Clearance of Acetyl Low Density Lipoprotein by Rat Liver Endothelial Cells

IMPLICATIONS FOR HEPATIC CHOLESTEROL METABOLISM*

(Received for publication, February 2, 1984)

Rune Blomhoff§, Christian A. Drevon†, Winnie Eskildt, Per Helgerud†, Kaare R. Norum‡, and Trond Berg‡

From the §Institute for Nutrition Research, School of Medicine and the †Department of Pharmacology, Institute of Pharmacy, University of Oslo, Norway

We have studied the hepatic uptake of human [14C]cholesteryl oleate labeled acetyl low density lipoprotein (LDL). Acetyl-LDL injected intravenously into rats was cleared from the blood with a half-life of about 10 min. About 80% of the injected acetyl-LDL was recovered in the liver after 1 h. Initially, most of the [14C]cholesterol was recovered in liver endothelial cells (about 60%). Some radioactivity (about 15%) was also recovered in the hepatocytes, while the Kupffer cells and stellate cells contained only small amounts of the label (less than 5%).

About 1 h after injection, radioactivity started to disappear from endothelial cells and appeared instead in hepatocytes. Radioactivity subsequently declined in hepatocytes as well. After a lag phase of 4 h, significant amounts of radioactivity were recovered in bile.

The in vitro uptake and hydrolysis of [14C]cholesteryl oleate-labeled acetyl-LDL were saturable in isolated rat liver endothelial cells. Native LDL does neither affect the uptake nor the hydrolysis of acetyl-LDL. Ammonia and monensin reduced the hydrolysis of acetyl-LDL in isolated liver endothelial cells. Furthermore, monensin at concentrations above 10 μM completely blocked the binding of acetyl-LDL to the liver endothelial cells, suggesting that the receptor for acetyl-LDL is trapped inside the cells.

The liver endothelial cells may be involved in the protection against atherogenic lipoproteins, e.g. liver endothelial cells may mediate uptake of cholesterol from plasma and transfer of cholesterol to the hepatocytes for further secretion into the bile.

LDL that has been reacted with acetic anhydride in vitro to form acetyl-LDL is taken up by macrophages from different sources and species by receptor-mediated endocytosis (for review see Ref. 1). Normal tissue macrophages express on the other hand few if any receptors for native LDL. Acetyl-LDL does not bind to the LDL receptor (2). Several ligands may exist in vivo for the acetyl-LDL receptor. For instance, LDL incubated with aortic endothelial cells (3) or malondialdehyde-modified LDL obtained after interactions between blood platelets and LDL (4) may be taken up by the same receptor as acetyl-LDL. Fogelman et al. (5) have reported that lymphocyte cultures may produce a substance which competes for the acetyl-LDL receptor. Furthermore, several modified proteins with increased negative charge (e.g. formaldehyde-treated albumin) may also be endocytosed via the receptor for modified LDL (6, 7).

The number of receptors for native LDL on nonmacrophage cells is suppressed when large doses of LDL are taken up by the cells. In contrast, the acetyl-LDL receptor remains constant in number even when the macrophages have accumulated massive amounts of cholesterol (8). Hence, macrophages may be converted into foam cells. This may have important implications for foam cell formation during the development of atherosclerosis.

In addition to macrophages, also cultured bovine aortic endothelial cells express acetyl-LDL receptors and degrade 125I-labeled acetyl-LDL (9).

Goldstein et al. (8) have shown that shortly after intravenous injection of 125I-acetyl-LDL into mice, most of the dose is recovered in liver. Moreover, Mahley et al. (10) reported that the nonparenchymal liver cells were far more active than the hepatocytes. In accordance with this, formaldehyde-treated albumin is also taken up by nonparenchymal liver cells (11–13). We recently found that formaldehyde-treated albumin was mainly removed by a subgroup of the nonparenchymal liver cells, the endothelial cells (6). Formaldehyde-treated albumin and acetyl-LDL are probably taken up via the same receptor (6, 7). Hence, liver endothelial cells may be involved in the protection against the atherogenic action of modified lipoproteins.

