Heterogeneity of dog interstitial fluid (peripheral lymph) high density lipoproteins: implications for a role in reverse cholesterol transport

L. Dory,[1,*] L. M. Boquet,[*] R. L. Hamilton,[**] C. H. Sloop,[*] and P. S. Roheim[*]

Department of Physiology, Louisiana State University Medical Center, 1542 Tulane Avenue, New Orleans, LA 70112-2822* and Department of Anatomy, Cardiovascular Research Institute, University of California San Francisco, 1375 S. Medical Sciences Building, San Francisco, CA 94143**

Abstract The heterogeneity of dog interstitial fluid (peripheral lymph) high density lipoprotein (HDL) was investigated and compared to plasma HDL. Interstitial fluid and plasma HDL of normal and cholesterol-fed dogs was subfractionated by ultracentrifugation and affinity and molecular weight sieving chromatography. Both plasma (P) and interstitial fluid (L) HDL can be subfractionated into a larger fraction (P-I and L-I) and a smaller one (P-II and L-II). Cholesterol feeding induces a large increase in the P-I and L-I component of HDL, but the increase in L-I is far greater in proportion than that of P-I. Furthermore, L-I of cholesterol-fed dogs appears to be almost exclusively discoid in shape, while only approximately 15% of particles in P-I are discoidal. The discoid HDL of L-I is reflected in its chemical composition: 28% unesterified cholesterol, 6% cholesteryl ester, 45% phospholipid, and 21% protein. It contains large amounts of apoE in addition to apoA-I and apoA-IV. We found that the association of apoE with discoid particles is frequent, but not necessary. Calculations based on known protein mass and quantitation of discoid particles on electron micrographs suggest that the concentration of discoid particles in the peripheral lymph of cholesterol-fed dogs is about fourfold that of the plasma of the same animal. These findings provide strong circumstantial evidence for the peripheral formation of discoid HDL, perhaps as an early event in reverse cholesterol transport. — **Dory, L., L. M. Boquet, R. L. Hamilton, C. H. Sloop, and P. S. Roheim.** Heterogeneity of dog interstitial fluid (peripheral lymph) high density lipoproteins: implications for a role in reverse cholesterol transport. *J. Lipid Res.* 1985. **26:** 519–527.

Supplementary key words discoidal HDL • apolipoproteins • cholesterol feeding

Epidemiologic studies have clearly established the negative correlation between plasma high density lipoprotein (HDL) cholesterol concentrations and the development of coronary heart disease in man (1, 2). Although this relationship and its independence from other risk factors is well documented, the mechanism of the apparently protective effects of HDL is not clear. It was first proposed by Glomset (3) that HDL may promote cholesterol efflux from peripheral tissues by a process usually referred to as reverse cholesterol transport. The ability of HDL or even specific HDL apolipoproteins to promote cholesterol efflux from cultured cells has been clearly demonstrated in a number of laboratories (4–8). Despite the potential importance of this process in the prevention of the development of coronary heart disease, there is little direct evidence in vivo for the interaction of HDL with peripheral cells and a resultant removal of cellular cholesterol by HDL. Compositional studies of interstitial fluid lipoproteins can thus provide indirect evidence for the interaction between plasma lipoproteins and peripheral cells. Such an interaction, as manifested by altered chemical composition, would be difficult to detect when examining plasma HDL because it represents a mixture of particles of hepatic, intestinal, and interstitial origin, modified to varying degrees by plasma enzymes. Our studies of the interstitial fluid lipoproteins have provided us with a unique opportunity to detect and characterize lipoproteins in direct contact with peripheral cells. Specifically, we have been able to show that interstitial fluid HDL of normal and of cholesterol-fed dogs differs from the plasma HDL of the same animal in a number of properties. These include a significantly higher proportion of unesterified cholesterol, phospholipid, and apoE and apoA-IV, increased heterogeneity in shape and size, and an overall increase in size (9, 10). Interstitial fluid HDL contains a variable but large number of discoid particles resembling nascent HDL secreted by the liver (11) or intestine (12). We have also shown recently that the discoid component of lymph HDL may be a precursor of plasma HDL_c in the cholesterol-fed dog (13).

