Lipoproteins in a nonrecirculating perfusate of rat liver

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Abstract Rat livers were perfused in a nonrecirculating system for 30–40 min with Krebs-Ringer bicarbonate–0.1% glucose solution gassed with 95% O_2–5% CO_2 at 37°C at a flow rate of 3 ml/g/min. The livers appeared normal as judged by O_2 uptake, bile flow, transaminase release, and net protein output (2.5 mg/g/hr). The perfusate was concentrated by ultrafiltration using Amicon PM-10 or PM-30 membranes. The concentrated perfusate was subjected to sequential ultracentrifugation at solution densities of 1.006, 1.04, 1.06, and 1.21, and the top fractions were analyzed for protein and lipid. The net release of protein in the four density classes, suitably corrected, averaged 39, 10, 5, and 20 μg/g/hr. The lipid composition of the perfusate lipoprotein fractions differed from that of serum mainly in the high percentage of free cholesterol, reflecting the lack of exposure to lecithin:cholesterol acyltransferase. When rat serum was fractionated in the same way, most of the lipoprotein in the d 1.006–1.06 range had a density greater than 1.04. It was concluded from these experiments that the liver secretes very low density lipoprotein (VLDL), high density lipoprotein (HDL), and a modified form of VLDL containing less lipid. Comparison of secretion rates and serum lipoprotein levels leads to the conclusion that the latter are largely determined by catabolic rates. When labeled amino acids were present, the perfusate HDL had a higher specific activity than VLDL. Addition of carrier whole serum did not alter recovery of labeled lipoproteins, but when these were isolated from Golgi membranes after a 40-min perfusion, more than twice as much label was recovered in HDL, suggesting the presence of precursors within the Golgi. The main advantages of the nonrecirculation perfusion technique are the avoidance of catabolic reactions, simplicity, and complete control over the composition of the perfusing medium.

Supplementary key words secretion · liver perfusion · VLDL · LDL · HDL · Golgi

Despite many studies of lipid output by the isolated perfused rat liver, the exact nature and quantity of the lipoproteins secreted in the three main density classes is not entirely clear. The major form in which triglyceride is released by the liver is VLDL (1). In the circulation, LDL is found in considerable amounts, and in rats and other mammals, HDL is the major lipoprotein density class (2). Except for a few experiments with liver slices (3), direct measurements of net HDL secretion have not been reported. Since the liver plays an important role in protein and lipoprotein catabolism as well as synthesis (4), it seemed worthwhile to reinvestigate the release of lipoprotein by rat liver under conditions in which no lipids were present in the medium and there was minimal opportunity for catabolic reactions. Such conditions were readily met by a nonrecirculating perfusion followed by ultrafiltration and ultracentrifugation of the filtrate at appropriate densities.

Liver perfusion by the simple method described in this paper does not impair hepatic function for short-term perfusions as judged by O_2 uptake, bile output, transaminase release, and net secretion of protein. The lipoproteins secreted were mainly VLDL and HDL, with smaller amounts in the 1.006–1.04 density range and very small quantities with densities between 1.04 and 1.06. Measurements of the incorporation of labeled amino acids into perfusate lipoproteins and those extracted from Golgi membranes after 40 min of perfusion are also reported.

MATERIALS AND METHODS

The rats used were males of the Holtzman strain, 250–400 g, fed ad lib. on laboratory chow. The perfusion medium was Krebs-Ringer bicarbonate–0.1% glucose equilibrated with 95% O_2–5% CO_2; the pH was 7.4. In a few experiments, an amino acid mixture was added (Eagle's minimal essential amino acids plus glutamine, Microbiological Associates, Inc., Bethesda, Md.).

O_2 uptake was measured with an oxygen electrode calibrated with known gas mixtures. Glutamic–oxaloacetic transaminase activity was measured with the GOT Stat-Pack from Calbiochem (La Jolla, Calif.). Proteins were measured by the procedure of Lowry et al. (5) with bovine serum albumin as the standard. Total lipids were mea-

Abbreviations: VLDL, very low density lipoprotein ($d < 1.006$); LDL, low density lipoprotein ($1.006 < d < 1.06$); HDL, high density lipoprotein ($1.06 < d < 1.21$).

