or a thioester linkage.

DISCUSSION

Interest in covalent fatty acid acylation of proteins has grown since the first reports of viral coat protein acylation in 1979 (25–27). Subsequently, a wide variety of eukaryotic cellular membrane proteins have also been shown to covalently incorporate fatty acids (21, 28–37). The apolipoproteins resemble cellular membrane proteins since they also reside at aqueous-lipid interfaces (38) and are noncovalently associated with lipids (39). Although the physiological role for covalent fatty acid acylation remains elusive, the fatty acids have been proposed to serve as a hydrophobic anchor (40). The data in the present study indicate that like apoA-I and apoE (13), newly secreted apoB-100 is covalently acylated with fatty acids. Both palmitate and stearate are present on apoB-100 and they can be removed with hydroxylamine treatment. However, unlike a previous report (10), the apoB-100 present in circulating LDL is not acylated with fatty acids. These findings indicate that fatty acid acylation may play a role in intracellular lipoprotein synthesis and assembly or possibly in intracellular apolipoprotein trafficking. Fisher and Gurin (10) were the first to suggest that apolipoproteins may be covalently bound to fatty acids. However, we were unable to confirm their finding of fatty acids attached to circulating apolipoproteins. Exhaustive delipidation was performed and this eliminated all fatty acid peaks from GLC–mass spectroscopic analyses. The current findings, therefore, indicate that only newly secreted apolipoproteins are fatty acid-acylated. Huang, Lee, and Singh have also just reported the incorporation of palmitate and stearate into apoB (41). Their data indicate the presence of a thioester bond.

The possible functions of fatty acid acylation of the apolipoproteins are numerous. Acylation may be important for initial association of apolipoproteins with the hydrophobic lipids during particle assembly. Interaction of the fatty acids with cellular cytoskeletal elements could facilitate intracellular transport of the nascent particles to the cell surface.

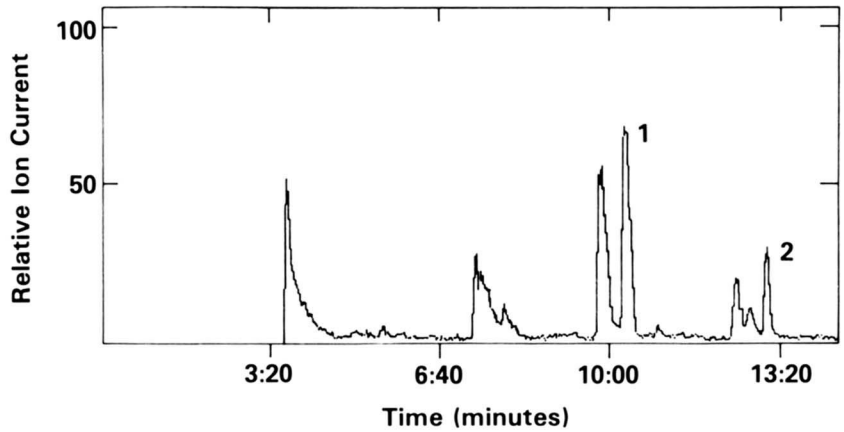

Fig. 4. For gas–liquid chromatography–mass spectrometry analysis, 100 μg of delipidated apoB isolated from Hep G2 cell media was separated by NaDodSO$_4$-PAGE and apoB-100 was eluted from the gel. After acid hydrolysis and n-hexane extraction, the sample was esterified with methanolic HCl. Peaks at 10:20 (peak 1) and 13:00 (peak 2) min represent fatty acids that coelute with palmitate and stearate standards, respectively.

In addition, these studies raise the possibility that apolipoproteins could contain diacylglycerol moieties that could serve as cellular signal transducers (42). Further studies directed toward these hypotheses may lead to an understanding of the normal physiologic role(s) of apolipoprotein fatty acid acylation as well as a mechanism for the development of inborn errors of apolipoprotein metabolism.

We thank Dr. Henry Krutzsch for his helpful suggestions and discussions and Ms. Joanie Mok Gault for her help in the preparation of the manuscript.

Manuscript received 1 February 1988 and in revised form 13 April 1988.