The aim of the present study was to determine the role of the different types of liver cells in the catabolism of acetyl-LDL. In particular, we wanted to trace the fate of cholesterol in the acetyl-LDL particle.

MATERIALS AND METHODS

Chemicals—[4-14C]Cholesteryl oleate (59.4 mCi/mmol) was obtained from New England Nuclear, and [U-14C]glucose (552 mCi/mmol) was obtained from Amersham International, England. Collagenase and Pronase were obtained from Sigma; enterotoxin and Nycodenz were purchased from P. A. Grunam, Norwegian Food Research Institute, As, Norway, and Nyegaard & Company A/S, Norway, respectively. Fluorescein amine-conjugated ovalbumin was donated by B. Smedsrd, Biomedicum, Uppsala, Sweden.

Preparation of Lipoproteins—LDL (density, 1.020–1.050 g/ml) was obtained from fasted human plasma and prepared by differential
Labeling of LDL with [14C]Cholesteryl Oleate and [14C]Sucrose—Acetylated LDL was labeled with [14C]cholesteryl oleate in the following way. Two portions of 50 μl (0.42 μmol) of [14C]cholesteryl oleate in acetone were added to 0.5 ml of a human plasma fraction (density more than 1.24 g/ml) containing the cholesteryl ester transfer protein (15). Following reconstitution of the acyltransferase with NAD, the solution was incubated at 37°C for 10 min. 100 μl of acetylated LDL (600 μg of LDL-protein) were added, and this mixture was incubated for 6-7 h at 37°C. The LDL fraction was then reisolated by ultracentrifugation and dialyzed for 24 h at 4°C against 3 x 4 liters of a 0.05 M phosphate buffer, pH 7.4, containing 0.15 M NaCl and 0.3 mM EDTA. About 90% of the originally added [14C]radioactivity was recovered in the final LDL preparation. 98.2 ± 0.5% (S.D.) (N = 5) of the 14C radioactivity in the labeled acetylated-LDL was present as cholesteryl ester and less than 2% as unesterified cholesterol. The specific activity of the [14C]cholesteryl oleate acetylated-LDL was 25-50 μCi/mg of protein, corresponding to about 50 μCi/mg of total cholesterol (16, 17). Acetylated LDL was labeled with [14C]sucrose by the procedure described by Pittman et al. (18).

Preparation of Hepatocytes—Total liver cell suspensions were prepared by a collagenase perfusion technique (19) from male Wistar rats (250-300 g). Hepatocytes were isolated from the total liver cell suspension by differential centrifugation (20). About 98% of the isolated hepatocytes were viable as determined by the trypan blue exclusion test. The hepatocyte suspensions were contaminated with 1.1 ± 1.9% (S.D.) (N = 7) endothelial cells, 4.6 ± 5.1% (N = 7) Kupffer cells, and 18.1% (S.D.) (N = 7) stellate cells. Endothelial cells, Kupffer cells, and stellate cells were identified as described later. Assuming that the hepatocytes and the nonparenchymal liver cells contain 1.6 mg of protein/106 cells and 0.14 mg of protein/106 cells (21), respectively, less than 2% of the protein in the hepatocyte fraction represents nonparenchymal cell protein.

Preparation of Liver Endothelial Cells—Total liver cell suspensions were treated with enterotoxin from Clostridium perfringens as described earlier (22, 23). The enterotoxin made the hepatocytes leaky, and these cells were separated from the nonparenchymal cells by centrifugation in a solution containing 20% (w/v) Nycodenz. Endothelial cells were separated from the other nonparenchymal liver cells by centrifugal elutriation (23). The endothelial cells became highly fluorescent following incubation with fluorescein amine-conjugated ovalbumin, which is selectively taken up by these cells (24). None of the endothelial cells showed positive cytochemical peroxidase reaction (25). The endothelial cell fractions were contaminated with 2.7 ± 1.6% (S.D.) (N = 5) Kupffer cells and 7.6 ± 5.8% (S.D.) (N = 5) stellate cells.

