Uptake and Degradation of Iodine-Labelled Chylomicron Remnant Particles by Monolayers of Rat Hepatocytes

By CLAES-HENRIK FLORÉN and ÅKE NILSSON

Department of Physiological Chemistry, University of Lund, Lund, Sweden

(Received 16 January 1978)

1. Rat chylomicrons were labelled with ^{125}I with 69–72% of the iodine in the protein moiety. Less than 1 nmol of iodine was incorporated per nmol of protein. Of the peptide radioactivity 44–56% was in apolipoprotein A-1, 30–40% in the C peptides and 11–15% in apolipoprotein B. The arginine-rich peptide, which accounted for about 14% of the chylomicron protein mass as determined by scanning of sodium dodecyl sulphate–polyacrylamide gels, contained very little radioactivity. 2. Chylomicron remnants generated with postheparin plasma from iodine-labelled chylomicrons showed a relative increase in the percentage of the arginine-rich peptide (76–90% of the apolipoprotein mass according to gel scanning). The major portion of the peptide iodine label was present in apolipoprotein A-1 (43–57%), B (22–32%) and C peptides (17–35%). 3. When iodine-labelled chylomicron remnants were added to rat hepatocytes in primary culture, labelled peptides were taken up and degraded by the hepatocytes by a saturable process. The V_{max} for the uptake was calculated to be 300 ng of protein/h per mg of cell protein and the apparent K_m as 7.7 μg of protein/mg of cell protein. A larger proportion of the ^{125}I -labelled lipids of the remnants (mainly polar lipids) was taken up. This suggests that these may also enter the cells by a mechanism that does not involve particulate uptake, such as phospholipid exchange. 4. The degradation of labelled peptides was inhibited by colchicine, concanavalin A, chloroquine and NH_4Cl , which also inhibit degradation of the cholesteryl ester portion. All these drugs exerted their inhibition mainly after the uptake of labelled peptide. No degradation occurred at 4°C, and also the uptake was markedly decreased. 5. The uptake of labelled chylomicron remnant peptide was 77 times as effective as that of labelled sucrose, which is likely to be taken up randomly by pinocytosis.

The cholesteryl ester of chylomicron remnants, which are formed by the action of lipoprotein lipase on chylomicrons (Redgrave, 1970; Mjøs *et al.*, 1975), is taken up mainly by the liver (Lossow *et al.*, 1962; Goodman, 1962; Redgrave, 1970). The main part of the total protein fraction (Stein *et al.*, 1974), of the B peptide (Faergeman *et al.*, 1975) and of the cholesteryl ester (Faergeman & Havel, 1975) of rat VLD lipoprotein is rapidly metabolized by the liver *in vivo*. This suggests that the remnant particles formed from these lipoproteins are metabolized as units. Cholesteryl ester-labelled chylomicron remnants are taken up and degraded more efficiently than native chylomicrons both in the perfused liver (Gardner & Mayes, 1976; Cooper, 1977) and in suspended (Nilsson, 1977) and cultured (Florén & Nilsson, 1977a,b) hepatocytes. In hepatocyte monolayers and *in vivo* (Nilsson, 1975; Florén *et al.*, 1977) the degradation of the remnant cholesteryl ester is inhibited

by colchicine and by chloroquine. This suggests that the degradation depends on intact microtubules and on lysosomal enzymes. The presence of VLD lipoprotein peptide in secondary lysosomes (Stein *et al.*, 1974) and the inhibition of VLD lipoprotein peptide degradation by chloroquine *in vivo* (Stein *et al.*, 1977) provide further evidence that remnants of lipoproteins rich in triacylglycerol may be taken up by an endocytic process by the hepatocytes and degraded by lysosomal enzymes.

If remnants are taken up as whole particles, catabolism of the peptide portion of chylomicron remnants in hepatocyte monolayers would be expected to have the same characteristics as that of the cholesteryl ester portion. In the present study the kinetics for the uptake of iodine-labelled remnant particles were studied in hepatocyte monolayers, and the effects on peptide degradation of chloroquine and of colchicine were examined. In addition the effects of the ammonium ion, which inhibits lysosomal degradation of protein in hepatocytes (Seglen & Reith, 1976), and of the lectin concanavalin A on both cholesteryl ester and peptide degradation, were

Abbreviations used: VLD lipoprotein, very-low-density lipoprotein ($d < 1.006$, S_r 20–400); SDS, sodium dodecyl sulphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

examined. Finally, the remnant uptake was compared with the uptake of sucrose (which is taken up randomly by pinocytosis) (Wagner *et al.*, 1971; Steinman *et al.*, 1976), to estimate the possible contribution of the latter process to the remnant uptake.

Materials and Methods

Materials

[1-¹⁴C]linoleic acid, [G-³H]cholesterol, [U-¹⁴C]-sucrose and sodium [¹²⁵I]iodide (carrier-free, 100 mCi/ml) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Leibovitz L-15 medium, foetal calf serum and Hepes were purchased from Flow Laboratories, Irvine, Ayrshire, U.K. Acid-soluble calf-skin collagen, concanavalin A (grade IV), tetramethylurea and chloroquine were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and Amberlite monobed resin MB-1, colchicine, iodine monochloride and sucrose from BDH Chemicals Ltd., Poole, Dorset, U.K. Porcine insulin was obtained from Novo Industri A/S, Bagsvaerd, Denmark, and collagenase [CLS, 143–180 units/mg (1 unit liberates 1 μ mol of amino acid from collagen in 5 h at 37°C, pH 7.4)] from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Solutions containing trypsin (1%, w/v) and EDTA (0.02%, w/v) in phosphate-buffered saline (NaCl 137 mM, KCl 2.7 mM, Na₂HPO₄·2H₂O 0.8 mM, KH₂PO₄ 1.5 mM) were obtained from Statens Bakteriologiska Laboratorium, Stockholm, Sweden, cholesterol from Merck, Darmstadt, West Germany, and heparin, 5000 units/ml (2.4 μ g of uronic acid/unit), from Vitrum AB, Stockholm, Sweden. Materials for polyacrylamide-gel electrophoresis were from Eastman Organic Chemicals, Rochester, New York, U.S.A.

