Secretion of nascent lipoproteins and apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs

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Abstract Triglyceride-rich very low density lipoproteins (VLDL) are the major lipoprotein in perfusates of normal guinea pig livers. Their component apoprotein B is mainly B-100 together with some B-95. This apoprotein is actively synthesized, as are C apoproteins and small amounts of apoprotein E. Only trace amounts of intermediate density lipoproteins (IDL, $1.015 < d < 1.05$ g/ml) are found in perfusates, but appreciable amounts of low density lipoproteins (LDL, $1.05 < d < 1.10$ g/ml) accumulate. These LDL are not newly synthesized, but rather appear to be gradually washed out of the liver. High density lipoproteins (HDL, $1.10 < d < 1.21$ g/ml) both discoidal and spheroidal, also accumulate, which contain newly synthesized apoproteins A-I, E and C. Fatty livers of guinea pigs fed cholesterol secrete less VLDL and more IDL than normals, but the combined amount of protein is unchanged. These lipoproteins contain newly synthesized apoprotein B, are enriched in cholesteryl esters and newly synthesized apoprotein E, and have reduced electrophoretic mobilities, making them resemble remnants. Large amounts of LDL also accumulate in perfusates of livers from cholesterol-fed animals, much of which does not appear to be newly synthesized, as judged from single pass perfusions. However, the LDL fraction is complex and includes particles that contain newly synthesized apoprotein B. Thus, these livers appear to secrete a spectrum of cholesteryl ester-rich particles, containing newly synthesized apoproteins B and E that span the density range of VLDL, IDL, and LDL. Livers of cholesterol-fed guinea pigs secrete large amounts of discoidal HDL with a free cholesterol-phospholipid molar ratio of 2:1. Accumulation of protein (almost entirely newly synthesized apoprotein E) in HDL is increased 25-fold over that in perfusates from normal guinea pig livers.—**Guo, L.S.S., R.L. Hamilton, R. Ostwald, and R. J. Havel.** Secretion of nascent lipoproteins and apolipoproteins by perfused livers of normal and cholesterol-fed guinea pigs. *J. Lipid Res.* 1982. **23:** 543–555.

Supplementary key words cholesterol fatty livers • abnormal nascent very low density and intermediate density lipoproteins • discoidal high density lipoproteins • electron microscopy

The guinea pig, unlike other experimental animals, has remarkably low concentrations of high density lipoproteins (HDL) in blood plasma (1, 2). Low density lipoproteins (LDL) are by far the major lipid-bearing fraction in the plasma of this species (1, 3). These LDL are more dense than those of most other species with a hydrated density between 1.05–1.10 g/ml (3). The fraction of cholesteryl esters of very low density lipoproteins (VLDL) converted to LDL is considerably larger in guinea pigs than in rats (4). Cholesteryl esters of guinea pig LDL, like those of humans, may be largely derived from plasma lecithin:cholesterol acyltransferase (LCAT) (4).

In response to dietary cholesterol, guinea pigs differ from other species thus far described in several interesting respects. Whereas most species respond to dietary cholesterol with a reduction of plasma HDL concentration (5), this density fraction increases many-fold after several weeks of cholesterol feeding to guinea pigs (6, 7). Furthermore, a distinct plasma HDL particle accumulates (6) that appears remarkably similar to the apoE-rich discoidal HDL found in humans with LCAT deficiency (8–11), and in perfusates of rat liver when LCAT is inhibited (12). During the first few weeks of cholesterol feeding, hepatic cholesterol acyl CoA acyltransferase (ACAT) increases several-fold (13), hepatic cholesteryl esters increase markedly (14), and plasma VLDL become enriched in cholesteryl esters (3).

To study the origins of normal nascent plasma lipoproteins and those abnormal lipoproteins that appear in the plasma of guinea pigs fed cholesterol, we have characterized and quantified the lipoproteins that accumulate in liver perfusates of these animals. We show here that many of the abnormal lipoproteins found in the plasma of these cholesterol-fed animals are newly synthesized by the abnormally fatty livers.

Abbreviations: VLDL, very low density lipoprotein(s); IDL, intermediate density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); apoB, apolipoprotein B; apoE, apolipoprotein E; apoA-I, apolipoprotein A-I; SDS, sodium dodecyl sulfate; LCAT, lecithin:cholesterol acyltransferase; ACAT, acyl CoA acyltransferase; TMU, tetramethylurea.

MATERIALS AND METHODS

Animals

Male albino guinea pigs weighing 200–250 g (Simonson Lab., Gilroy, CA) were fed Purina chow ad libitum (normal) or the Purina chow supplemented with 5% cottonseed oil and 1% cholesterol (7). Animals were usually maintained on the cholesterol diet for 8–12 weeks and those weighing 550–650 g were selected as liver donors. In some experiments, animals fed cholesterol for a shorter period (10–28 days) were used.

Recirculating liver perfusion

Perfusions were carried out in pairs by the in situ method with a "lung" of 7.6 meters of silastic tubing, as previously described (12). The perfusion medium (1 volume thrice-washed guinea pig erythrocytes and 3 volumes Krebs-Henseleit buffer containing 0.15% glucose, pH 7.4) was pumped through the liver at a rate of 12–14 $ml \cdot min^{-1}$. Each liver was initially flushed with 40 ml of perfusate and then perfused in a recirculating system. Usually, fresh perfusate (40 ml) was replaced after the first 30 min of perfusion, and the perfusion was continued for 5 hr. Because of losses of perfusate volume during the perfusion (due largely to secretion of 10–15 ml bile in 5 hr), Krebs-Henseleit buffer was added at 30-min intervals to the reservoir to maintain the initial volume of about 50 ml. To study hepatic biosynthesis of apolipoproteins, 100 μCi of [4,5-^{3}H]lysine (Amersham, Arlington Heights, IL) in perfusate buffer was added to the reservoir at 30-min intervals (1.0 mCi/liver). In some experiments, hourly samples of perfusate (1.2 ml) were withdrawn for analyses of accumulated lipid and apolipoprotein.

Single pass liver perfusion

The perfusion apparatus and the silastic tubing "lung" were the same used for the recirculating system. The perfusion medium was Krebs-Henseleit buffer containing normal guinea pig erythrocytes (7% hematocrit), 0.15% glucose, pH 7.4. About 900 ml of the medium containing 1 mCi of [4,5-^{3}H]lysine was pumped thruogh each liver at a rate of 9 $ml \cdot min^{-1}$. Livers were flushed first with 30 ml of perfusion medium (3.3 min) and then perfused for 1.5 hr. The perfusate was collected and chilled immediately in ice. Erythrocytes were removed by centrifugation (20,000 rpm, 10 min) and the perfusate plasma was concentrated to about 50 ml by ultrafiltration (Xm 50 membrane, Amicon Co., Danvers, MA).