REFERENCES

1. Gordon, J. I., H. F. Sims, S. R. Lentz, C. Edelstein, A. M. Scanu, and A. W. Strauss. 1983. Proteolytic processing of human preproapolipoprotein A-I: a proposed defect in the conversion of proA-I to A-I in Tangier's disease. *J. Biol. Chem.* **258:** 4037–4044.
2. Stoffel, W., E. Kruger, and R. Deutzmann. 1983. Cell-free translation of human liver apolipoprotein A-I and A-II mRNA: processing of primary translation products. *Hoppe-Seyler's Z. Physiol. Chem.* **364:** 227–237.
3. Zannis, V. I., S. K. Karathanasis, H. Keutmann, G. Goldberger, and J. L. Breslow. 1983. Intracellular and extracellular processing of human apolipoprotein A-I: secreted apolipoprotein A-I isoprotein 2 is a propeptide. *Proc. Natl. Acad. Sci. USA* **80:** 2574–2578.
4. Bojanovski, D., R. E. Gregg, and H. B. Brewer, Jr. 1984. Tangier disease: in vitro conversion of proapoA-I Tangier to mature apoA-I Tangier. *J. Biol. Chem.* **259:** 6049–6051.
5. Swaminathan, N., and F. Aladjem. 1976. The monosaccharide composition and sequence of the carbohydrate moiety of human serum low density lipoproteins. *Biochemistry.* **15:** 1516–1522.
6. Lee, P., and W. C. Breckenridge. 1967. The carbohydrate composition of human apo low density lipoprotein from normal and type II hyperlipoproteinemic subjects. *Can. J. Biochem.* **54:** 42–49.
7. Brewer, H. B., Jr., R. Shulman, P. Herbert, R. Ronan, and K. Wehrly. 1974. The complete amino acid sequence of alanine apolipoprotein (apoC-III), an apolipoprotein from human plasma very low density lipoproteins. *J. Biol. Chem.* **249:** 4975–4984.
8. Zannis, V. I., and J. L. Breslow. 1981. Human very low density lipoprotein E isoprotein polymorphism is explained by genetic variation and post-translational modification. *Biochemistry.* **20:** 1033–1041.
9. Ghiselli, G., M. F. Rohde, S. Tanenbaum, S. Krishnan, and A. M. Gotto. 1985. Origin of apolipoprotein A-I polymorphism in plasma. *J. Biol. Chem.* **29:** 15662–15668.
10. Fisher, W. R., and S. Gurin. 1964. Structure of lipoproteins: covalently bound fatty acids. *Science.* **143:** 362–363.
11. Segrest, J. P., R. L. Jackson, J. D. Morrisett, and A. M. Gotto. 1974. A molecular theory of lipid-protein interactions in the plasma lipoproteins. *FEBS Lett.* **38:** 247–253.
12. Assmann, G., and H. B. Brewer, Jr. 1974. Lipid-protein interactions in high density lipoproteins. *Proc. Natl Acad. Sci. USA.* **71:** 989–993.
13. Hoeg, J. M., M. S. Meng, R. Ronan, T. Fairwell, and H. B. Brewer, Jr. 1986. Human apolipoprotein A-I: post-translational modification by fatty acid acylation. *J. Biol. Chem.* **261:** 3911–3914.
14. Osborne, J. C., and H. B. Brewer, Jr. 1977. The plasma lipoproteins. *Adv. Protein Chem.* **31:** 253–337.
15. Knowles, B. B., C. C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science.* **209:** 497–499.
16. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
17. Gabelli, C., D. G. Stark, R. E. Gregg, and H. B. Brewer, Jr. 1986. Separation of apolipoprotein B species by agarose-acrylamide gel electrophoresis. *J. Lipid Res.* **27:** 457–460.
18. Meng, M. S., R. E. Gregg, E. J. Schaefer, J. M. Hoeg, and H. B. Brewer, Jr. 1983. Presence of two forms of apolipoprotein B in patients with dyslipoproteinemia. *J. Lipid Res.* **24:** 803–809.
19. Bilheimer, D. L., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
20. Towbin, H., T. Staehlin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* **76:** 4350–4354.
21. Omary, M. B., and I. S. Trowbridge. 1981. Covalent binding of fatty acids to the transferrin receptor in cultured human cells. *J. Biol. Chem.* **256:** 4715–4718.
22. Ryhage, R., and E. Stenhagen. 1960. Mass spectrometry in lipid research. *J. Lipid Res.* **1:** 361–390.
23. Edge, S. B., J. M. Hoeg, P. D. Schneider, and H. B. Brewer, Jr. 1985. Apolipoprotein B synthesis in man: liver synthesis only apolipoprotein B-100. *Metabolism.* **34:** 726–730.
24. Cardin, A. D., K. R. Witt, J. Chao, H. J. Margolius, V. H. Donaldson, and R. L. Jackson. 1984. Degradation of apolipoprotein B-100 of human plasma low density lipoproteins by tissue and plasma kallikreins. *J. Biol. Chem.* **259:** 8522–8528.
25. Schmidt, M. F. G., and M. J. Schlesinger. 1979. Fatty acid binding to vesicular stomatitis virus glycoprotein: a new type of post-translational modification of the viral glycoprotein. *Cell.* **17:** 813–819.
26. Schmidt, M. F. G., M. Bracha, and M. J. Schlesinger. 1979. Evidence for covalent attachment of fatty acids to Sindbis virus glycoproteins. *Proc. Natl. Acad. Sci. USA.* **76:** 1687–1691.
27. Henderson, L. E., H. C. Krutzsch, and S. Droszlan. 1983. Myristyl amino-terminal acylation of murine retrovirus proteins: an unusual post-translational protein modification. *Proc. Natl. Acad. Sci. USA.* **80:** 339–343.
28. Carr, S. A., K. Biemann, S. Shoji, D. C. Parmelee, and K. Titani. 1982. *n*-Tetradecanonoyl is the NH_2-terminal blocking group of the catalytic subunit of cyclic AMP-dependent protein kinase from bovine cardiac muscle. *Proc. Natl. Acad. Sci. USA.* **79:** 6128–6131.
29. Schlesinger, M. J., A. I. Magee, and M. F. G. Schmidt. 1980. Fatty acid acylation of proteins in cultured cells. *J. Biol. Chem.* **255:** 10021–10024.
30. Aitken, A., P. Cohen, S. Santikarn, D. H. Williams, A. G. Calder, A. Smith, and C. B. Klee. 1982. Identification of the NH_2-terminal blocking group of calcineurin B as myristic acid. *FEBS Lett.* **150:** 314–318.
31. Olson, E. N., L. Glaser, and J. P. Merlie. 1984. α And β subunits of the nicotinic acetylcholine receptor contain covalently bound lipid. *J. Biol. Chem.* **259:** 5364–5367.