Methods

Preparation of chylomicrons and chylomicron remnants. Male white Sprague–Dawley rats, weighing 250–300 g, were obtained from Anticimex AB, Stockholm, Sweden. Thoracic duct cannulations (Bollman *et al.*, 1948) were performed as described in detail by Nilsson (1977). In experiments with cholesterol-labelled lipoproteins, rats were fed 0.75 ml of corn oil containing 500 μ Ci of [³H]cholesterol, 250 μ Ci of [¹⁴C]linoleic acid and 50 μ mol of non-radioactive cholesterol. In the experiments with iodinated lipoprotein particles, the rats were fed 1 ml of glycerol trioleate and 50 μ mol of cholesterol. Lymph was collected and stored at 4°C with 2.0 mM-disodium EDTA present (Ontko, 1970). Chylomicrons ($S_r > 400$) were prepared as described by Minari & Zilversmit (1963). The density of the chyle was adjusted to $d = 1.063$ by adding a stock solution

of KBr and NaCl ($d = 1.35$). This was overlaid with 5 ml of $d = 1.041$ (20.46 g of NaCl and 25.83 g of KBr/l), 5 ml of $d = 1.019$ (10.58 g of NaCl and 1.50 g of KBr/l) and enough (≈ 8 ml) $d = 1.006$ (1.1%, w/v, NaCl) containing 0.01% (w/v) disodium EDTA to fill the tube (25 ml). The tubes were centrifuged in an MSE 65TC centrifuge with a 3 $\times$ 25 ml swing-out rotor at 29000 rev./min ($g_{av.} = 90000$) for 45 min at 4°C. The top layer was collected and recentrifuged twice as above. After this procedure chylomicrons were free from albumin as shown by SDS–polyacrylamide-gel electrophoresis (Fig. 1a). Chylomicron remnants ($S_r > 200$) were prepared by injecting [³H]cholesterol and [¹⁴C]linoleic acid-labelled chyle into functionally hepatectomized rats (Redgrave, 1970) as described by Florén & Nilsson (1977b) or by treating iodine-labelled chylomicrons ($S_r > 400$) with postheparin plasma. Postheparin plasma was obtained as described by Nilsson (1977). Iodine-labelled chylomicrons were mixed with postheparin plasma and 5% bovine serum albumin (Cohn fraction V, Serva Feinbiochemica, Heidelberg, West Germany), incubated and centrifuged as described by Florén & Nilsson (1977b). As serum albumin was present in the chylomicron remnant fraction, the particles were recentrifuged in a Minari & Zilversmit (1963) gradient as described above for 90 min at 4°C. In spite of this, 10–47% of the protein mass of the remnants was still serum albumin as determined by scanning of photonegatives from polyacrylamide gels. This percentage was not decreased by a further centrifugation in a Minari–Zilversmit gradient. Consequently chylomicron remnants were used in incubation studies after one wash only. The remnants ($S_r > 200$) were collected with a Pasteur pipette under sterile conditions and were used within 48 h.

Preparation of iodine-labelled chylomicrons. Chylomicrons ($S_r > 400$) obtained after three consecutive Minari–Zilversmit gradients and free from contaminating serum albumin were iodinated with ¹²⁵I. The procedure followed was the iodine monochloride method described by McFarlane (1958). Chylomicrons were dialysed for 16 h at 4°C against 0.9% (w/v) NaCl, then concentrated in a Minicon B-15 [Amicon B.V., Oosterhout (N.B.), Holland]. An equal volume of 0.8 M-glycine/NaOH buffer, pH 10.0, was added to the chylomicron solution. ICI dissolved in 100 μ l of 2 M-NaCl was mixed with 2.5 mCi of Na¹²⁵I (100 mCi/ml). This was added to the chylomicron solution buffered with glycine/NaOH (total volume 5.4–7.2 ml) while being mixed vigorously. The iodine-labelled chylomicrons were dialysed extensively at 4°C (3 litres changed 10 times) for 24–48 h against 0.9% (w/v) NaCl/0.01% (w/v) disodium EDTA, pH 7.4, to remove free ¹²⁵I.

Chylomicrons were labelled by mixing iodine monochloride in a ratio of 3.0–5.0 nmol of iodine/

nmol of protein. More than 92% of the label was precipitated with 10% (w/v) trichloroacetic acid; of this 27–33% was extracted with chloroform/methanol (2:1, v/v). As most of the protein label was in apolipoprotein A-1 and in the Capolipoproteins (Fig. 1b, Table 1) a mean mol.wt. of 20000 for chylomicron apolipoproteins was used to calculate the ratio nmol of iodine incorporated/nmol of protein. From this calculation, a mean ratio of 0.7 was obtained in five experiments (Table 1).

High-density lipoproteins ($1.070 < d < 1.21$) from rat plasma were prepared as described by Florén & Nilsson (1977b).

Hepatocytes in primary culture. Hepatocytes were prepared by a collagenase procedure (Berry & Friend, 1969) using the conditions of Seglen (1973). The cells were cultured in primary monolayers (Bisell *et al.*, 1973; Bonney *et al.*, 1974) on collagen-coated 60mm Petri dishes (Falcon no. 3002) according to Lin & Snodgrass (1975). Further details have been given by Florén & Nilsson (1977a,b). After incubation with lipoproteins the medium was collected and the cells were washed twice with 0.5 ml of NaCl (0.85%, w/v). Further washings did not result in any significant release of labelled material. Cells incubated with chylomicron remnants labelled with [^3H]cholesterol and [^{14}C]linoleic acid were scraped off with a rubber policeman during repeated additions (4×1 ml) of methanol/water (2:1, v/v). Cells incubated with iodine-labelled chylomicron remnants were washed twice with 0.5 ml of 0.85% (w/v) NaCl and then trypsinized off. As a control, triplicate incubations with lipoproteins were done in cell-free but collagen-coated dishes in each experiment. Trypsinization of monolayers was done with 0.02% (w/v) EDTA/0.1% (w/v) trypsin in phosphate-buffered saline as described by Floren & Nilsson (1977b).

The experiments at 4°C were carried out as follows. Plates were preincubated with ice-cold medium for 30 min. After that fresh ice-cold medium and chylomicron remnants were added and incubated at 4°C for 4 h with shaking (25 cycles/min). The medium was removed at 4°C, the cells were washed twice with ice-cold 0.85% NaCl and three times with ice-cold buffer (3 ml) containing 50 mM-Tris/HCl, pH 7.4, 0.15 M-NaCl and 6 mg of bovine serum albumin. The plates were then incubated at 4°C with 3 ml of ice-cold Tris/HCl buffer containing albumin (2 mg/ml) and finally washed once with 3 ml of the same buffer. The cells were then either removed with a rubber policeman, or trypsinized as described above.

After the incubations with [^{14}C]sucrose the medium was removed and the cells were washed 12 times with 2 ml of 0.85% (w/v) NaCl. Three portions (1 ml) of 1 M-NaOH were added and the cells removed from dishes with a rubber policeman. The mixture was sonicated for 30 s with a Branson Sonifier B-12 at setting 4 and was left for 16 h at 20°C. A portion

(800 μl) was added to a counting vial and neutralized with 200 μl of 4 M-HCl. Instagel (10 ml) (Packard Instrument Co., Downers Grove, IL, U.S.A.) was then added and the radioactivity in 1 ml of the medium in 10 ml of Instagel was measured.

SDS-polyacrylamide-gel electrophoresis. After delipidation of lipoproteins (see below) the radioactivity of the different apolipoproteins was assessed by SDS-polyacrylamide-gel electrophoresis according to Neville (1971). Gels were stained with 0.25% (w/v) Coomassie Blue in acetic acid/methanol/water (7:50:43, by vol.), then destained in acetic acid/methanol/water (7:25:68, by vol.) together with 25 g/l of the monobed resin Amberlite MB-1. After destaining, the gels were photographed and then cut into approximately 1 mm-thick slices with a razor blade. The ^{125}I radioactivity in the slices was measured with an Intertechnique CG automatic gamma spectrometer. To measure semi-quantitatively the mass distribution of protein in different remnant apolipoproteins photonegatives from gels were scanned in a Kipp and Zonen (Delft, Holland) densitometer DD2.