Preparation of Kupffer Cells—Nonparenchymal liver cells were prepared from the total liver cell suspension by incubation with Pronase (26) or enterotoxin (23), both of which destroy the hepatocytes. The Kupffer cells were purified further by centrifugal elutriation (23, 25). Kupffer cells were identified cytochemically by positive peroxidase reaction (25). The Kupffer cell fraction isolated by the Pronase method was contaminated with 0.2 ± 0.3% (S.D.) (N = 6) hepatocytes, 5.1 ± 0.6% (S.D.) (N = 6) endothelial cells, and 0.5 ± 1.6% (S.D.) (N = 6) stellate cells, and the Kupffer cell fraction isolated by the enterotoxin method was contaminated with 29.4 ± 6.8% (S.D.) (N = 6) endothelial cells and 13.9 ± 4.1% (S.D.) (N = 4) stellate cells.

Preparation of Stellate Cells—Nonparenchymal liver cells were prepared from the total liver cell suspension by differential centrifugation (25). The stellate cells were isolated further by Percoll density gradient centrifugation (27). The fractions with densities between 1.025 and 1.035 g/ml contained only stellate cells. The stellate cells were identified by fluorescence microscopy, due to their content of retinyl ester (27).

Cellular Uptake and Binding of Acetyl-LDL—Suspensions of liver endothelial cells (2-5 x 10⁶ cells/ml) were incubated in Erlenmeyer flasks in a minimal salt solution (20) containing 1% albumin. To assess cell-associated radioactivity, aliquots were withdrawn and cells were separated from the medium by centrifuging the cells through a 90% mixture of dibutyl phthalate and dinonyl phthalate (5:1) (11).

Analytical Procedures—Free and esterified [14C]cholesterol were analyzed by thin layer chromatography (28) after lipid extraction (29). Protein was determined according to Lowry et al. (30) using bovine serum albumin as standard.

Experimental Design—0.7-1.0 μCi of [14C]cholesteryl oleate acetylated-LDL (50-100 μg of LDL-protein) was injected into the right femoral vein. After different periods of time, a blood sample was taken and the liver was perfused as described earlier (19). A liver lobus was tied off after the preperfusion with a Ca²⁺-free buffer. The liver cells were suspended in 60 ml of a HEPES-buffered minimal salt solution (20). Hepatocytes were isolated from 20 ml of the total cell suspension by differential centrifugation (20). Stellate cells were prepared from the pooled supernatants during the differential centrifugation (27). The rest of the total cell suspension (40 ml) was treated with enterotoxin, and endothelial cells and Kupffer cells were separated by centrifugal elutriation (23). When converting radioactivity in aliquots of cell suspensions to total liver values, it was assumed that the liver contains 150 x 10⁶ cells/g wet weight, and that the hepatocytes, endothelial cells, Kupffer cells, and stellate cells constitute 66, 19, 10, and 5% of the hepatic cells, respectively (27). The total weight of liver and plasma was assumed to be 4.1 and 3.2% of total body weight (34). All experiments have been performed at least three times with similar results.

RESULTS

Plasma Clearance and Hepatic Uptake of [14C]Cholesteryl Oleate Acetyl-LDL—Fig. 1 shows the result of an experiment in which 0.7-1.0 μCi of [14C]cholesteryl oleate acetyl-LDL (50-100 μg of LDL-protein) was injected into the right femoral vein of 13 rats. The radioactivity was cleared from the blood with a half-life of about 10 min. Most of the label was taken up by the liver, which after 1 h contained about 80% of the injected dose. After 3-4 h, some of the radioactivity was released from the liver. After 10 h, less than 30% of the injected radioactivity remained in the liver.

The Participation of Different Liver Cells in Hepatic Uptake of Acetyl-LDL—The livers were separated into hepatocytes, endothelial cells, Kupffer cells, and stellate cells following the injection of [14C]cholesteryl oleate acetyl-LDL (Fig. 2). Initially most of the radioactivity was found in endothelial cells, which contained about 60% of the administered dose after 1 h. Also the hepatocytes contained initially significant amounts of radioactivity (about 15% after 1 h), while less than 5% was recovered in Kupffer cells and stellate cells.