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sured after extraction with chloroform–methanol 2:1 (6). The chloroform extract was analyzed for total lipid using the direct charring method (7) with tripalmitin as a standard. Neutral lipids were separated on a 0.5 × 15 cm Florisil column (8). Cholesterol and cholesteryl esters were measured by the ferric ion–sulfuric acid method (9). Triglycerides were measured by the charring reaction (7); phospholipids were determined by the method of Bartlett (10) on the original chloroform extract.

Golgi membranes were obtained and extracted as previously described (11, 12) from livers perfused for 40 min with medium containing 0.2 μCi/ml of a mixture of ^{3}H-labeled amino acids (reconstituted algal protein hydrolysate, NET-250, from New England Nuclear Corp., Boston, Mass.). The labeled amino acids were diluted with 8.5 μg/ml of unlabeled amino acids to give an average specific activity of 23.5 μCi/mg. After isolation and dialysis overnight at 5°C against 0.15 M NaCl–0.002 M EDTA, pH 7.6, lipoproteins were precipitated with 5% trichloroacetic–1% phosphotungstic acids and resuspended in ether–ethanol 3:1 according to previous methods (12). After dissolving in 0.5 N NaOH, the proteins were counted in Aquasol liquid scintillation fluid for a minimum of 3,000 total counts.

Perfusates were concentrated at 5°C to 25–35 ml by ultrafiltration at no more than 24 psi in an Amicon TCF-10 apparatus using PM-30 or PM-10 membranes after centrifugation to remove red cells. After addition of EDTA to a final concentration of 10^{-3} M, lipoproteins were isolated by ultracentrifugation at 5°C after adjusting the solution density with a solution of NaBr in 0.05 M phosphate buffer, pH 7.2. VLDL was isolated by centrifugation for 18 hr at 40,000 rpm in a Spinco Ti-50 rotor in 9-ml polycarbonate tubes. The top 1 ml in each tube was carefully removed with a 0.5-ml Lang-Levy micropipet. The infranatant solution was adjusted to d 1.06 and ultracentrifuged as before. In some experiments, an intermediate density of 1.04 was employed before isolation at d 1.06. After removal of the top 1 ml as before, the remaining solution was adjusted to d 1.21 and ultracentrifugation was carried out for 20–24 hr at 43,000 rpm. Before study, all lipoprotein solutions were dialyzed overnight at 5°C as described above.

In most experiments, rats were anesthetized with 60 mg/kg of Nembutal administered intraperitoneally, and surgery was carried out as previously described (13). In other experiments, rats were killed by cervical fracture, and cannulation of the portal vein and vena cava was achieved within 2–5 min. Perfusion was by gravity flow, adjusted by a clamp, with a pressure of 50 cm of water. 95% O_2–5% CO_2 was bubbled constantly through the reservoir. After a preliminary 5–7-min perfusion to remove residual blood, the perfusate was collected in a flask surrounded by ice, and all subsequent manipulations were carried out at 4–5°C. To prevent bubble formation, the perfusion was carried out in a constant temperature room at 37 ± 2°C.

TABLE 1. Physiological parameters of nonrecirculating perfused liver

Parameter	Mean ± SEM	No. of Expts.
Oxygen uptake, moles/g/hr	135 ± 8.86	6
Bile output, ml/g/hr	0.051 ± 0.0051	9
Glutamic–oxaloacetic transaminase, mU/g/hr	9.1 ± 1.63	6
Total protein output, mg/g/hr	2.50 ± 0.134	11

RESULTS

Control experiments

Experiments were carried out to answer the following questions concerning the state of the liver and the technique employed for quantitative lipoprotein analysis.