Analytical. Lipids were extracted and separated by t.l.c. as described by Nilsson (1977). Cholesterol was determined according to Zak *et al.* (1954) after saponification of the lipid extract (Abell *et al.*, 1952). Triacylglycerols and their fatty acid composition were determined by g.l.c. of the fatty acid methyl esters (Åkesson *et al.*, 1970). Cellular protein was measured according to Lowry *et al.* (1951), with bovine serum albumin as standard. The presence of protein in collagen in each dish ($\approx 60 \mu\text{g}$) was corrected for. The protein contents of the chylomicrons and the chylomicron remnants were assessed by the Lowry method after precipitation with trichloroacetic acid and delipidation with diethyl ether/ethanol (3:1, v/v) as described by Tornqvist & Belfrage (1976). Before precipitation with trichloroacetic acid, chylomicrons, suspended in 0.9% NaCl, were extracted for 16 h with an equal volume of diethyl ether at 4–6°C.

To determine the amount of radioactive iodine in the lipoproteins, these were precipitated with ice-cold 7.5% (w/v) (final concn.) trichloroacetic acid in the presence of 0.5 ml of 10% (w/v) bovine serum albumin for 1 h. The precipitate was washed once with ice-cold 10% (w/v) trichloroacetic acid. To assess the amount of iodine bound to lipid a portion was mixed with 0.5 ml of 10% (w/v) bovine serum albumin, extracted with chloroform/methanol (2:1, v/v) and washed according to the method of Folch *et al.* (1957).

In experiments with iodine-labelled chylomicron remnants, trypsin-digested cells were analysed for the radioactivity in total iodine and were then extracted with chloroform/methanol (1:1, v/v) (Bligh & Dyer, 1959). The remaining radioactivity after lipid extraction is regarded as radioactivity in interiorized but

undigested lipoprotein peptides. The total radioactivity released by trypsin treatment of washed cells minus that extracted with chloroform/methanol (1:2, v/v) (Bligh & Dyer, 1959) is regarded as surface-bound lipoprotein peptide radioactivity (Stein & Stein, 1975).

The proteins of the media were precipitated with ice-cold trichloroacetic acid (final concn. 10.7%, w/v) after the addition of 0.5 ml of 10% (w/v) bovine serum albumin as carrier protein. After 1 h at 0°C the precipitate was sedimented and washed once with ice-cold 10% (w/v) trichloroacetic acid. To remove free iodide from the supernatants these were pooled (total volume 3 ml) and 30 μ l of 40% (w/v) KI and 120 μ l of 30% (v/v) H₂O₂ were added. After 10 min iodine was extracted with 6 ml of chloroform (Bierman *et al.*, 1974). The aqueous phase was removed and the chloroform phase washed once with 2 ml of 0.9% (w/v) NaCl. The radioactivity in the aqueous phases was determined. The same procedure was undertaken with the cell-free controls. The difference between cells incubated with lipoproteins and cell-free controls is regarded as an estimate of the hydrolysis of lipoprotein protein (Bierman *et al.*, 1974; Goldstein & Brown, 1974). A sample of the chloroform phase was analysed for radioactivity and corrected for quenching due to chloroform.

To determine the degree of chylomicron non-lipid radioactivity precipitated by tetramethylurea, chylomicrons (102–123 μ g of protein/ml, 250 μ l) were added to an equal volume of freshly distilled tetra-

methylurea as described by Kane *et al.* (1975). A portion of the tetramethylurea/water phase was removed and the remainder extracted with chloroform/methanol (2:1, v/v). The percentage of non-lipid radioactivity precipitated by tetramethylurea was calculated by difference. The same procedure was adopted to measure tetramethylurea-precipitable radioactivity in medium containing chylomicron remnants before and after incubation with hepatocytes.

Results

Labelling of chylomicrons and chylomicron remnants with iodine

In agreement with Mjøs *et al.* (1975) and Glickman & Green (1977) the major apolipoprotein in chylomicrons was apolipoprotein A-1, which constituted $60 \pm 4\%$ (means $\pm$ S.E.M., $n = 3$) of protein mass as determined by gel scanning (Table 1). Other peptides present were arginine-rich peptide $14 \pm 4\%$, B peptide $5 \pm 3\%$ and the C peptides $20 \pm 7\%$ (means $\pm$ S.E.M., $n = 3$).

Chylomicrons labelled with iodine had approximately 70% of the iodine label in the protein moiety (Table 1). As shown in Table 1 and Fig. 1 most of the radioactivity was in apolipoprotein A-1, B and in the C apolipoproteins. The band corresponding to the arginine-rich peptide contained very little radioactivity. Of the ¹²⁵I present in the lipid portion of

Table 1. *Labelling of chylomicrons with ¹²⁵I*

Chylomicrons ($S_r > 400$) were prepared and iodinated as described under 'Methods'. To determine the distribution of apolipoproteins in chylomicrons, polyacrylamide gels were photographed and the negatives scanned as described under 'Methods'. As most radioactivity is present in apolipoprotein A-1 and the C apolipoproteins, a mean molecular weight of 20000 for chylomicron apolipoproteins was used for calculation of the ratio nmol of iodine incorporated/nmol of protein. TMU, tetramethylurea; A-1, apolipoprotein A-1; ARP, arginine-rich peptide; B, apolipoprotein B; C, apolipoproteins C; n.d., not determined.

Expt. no.	Added chylomicron triacylglycerol (mg)	Added chylomicron protein					Added I ¹²⁵ I (nmol)	¹²⁵ I incorporated (% of total)
		(μ g)	A-1 (%)	ARP (%)	B (%)	C (%)		
1	114	1150	68	17	1	14	287	17.9
2	92	850	58	18	10	10	211	28.8
3	92	850	55	7	5	34	211	27.8
4	117	1000	n.d.	n.d.	n.d.	n.d.	150	14.1
5	117	1000	n.d.	n.d.	n.d.	n.d.	150	14.8

Expt. no.	Lipid radioactivity (%)	I incorporated (nmol/nmol of protein)	Protein radioactivity (%)			TMU- precipitable radioactivity
			A-1	B	C	
1	28.8	0.7	52	11	40	n.d.
2	27.4	1.1	44	15	40	29.3
3	32.6	1.0	56	13	30	24.7
4	30.9	0.3	n.d.	n.d.	n.d.	19.2
5	30.6	0.3	n.d.	n.d.	n.d.	35.7

Fig. 1. *SDS-polyacrylamide-gel electrophoresis of iodine-labelled chylomicron peptides*

Iodine-labelled chylomicrons were delipidated and loaded on a polyacrylamide gel as described under 'Methods'. The chylomicron apolipoproteins loaded contained $26\text{ }\mu\text{g}$ of protein and ^{125}I ($1.2 \times 10^6\text{ c.p.m.}$). After running the gel it was cut in slices ($\approx 1\text{ mm}$) and the radioactivity counted. (a) The gel after staining with Coomassie Blue; (b) radioactivity profile of the separating gel. The inset in (b) shows a scan of a photonegative of the gel for comparison. The spacer gel did not stain with Coomassie Blue and contained very little radioactivity. The identity of each band and peak is determined by comparison with reference proteins of known molecular weight and data from other work (Fielding & Fielding, 1976; Breslow *et al.*, 1977; Fainaru *et al.*, 1977). Also apolipoprotein A-1 co-migrated with the major apolipoprotein of rat high-density lipoprotein. When loading gels with larger amounts of chylomicron protein a band corresponding to an apparent molecular weight of around 45000 appeared. No significant radioactivity was present in this band. ARP, arginine-rich peptide; A-1, apolipoprotein A-1; Alb., albumin; B, apolipoprotein B; C, apolipoproteins C.

chylomicrons more than 50% was in the polar lipid fraction.