Isolation of lipoproteins

Perfusate lipoproteins were isolated by sequential ultracentrifugation (15) at 4°C in a 40.3 rotor (Beckman Instruments, Inc., Palo Alto, CA) at the following densities: VLDL, $d < 1.015$ g/ml; IDL, $1.015 < d < 1.05$ g/ml; LDL, $1.05 < d < 1.10$ g/ml; HDL, $1.10 < d < 1.21$ g/ml. The lipoprotein fractions were recentrifuged at their upper density limits. All fractions were dialyzed for 24–40 hr at 4°C against 0.9% NaCl containing 0.02% NaN_3 and 0.04% EDTA, pH 7.4.

The densities of the perfusate fractions were chosen for the following reasons: *1*) VLDL, $d < 1.015$ g/ml, is the density of the perfusate at the end of the experiments; *2*) "IDL," $1.015 < d < 1.05$ g/ml was chosen because only trace amounts of lipoproteins are present in this density interval in perfusates of normal livers, whereas almost all perfusate "LDL" of normal guinea pig liver is recovered between densities of 1.05 and 1.10 g/ml; *3*) the HDL fraction of $1.10 < d < 1.21$ g/ml contains no detectable LDL and no apoB.

Measurement of radioactivity

To measure incorporation of radioactivity into lipoprotein fractions, a portion was dissolved in 10 ml of Aquasol (New England Nuclear, Boston, MA) containing 0.5% glacial acetic acid; ^{3}H was counted by liquid scintillation spectrometry. ^{3}H was measured in apolipoproteins separated by polyacrylamide gel electrophoresis in SDS by a modified method of Young (16). Stained gels were sliced into 2-mm discs with a gel slicer (Hoefer Scientific Instruments, San Francisco, CA). Individual slices were allowed to dissolve in 0.4 ml of 30% H_2O_2 at 25°C overnight; 10 ml of Aquasol containing 0.5% glacial acetic acid was then added.

Analytical techniques

Isolated perfusate lipoproteins were examined by agarose gel electrophoresis (17). Apolipoproteins were separated in 10% and 3% polyacrylamide gels containing 0.1% SDS. For 10% gels, lipoproteins (30–50 μg of protein) were delipidated by incubation in 1% SDS, 5% 2-mercaptoethanol at 90°C for 3 min and then subjected to electrophoresis (18). The gels were stained in 0.25% Coomassie blue in ethanol–water–acetic acid 9:9:2 (by vol) and destained in 10% acetic acid. For 3% gels, lipoproteins were delipidated by extraction twice with 20 vol of ethanol–diethyl ether 3:1 (vol/vol). Apoproteins were dissolved in 0.02 M phosphate buffer (pH 7.2) containing 1% SDS, 1% 2-mercaptoethanol (2 mg of protein/ml buffer). Gels were fixed, stained, and destained according to Kane, Hardman, and Paulus (19). Lipoprotein-protein was determined by a modified procedure of Lowry et al. (20) using bovine serum albumin as standard. Content of lipids of perfusate and plasma lipoproteins was measured by standard techniques (21). Concentration of cholesterol in perfusates was determined by an enzymatic procedure (22) or by gas–liquid

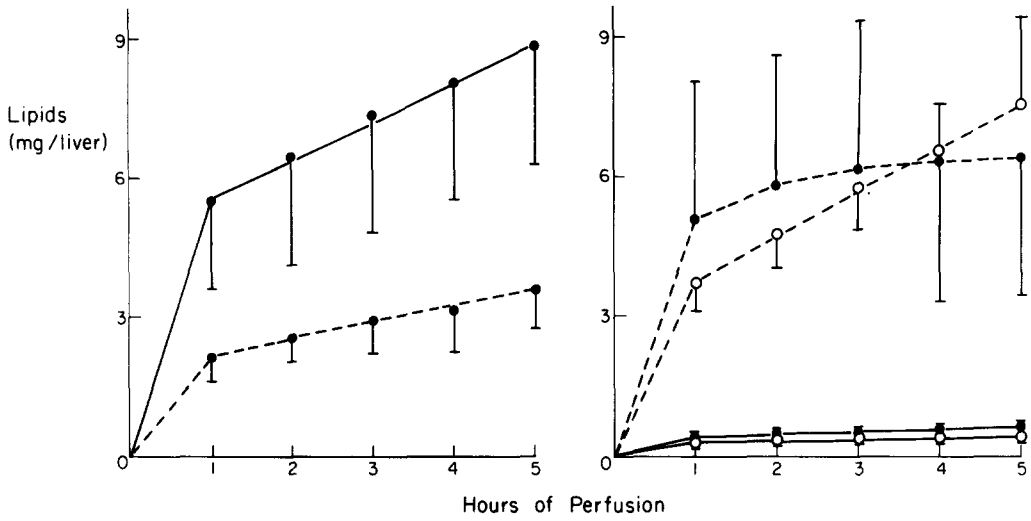

Fig. 1. Left: Accumulation of triglycerides in perfusates during the recirculating perfusions of normal (solid line) and cholesterol-fed fatty (dotted lines) livers. Right: Accumulation of cholesterol (○) and cholesteryl esters (●) in perfusates during recirculating perfusions for 5 hr of normal (solid line) and cholesterol-fed fatty (dotted line) livers. Circles and bars represent means and SD for four livers. All livers were perfused by recirculating the medium for 30 min before the perfusate was replaced with fresh medium.

chromatography (23) with β-sitosterol (Applied Science, State College, PA) as an internal standard. ApoE and apoA-I in perfusates were quantified by an immunoelectrophoretic technique in the presence of 0.1% Triton (24). Concentration of purified apolipoproteins used for immunoelectrophoretic standards was determined by amino acid analysis (25). Contents of apoB in perfusate IDL and LDL were estimated by measuring protein mass insoluble in 4.2 M 1,1,3,3-tetramethylurea (TMU) (26). To estimate the amount of lipoprotein-bound apoE, 1 ml of fresh perfusate from controls and fatty livers was fractionated on a 10% agarose gel column (1.2 × 50 cm). ApoE in pooled column fractions was quantified by the immunoelectrophoretic technique. Recovery of apoE from the column was about 90%.

Electron microscopy

Negatively stained preparations of perfusate lipoproteins were prepared and sized as described (27). They were examined and photographed at 60,000 diameters and 80 kV in a Siemens 101 electron microscope (Siemens Corp., Medical/Industrial Groups, Iselius, NJ).