(*a*) *Was the liver in a satisfactory functional state?* Oxygen uptake, bile output, total protein output, and transaminase release into the medium were measured (**Table 1**). Bile output and O_2 uptake were comparable to reported values of 0.067 ml/g/hr and 136 μmoles/g/hr in recirculation perfusions containing red cells (14). Transaminase release, although higher than the negligible release reported by Bartošek, Guaitani, and Garattini (15) for the first 30 min of whole blood perfusion, was extremely low compared with values of 100–300 mU/g/hr after that time. Of the total protein output of 2.5 mg/g/hr (Table 1), about one-third was albumin, as judged by solubility in 95% ethanol–1% trichloroacetic acid. We have previously reported values of 0.6 mg/g/hr for albumin synthesis in recirculating perfusions containing red cells (16). The histological appearance of the liver was normal after 30 or 60 min of perfusion. No decline in O_2 uptake was noted between 30 and 60 min, but total protein output declined about 10% during that time. Most perfusions were carried out for 30 min, though results are expressed per hour. No effects of added amino acids on protein output were noted in 30 min. A release of amino acids occurred, amounting to 1–2 mg/g/hr as measured by protein analyses on the PM-10 ultrafiltrate.

(*b*) *To what extent did the observed lipoprotein output reflect residual plasma protein washed out during the perfusion?* This was estimated in three ways. From the hemoglobin content of the perfusate, the estimated residual plasma was 0.002–0.02 ml, averaging 0.01 ml. Rat serum contains about 1.3 mg/ml of HDL protein, so that 13–26 μg of HDL protein could be accounted for in an average perfusion. The output of HDL by a 12-g liver in 30 min averaged 120 μg, so that a maximum of 22% of the HDL output could be accounted for in this way, assuming no

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TABLE 2. Lipoprotein output by perfused rat liver

	VLDL	LDL	HDL
	μg/g/hr	μg/g/hr	μg/g/hr
Protein	39 ± 1.9 (11)	15 ± 1.8 (11)	20 ± 1.5 (15)
Lipid	186 ± 2.1 (9)	60 ± 7.5 (9)	20 ± 2.6 (8)
Lipid/protein ratio	5.1 ± 0.5 (8)	4.0 ± 0.7 (7)	1.0 ± 0.3 (8)
Triglyceride	105.4 ± 11.4 (6)	33.0 ± 6.0 (6)	7.3 ± 0.8 (5)
Cholesteryl ester (as cholesterol)	3.5 ± 0.7 (5)	3.3 ± 1.1 (6)	1.3 ± 0.5 (6)
Cholesterol	11.6 ± 2.1 (6)	5.9 ± 1.3 (6)	3.4 ± 0.6 (6)
Phospholipid	35.8 ± 7.9 (7)	11.8 ± 2.8 (7)	4.7 ± 1.0 (7)

Values are means ± SEM. Numbers in parentheses are the numbers of observations. The lipid/protein ratios were averaged for each individual experiment. The lipoprotein outputs shown in this table were corrected for operational losses. Using protein as an example, the mean measured output (μg/g/hr) in VLDL, LDL, and HDL was 26, 15, and 20, respectively. Correction for 83% recovery in the concentration step yields 39, 12.9, and 20.1; correction for 10% of LDL in HDL, 39, 14.2, and 18.8. Since the latter two figures are quite close to the measured values, the uncorrected, measured values for LDL and HDL output are shown in the table.

losses during the isolation. VLDL plus LDL output by a 12-g liver averaged 240 μg. Since 10 μl of rat serum contains about 1.3 μg of VLDL plus LDL protein, less than 1% of this output can be accounted for by residual serum. The second and third methods used to estimate residual serum protein contamination were to inject the donor rat with ^{125}I-labeled HDL or albumin, labeled in vitro by the method of McFarlane (17). After 3 min, a blood sample was obtained and the perfusion was begun. After 7 min, the perfusate was collected and lipoproteins were isolated as described In the first experiment, the specific activity of the serum HDL at the time perfusion began was 713 cpm/μg. A total of 6,780 cpm was present in perfusate HDL containing 142 μg of protein; residual serum HDL was therefore 9.5 μg or 6.7% of the total. In the second experiment, the donor rat serum contained 270 cpm/μl of labeled albumin. The concentrated perfusate prior to ultracentrifugation contained 5,373 cpm, equivalent to 19.9 μl of serum. From the red cell hemoglobin, 9.7 μl of serum was calculated, indicating additional serum protein outside the capillary bed. The calculated amount of VLDL plus LDL in 20 μl of serum is 2.6 μg of protein, 0.7% of the amount found in the experiment. The calculated amount of HDL in 20 μl of serum is 26 μg, 15% of that found. On the basis of these control experiments, residual serum lipoprotein does not account for an appreciable fraction of the lipoprotein released.