During the conversion of chylomicrons into chylomicron remnants, there was a relative increase in the percentage of the arginine-rich peptide (Fig. 2a). If contamination of chylomicron remnants with serum albumin (10–47%) was disregarded gel scanning indicated an approximate chylomicron remnant protein composition of $4 \pm 1\%$ apolipoprotein B, $83 \pm 4\%$ arginine-rich peptide, $6 \pm 2\%$ apolipoprotein A-1 and $8 \pm 4\%$ C peptides (means $\pm$ S.E.M., three different preparations). The distribution of iodine label in the protein moiety is shown in Fig. 2(b): $26 \pm 3\%$ was present in apolipoprotein B, $50 \pm 4\%$ in apolipoprotein A-1 and $24 \pm 6\%$ in the C peptides (means $\pm$ S.E.M., $n = 3$). Hardly any radioactivity was present in the arginine-rich peptide. $95.8 \pm 0.5\%$ of the iodine label was precipitated with trichloroacetic acid; of this $33.7 \pm 8.2\%$ was soluble in chloroform/methanol. Of the lipids $67.9 \pm 4.6\%$ was in the polar lipid fraction (means $\pm$ S.E.M., $n = 4$).

Uptake and degradation of iodine-labelled chylomicron remnants by hepatocytes in primary culture

The time course for uptake and degradation of chylomicron remnant protein is shown in Table 2. The cell surface-bound protein radioactivity increased during the first 2 h of incubation to a constant level, but the interiorization and hydrolysis of the protein continued to increase over the 4 h period studied. A larger proportion of the lipid-bound ^{125}I than of the protein-bound iodine had been taken up by the cells in such a manner that it could not be released by trypsin. On the other hand, less lipid than protein radioactivity was surface-bound. The data may be explained if the lipids participate in exchange reactions, which have been shown to occur between chylomicrons and liver phospholipids (Edgren & Zilversmit, 1965).

When increasing amounts of iodine-labelled chylomicron remnants were added to hepatocyte monolayers the uptake approached a saturation level (Fig. 3a and b). As shown in Table 2, hydrolysis of the

Fig. 2. SDS-polyacrylamide-gel electrophoresis of iodine-labelled chylomicron remnant proteins
Chylomicron remnants, generated from iodine-labelled chylomicrons, were delipidated and loaded on a polyacrylamide gel as described under 'Methods'. The chylomicron remnants loaded contained 39 μ g of protein and 125 I (113 000 c.p.m.). After running the gel it was cut in slices (≈ 1 mm each) and radioactivity in each slice was counted. (a) The gel after staining with Coomassie Blue; (b) radioactivity profile of the separating gel. The inset (b) shows a scan of a photonegative of the gel for comparison. The spacer gel did not stain with Coomassie Blue and contained very little radioactivity. The identity of each band and peak is determined by comparison with reference proteins of known molecular weight and data from other work (Fielding & Fielding, 1976; Breslow *et al.*, 1977; Fainaru *et al.*, 1977). Also apolipoprotein A-1 co-migrated with the major apolipoprotein of rat high-density lipoprotein. ARP, arginine-rich peptide; Alb., albumin; C, apolipoproteins C.

Table 2. Time course for uptake of protein and lipid of iodine-labelled chylomicron remnants by hepatocyte monolayers
Chylomicron remnants were incubated with hepatocyte monolayers (1.7 mg of cellular protein) for 1–4 h. After interruption of the incubations, the cells were treated with trypsin to determine the protein and lipid radioactivity of the cells that was cell-surface bound and truly interiorized (see under 'Methods'). The chylomicron remnants added contained 30 μ g of protein, 10.8 μ g of cholesteryl ester and 20.3 μ g of triacylglycerol. During preparation of chylomicron remnants from chylomicrons, the hydrolysis of the triacylglycerol portion was 94.8%. The added 125 I-labelled protein radioactivity was 55 600 c.p.m. and the added 125 I-labelled lipid radioactivity was 27 500 c.p.m. Of the lipid radioactivity 71% was in polar lipids, 5% in cholesterol and diacylglycerol, 8% in fatty acids, 6% in triacylglycerol and 10% in cholesteryl ester. The Table shows percentage total uptake, surface binding, interiorization and hydrolysis of added chylomicron remnant iodine-labelled protein, and percentage total uptake, surface binding and interiorization of added iodine-labelled lipids. Values are means $\pm$ S.E.M. for two or three dishes.

Incubation time (h)	% of total uptake		% surface-bound		% interiorized		Hydrolysis of protein (%)
	Protein	Lipid	Protein	Lipid	Protein	Lipid	
1	9.4	4.7	7.3 $\pm$ 0.8	2.5 $\pm$ 0.3	1.7 $\pm$ 0.1	2.2 $\pm$ 0.1	0.4 $\pm$ 0.0
2	14.0	11.3	10.1 $\pm$ 0.7	5.1 $\pm$ 0.9	3.1 $\pm$ 0.1	6.2 $\pm$ 0.4	0.8 $\pm$ 0.1
3	15.4	14.0	10.2 $\pm$ 0.8	4.2 $\pm$ 0.3	4.1 $\pm$ 0.3	9.8 $\pm$ 0.2	1.1 $\pm$ 0.1
4	16.2	19.7	10.5 $\pm$ 0.4	6.2 $\pm$ 0.6	4.3 $\pm$ 0.2	13.5 $\pm$ 0.8	1.4 $\pm$ 0.1

protein part, compared with surface binding and interiorization, was low. However, if incubations are extended in time (Table 4), hydrolysis of the protein moiety still increases while the part interiorized decreases, indicating that hydrolysis of remnant

apoproteins is slower than for the cholesteryl ester part (Florén & Nilsson, 1977b). The values for the total uptake (surface binding, interiorization and hydrolysis) of the protein moiety conformed to a linear double-reciprocal plot (Fig. 3b). The $V_{\max}$.