RESULTS

Secretion of lipids and apolipoproteins

Guinea pigs fed cholesterol for 8–12 weeks developed severe hypercholesterolemia and fatty livers. The weight of the livers was increased 2- to 3-fold (such livers contain about 30-fold more cholesteryl esters, 3-fold more unesterified cholesterol, and about twice as much triglyceride as normals (14)). During the last 4 hr of perfusion, the rate of accumulation of triglycerides in the perfusates of fatty livers from cholesterol-fed animals was significantly lower than in those fed chow (365 vs. 780 $g \cdot hr^{-1}$ (**Fig. 1**, left). However, during these 4 hr, much more unesterified cholesterol (1035 vs. 36 $\mu g \cdot hr^{-1} \cdot liver^{-1}$) and cholesteryl esters (323 vs. 54 $\mu g \cdot hr^{-1} \cdot liver^{-1}$) accumulated in perfusates of the fatty livers (Fig. 1, right). Accumulation of lipids in perfusates was linear between 1 and 5 hr with the exception that cholesteryl esters remained constant in perfusates of the fatty livers after the first hour. A rapid initial release of lipids into the perfusate was found in both types of livers during the first hr of perfusion (Fig. 1), although all livers were perfused by recirculating the medium for 30 min before the perfusate was replaced with fresh medium.

Accumulation of apoE and apoA-I was linear throughout the perfusion except for a large initial release of apoE from the fatty livers during the first hour (**Fig. 2**). Thereafter, the rate of accumulation of apoE was greater in perfusates of the fatty livers than in controls (422 vs. 165 $\mu g \cdot hr^{-1} \cdot liver^{-1}$). As determined by chromatography on columns of 10% agarose gel, about 95% of the apoE recovered from whole perfusates of both control and fatty livers was associated with lipoprotein particles (data not shown). Accumulation of apoA-I in perfusates was much lower than that of apoE in both normals and fatty livers, although the rate was doubled in the fatty livers as compared to controls (15.2 vs. 7.5 $\mu g \cdot hr^{-1} \cdot liver^{-1}$).

Yield of perfusate lipoproteins

VLDL was the major lipoprotein recovered from perfusates of normal livers after 5 hr recirculating perfusion

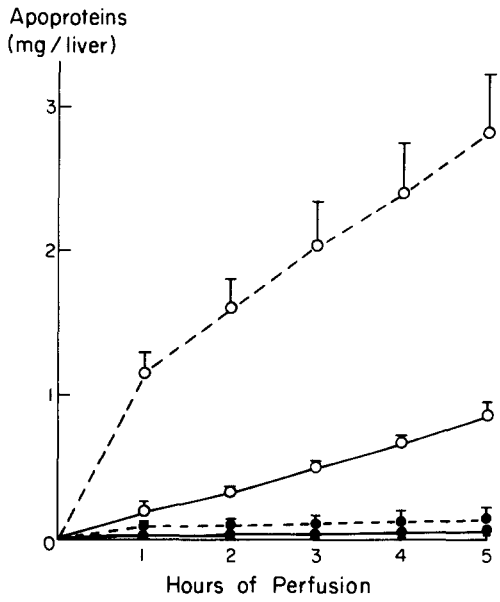

Fig. 2. Accumulation of apoE (○) and apoA-I (●) in perfusates during recirculating perfusions of normal (solid line) and cholesterol-fed fatty (dotted line) livers. Circles and bars represent means and SD for four livers. All livers were perfused by recirculating the medium for 30 min before the perfusate was replaced with fresh medium.

(**Table 1**). Because only trace amounts of lipoprotein with the same electrophoretic mobility and similar chemical composition as VLDL (**Table 2**) were present in the IDL fraction ($1.015 < d < 1.05$ g/ml), this material was routinely included together with the VLDL by ultracentrifugation at 1.05 g/ml, yielding a single pre-β band on agarose gel electrophoresis (**Fig. 3**). In earlier studies carried out without a 30-min recirculating flush, variable amounts of LDL (0.5–1.2 mg lipoprotein protein) were recovered from perfusates of normal livers. The amount of LDL protein recovered was much less variable and accounted for only about 20% of the total lipoprotein protein recovered after the flush procedure was established (Table 1). This LDL had the same chemical composition (**Table 3**), cholesteryl ester fatty acids (**Table 4**), particle size, and apoprotein pattern (see below) as serum LDL. The flush procedure did not change the overall amount of nascent HDL recovered (9% of total lipoprotein protein) from normal livers.

Yield of perfusate lipoproteins from the fatty livers was remarkably different from that of the controls. Whereas the amounts of IDL, LDL, and HDL recovered were strikingly increased, accumulation of VLDL was significantly lower ($P < 0.01$) (Table 1). During the course of cholesterol feeding, the ratio VLDL/IDL protein recovered from liver perfusates fell from 7.5:1 in controls (n = 4) to 3.6:1 after 10 days (n = 4), 0.7:1 after 1 month (n = 4), and finally, to 0.3:1 after 3 months (n = 6). These VLDL and IDL particles, unlike those from control perfusates, exhibited β-mobilities upon agarose gel electrophoresis (Fig. 3). Perfusate LDL from normal livers exhibited a single band of β-mobility, wherease perfusate LDL from fatty livers usually contained one major and one minor band of β- and α-mobilities, respectively. Perfusate HDL from fatty livers had a slightly lower mobility than HDL from control livers (Fig. 3).

Composition of perfusate lipoproteins

Both VLDL and IDL from cholesterol-fed guinea pig livers contained much more cholesterol and cholesteryl esters than normals (Table 2). VLDL contained pro-

TABLE 1. Yield of lipoprotein-protein from perfused livers of normal and cholesterol-fed guinea pigs[a]

	Control	Cholesterol-fed	
	Recirculating	Recirculating	Single Pass
	mg/liver/5 hr	*mg/liver/5 hr*	*mg/liver/1.5 hr*
VLDL[b]	0.75 ± 0.17[c]	0.20 ± 0.11	0.13 (0.12–0.15)
IDL ($1.015 < d < 1.05$ g/ml)	—	0.62 ± 0.29	0.94 (0.71–1.16)
LDL ($1.05 < d < 1.10$ g/ml)	0.22 ± 0.07	1.23 ± 0.50	3.98 (3.98–3.99)
HDL ($1.10 < d < 1.21$ g/ml)	0.09 ± 0.03	2.26 ± 0.84	1.14 (0.94–1.33)

[a] All animals were fasted overnight (17–18 hr). Recirculating liver perfusion was carried out for 5 hr after a 30-min recirculating flush. For single pass perfusions, livers were flushed with 30 ml of perfusion medium and then perfused for 1.5 hr.

[b] In liver perfusates of the normal animals, only trace amounts of lipoprotein were present between densities 1.015 and 1.05 g/ml; VLDL were routinely isolated at $d < 1.05$ g/ml. In cholesterol-fed animals, VLDL were isolated at $d < 1.015$ g/ml.

[c] Values represent mean ± SD from six livers, or mean and range (in parentheses) of two livers.