The purpose of the studies reported here was to isolate and characterize distinct, homogeneous subfractions of

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EDTA, ethylene diamine tetraacetic acid; apo, apolipoprotein; UC, unesterified cholesterol.

[1]To whom reprint requests should be addressed.

lymph HDL, compare them to equivalent plasma HDL subfractions (if present), and examine the implications of our findings with respect to reverse cholesterol transport.

MATERIALS AND METHODS

Animals, diets, and lymph collection

Mongrel dogs (free of heartworms) were fed either normal dog chow (Purina) or dog chow supplemented with 10% sucrose, 0.3% cholic acid, 3% cholesterol, 20% lard, and 0.5% propylthiouracil for 30–40 days (14). Both control and cholesterol-fed dogs were fasted overnight prior to the surgery for lymph collection. The surgical procedure and lymph collection were carried out as previously described (10).

Lipoprotein isolation techniques

Non-apoB-containing (HDL) lipoproteins, especially in cholesterol-fed dogs, extend beyond the traditional density cut of d > 1.063 g/ml (14). HDL were isolated at the density interval of 1.05–1.21 g/ml. The d < 1.05 g/ml lipoproteins were removed by a 24-hr run at 100,000 *g* in an SW-40 (Beckman) rotor at 5°C. The infranatants were then adjusted to d 1.22 g/ml by addition of solid KBr, and a substantial volume of KBr of d 1.21 g/ml was layered over. The d 1.05–1.21 g/ml fraction was isolated by a 40-hr run in the same rotor under the same conditions. The subsequent fractionation procedure is summarized in **Fig. 1**. The apoB-containing lipoproteins present in the d 1.05–1.21 g/ml fraction were removed by affinity chromatography using Con A-Sepharose (15). The d 1.05–1.21 g/ml fraction was extensively dialyzed against the equilibrating buffer consisting of 0.05 M Tris-HCl, pH 7.0, containing 1.0 M NaCl and 1 mM each of $CaCl_2$, $MgCl_2$, and $MnCl_2$. The affinity chromatography was carried out in a 2.5 × 15 cm column with a flow rate of 0.3 ml/min. The unretained fraction (HDL mixture) was concentrated in dialysis bags against Aquacide and applied to a 1 × 120 cm column of 10% agarose (A-0.5m, Bio-Rad) equilibrated in 0.15 M NaCl, 0.1% Na azide, and EDTA, pH 7.4. All chromatographic steps were carried out at 4°C.

Heparin-Sepharose affinity chromatography was carried out as described by Shelburne and Quarfordt (16). The heparin-Sepharose 4B was packed in a 1.5 × 20 cm column and equilibrated in 2 mM phosphate buffer, pH 7.4, containing 0.05 M NaCl and 0.01% EDTA, Na azide. The lipoprotein mixture was extensively dialyzed against the same buffer and applied to the column. The unretained lipoproteins (apoE-poor) were washed off with several volumes of the same buffer. The retained apoE-rich lipoproteins were then eluted in a single step by a 2 mM phosphate buffer, pH 7.4, containing 1.0 M NaCl.