At intervals later than 1 h, the distribution of radioactivity in liver cells changed gradually. In addition to the label which initially was found in the hepatocytes, radioactivity apparently was transferred from the endothelial cells to the hepatocytes. The hepatocytes showed peak activity 3-4 h after injection (about 40% of injected dose). After 10 h, hepatocytes and endothelial cells contained only about 15% of the injected dose each. Hence, the data suggest that the radioactivity is initially taken up by the endothelial cells and subsequently transferred to the hepatocytes. However, radioactivity is soon released from the hepatocytes as well.

Hydrolysis of [14C]Cholesteryl Oleate following Intravenous Injection of Labeled Acetyl-LDL—The hydrolysis in the dif-
Injection of \[^{14}C\]Cholesteryl Oleate Acetyl-LDL—If lipoprotein clearance via the acetyl-LDL receptor has any implications for the hepatic cholesterol catabolism, one would expect that cholesterol or cholesterol metabolites appeared in the bile soon after its transfer from the endothelial cells to the hepatocytes. In a group of rats a catheter was placed in the common bile duct, and the appearance of \[^{14}C\] in the bile was determined.

Fig. 3 shows results from experiments in which rats were given 0.4 \(\mu\)Ci of \[^{14}C\]cholesterol oleate acetyl-LDL (50 \(\mu\)g of LDL-protein). Appearance of radioactivity in bile showed a lag of 3–4 h. After about 24 h, 10% of the injected dose was recovered in the bile. When bile fractions were extracted according to Folch et al. (29) most of the radioactivity (85–90%) was recovered in the water phase, suggesting that the radioactive product may be bile salts.

In Vitro Uptake and Hydrolysis of \[^{14}C\]Cholesteryl Oleate Acetyl-LDL in Isolated Liver Endothelial Cells—Suspensions of isolated rat liver endothelial cells (2.6 \(\times\) 10^6 cells/ml) were incubated in the presence of \[^{14}C\]cholesterol oleate acetyl-LDL (3 \(\mu\)g of LDL-protein/ml) at 37 °C. Cell-associated radioactivity and hydrolysis of cholesterol oleate were assessed after different time points (Fig. 4). After 25 min, about 60% of the radioactivity was cell-associated. It subsequently declined to about 45% after 120 min. The hydrolysis of cholesterol oleate showed a lag phase of about 20 min. The hydrolysis was then linear for at least 120 min. After 120 min, 25% of the \[^{14}C\]cholesterol oleate added to the cells was hydrolyzed. Since there is a possibility that hydrolyzed cholesterol
ester may be re-esterified by acyl-CoA:cholesterol acyltransferase, our data represent minimum hydrolysis.

**Competition Experiments**—We have tested the effect of unlabeled acetyl-LDL on uptake and hydrolysis of \([^{14}C]\)cholesteryl oleate acetyl-LDL (3 µg of LDL-protein/ml) in isolated liver endothelial cells. As shown in Fig. 5, both uptake and hydrolysis were reduced by unlabeled acetyl-LDL. Half-maximum reduction was observed at a concentration of about 20 µg/ml. The specific uptake (i.e., the uptake which could be displaced by excess unlabeled acetyl-LDL) represents about 60% of the total uptake in the cells. However, hydrolysis could be reduced by 85% by addition of excess (100–600 µg of LDL-protein/ml) unlabeled acetyl-LDL. On the other hand, native LDL had no significant effect either on the uptake or on the hydrolysis of \([^{14}C]\)cholesteryl oleate acetyl-LDL.

**Effect of Ammonia and Monensin on Uptake and Hydrolysis of Acetyl-LDL**—We have tested the effect of ammonia and monensin on uptake and hydrolysis of \([^{14}C]\)cholesteryl oleate acetyl-LDL (4 µg/ml of LDL-protein) in isolated liver endothelial cells (Figs. 6 and 7). Cell-associated radioactivity after 20–40 min was reduced by about 35% in the presence of 10 mM NH₄Cl and by about 25% in the presence of 5 µM monensin. The effects on hydrolysis were more profound. Ammonia (10 mM) and monensin (5 µM) reduced the hydrolysis after 40 min by about 90 and 80%, respectively.