TABLE 2. Effect of cholesterol feeding on composition of perfusate lipoproteins

	Days Fed Cholesterol			
	0	10	28	84
	% of mass			
VLDL (d < 1.015 g/ml)				
Triglycerides	71.1 ± 4.2[a]	57.6 ± 2.3	45.8 ± 7.5	36.3 ± 0.0
Cholesteryl esters	0.3 ± 0.1	13.3 ± 0.7	23.9 ± 3.9	32.2 ± 0.4
Cholesterol	3.2 ± 0.1	7.0 ± 0.4	9.0 ± 1.0	13.8 ± 0.3
Phospholipids	16.3 ± 2.5	13.2 ± 0.7	12.8 ± 2.3	11.6 ± 0.6
Protein	8.9 ± 1.6	8.8 ± 0.8	8.5 ± 6.0	6.0 ± 0.4
IDL (d 1.015–1.05 g/ml)				
Triglycerides	52.5 ± 4.8	10.5 ± 4.2	6.2 ± 0.6	6.3 ± 3.0
Cholesteryl esters	2.2 ± 0.9	47.3 ± 5.6	48.2 ± 5.0	48.1 ± 3.3
Cholesterol	5.2 ± 3.6	12.0 ± 0.8	15.6 ± 1.8	18.5 ± 0.5
Phospholipids	18.3 ± 2.0	16.3 ± 1.1	14.8 ± 1.2	15.2 ± 0.2
Protein	21.8 ± 1.5	13.8 ± 0.7	14.8 ± 1.6	11.9 ± 0.1
LDL (d 1.05–1.10 g/ml)				
Triglycerides	9.0 ± 0.6	7.1 ± 2.5		2.8 ± 0.6
Cholesteryl esters	42.0 ± 3.2	34.4 ± 9.5		28.2 ± 2.0
Cholesterol	5.1 ± 0.8	15.0 ± 1.0		24.3 ± 2.3
Phospholipids	16.8 ± 0.2	21.1 ± 4.2		23.0 ± 3.1
Protein	27.1 ± 1.8	22.4 ± 1.8		21.6 ± 3.9
HDL (d 1.10–1.21 g/ml)				
Triglycerides	12.9[b]	4.5 (3.6–5.4)	4.1 ± 2.4	0.8 ± 0.07
Cholesteryl esters	19.1	4.4 (3.9–4.9)	2.5 ± 1.1	1.4 ± 0.5
Cholesterol	3.8	22.0 (21.4–22.7)	27.3 ± 1.0	31.4 ± 1.5
Phospholipids	19.1	33.5 (32.4–34.7)	30.1 ± 2.9	34.3 ± 0.5
Protein	45.0	35.4 (33.6–37.3)	36.0 ± 2.7	32.2 ± 1.6

[a] Values are expressed as mean ± SD from three livers, or mean and range (in parentheses) of two livers.
[b] Single determination of pooled fraction from two livers.

gressively increasing amounts of cholesteryl esters, from only 0.3% of mass normally to reach 33% of mass after 3 months on the cholesterol diet. However, the mass of total core lipids (cholesteryl esters plus triglycerides) remained unchanged (Table 2). IDL showed a much more rapid shift in composition; by 10 days, the core composition was shifted markedly from mostly triglyceride to mostly cholesteryl esters (Table 2). The LDL composition also was changed by cholesterol feeding, although much of the effect was due to the incomplete separation of large discoidal particles like the HDL (see Fig. 5).

Perfusate HDL from fatty livers contained somewhat less protein by mass compared to controls and showed a striking shift in lipid composition (Table 2). The core lipids were reduced from 30% of mass in normal per-

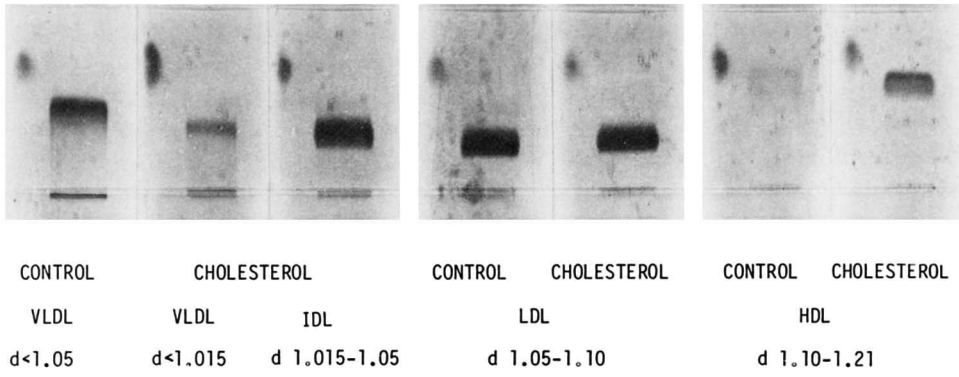

Fig. 3. Agarose gel electrophoretograms of perfusate lipoproteins from normal and cholesterol-fed fatty livers. Marker at left in each gel is human serum albumin.

TABLE 3. Composition of plasma and perfusate LDL (1.05 < d < 1.10 g/ml) from normal guinea pigs

	Triglyceride	Cholesteryl Esters	Free Cholesterol	Phospholipids	Protein
			% of mass		
Plasma LDL	7.6 ± 2.0[a]	41.5 ± 1.6	5.7 ± 0.4	16.7 ± 0.8	28.1 ± 2.7
Perfusate LDL	9.0 ± 0.6	42.0 ± 3.2	5.1 ± 0.8	16.8 ± 0.2	27.1 ± 1.8

[a] Values are expressed as mean ± SD from three preparations.

fusate HDL to 9% in 10 days and to only 2% after 3 months. By that time, the molar ratio of cholesterol to phospholipids had risen from 0.4:1 to 2:1.

Electron microscopy

The triglyceride-rich VLDL and IDL from perfused livers of the control guinea pigs appeared spherical in negative stains (**Fig. 4**, top: left and middle). They were variable in size and closely resembled plasma lipoproteins of the same density range (not shown). LDL were spherical particles of 185 Å mean diameter undistinguishable from plasma LDL (**Fig. 5**, top: left and right). HDL from the control perfusates contain both discs and apparently spherical particles the same size and appearance as plasma HDL (Fig. 4, top: right). Cholesteryl ester-rich VLDL and IDL from fatty livers contained many particles with angular surfaces (Fig. 4, middle: left and middle). LDL were a polymorphic mixture of spherical, angular and large discoidal particles (Fig. 5, bottom). The cholesteryl ester-poor HDL appeared to be composed almost entirely of discs that formed rouleaux in the negatively stained preparations (Fig. 4, middle: right). Both angular and discoidal particles were seen in unfractionated whole perfusates (Fig. 4, bottom).