32. O'Brien, P. J., and M. Zatz. 1984. Acylation of bovine rhodopsin by [^{3}H]palmitic acid. *J. Biol. Chem.* **259:** 5054–5057.
33. Kaufman, J. F., M. S. Krangel, and J. L. Strominger. 1984. Cysteines in the transmembrane region of major histocompatibility complex antigens are fatty acid acylated via thioester bonds. *J. Biol. Chem.* **259:** 7230–7238.
34. Berger, M., and M. F. G. Schmidt. 1984. Cell-free fatty acid acylation of Semliki forest viral polypeptides with microsomal membranes from eukaryotic cells. *J. Biol. Chem.* **259:** 7245–7252.
35. Bolanowski, M. A., B. J. Earles, and W. J. Lennarz. 1984. Fatty acylation of proteins during development of sea urchin embryos. *J. Biol. Chem.* **259:** 4934–4940.
36. Olson, E. N., D. A. Towler, and L. Glaser. 1985. Specificity of fatty acid acylation of cellular proteins. *J. Biol. Chem.* **260:** 3784–3790.
37. Olson, E. N., and G. Spizz. 1986. Fatty acid acylation of cellular proteins: temporal and subcellular differences between palmitate and myristate acylation. *J. Biol. Chem.* **261:** 2458–2466.
38. Brewer, H. B., Jr. 1981. Current concepts of the molecular structure and metabolism of human apolipoproteins and lipoproteins. *Klin. Wochschr.* **59:** 1023–1035.
39. Gofman, J. W., F. Glazier, A. Tomplin, B. Strisower, and O. De Lalla. 1954. Lipoproteins, coronary heart disease, and atherosclerosis. *Physiol. Rev.* **34:** 589–607.
40. Huang, L. 1985. Incorporation of acylated antibody into planar lipid multilayers: characterization and cell binding. *Biochemistry.* **34:** 29–34.
41. Huang, G., D. M. Lee, and S. Singh. 1988. Identification of the thiol ester-linked lipids in apolipoprotein B. *Biochemistry.* **27:** 1395–1400.
42. Low, M. G., and A. R. Saltiel. 1988. Structural and functional roles of glycosylphosphatidylinositol in membranes. *Science.* **239:** 268–275.