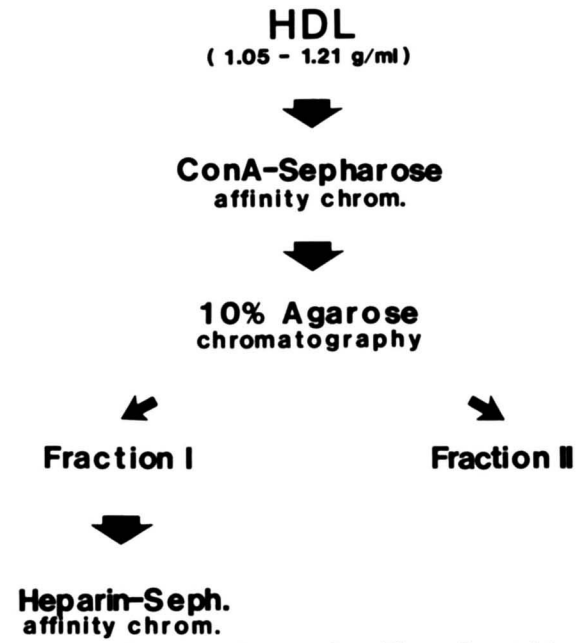


Fig. 1. HDL fractionation procedure. The apoB-containing lipoproteins from the ultracentrifugally isolated plasma or interstitial fluid HDL were removed by Con A-Sepharose affinity chromatography. The HDL mixture was then subfractionated according to size by 10% agarose column chromatography. The larger, more heterogeneous HDL subfraction obtained in this step (Fraction I) was further subfractionated into apoE-rich and apoE-deficient HDL by heparin-Sepharose affinity chromatography. For details, see Materials and Methods.

Analytical techniques

The protein content of the various lipoprotein fractions was determined by the method of Lowry et al. (17), and the total and unesterified cholesterol content by an en-

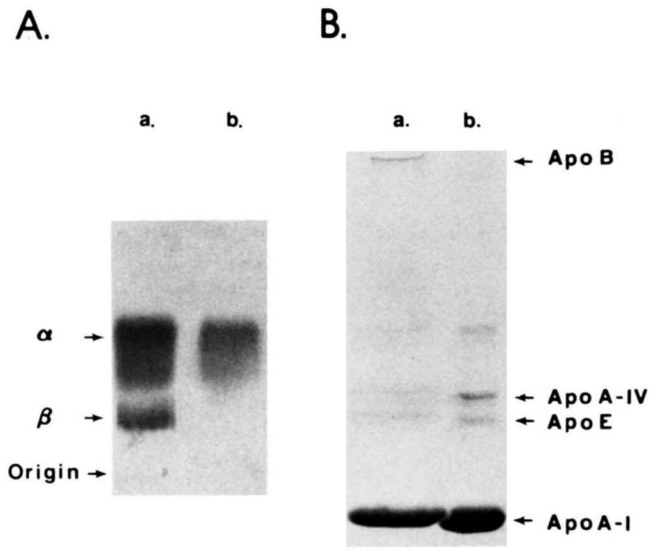


Fig. 2. Agarose electrophoresis (A) and SDS-PAGE (B) of interstitial fluid HDL (d 1.05–1.21 g/ml) before (a) and after (b) Con A-Sepharose affinity chromatography. Note the absence of β-migrating lipoproteins (A) or apoB (B) in HDL obtained after this step.