Apoprotein composition of lipoproteins from perfused livers

Analytical SDS gel electrophoretograms (**Fig. 6**, left) showed that perfusate VLDL from normal livers contained mainly apoB, apoE, and apoC, whereas apoB was the principal apoprotein of perfusate LDL. The major apoproteins of perfusate HDL were apoE, apoA-I, and apoC. The prominent changes in perfusate lipoproteins from the fatty livers were a substantial increase in the proportion of apoE and a decrease of apoC. Similar apoprotein patterns of lipoproteins obtained from fatty livers were present in the single pass perfusions, except that the content of apoC was even lower (not shown). In 3% polyacrylamide gels (Fig. 6, right), apoB from the perfusate VLDL of normal livers separated into one major and one minor closely migrating band with mobilities corresponding to the B-100 and B-95 proteins of human and rats. Unlike the case in the rat (28), no B-48 was detected (19, 29) in liver perfusate lipoproteins from normal or cholesterol-fed guinea pigs.

TABLE 4. Fatty acid composition of cholesteryl esters in plasma and perfusate LDL from normal guinea pigs

Fatty Acid	Plasma	Perfusate
14:0	0.4	0.6
16:0	9.3	9.3
16:1	1.1	1.2
18:0	1.3	1.1
18:1	6.2	4.7
18:2	76.4	74.8
18:3	1.3	3.5
20:4	1.4	1.4

Incorporation of [^{3}H]lysine into lipoprotein proteins

After recirculating perfusions of control livers for 5 hr, both VLDL and HDL were highly labeled and comprised 89% and 8% of the total recovered radioactivity, respectively (**Table 5**). The specific activity of LDL protein was only one-eighth that of VLDL.

In recirculating perfusions of the fatty livers, all four lipoprotein fractions were intensely labeled (Table 5), whereas in the single pass perfusions, LDL had significantly lower specific activity than the other fractions. The low specific activity of LDL, together with the high yield of this lipoprotein (Table 1), suggests that most of this fraction represents a wash-out of trapped particles. Distribution of radioactivity among the different lipoprotein fractions was similar in recirculating and in single pass perfusions (Table 5).

The incorporation of [^{3}H]lysine into electrophoretically separated apoproteins was measured in slices of 10% polyacrylamide gels (**Table 6**). The major difference seen between perfusate VLDL from the normal and the fatty livers was a large increase of radioactivity associated with apoE with a reduced amount in apoC. Nevertheless, apoB contained most of the radioactivity in the VLDL and IDL (57–68%) from both types of livers. In LDL from normal livers, about one-half of ^{3}H was associated with the apoB, whereas in LDL fractions from fatty livers, only 26–39% of the label was in apoB. VLDL, IDL, and LDL from single pass perfusions showed no evidence of radioactivity in the apoC region. In perfusate HDL from the normal livers, most of the radioactivity

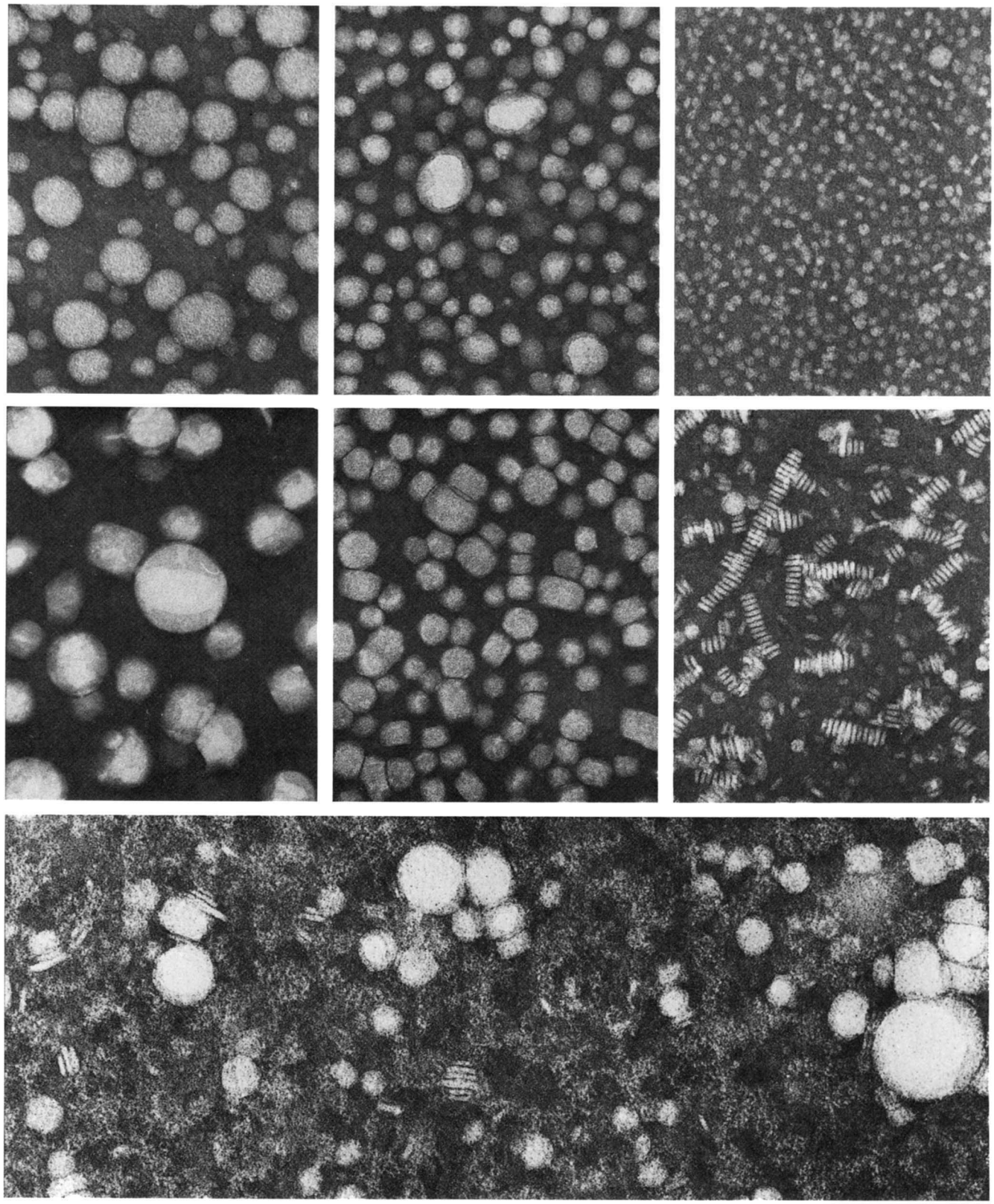

Fig. 4. Negatively stained preparations of perfusate lipoproteins (×180,000). Top: VLDL, d < 1.015 g/ml (left); IDL, 1.015 < d < 1.05 g/ml (middle); HDL, 1.10 < d < 1.21 g/ml (right) from normal livers. Middle: VLDL, d < 1.015 g/ml (left); IDL, 1.015 < d < 1.05 g/ml (middle); and HDL, 1.10 < d < 1.21 g/ml (right) from cholesterol-fed fatty livers. Bottom: Negatively stained preparation of whole perfusate after recirculating perfusion for 5 hr of a cholesterol-fed fatty liver.