TABLE 3. Percentage distribution of protein in four lipoprotein density ranges[a]

Density Range	Perfusate	Serum
	% of total protein of lipoprotein	
<1.006	35.1 ± 1.8	8.9 ± 0.9
1.006–1.04	23.5 ± 1.7	2.7 ± 0.4
1.04–1.06	10.7 ± 1.0	4.2 ± 0.5
1.06–1.21	30.7 ± 1.7	84.2 ± 1.6
Ratio, $\frac{1.006–1.04}{1.04–1.06}$	2.22 ± 0.18[b]	0.63 ± 0.053

[a] Values are means ± SEM of four experiments. No corrections for manipulative losses such as given in Table 2 have been made. However, each lipoprotein fraction was recentrifuged an additional time, under the same conditions as in the original isolation, before analysis.

[b] Significantly different from the ratio for serum ($P < 0.001$).

(*c*) *What was the extent of lipoprotein recovery during the concentration process?* VLDL, LDL, and HDL were prepared from rat serum as described, and each fraction was recentrifuged an additional time. Approximately 1 mg of protein was diluted to 500 ml with 0.15 M NaCl and concentrated to 30 ml with PM-30 membranes. The average recovery of lipoprotein protein was 80 ± 1.5% in five experiments with new membranes, increasing to 85% with used membranes. The recovery of lipid was not significantly different, nor were significant differences noted among the three lipoprotein classes. Albumin recoveries were slightly higher (85–90%).

(*d*) *Was the sequential lipoprotein isolation technique quantitatively adequate?* The ultracentrifugal isolation time and speed were comparable to those reported by Lasser et al. (18) except for HDL, for which we employed 3 × 10^6 *g*-hr rather than 4.6 × 10^6. However, recentrifugation of the d > 1.21 fraction did not yield any significant additional quantity of lipid. The situation with regard to HDL is more complicated than with VLDL or LDL because the HDL after a single centrifugation is appreciably contaminated with other proteins. For quantitative purposes, rather than recentrifuging the HDL we have chosen to correct the protein values found after a single centrifugation in the following way. Using albumin as a representative contaminant, control experiments were carried out that indicated that 0.37% of added albumin was recovered in the top 1 ml after centrifugation at d 1.21. The amount of protein found in the HDL was therefore corrected by subtracting 0.37% of the total perfusate protein. The validity of this approach appears to be confirmed by the fact that the lipid/protein ratio for HDL was 1.0, as reported by Koga, Horwitz, and Scanu (19).

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TABLE 4. Effect of recentrifugation at d 1.006 on lipoprotein fractions recovered from liver perfusate

	Density Fraction									
	1.006_1		1.006_2		1.04		1.06		1.21	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
					$\mu g/g/hr$					
Protein output	24	25	3.5	4.0	4.2	6.4	4.9	5.4	12.9	10.0
Lipid output	293	273	13.7	20.8	20.6	31.4	16.7	21.6	22.8	16.1
Lipid/protein ratio	12.2	10.9	3.9	5.2	4.9	4.9	3.4	4.0	1.8	1.6

These rats, 414 and 369 g, respectively, were fed a 67% sucrose–20% casein diet (22) for 3 wk prior to liver perfusion. Lipoprotein isolations were carried out as described in the text except that the first centrifugation at d 1.006 was repeated before raising the density to 1.04. The values shown are those observed—no corrections were made. Total lipids were determined by charring aliquots of the chloroform–methanol extract using tripalmitin as the standard (7).

When HDL fractions were recentrifuged, the amount of protein found was in reasonable agreement (±20%) with that expected from the above calculation when recentrifugation losses were taken into account (20).

The question of contamination of LDL and HDL by virtue of incomplete removal of the previous density class was investigated by sequential centrifugation of VLDL or LDL and determination of the amount of protein appearing at d 1.006, 1.06, and 1.21. These lipoproteins were purified by recentrifugation before use. The experiments indicated that 13 ± 3% of VLDL was recovered at d 1.06 and 10% at d 1.21. For LDL, 10% was recovered at d 1.21. The analytical results were therefore corrected for these effects (see footnote to Table 2). In addition, the possible appearance of HDL protein in the 1.006–1.06 density class was investigated by injecting ^{125}I-labeled HDL into a rat, collecting serum after 3 min, and centrifuging at d 1.06 followed by centrifugation at d 1.21. The d < 1.06 fraction contained 1.5×10^5 cpm compared with 1.85×10^6 cpm in HDL. Assuming that none of the HDL peptides exchanged with VLDL, no more than 7.5% of HDL protein can be accounted for in the lower density fractions.