Fig. 3. Effect of increasing concentrations of iodine-labelled chylomicron remnants on binding, interiorization and hydrolysis of the protein and lipid, moieties

Iodine-labelled chylomicron remnants (25–200 μl) were incubated for 4 h with hepatocyte monolayers (3.1 mg of cellular protein). The added chylomicron remnants contained (per 100 μl): 6.2 μg of cholesteryl ester, 168 μg of triacylglycerol and 17.3 μg of protein. The added protein ¹²⁵I radioactivity was 81 000 c.p.m. and the added ¹²⁵I lipid radioactivity was 17 000 c.p.m. During preparation of chylomicron remnants, hydrolysis of original chyle triacylglycerol was 65.7%. (a) Percentage of radioactivity (c.p.m.) of added chylomicron remnant iodine-labelled protein that was cell-surface bound (Δ), interiorized (○) and hydrolysed (▲). The Figure also shows c.p.m. of added lipid-bound iodine: □, cell-surface bound and ■, interiorized. Of the added lipid radioactivity 76% was in polar lipids, 8% in cholesterol and diacylglycerol, 4% in fatty acids, 5% in triacylglycerol and 7% in cholesteryl ester. Mean values ± S.E.M. of triplicate observations are plotted. (b) A double-reciprocal plot from values obtained for total uptake of iodine-labelled chylomicron remnant protein (cell-surface bound, interiorized and hydrolysed). The reciprocal of the amount (in μl) of chylomicron remnant added was plotted against the velocity (c.p.m. of total uptake during a 4 h incubation period).

calculated from this plot was 300 ng of remnant protein/h per mg of cell protein (albumin present in remnants is disregarded in this calculation). The added remnants contained 35.8 μg of cholesteryl

ester/100 μg of remnant protein and, as determined by scanning, approximately 47% of the added protein was albumin. The V_{max} for the cholesteryl ester uptake with this remnant preparation was thus 200 ng/h per mg of cell protein, if it is assumed that the cholesteryl ester and the peptides are taken up as a unit. This is within the range found in experiments with cholesteryl ester-labelled chylomicron remnants. In these experiments with remnants with different extents of hydrolysis of the triacylglycerol portion, the V_{max} for the cholesteryl ester uptake has varied between 80 and 320 ng/h per mg of cell protein and the apparent K_m between 1.4 and 5.9 μg of cholesteryl ester/mg of cell protein (Florén & Nilsson, 1977b; C.-H. Florén & Å. Nilsson, unpublished work). The apparent K_m of iodine-labelled chylomicron remnants was calculated to be 7.7 μg of remnant apolipoprotein/mg of cell protein. This would correspond to 5.1 μg of cholesteryl ester/mg of cell protein, which is within the range of the earlier experiments. At all concentrations of remnant particles the percentage uptake of iodine-labelled lipid exceeded that of the protein (Fig. 3a). The major portion of the ¹²⁵I-labelled lipid radioactivity was, however, in polar lipids and it was not possible to follow separately the uptake of the cholesteryl ester, which does not participate in exchange reactions.

Since some chylomicron remnant peptides may be more loosely bound to the particles different apolipoproteins might be taken up and degraded by the hepatocytes at different rates. Apolipoprotein B is considered to be a firmly bound structural component of triacylglycerol-rich lipoproteins (see Eisenberg & Levy, 1975). The ratio between tetramethylurea-insoluble (apolipoprotein B) and -soluble non-lipid radioactivity in the medium was measured to determine whether it remained constant during the incubation with hepatocytes. There was, if anything, a slight decrease in the proportion of B peptide rather than a selective uptake of other peptides (Table 4).

Effects of colchicine, chloroquine and NH₄Cl

Florén & Nilsson (1977a,b) have shown that colchicine and chloroquine inhibit the hydrolysis of chylomicron remnant cholesteryl ester after interiorization of the cholesteryl ester by hepatocytes. In the present study 25 μM-chloroquine and 33 μM-colchicine (Table 3) also inhibited the hydrolysis of chylomicron remnant protein. Inhibition of hydrolysis was, however, not complete, hydrolysis amounting to about 30–50% of the controls. The unhydrolysed protein remained intracellular, and surface binding of the iodine-labelled chylomicron remnants was unaffected (Table 3).

NH₄Cl (10 mM) partially inhibits the degradation of endogenous protein (Seglen & Reith, 1976) and of ¹²⁵I-labelled asialofetuin (Tolleshaug *et al.*, 1977) in

Table 3. *Effects of colchicine and chloroquine on binding, interiorization and hydrolysis of chylomicron remnant protein labelled with iodine*

Chylomicron remnants were added to hepatocyte cultures in three different experiments (1.7–2.5 mg of protein per dish). Chylomicron remnants added contained 16.1–29.5 μ g of protein and 10.1–10.8 μ g of cholesteryl ester. During preparation of chylomicron remnants hydrolysis of original chyle triacylglycerol was 94.8–95.9%. Chylomicron remnants contained 55 600–92 500 c.p.m. of 125 I in the protein moiety. Hepatocyte monolayers with chloroquine present were preincubated 30 min before remnants were added; colchicine and NH_4Cl were added together with remnants. All incubations continued for 4 h at 37°C. Incubations were interrupted with trypsinization as is described under 'Methods'. Results are given as % of control values (= 100%). All figures are means $\pm$ S.E.M. Total number of dishes is given in parentheses.

Addition	Cell-surface bound (%)	Interiorized (%)	Hydrolysed (%)
Control	100	100	100
33 μM -Colchicine (5)	102 $\pm$ 9	131 $\pm$ 3	58 $\pm$ 6
25 μM -Chloroquine (5)	108 $\pm$ 4	137 $\pm$ 4	68 $\pm$ 4
10 mM- NH_4Cl (3)	110 $\pm$ 5	118 $\pm$ 1	51 $\pm$ 14

Table 4. *Determination of percentage of tetramethylurea-insoluble non-lipid radioactivity during extended incubation periods of chylomicron remnants with hepatocyte monolayers*

Chylomicron remnants were added to hepatocyte monolayers (1.6 mg of cellular protein) and incubated for 4, 12 and 24 h. Incubations were interrupted with trypsinization as described under 'Methods'. Chylomicron remnants added contained 20 μ g of protein, 8.1 μ g of cholesteryl ester and 58 μ g of triacylglycerol. During preparation of chylomicron remnants from original chylomicrons, hydrolysis of the triacylglycerol portion was 87.5%. In the labelled protein moiety added, iodine radioactivity was 68 900 c.p.m. Tetramethylurea-insoluble non-lipid radioactivity as % of total non-lipid radioactivity was determined as described under 'Methods'. 50 μ l of unlabelled chylomicron remnants (1.3 μ g of cholesteryl ester, 19 μ g of triacylglycerol), obtained from hepatectomized rats, was added as a carrier to 200 μ l of medium to be analysed before mixing with tetramethylurea. TCA, trichloroacetic acid; TMU, tetramethylurea; n.d., not determined. All figures are means $\pm$ S.E.M. of three dishes.