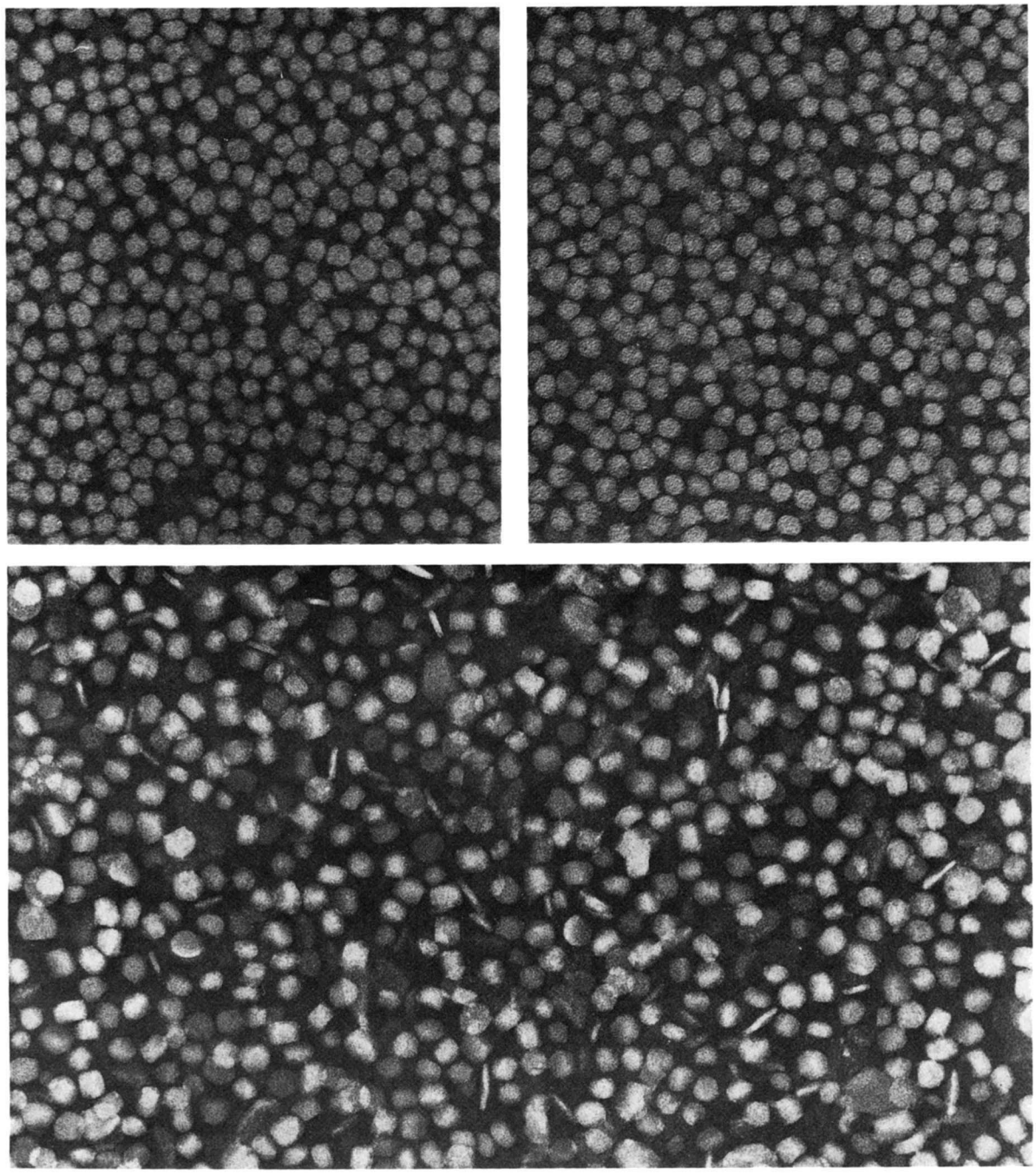

Fig. 5. Negatively stained preparations of serum LDL ($1.05 < d < 1.10$ g/ml, top left) and perfusate LDL ($1.05 < d < 1.10$ g/ml, top right) from normal guinea pigs, and perfusate LDL ($1.05 < d < 1.10$ g/ml, bottom) from cholesterol-fed fatty livers (×180,000).

was associated with apoA-I, apoC, and apoE. About 90% of total radioactivity was associated with apoE in perfusate HDL from the fatty livers. IDL and LDL from fatty livers contained about 73% and 78% apoB, respectively, estimated by measuring protein mass insoluble in 4.2 M TMU (**Table 7**). These apoB components had similar specific activities, suggesting that they were both newly synthesized by the liver. Content of apoE, measured by immunoelectrophoresis, was closely similar (22–23%) in these two fractions (Table 7).

DISCUSSION

In this study, our initial attempts to determine the nature of nascent lipoproteins secreted by perfused

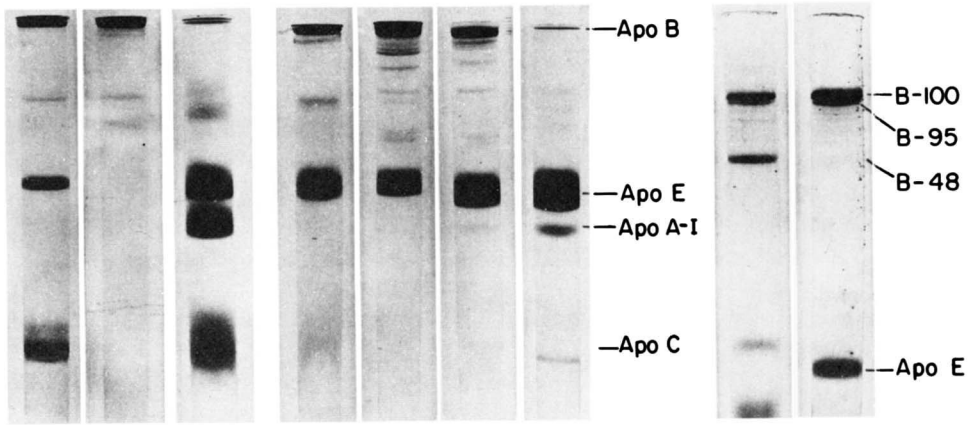

Fig. 6. Analytical SDS gel electrophoretogram of perfusate lipoproteins. Left: 10% polyacrylamide gels. From left: VLDL plus IDL ($d < 1.05$ g/ml); LDL ($1.05 < d < 1.10$ g/ml); HDL ($1.10 < d < 1.21$ g/ml) from normal livers; VLDL ($d < 1.015$ g/ml); IDL ($1.015 < d < 1.05$ g/ml); LDL ($1.05 < d < 1.10$ g/ml); HDL ($1.10 < d < 1.21$ g/ml) from cholesterol-fed fatty livers. (Approximately 30 μg protein was applied to each gel). Right: 3% polyacrylamide gels. From left: liver perfusate VLDL ($d < 1.015$ g/ml) from rats; liver perfusate VLDL ($d < 1.015$ g/ml) from normal guinea pigs. (Approximately 10 μg apo B was applied to each gel.)