Lipoprotein secretion

The quantitative results of these perfusion experiments are given in **Table 2.** For the protein moiety, a total of 74 μg/g/hr of lipoprotein was secreted, of which 53, 20, and 27% appeared in VLDL, LDL (1.006 < d < 1.06), and HDL, respectively. The total lipid output averaged 266 μg/g/hr, of which 70, 23, and 7% appeared in VLDL, LDL, and HDL. It is clear that HDL is not quantitatively important in lipid secretion by rat liver. It should be noted that the lipid composition of the perfusate lipoproteins differs from that of serum (19), particularly in the relatively high proportion of unesterified cholesterol and in the presence of appreciable triglyceride in HDL.

The question of the nature of the lipoprotein secreted in the LDL density range was investigated further because Levy, Bilheimer, and Eisenberg (21) have evidence that, in man, LDL is derived from VLDL catabolism. In four experiments, shown in **Table 3,** it was found that two-thirds of the lipoprotein protein secreted in the d 1.006–1.06 fraction had a density less than 1.04, whereas in serum, two-thirds appeared in the d 1.04–1.06 fraction. The latter observation is in agreement with the conclusion of Lasser et al. (18) that most rat LDL is in the d 1.04–1.05 fraction.

To check the possibility that the d 1.006–1.04 fraction could be accounted for by incomplete removal of VLDL in the first centrifugation step, in two experiments centrifugation at d 1.006 was repeated before the density was changed. The results are shown in **Table 4.** In these experiments, the rats were fed a high sucrose diet to enhance triglyceride output. The data show that the first centrifugation at d 1.006 removed 87% of the protein and 94% of the lipid. The d < 1.006 fraction in the second centrifugation showed a significantly lower lipid/protein ratio. Raising the density to 1.04 resulted in considerably more lipid recovery, averaging 1.5 times that found after the second centrifugation at d 1.006. The total lipoprotein protein output in fractions 1.006_2, 1.04, and 1.06 averaged 14 μg/g/hr, close to that found in the 1.006–1.06 fraction reported in Table 2.

Labeling of nascent and secreted lipoproteins

The labeling pattern of VLDL, LDL, and HDL secreted during perfusion with labeled amino acids was determined. At 40 min, perfusion was discontinued and Golgi membranes were isolated and extracted as previously described (12). The lipoprotein fractions, whether from perfusate or Golgi, were isolated with and without addition of 2 ml of carrier rat serum. The perfusate results, shown in **Table 5,** indicate that 32, 20, and 48%, respectively, of the labeled lipoprotein appeared in VLDL, LDL, and HDL. If the corrections given in Table 2 are applied to these results, the percentages of labeled protein in these fractions would be 41, 17, and 41%. This distribution is not greatly different from that found by Windmueller, Herbert, and Levy (23) after 5 hr of recirculating perfusion with whole blood and labeled lysine, i.e., 44, 11, and 45%. When compared with the analyti-

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TABLE 5. Distribution of labeled lipoprotein in liver perfusate

Expt. No.	Lipoprotein Labeling					
	VLDL		LDL		HDL	
	No Serum	Plus Serum	No Serum	Plus Serum	No Serum	Plus Serum
	cpm		*cpm*		*cpm*	
1	3,573	3,160	2,106	2,334	4,220	4,218
2	1,413	1,290	833	967	2,061	2,153
3	1,266	1,147	766	595	1,755	2,257
	(13.0)[a]		(12.9)		(23.0)	
4	1,354	1,237	660	673	2,073	2,133
	(9.6)		(10.8)		(15.2)	
5	1,092	1,146	907	927	2,000	2,729
	(8.7)		(13.3)		(14.7)	
Ratio, cpm no serum/cpm plus serum[b]	0.93 ± 0.0294		1.02 ± 0.0653		1.14 ± 0.0680	
Percentage of total labeled lipoprotein in fraction (no serum)	32 ± 1.48		20 ± 1.18		48 ± 1.45	

[a]Values in parentheses give the specific activity of the protein, in cpm/μg. All values shown in this table are the measured values—no corrections such as those given in Table 2 have been applied.