Incubation time (h)	Hydrolysis of iodine-labelled protein (%)	Interiorization of iodine-labelled protein (%)	Cell-surface binding of iodine-labelled protein (%)	TCA-soluble iodide radioactivity (c.p.m.)	TCA-soluble non-iodide radioactivity (c.p.m.)	TMU-insoluble non-lipid radioactivity (%)
Added chylomicron remnant	—	—	—	5294	399	n.d.
Cell-free controls (4)	—	—	—	9228 $\pm$ 135	1297 $\pm$ 49	30.7 $\pm$ 1.6
4	3.1 $\pm$ 0.3	8.1 $\pm$ 0.5	20.1 $\pm$ 0.7	15 632 $\pm$ 309	2494 $\pm$ 120	26.2 $\pm$ 2.6
12	9.9 $\pm$ 0.4	10.7 $\pm$ 0.3	26.2 $\pm$ 1.5	24 900 $\pm$ 148	5002 $\pm$ 161	23.8 $\pm$ 1.8
24	20.0 $\pm$ 0.4	7.4 $\pm$ 0.3	26.7 $\pm$ 0.9	31 167 $\pm$ 763	8872 $\pm$ 191	23.5 $\pm$ 1.3

suspended rat hepatocytes. It inhibited degradation of iodine-labelled chylomicron remnants after uptake by the cells (Table 3). It also inhibited the hydrolysis of chylomicron remnant cholesteryl ester to about the same extent (40.0 $\pm$ 1.0%, mean $\pm$ S.E.M. of five dishes from two independent experiments).

Incubations of hepatocyte monolayers with chylomicron remnants at 4°C

When the experiments were performed at 4°C the hydrolysis of both the cholesteryl ester and the protein component was completely inhibited. The radioactivity in cholesteryl ester and in peptide associated with the cells was decreased by 73–78%, compared with results for controls incubated at 37°C.

Effects of concanavalin A

The effects of concanavalin A on the uptake and degradation of doubly labelled (^3H)cholesterol, [^{14}C]linoleic acid) chylomicron remnants in hepatocyte monolayers are shown in Fig. 4. Concanavalin A almost completely inhibited the degradation of the cholesteryl ester whereas the amount of [^3H]cholesteryl ester associated with cells remained constant. Although the major inhibition of the hydrolysis occurred after the uptake, there was thus some decrease also in the total uptake of [^3H]cholesteryl ester. There was a decrease in the ratio of radioactivity in the cellular ^{14}C -labelled polar lipid/triacylglycerol, suggesting that there was a decreased hydrolysis of triacylglycerol and thereby a decreased use of [^{14}C]fatty acid for phospholipid synthesis. In

Fig. 4. Effects of concanavalin A on uptake and degradation of cholesteryl ester-labelled chylomicron remnants

Concanavalin A was preincubated with hepatocyte monolayers (3.9 mg of cellular protein per dish) for 2 h. Chylomicron remnants were then added (0.69 µg of cholesteryl ester, 6.35 µg of triacylglycerol, 21 700 d.p.m. of ³H as cholesteryl ester, 6200 d.p.m. as [³H]cholesterol). In the added chylomicron remnants hydrolysis of original chyle triacylglycerol was 84.4%. The Figure shows means $\pm$ S.E.M. for duplicate dishes. In a similar experiment chylomicron remnants (0.89 µg of chylomicron remnant cholesteryl ester) were added to cells (3.0 mg of protein) preincubated with concanavalin A (1 mg/ml of medium). The increase in cell association of chylomicron remnant cholesteryl ester was $8.7 \pm 0.5\%$ and the decrease in chylomicron remnant cholesteryl ester hydrolysis was $99.3 \pm 3.3\%$ (means $\pm$ S.E.M., two dishes) compared with controls. ●, % cell association of chylomicron remnant cholesteryl ester; ▲, % hydrolysis of chylomicron remnant cholesteryl ester; ■, ratio cellular [¹⁴C]fatty acid radioactivity in phospholipids/cellular [¹⁴C]fatty acid radioactivity in triacylglycerol.

the highest concentration used (1 mg of concanavalin A/ml) an increased cellular granularity was noticed in phase-contrast microscopy. The cells, however, still adhered to the dishes and excluded Trypan Blue. Concanavalin A (100 µg/ml) did not significantly influence the rate of incorporation of ³H₂O into cholesterol and into glycerolipids. In 4 h incubations without serum, the rate of incorporation of ³H into cholesterol was 16.7 ± 0.9 ($n = 3$, mean $\pm$ S.E.M.) ng-atoms of ³H/mg of protein without and 19.8 ± 1.0 with concanavalin A present. The incorporation into glycerolipids was 98 ± 2.3 and 106.7 ± 0.7 ng-atoms/mg of protein with and without concanavalin A respectively. As concanavalin A might bind to chylomicron remnants (as is the case with low-density lipoproteins) (McConathy & Alaupovic, 1974), cells were preincubated with concanavalin A and then washed, before chylomicron remnants were added. The inhibition of degradation of chylomicron remnant cholesteryl ester was, however, the same as when it was added together with the remnants.

Concanavalin A (100 µg/ml) also inhibited the hydrolysis of iodine-labelled chylomicron remnant peptides by $55.8 \pm 6.3\%$ with concanavalin A (mean $\pm$ S.E.M., $n = 3$) present. Cell association of iodine-labelled protein increased from $16.9 \pm 0.6\%$ (controls, $n = 3$) to $24.3 \pm 1.5\%$ (concanavalin A, 100 µg/ml; $n = 3$, means $\pm$ S.E.M.) of that added.

Uptake of [¹⁴C]sucrose

The uptake of [¹⁴C]sucrose by the cultured hepatocytes over 4 h was low, and the percentage taken up did not vary when the sucrose concentration was increased from 6 to 30 µM. The uptake was 0.45% and with a medium volume of 2500 µl this represents 11.3 µl cleared of sucrose in 4 h (3.5 µl/mg of protein). By contrast, in incubations of hepatocyte monolayers with cholesteryl ester-labelled chylomicron remnants (0.34–0.82 µg as cholesteryl ester added per plate) an uptake (internalized cholesteryl ester plus cholesteryl ester hydrolysed) of 17.5–34.7% of that added was measured (Florén & Nilsson, 1977b). This would correspond to clearance of 437–934 µl of medium/4 h (269 ± 38 µl/mg of protein, six independent experiments, mean $\pm$ S.E.M.).

Discussion

Because of the high lipid content in chylomicrons it is not possible to get a pure labelling of the protein moiety without significant ¹²⁵iodine-labelling of the lipids. With chylomicrons obtained by feeding with glycerol trioleate rather than more unsaturated fat (Poulis & Fidge, 1977) and with a suitable ratio of iodine monochloride to chylomicron protein it was, however, possible to get 65% or more of the labelling in the protein portion. The remainder was mainly in the surface coat of polar lipids and it was thus not possible to compare directly the peptide uptake by the cells with that of a non-exchangeable lipid component as cholesteryl ester. Although a high ratio of iodine monochloride to lipoprotein favours the incorporation of ¹²⁵I into peptide rather than lipid (Noel *et al.*, 1975), less than 1 nmol of iodine was incorporated/nmol of protein under the conditions used. The degree of iodination did, however, vary for different peptides. Of special interest is the low degree of iodination of the arginine-rich peptide. This is good in the sense that any possible function of this peptide in the binding of remnants to hepatocytes would probably remain unaffected by the iodination of the chylomicrons from which remnants were prepared. On the other hand it was, of course, impossible to follow the uptake and degradation of this quantitatively important peptide separately.