guinea pig livers were confounded by continued release of non-nascent lipoproteins that were probably trapped in extravascular spaces or adsorbed to cell surfaces. We found that in the normal guinea pig liver, a large amount of LDL was released during the first hour of a recirculating flush and that smaller amounts continued to be released for several hours. This appeared to be even more of a problem with the LDL of the cholesterol-fed animals. Even after a recirculating flush of 30 min, larger amounts of lipoprotein were released during the first hour of continued perfusion than during the remaining 4 hrs. In single pass perfusions of the fatty livers, LDL was the major lipoprotein fraction by mass, but it contained the least radioactivity in apoproteins. Although this wash-out phenomenon of non-nascent lipoproteins from perfused livers has been noted in previous reports, it has not been emphasized (30–32). For example, early wash-out appears to account for much of the increased release of apoE from recirculating perfused livers of cholesterol-fed rats (30). The much larger mass but much lower specific activity of apoA-I released from single pass perfusions of livers of nephrotic rats may also be due in part to wash-out of cold apoA-I (31). Most of the LDL released from perfused porcine livers represents preformed particles (32), much the same as we have found in normal guinea pig livers. Our findings, together with those cited above, indicate that the use of liver perfusions (both recirculating and single pass) to characterize and quantify nascent lipoprotein and apoprotein synthesis and secretion must be evaluated carefully. It appears likely that the problem of wash-out may be greater in livers of cholesterol-fed animals, in livers of larger size and in hyperlipidemias.

VLDL ($d < 1.05$ g/ml) and HDL ($1.10 < d < 1.21$

TABLE 5. Incorporation of [^{3}H]lysine into perfusate lipoproteins

	Specific Activity			Distribution of Radioactivity		
	Control	Cholesterol-fed		Control	Cholesterol-fed	
	Recirculating	Recirculating	Single Pass	Recirculating	Recirculating	Single Pass
		cpm/μg protein			%	
VLDL[a]	584 ± 60[b]	576 ± 36	61 (44–77)	89 ± 5	6 ± 3	4 (3–5)
IDL ($1.015 < d < 1.05$ g/ml)		356 ± 74	40 (21–59)		11 ± 3	16 (15–18)
LDL ($1.05 < d < 1.10$ g/ml)	69 ± 25	325 ± 82	16 (13–18)	3 ± 1	21 ± 7	32 (31–33)
HDL ($1.10 < d < 1.21$ g/ml)	397 ± 71	581 ± 58	87 (59–115)	8 ± 4	62 ± 7	48 (46–49)

[a] In liver perfusate of the normal animals, only trace amounts of lipoprotein were present in density of $1.015 < d < 1.05$ g/ml; VLDL were routinely isolated at $d < 1.05$ g/ml. In cholesterol-fed animals, VLDL were isolated at $d < 1.015$ g/ml.

[b] Values represent mean ± SD from four livers for normal and six livers for cholesterol-fed animals. For single pass perfusion, values represent mean and range (in parentheses) of two livers.

TABLE 6. Distribution of ^3H in perfusate apolipoproteins (percent of recovered ^3H)[a]

Apolipoprotein	Control	Cholesterol-fed	
	Recirculating	Recirculating	Single Pass
VLDL[b]			
B	68 ± 5[c]	57 ± 3[c]	66 (58–74)[c]
E	5 ± 4	25 ± 3	34 (26–42)
C	27 ± 2	18 ± 4	0
IDL (1.015 < d < 1.05 g/ml)			
B	—	65 ± 6	57 (49–65)
E	—	32 ± 3	43 (35–51)
C	—	3 ± 1	0
LDL (1.05 < d < 1.10 g/ml)			
B	53 ± 16	39 ± 14	26 (26–27)
E	10 ± 6	58 ± 14	74 (73–74)
A-I	0	1 ± 1	0
C	37 ± 11	2 ± 0.5	0
HDL (1.10 < d < 1.21 g/ml)			
B	2 ± 2	1 ± 0.5	2 (1–2)
E	22 ± 8	91 ± 3	91 (86–96)
A-I	39 ± 12	4 ± 2	6 (3–9)
C	36 ± 15	4 ± 1	2 (0–3)

[a] Apolipoproteins were separated by electrophoresis on SDS polyacrylamide gel and measured in gel slices.

[b] In liver perfusates of the normal animals, only trace amounts of material were present in density of 1.015 < d < 1.05 g/ml; VLDL were routinely isolated at d < 1.05 g/ml. In cholesterol-fed animals, VLDL were isolated at d < 1.015 g/ml.

[c] Values represent mean ± SD from three livers for normal and five livers for cholesterol-fed animals. For single pass perfusion, values represent mean and range (in parentheses) of two livers.

g/ml) are major nascent lipoproteins secreted by the liver of normal guinea pigs. This is shown by linear accumulations of triglyceride and apoE after the first hour of perfusion (Figs. 1 and 2) and the active incorporation of [^3H]lysine into the apoproteins of these fractions (Table 6). Furthermore, the composition of the perfusate VLDL (Table 2) was similar to that of lipoprotein particles (d < 1.007 g/ml) isolated from Golgi apparatus of guinea pig liver by Chapman, Mills, and Taylaur (33). Several observations indicate that perfusate LDL were mainly plasma LDL that were washed out of the liver. First, as compared to VLDL and HDL, perfusate LDL was the least intensely labeled apoprotein fraction. LDL contained about 20% of the accumulated lipoprotein (Table 1), but only 3% of the recovered radioactivity (Table 5). Second, the rate of accumulation of LDL decreased sharply after the first hour. Third, perfusate LDL had the same composition (Table 3), cholesteryl ester fatty acid composition (Table 4), and electron microscopic size as plasma LDL. The presence of small

TABLE 7. Contents of apolipoproteins B and E and incorporation of [^3H]lysine into apolipoprotein B of IDL and LDL from perfused fatty livers of cholesterol-fed guinea pigs[a]

	ApoB	ApoE	Specific Activity of ApoB
	% of protein		*cpm/μg protein*
IDL (1.015 < d < 1.05 g/ml)	73.3 ± 6.7[b]	22.2 ± 3.8	226 ± 110
LDL (1.05 < d < 1.10 g/ml)	77.7 ± 7.2	23.2 ± 6.3	213 ± 73

[a] Content of apoB was estimated as protein insoluble in 4.2 M TMU (26) and content of apoE was quantified by immunoelectrophoresis. ^3H of apoB was calculated by subtracting ^3H of TMU-soluble protein from total ^3H.