**Effect of Monensin on Binding of Acetyl-LDL to Isolated Liver Endothelial Cells**—Liver endothelial cells (5 × 10⁵ cells/ml) were incubated with various concentrations of monensin at 37 °C for 15 min. The cells were then cooled to 4 °C and incubated further in the presence of \([^{14}C]\)cholesteryl oleate acetyl-LDL (4 µg of LDL-protein/ml) for 90 min (Fig. 8). Cell-associated radioactivity would after this procedure only represent ligand bound to the cell surface. 5 µM monensin reduced the binding of acetyl-LDL to the cells to about 65% of control values. Concentrations above 10 µM almost completely blocked the binding of acetyl-LDL to the endothelial cells.

**DISCUSSION**

The present study shows that \([^{14}C]\)cholesteryl oleate LDL which has been reacted with acetic anhydride to form acetyl-LDL is rapidly cleared from the plasma and that most of the modified lipoprotein is recovered in the liver. This is in accordance with earlier reports (6, 8, 10, 11).

Acetyl-LDL has been shown to be taken up by macrophages (1) and was, therefore, expected to be taken up by the Kupffer cells (1, 10, 11). On the other hand, we have recently found that intravenously injected formaldehyde-treated albumin is primarily taken up by the liver endothelial cells (6). This ligand is probably removed by the same receptor as acetyl-LDL, since the formation of both these modified ligands from their native compounds involves an increase in net negative charge.

Our results confirm and extend a report by Nagelkerke et al. (31) which was published after most of the present experiments were finished. Nagelkerke et al. (31) studied the hepatic uptake of \([^{31}P]\)-labeled acetyl-LDL in vivo and in vitro and found that the ligand was endocytosed and degraded...
Hepatic Metabolism of Acetyl-LDL

primarily by liver endothelial cells. In vitro degradation of 125I-labeled acetyl-LDL was reduced to 10% of control values in the presence of 10 mM ammonia or 50 μM monensin. The methods used by us differ from those used by Nagelkerke et al. (31) mainly in two ways. We have used [14C]cholesterol oleate-labeled acetyl-LDL while they used 125I-labeled acetyl-LDL. Secondly, they reported a yield of endothelial cells of 1.5-4.5%, while our methods enable us to recover 40-60% of the liver endothelial cells. In spite of these methodological differences the results are strikingly similar, and it may be concluded that both the protein moiety and the cholesteryl ester part of the acetyl-LDL are endocytosed by the endothelial cells of the liver.

In addition, use of cholesteryl ester-labeled acetyl-LDL enabled us to trace the route of cholesterol following uptake in endothelial cells as part of the acetyl-LDL particle. We found that some [14C]cholesterol after uptake in endothelial cells was transferred to the liver parenchymal cells and then to the bile canaliculi. However, a significant amount of cholesterol was also released from the liver in addition to that recovered in bile. Unesterified cholesterol rapidly exchanges between cellular membranes and lipoproteins (32, 33). Therefore, the transfer of [14C]cholesterol from endothelial cells to hepatocytes may be due to a general diffusion of unesterified cholesterol. On the other hand, it seems that the transfer of cholesterol between liver cells is fairly specific. Cholesterol which is taken up in hepatocytes as part of the chylomicron remnant particles is not transferred to any of the nonparenchymal liver cells (34). Furthermore, cholesterol which is taken up in liver endothelial cells as part of the acetyl-LDL particles, is transferred to hepatocytes, but not to Kupffer cells or stellate cells.

Some other molecules are also known to be transferred between the individual liver cells; hepatic lipase which is synthesized in the hepatocytes is subsequently transferred to the liver endothelial cells (35), and retinol (vitamin A) which is taken up in hepatocytes as part of the chylomicron remnant particles is soon transferred to the stellate cells in liver for storage (27, 34).