As in earlier experiments with cholesteryl ester-labelled chylomicron remnants the uptake approached a saturation level when increasing amounts

of particles were added (Fig. 3). If this represented an uptake by the whole particle rather than binding and degradation of more loosely associated peptides like the A-1 (Fainaru *et al.*, 1975) and the C peptides, which may be transferred between different lipoprotein classes, the radioactivity ratio between this peptide group and the 'structural' B peptide would remain constant. The distribution of tetramethylurea-soluble and -insoluble radioactivity in remnant particles was therefore examined before and after incubation with the cells. The ratio was found to remain relatively unchanged after up to 24 h incubation, when more than 50% of the protein radioactivity had disappeared from the medium. There was thus no significant enrichment of the 'structural' B peptide in the particles remaining in the medium, and thus no evidence for a selective uptake of the more loosely associated proteins. However, there was some discrepancy between the amount of B peptide in chylomicrons as measured by scanning and by precipitation with tetramethylurea (Table 1). The reason for this is not clear.

Chloroquine, which inhibits lysosomal degradation of protein (Wibo & Poole, 1974) and of low-density lipoprotein (Goldstein *et al.*, 1975) in cultured cells, also inhibits the degradation of chylomicron remnant cholesteryl ester *in vivo* (Florén *et al.*, 1977) and in cultured hepatocytes (Florén & Nilsson, 1977a,b). Colchicine has the same effects on the degradation of remnant cholesteryl ester. Since chloroquine accumulates in lysosomes and drastically alters lysosomal structure and function the inhibition has been interpreted as evidence for degradation by an endocytic process, followed by lysosomal hydrolysis. *In vivo*, chloroquine inhibits the degradation of very-low-density lipoprotein peptide in the liver (Stein *et al.*, 1977). The inhibition of peptide degradation, which normally occurs at a slower rate than the cholesteryl ester degradation, was more complete than that of the cholesteryl ester degradation. In the present study chloroquine and colchicine had the same effects on peptide degradation, as was earlier shown on cholesteryl ester degradation. Neither chloroquine (25 μM) nor colchicine (33 μM) inhibited the hydrolysis of peptide by more than 50%. As estimated by the measurement of the increase in the trichloroacetic acid-soluble non-iodide radioactivity in the medium the percentage hydrolysis over 4 h was significantly less than that of the cholesteryl ester in earlier studies, whereas the portions that were associated with cells but not degraded were similar. There was, however, also a significant increase in the trichloroacetic acid-soluble iodide radioactivity during incubation, which exceeded that in the cell-free controls, suggesting that there may be a further oxidative metabolism of released tyrosine or deiodination of protein (Table 4). It is thus possible that the hydrolysis of protein is underestimated if it is measured as the release of

iodinated tyrosine. Since the metabolism of iodine-labelled lipids and the release of loosely bound ^{125}I may contribute to the released ^{125}I , it is, however, not possible to quantify exactly the oxidative degradation of formed ^{125}I -labelled tyrosine from the present data.

In earlier studies it was demonstrated that NH_4Cl inhibits lysosomal degradation of protein (Seglen & Reith, 1976) and of desialylated fetuin taken up by endocytosis in hepatocytes (Tolleshaug *et al.*, 1977). This has been ascribed to accumulation of ammonia in the lysosomes and thereby increased intralysosomal pH. If the degradation of remnants occurs by endocytosis and lysosomal hydrolysis the degradation of both peptide and cholesteryl ester would therefore be expected to be inhibited by NH_4Cl . This was actually found to be the case. The degree of inhibition, i.e. 35–50% with 10 mM- NH_4Cl , is about the same as that noted by Tolleshaug *et al.* (1977) for the degradation of asialofetuin, which is known to be degraded by an endocytic process.

Goldstein *et al.* (1976) have shown that binding of low-density lipoproteins to cultured human fibroblasts was equal at 4°C and 37°C, whereas no degradation of low-density lipoproteins occurs in the cold. In the present experiments the degradation of both chylomicron remnant peptides and cholesteryl ester was completely inhibited at 4°C. The amount of undegraded remnants that was cell-associated decreased, however, also at 4°C. It is therefore not clear whether it is possible to study the binding of remnants at 4°C to characterize binding kinetics in a situation when no further metabolism occurs, since the 'receptor function' also might be influenced by temperature.

As sucrose is likely to be taken up by random pinocytosis (Wagner *et al.*, 1971; Steinman *et al.*, 1976), comparison between uptake of sucrose and chylomicron remnants was made to determine to what extent non-adsorptive pinocytosis contributes to interiorization and degradation of chylomicron remnants. Since the uptake of remnants was 77 times as efficient as that of sucrose, this contribution must, however, be small. Instead it is probable that chylomicron remnants are interiorized by a mechanism preceded by a specific binding to the hepatocyte. The relations between sucrose and remnant uptake are about the same as that between sucrose and low-density lipoprotein by cultured human fibroblasts (Miller *et al.*, 1977).

Concanavalin A, which is a lectin with affinity for the D-gluc- and D-manno-pyranose groups of cell-surface glycoproteins, might interfere with chylomicron remnant degradation in a number of ways. The binding to the cell surface might block carbohydrate groups which participate in the binding of lipoproteins to the cell surface. For instance, Drevon *et al.* (1977) showed that concanavalin A significantly decreases the uptake of high-density lipoproteins by hepatocyte suspensions and suggested that a receptor

of carbohydrate nature exists. Concanavalin A might also bind to the lipoprotein, particularly if this contains B peptide (McConathy & Alaupovic, 1974). Finally the binding of concanavalin A to the cells may lead to an accumulation of plasma membrane material as endocytic vesicles, which do not undergo fusion with lysosomes, as is the case in macrophages (Edelson & Cohn, 1974). In the present study concanavalin A somewhat reduced the total uptake of cholesteryl ester-labelled chylomicron remnants. Both the presence of and the preincubation of the cells with concanavalin A caused, however, a much more significant inhibition of the hydrolysis of cholesteryl ester after the uptake. This suggests that the remnant uptake does not depend on a concanavalin A-binding cell-surface receptor or on concanavalin A-binding structures on the lipoprotein particle. Instead the data are compatible with a degradation by endocytosis mediated by another type of receptor, succeeded by lysosomal degradation for which a concanavalin A-sensitive fusion with the endocytic vesicle (Edelson & Cohn, 1974) is necessary.

The structures on the cell surface and on the remnant particle that participate in the binding of the particle to the cell surface are presently unknown. The very significant relative increase in the percentage of arginine-rich peptide in the remnant particles which was seen earlier (Mjøs *et al.*, 1975) as well as in the present study provides, however, groups that may interact strongly with negatively charged cell-surface components such as glycosaminoglycans (Mahley & Innerarity, 1977; Shelburne & Quarfordt, 1977). The hypothesis that this contributes to the high affinity of remnants for hepatocytes has hitherto not been tested.