[b] Values represent mean ± SD from four livers.

amounts of radioactivity in apoB of perfusate LDL suggests that some LDL may be secreted directly by liver of guinea pigs, as reported for perfused porcine liver (32). In addition, small quantities of nascent VLDL may be metabolized to LDL and, as also reported for perfused porcine liver (32), preformed LDL may be gradually washed out of the liver during perfusion.

The amount of nascent HDL recovered from perfusates of normal guinea pig livers was barely sufficient for chemical analyses. However, the major HDL apoproteins of A-I, E, and C were present in this fraction and all were intensely radioactive when [^{3}H]lysine was added to the perfusate. It is possible that this fraction was rapidly metabolized in the recirculating perfusions. By electron microscopy, discoidal particles similar to nascent HDL from perfused rat livers (12) were seen, as well as particles similar in size and shape to plasma HDL.

Nascent plasma lipoproteins secreted by fatty livers of cholesterol-fed guinea pigs differed greatly in composition and quantity from those secreted from normal livers. The triglyceride-rich pre-beta migrating VLDL of normal livers disappeared completely. The small amount of lipoprotein that floated in the VLDL density range had β-mobility, large amounts of apoE, and much more cholesteryl ester in its core. IDL became major nascent particles. All of their properties, except for a slightly increased density and reduced triglyceride content, closely resembled the abnormal VLDL, including intense labeling of apoB. The LDL density fraction from the fatty livers also contained nascent apoB-containing particles, but the percent of radioactivity in apoB was substantially less, especially in the single pass perfusions. These data are consistent with our finding that much of this material is preformed plasma lipoproteins that are gradually washed out of the liver. Because the livers of these cholesterol-fed guinea pigs are loaded with cholesteryl esters and release much less triglyceride than normals, and because the apoB in both VLDL and IDL fractions is equally and intensely labeled ([^{3}H]lysine Tables 6 and 7), it is probable that a spectrum of cholesteryl ester-rich particles is secreted instead of triglyceride-rich nascent VLDL. The similarity of the apoprotein labeling in the single pass and recirculating perfusions would seem to be inconsistent with substantial conversion of VLDL to IDL during recirculating perfusions. Although these particles share many of the characteristics of "remnants" of plasma chylomicrons, they clearly represent nascent hepatic lipoproteins, which contain none of the B-48 protein associated with intestinal particles (28). Similar apoE and cholesteryl ester-enriched particles have been found in liver perfusates (30) and in isolated hepatic Golgi fractions (34, 35) of cholesterol-fed rats. Thus, the shift of the hepatic lipoprotein secretory mechanism from triglyceride-rich particles of VLDL density towards particles of IDL and even LDL density that are cholesteryl-ester enriched may represent a general response to excess cholesterol deposition in the liver, albeit more pronounced in the guinea pig.

The LDL fraction from the fatty livers is more complicated to interpret. In contrast to the LDL fraction from liver perfusates of normal guinea pigs, that of cholesterol-fed guinea pigs is heterogeneous, with three different morphologic species of particle. One is spherical, suggesting that it is more like normal plasma LDL (Fig. 5). A second particle has angular surfaces, similar to some of the abnormal particles in the VLDL and IDL fractions. Also present are a variable number of discs that appear larger than those of the HDL fraction and they presumably account for the faintly staining alpha migrating components on agarose electrophoresis. Much of the total radioactivity in this LDL fraction may be contained in apoE on these discoidal particles, especially in the single pass perfusions (Table 6). However, apoB contains substantial amounts of radioactivity in LDL from both single pass and recirculating liver perfusions (almost equal in specific activity to apoB of IDL in recirculating perfusions), indicating that some of these LDL particles probably are newly synthesized or partially metabolized, or both.

Another major alteration produced by cholesterol-feeding was the large amount of nascent discoidal HDL secreted by the fatty livers. The active incorporation and virtually identical distribution of apolipoprotein radioactivity in nascent HDL from both recirculating and single pass perfusions established that the discs were newly synthesized. Discoidal particles were also clearly evident in negative stains of whole perfusates, indicating they were not simply an artifact of ultracentrifugation. These nascent HDL differ from those described in perfusates of normal rat livers (12, 31). The unusually high molar ratios of cholesterol to phospholipid may explain why the cholesterol in these particles was not esterified by LCAT. LCAT esterifies cholesterol very poorly in lecithin liposomes when the molar ratio of cholesterol to lecithin exceeds ~0.5 (36). Although LCAT activity reportedly is present in plasma of both normal and cholesterol-fed guinea pigs (13, 37), it is possible that LCAT is not secreted by these fatty livers. Discoidal HDL are recovered from perfusates of livers of guinea pigs after only 10 days on the cholesterol diet, yet the discs do not accumulate in plasma of these animals until 8–10 weeks. This suggests that LCAT may operate more effectively in the intact animal than in our liver perfusion system. Rates of accumulation of lipoproteins during single pass perfusions may give more reliable estimates of rates of secretion of lipoproteins and apolipoproteins than recirculating perfusions, provided that the lipoproteins can be shown to be newly synthesized. We found labeling

of C apoproteins to be much lower in single pass than in recirculating perfusions. C apoproteins can inhibit the uptake of triglyceride-rich lipoproteins in perfused rat livers (38). This suggests that the accumulation of C apoproteins in recirculating perfusions, as observed in guinea pigs and rats (12), may reflect the progressive effect of continuing secretion of small amounts of C proteins upon the hepatic uptake of nascent particles.

We have shown that plasma levels of apoA-I and apoE are elevated in guinea pigs fed cholesterol (24). Increases of plasma apoA-I (2-fold) in animals fed cholesterol for 8 to 10 weeks may correspond directly to a 2-fold increase of apoA-I secretion from the fatty livers (Fig. 2). Increases of plasma apoE (22-fold), on the other hand, suggest that removal of this apolipoprotein is reduced, because the fatty livers from these animals appeared to secrete only 2.5-fold more apoE than normals (Fig. 2). Recent studies by Kovanen et al. (39) have demonstrated that saturation and suppression of hepatic lipoprotein receptors contribute to the rapid development of hypercholesterolemia in cholesterol-fed rabbits. Hepatic uptake of abnormal lipoproteins enriched in apoE may also be reduced in the cholesterol-fed guinea pigs and contribute to the remarkable accumulation of this apoE and cholesterol in plasma.

An interesting finding of this study is that many of the abnormal plasma lipoproteins of cholesterol-fed guinea pigs, as described by Sardet, Hansma, and Ostwald (6), were shown to originate from the abnormal fatty livers of these animals. The lipid and protein composition and electron microscopic appearance of comparable lipoprotein fractions obtained from plasma and liver perfusates of the same animal did not differ detectably, except for an increased amount of apoA-I in the HDL from plasma (data not shown). Further work will be required to establish the magnitude of the intestinal contribution to the plasma lipoproteins of these animals.

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