The finding that liver endothelial cells in vivo are the major cell type that takes up acetyl-LDL is unexpected on the basis of previously published data on uptake of 125I-labeled acetyl-LDL by vascular endothelial cells (9). Only a small amount of acetyl-LDL was metabolized by vascular endothelial cells as compared to that which is metabolized by macrophages in vitro (1). Our in vivo results may be a consequence of the anatomical relationship between endothelial cells and Kupffer cells in situ.

Modified LDL has been shown to convert macrophages to foam cells (8). Hence, receptor-mediated endocytosis of such lipoproteins in macrophages may play a major role in the development of atherosclerosis. On the other hand, it follows from the present study that liver endothelial cells may be a cell type involved in the protection against the atherogenic action of the modified LDL, i.e. the liver endothelial cells may mediate uptake of cholesterol from plasma and transfer of cholesterol to the hepatocytes for further metabolism.

In accordance with Nagelkerke et al. (31) we have shown that the uptake of acetyl-LDL in liver endothelial cells is saturable and that native LDL does not compete for the acetyl-LDL receptor. From in vitro studies it follows that after a short lag (15-20 min), the protein part (31) as well as the cholesteryl ester part (the present study) are hydrolyzed. Protein degradation and cholesteryl ester hydrolysis are almost completely inhibited in the presence of 10 mM NH4Cl or 5 μM monensin. Ammonia and monensin inhibit the acidicification of newly formed endosomes which may be a prerequisite for transfer of ligand to lysosomes (36, 37). The acidification of endosomes will in most cases studied (36, 38, 39) promote the dissociation of ligand from the receptor. Therefore, inhibition of the acidification of endosomes may trap the receptor inside the cell and reduce the number of functional receptors on the cell surface (40, 41). In fact, following a short preincubation at 37 °C in the presence of monensin, almost no binding of acetyl-LDL to the liver endothelial cells was observed. Hence, the receptor for acetyl-LDL seems to follow the pathway which is general for most other receptors studied (e.g. asialoglycoprotein receptor (36), LDL-receptor (41), insulin receptor (42), and transferrin receptor (43)). This pathway comprises internalization of receptor-ligand complex into a primary endosome, acidification of newly formed endosome resulting in dissociation of the ligand-receptor complex, delivery of ligand to the lysosomes, and recycling of the receptor back to the cell surface.

Because of its potential importance in protection against atherosclerosis, more attention should be paid to the mechanism and regulation of endocytosis of acetyl-LDL in liver endothelial cells.

Acknowledgment—Kari Holte has provided excellent technical assistance.

REFERENCES

1. Brown, M. S., and Goldstein, J. L. (1983) Annu. Rev. Biochem. 52, 225-261
2. Basu, S. K., Goldstein, J. L., Anderson, R. C. W., and Brown, M. S. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3178-3182
3. Hennekens, T., Mahoney, E. M., and Steinberg, D. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6499-6503
4. Haberland, M. E., Fogelman, H. A., and Edwards, P. A. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 1712-1716
5. Fogelman, A. M., Seager, J., Haberland, M. E., Hokom, M., Tanaka, R., and Edwards, P. A. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 922-926
6. Blomhoff, R., Eskild, W., and Berg, T. (1984) Biochem. J. 218, 81-86
7. Brown, M. S., Basu, S. K., Falck, J. R., Ho, Y. K., and Goldstein, J. L. (1980) J. Supramol. Struct. 13, 67-81
8. Goldstein, J. L., Ho, Y. K., Basu, S. K., and Brown, M. S. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 333-337
9. Stein, O., and Stein, Y. (1980) Biochim. Biophys. Acta 620, 631-635
10. Mahley, R. W., Weisgraber, K. H., Innerarity, T. L., and Windmueller, H. G. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1746-1750
11. Nilsson, M., and Berg, T. (1977) Biochim. Biophys. Acta 497, 171-182
12. Wandel, M., Berg, T., Eskild, W., and Norum, K. R. (1982) Biochim. Biophys. Acta 721, 469-477
13. Eskild, W., and Berg, T. (1982) in Sinusoidal Liver Cells (Knook, D. L., and Wisse, E., eds) pp. 265-262, Elsevier Biomedical Press, Amsterdam
14. Havel, R. J., Eder, H. A., and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345-1353
15. Pattanaik, N. M., Montes, A. A., and Steinberg, D. B. (1978) Biochim. Biophys. Acta 530, 428-438
16. Goldstein, J. L., and Brown, M. S. (1977) Annu. Rev. Biochem. 46, 897-930
17. Skipci, V. P. (1972) in Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism (Nelson, G. J., ed) pp. 471-583, Wiley-Interscience, New York
18. Pittman, R. C., Green, S. R., Attie, A. D., and Steinberg, D. (1979) J. Biol. Chem. 254, 6876-6879
19. Seglen, P. (1976) Methods Cell Biol. 13, 29-59
20. Tolleshaug, H., Berg, T., Nilsson, M., and Norum, K. R. (1977) Biochim. Biophys. Acta 499, 73-84
21. Munthe-Kaas, A. C., Berg, T., and Seljelid, R. (1976) Exp. Cell Res. 99, 146-154
Hepatic Metabolism of Acetyl-LDL