[b]The concentrated perfusate was divided in half, and 2 ml of rat serum was added to one half before isolation of the lipoproteins.

cal protein distribution given in Table 2 of 53, 20, and 27%, it can be seen that somewhat more label is in HDL than would be expected, implying a higher specific activity. This was confirmed by direct measurement of specific activity. In the three experiments shown in Table 5 (experiments 3, 4, and 5), and in one additional experiment, the average ratio of the specific activity of HDL to the mean of VLDL and LDL was 1.59 ± 0.15, significantly different from 1.0 ($P < 0.05$). When all three fractions were recentrifuged in another experiment, the ratio was 1.73. Addition of carrier serum did not increase the yield of labeled lipoprotein recovered from the perfusate.

The lipoproteins isolated from the Golgi at the end of the perfusion period (**Table 6**) were distributed in the VLDL, LDL, and HDL fractions by 60, 15, and 25%, respectively, or 69, 12, and 19% corrected according to Table 2. The values for VLDL and HDL are significantly different from those for the perfusate. The addition of serum increased the recovery of labeled HDL by 260%.

DISCUSSION

The nonrecirculating perfusion technique has permitted a study of hepatic lipoprotein secretion under conditions in which catabolic reactions were kept to a minimum. Although the perfusion conditions are far from physiological with regard to pressure, flow rate, and medium composition, the livers performed well up to 1 hr with respect to O_2 uptake, bile flow, and albumin secretion. Addition of albumin to the medium enables one to perfuse for longer periods but would have interfered with the isolation of HDL in the present experiments. Fröhlich, Hansen, and Scholz (24) found excellent gluconeogenesis by livers perfused in this manner.

The quantitative values for lipoprotein output are in the range of those previously reported for d < 1.063 lipoproteins by immunochemical estimation (13). No values for net HDL output have been reported in a perfusion system, but with liver slices I have reported values of 18 μg/g/hr of HDL-immunoreactive protein (3). Ruderman et al. (25) reported a release of VLDL protein that can be calculated at 58 μg/g/hr and a release of VLDL triglyceride of 290 μg/g/hr in a recirculating perfusion with medium containing albumin but no hemoglobin. Though higher than the present values, they had an uptake of 13 μmoles of linoleate/hr, which would be expected to increase VLDL output. Windmueller and Spaeth (26), using erythrocytes and albumin, reported a total fatty acid secretion of 450 μg/g/hr, but their rats were on a high carbohydrate diet, which enhances triglyceride release. It should be noted that livers perfused in the absence of whole plasma release less lipid (26, 27).

The lipid composition of the perfusate lipoprotein fractions differed from that of rat serum lipoproteins (19) in several ways. In all fractions, the percentage of free to esterified cholesterol was high. This would be expected from the fact that the secreted lipoproteins had little chance for exposure to lecithin:cholesterol acyltransferase present in serum. Though the liver secretes this enzyme (28), the nonrecirculating perfusion technique offers minimal opportunity for the reaction to occur. Roheim et al. (29) indicated that in the rat at least some cholesteryl ester was made in the liver itself; the present experiments confirm this conclusion but indicate that some ester is present in every density class. The lipid/protein ratio of VLDL in the perfusate was 5.1 while Koga et al. (19) found a value of 5.8. Our fraction of d 1.006–1.06 had a ratio of 4.0, which was not significantly different from VLDL, probably owing to the moderately large individual variability.

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TABLE 6. Distribution of labeled lipoprotein from liver Golgi

Expt. No.	Lipoprotein Labeling					
	VLDL		LDL		HDL	
	No Serum	Plus Serum	No Serum	Plus Serum	No Serum	Plus Serum
	cpm		*cpm*		*cpm*	
2	157	156	67	37.	46	144
3	357	334	55	89	149	359
4	65	73	14	19	33	75
5	141	133	30	32	69	190
Ratio, cpm no serum/cpm plus serum	1.00 ± 0.043		1.15 ± 0.23		2.64[a] ± 0.192	
Percentage of total labeled lipoprotein in fraction (no serum)	60[b] ± 1.44		15 ± 3.46		25[b] ± 2.78	

[a]Significantly different from 1.00 ($P < 0.001$).
[b]Significantly different from the mean value for perfusate lipoprotein shown in Table 3 ($P < 0.001$).