Miss Gertrud Ohlsson and Mrs Hildegund Lundberg provided skilful technical assistance. We thank Dr. Rolf Ekman, Department of Clinical Chemistry, University Hospital, Lund, for providing reference proteins of known molecular weight. The work was supported by grants from: the Swedish Medical Research Council (grant no. 03X-3969); the Medical Faculty, University of Lund; Albert Pahlssons Stiftelse; Alfred Österlunds Stiftelse; Svenska Livförsäkringsbolagens Nämnd för Medicinsk Forskning and Segerfalks Stiftelse.

References

- Abell, L. L., Levy, B. B., Brodie, B. B. & Kendall, F. E. (1952) *J. Biol. Chem.* **195**, 357–366
- Åkesson, B., Elovson, J. & Arvidson, G. (1970) *Biochim. Biophys. Acta* **210**, 15–27
- Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520
- Bierman, E. L., Stein, O. & Stein, Y. (1974) *Circ. Res.* **35**, 136–150
- Bisell, D. M., Hammaker, L. E. & Meyer, U. A. (1973) *J. Cell Biol.* **59**, 722–734
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917
- Bollman, J. L., Cain, J. C. & Grindlay, J. H. (1948) *J. Lab. Clin. Med.* **33**, 1349–1352
- Bonney, R. J., Becker, J. E., Walker, P. R. & Potter, V. R. (1974) *In Vitro* **9**, 399–413
- Breslow, J. L., Lothrop, D. A., Clowes, A. W. & Lux, S. E. (1977) *J. Biol. Chem.* **252**, 2726–2733
- Cooper, A. D. (1977) *Biochim. Biophys. Acta* **488**, 464–474
- Drevon, C. A., Berg, T. & Norum, K. R. (1977) *Biochim. Biophys. Acta* **487**, 122–136
- Edgren, B. & Zilversmit, D. B. (1965) *Proc. Soc. Exp. Biol. Med.* **119**, 64–71
- Edelson, P. J. & Cohn, Z. A. (1974) *J. Exp. Med.* **140**, 1364–1386
- Eisenberg, S. & Levy, R. I. (1975) *Adv. Lipid Res.* **13**, 1–89
- Faergeman, O. & Havel, R. J. (1975) *J. Clin. Invest.* **55**, 1210–1218
- Faergeman, O., Sata, T., Kane, J. P. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 1396–1403
- Fainaru, M., Glangaud, M. C. & Eisenberg, S. (1975) *Biochim. Biophys. Acta* **386**, 432–443
- Fainaru, M., Felker, T. E., Hamilton, R. L. & Havel, R. J. (1977) *Metabolism* **26**, 999–1004
- Fielding, C. J. & Fielding, P. E. (1976) *J. Lipid Res.* **17**, 419–423
- Florén, C.-H. & Nilsson, Å. (1977a) *Biochem. Biophys. Res. Commun.* **74**, 520–528
- Florén, C.-H. & Nilsson, Å. (1977b) *Biochem. J.* **168**, 483–494
- Florén, C.-H., Nordgren, H. & Nilsson, Å. (1977) *Eur. J. Biochem.* **80**, 331–340
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Gardner, R. S. & Mayes, P. A. (1976) *Biochem. Soc. Trans.* **4**, 715–716
- Glickman, R. M. & Green, P. H. R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2569–2573
- Goldstein, J. L. & Brown, M. S. (1974) *J. Biol. Chem.* **249**, 5153–5162
- Goldstein, J. L., Brunschede, G. Y. & Brown, M. S. (1975) *J. Biol. Chem.* **250**, 7854–7862
- Goldstein, J. L., Basu, S. K., Brunschede, G. Y. & Brown, M. S. (1976) *Cell* **7**, 85–95
- Goodman, D. S. (1962) *J. Clin. Invest.* **41**, 1886–1896
- Kane, J. P., Sata, T., Hamilton, R. L. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 1622–1634
- Lin, R. C. & Snodgrass, P. J. (1975) *Biochem. Biophys. Res. Commun.* **64**, 725–734
- Lossow, W. J., Brot, N. & Chaikoff, I. L. (1962) *J. Lipid Res.* **3**, 207–215
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mahley, R. W. & Innerarity, T. L. (1977) *J. Biol. Chem.* **252**, 3980–3986
- McConathy, W. J. & Alaupovic, P. (1974) *FEBS Lett.* **41**, 174–178
- McFarlane, A. S. (1958) *Nature (London)* **182**, 53
- Miller, N. E., Weinstein, D. B. & Steinberg, D. (1977) *J. Lipid Res.* **18**, 438–450
- Minari, O. & Zilversmit, D. B. (1963) *J. Lipid Res.* **4**, 424–436
- Mjøs, O. D., Faergeman, O., Hamilton, R. L. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 603–615

- Neville, D. M., Jr. (1971) *J. Biol. Chem.* **246**, 6328–6334
- Nilsson, Å. (1975) *Biochem. Biophys. Res. Commun.* **66**, 60–66
- Nilsson, Å. (1977) *Biochem. J.* **162**, 367–377
- Noel, S.-P., Dolphin, P. J. & Rubinstein, D. (1975) *Biochem. Biophys. Res. Commun.* **63**, 764–772
- Ontko, J. A. (1970) *J. Lipid Res.* **11**, 367–375
- Poulis, P. & Fidge, N. H. (1977) *Lipids* **12**, 288–292
- Redgrave, T. G. (1970) *J. Clin. Invest.* **49**, 465–471
- Seglen, P. O. (1973) *Exp. Cell. Res.* **82**, 391–398
- Seglen, P. O. & Reith, A. (1976) *Exp. Cell Res.* **100**, 276–280
- Shelburne, F. A. & Quarfordt, S. H. (1977) *J. Clin. Invest.* **60**, 944–950
- Stein, O. & Stein, Y. (1975) *Biochim. Biophys. Acta* **398**, 377–384
- Stein, O., Rachmilewitz, D., Sanger, L., Eisenberg, S. & Stein, Y. (1974) *Biochim. Biophys. Acta* **360**, 205–216
- Stein, Y., Ebin, V., Bar-On, H. & Stein, O. (1977) *Biochim. Biophys. Acta* **486**, 286–297
- Steinman, R. M., Brodie, S. E. & Cohn, Z. A. (1976) *J. Cell Biol.* **68**, 665–687
- Tolleshaug, H., Berg, T., Nilsson, M. & Norum, K. R. (1977) *Biochim. Biophys. Acta* **499**, 73–84
- Tornqvist, H. & Belfrage, P. (1976) *J. Lipid Res.* **17**, 542–545
- Wagner, R., Rosenberg, M. & Estensen, R. (1971) *J. Cell Biol.* **50**, 804–817
- Wibo, M. & Poole, B. (1974) *J. Cell Biol.* **63**, 430–440
- Zak, B., Moss, N., Boyle, A. J. & Zlatkis, A. (1954) *Anal. Chem.* **26**, 776–777