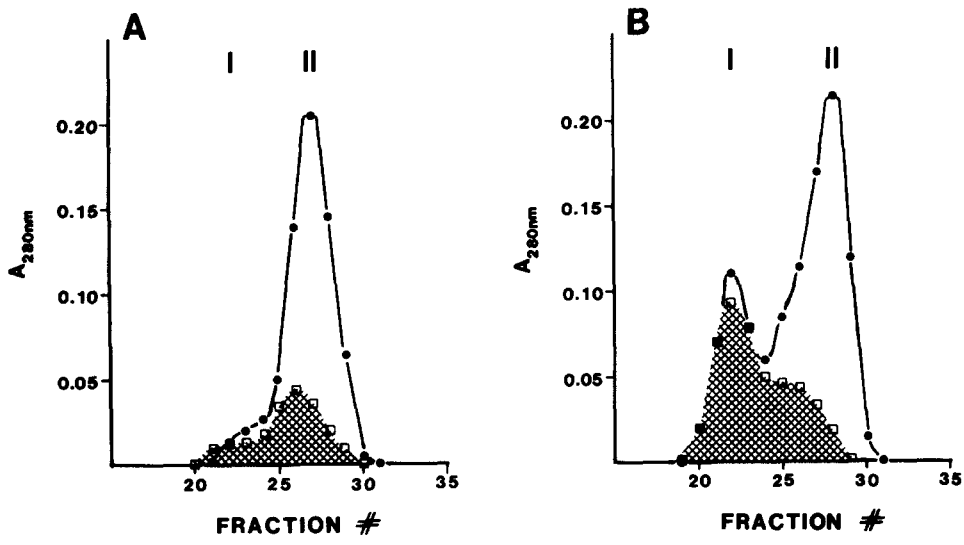


Fig. 3. Ten percent agarose (A-0.5m) column chromatography of interstitial fluid (shaded area □ — □) or plasma (clear area ● — ●). HDL mixture obtained from a typical normal (A) or cholesterol-fed (B) dog. Fractions I and II were pooled separately as indicated.

zymatic method using cholesterol oxidase with or without cholesteryl ester hydrolase, respectively, in the assay mixture (18). The phospholipid content was determined by the method of Bartlett (19). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (20). Electron microscopy was performed on samples negatively stained with 2% phosphotungstic acid, pH 6.4, as previously described (21).

RESULTS

Fractionation of HDL

As shown in **Fig. 2**, the contaminating apoB-containing lipoproteins from the d 1.05–1.21 g/ml fraction are removed by Con A-Sepharose affinity chromatography. The apoB-containing lipoproteins remain bound to Con A and are eluted subsequently with 0.2 M of 1-O-methyl α-D-glucopyranoside in the equilibrating buffer (15). The electrophoretic mobility and the apolipoprotein composition of HDL before and after Con A-Sepharose chromatography are shown in Fig. 2.

This heterogeneous mixture of non-apoB HDL is subfractionated according to size by chromatography in 10% agarose. **Fig. 3** shows a typical elution pattern of the plasma and interstitial fluid HDL mixture from a cholesterol-fed as well as from a control dog. It is apparent that in the control dog (Fig. 3A), HDL fraction II (smaller size HDL) is the predominant species, both in plasma and interstitial fluid. Fraction I represents only a minor component. Cholesterol feeding, on the other hand, induces a large increase in fraction I of both plasma and interstitial fluid HDL. Fraction II remains the dominant HDL species of plasma, but fraction I becomes the dominant fraction in interstitial fluid (Fig. 3B).

The total protein and cholesterol mass distribution between the two fractions is shown in **Table 1**. It can be seen that, in cholesterol-fed dogs, interstitial fluid HDL fraction I (L-I) contains over 50% of the total protein and 66% of the total cholesterol mass present in unfractionated HDL, whereas plasma HDL fraction I (P-I) of the same animal contains only 12% of the total protein and 22% of the total cholesterol mass. On the other hand, in control dogs over 90% of the protein and 85% of the cholesterol mass of interstitial fluid or plasma HDL is present in fraction II (not shown).

Physico-chemical properties of HDL subfractions

Fig. 4 shows typical electron micrographs of plasma and interstitial fluid HDL subfractions of a cholesterol-fed dog. L-I appears to be morphologically homogeneous, with 80–90% of the particles of discoid shape. P-I, on the other hand, is not homogeneous; it appears to be a mixture of discoid and mostly larger, spherical HDL. On the

TABLE 1. Protein and total cholesterol mass distribution in fractions I and II of interstitial fluid and plasma HDL (1.05–1.21 g/ml) of cholesterol-fed dogs as separated by column chromatography in 10% agarose

	Interstitial Fluid		Plasma	
	I	II	I	II
Protein (%)	51 ± 5[a]	49 ± 5	12 ± 2	88 ± 2
Cholesterol (%)	66 ± 3	34 ± 3	22 ± 3	78 ± 3

[a]Mean ± SEM of three experiments.