However, rats on a high sucrose diet did show a difference in lipid/protein ratio between VLDL and the d 1.006–1.04 fraction (Table 4). The lipid/protein ratio of 1.0 for HDL agrees with that for serum (19). However, from the data in Table 2 it can be calculated that the HDL lipid contained 44% triglyceride, whereas in serum it is only 9%. This result can be attributed to the contamination of the HDL fraction by VLDL and LDL in the sequential isolation method. Such contamination does not significantly affect the quantitative protein results, but the relatively large amount of triglyceride present in VLDL and LDL would seriously alter the observed HDL composition. Though only 7% of the total lipid was in HDL, about 18% of the total cholesterol output was in this fraction.

The question of the possible primary secretion of LDL by rat liver has not been resolved by the present experiments. Most of the lipoprotein secreted in the d 1.006–1.06 range has a density less than 1.04, whereas in serum most has a density greater than 1.04 (Table 3 and Ref. 18). Since a triglyceride lipase has been reported to be present in hepatocyte surface membrane (30), it is possible that conversion of VLDL to LDL may begin during the secretory process itself. However, we have no definitive evidence at this time concerning the nature of the protein moiety of the d 1.006–1.06 fraction. Windmueller et al. (23) in their perfusion studies using whole blood and labeled lysine found that most of the label was in the density range 1.035–1.063 in the total 1.006–1.063 fraction. Because in their earlier work most of rat LDL was recovered in the d < 1.035 fraction, they assumed that most of the 10.9% of total lipoprotein label in the entire LDL fraction may have represented contamination by HDL, but this has not been proved. In our earliest experiments (13) in which livers were perfused with red cells suspended in medium, we found that 30% of the total d < 1.063 lipoprotein had S_f values in the 4–28 range in the absence of heparin. Since an S_f rate of 4 corresponds approximately to a hydrated density of less than 1.04 (31), these early results correspond well to the data in Table 2 in which 25% of the total lipoprotein of d < 1.06 (protein plus lipid) represented LDL. This finding also suggests that catabolism may not be a serious problem in recirculating perfusions provided the concentration of lipoprotein in the medium is low and heparin is absent.

The short-term labeling experiments (Table 5) indicated that the specific activity of perfusate HDL was higher than that of VLDL or LDL. This may merely reflect a smaller hepatic HDL pool size. It is not possible to directly equate isotope incorporation rates and synthesis rates from a single time value. The higher HDL specific activity was not due to contamination with some extremely highly labeled protein unless such a contaminant was bound tightly to HDL, because the difference persisted after recentrifugation of the HDL fraction. The distribution of labeled lipoprotein isolated from the Golgi showed that more was present in VLDL and less in HDL than in the perfusate. Since the perfusate labeling represents cumulative secretion over the 40-min period whereas the Golgi extract represents the situation at a single time, a direct comparison cannot be made. The addition of carrier rat serum doubled the label recovered in HDL from the Golgi (Table 6) but not from the perfusate. We have reported such a phenomenon in lipoproteins isolated from Golgi labeled in vitro with sialic acid (12). This phenomenon requires further investigation. At present, it suggests a precursor pool of apoproteins in the Golgi. Failure to demonstrate this in the perfusate suggests that these labeled peptides are not secreted. The early finding by Radding, Bragdon, and Steinberg (32) that addition of serum to the incubation medium containing liver slices greatly increased the recovery of labeled lipoprotein may have been due to release of lipases or a similar artifact of the slice technique.

We conclude that the nonrecirculating perfusion technique has sufficient advantages in studies of hepatic lipoprotein secretion to warrant its use when precise control of medium composition and avoidance of catabolic reactions are important experimental considerations. Comparison of

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secretion rates and serum lipoprotein levels leads to the conclusion that the latter are largely determined by catabolic rates.

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