22. Berg, T., Tolleshaug, H., Ose, T., and Skjelvåle, R. (1979) Kupffer Cell Bull. 2, 21–25
23. Blomhoff, R., Smedså, B., Eskild, W., Granum, P. E., and Berg, T. (1984) Exp. Cell Res. 150, 194–204
24. Smedså, B., Eriksson, S., Fraser, J. R. E., Laurent, T. C., and Pertot, H. (1982) in Sinusoidal Liver Cells (Knook, D. L., and Wisse, E., eds) pp. 263–270, Elsevier Biomedical Press, Amsterdam
25. Wisse, E. (1974) J. Ultrastruct. Res. 46, 393–426
26. Berg, T., and Boman, D. (1973) Biochim. Biophys. Acta 321, 585–596
27. Blomhoff, R., Holte, K., Næs, L., and Berg, T. (1984) Exp. Cell Res. 150, 186–193
28. Drevon, C. A., Engelhorn, S. C., and Steinberg, D. (1980) J. Lipid Res. 21, 1065–1071
29. Folch, J., Lees, M., and Sloane Stanley, G. L. (1957) J. Biol. Chem. 226, 497–509
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
31. Nagelkerke, J. F., Barto, K. P., and van Berkel, T. J. C. (1983) J. Biol. Chem. 258, 12221–12227
32. Rothblat, G. H., and Phillips, M. C. (1982) J. Biol. Chem. 257, 4775–4782
33. Murphy, J. (1962) J. Lab. Clin. Med. 60, 86–109
34. Blomhoff, R., Helgerud, P., Rasmussen, M., Berg, T., and Norum, K. R. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 7926–7930
35. Jansen, H., and Hülsmann, W. C. (1986) Trends Biochem. Sci. 5, 265–268
36. Harford, J., Wolkoff, A. W., Ashwell, G., and Klausner, R. D. (1983) J. Cell Biol. 96, 1824–1828
37. Tyc, B., and Maxfield, F. R. (1982) Cell 28, 643–651
38. Maxfield, F. R. (1982) J. Cell Biol. 95, 676–681
39. Gonzalez-Noriega, A., Grub, J. H., Talkad, V., and Sly, W. S. (1980) J. Cell Biol. 85, 839–852
40. Berg, T., Blomhoff, R., Næs, L., Tolleshaug, H., and Drevon, C. A. (1983) Exp. Cell Res. 148, 319–330
41. Basu, S. K., Goldstein, J. L., Anderson, R. G. W., and Brown, M. S. (1981) Cell 24, 493–502
42. Posner, B. I., Josenberg, Z., and Bergeron, J. J. M. (1978) J. Biol. Chem. 253, 4067–4073
43. Dautry-Varsat, A., Ciechanover, A., and Lodish, H. F. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2258–2262