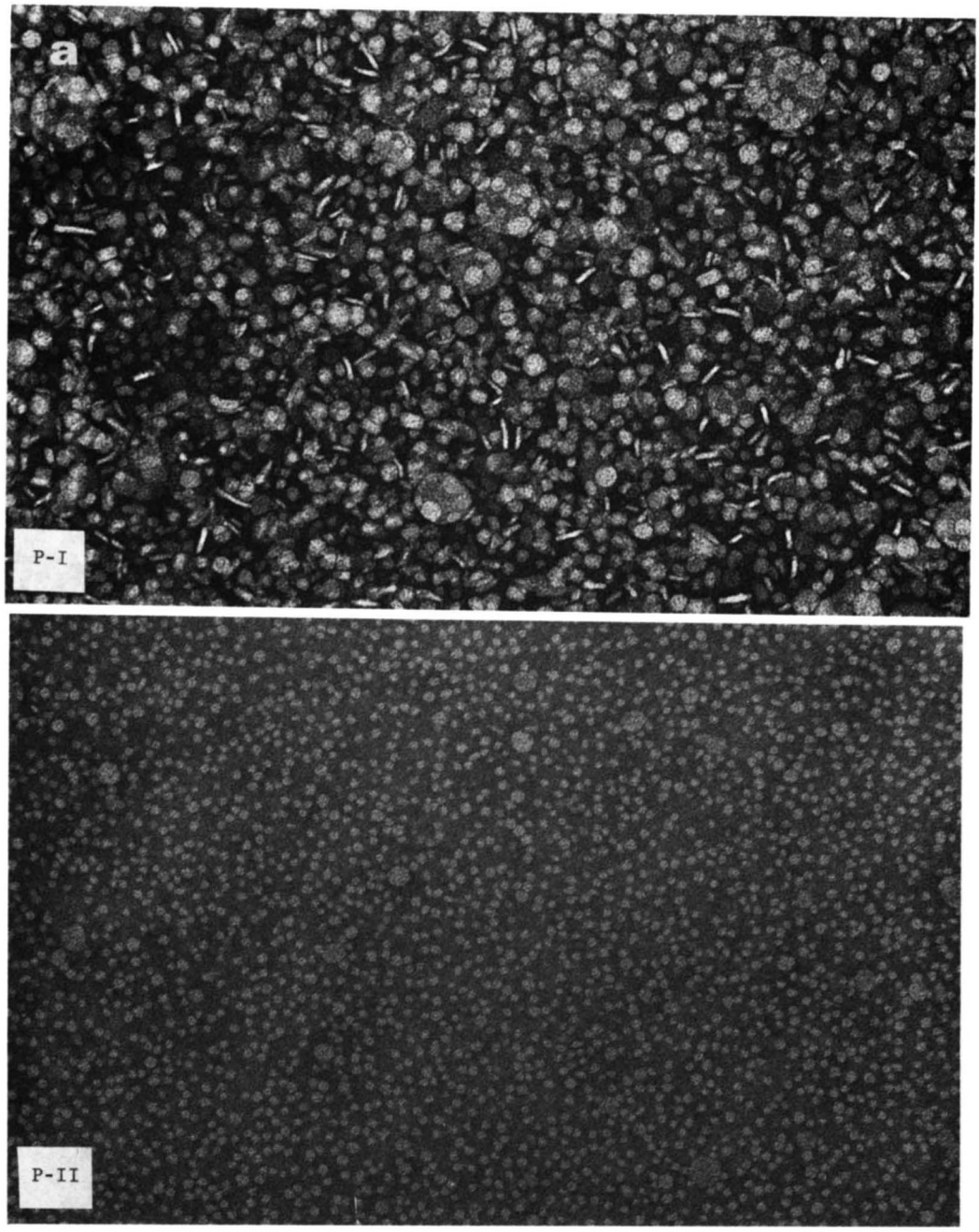
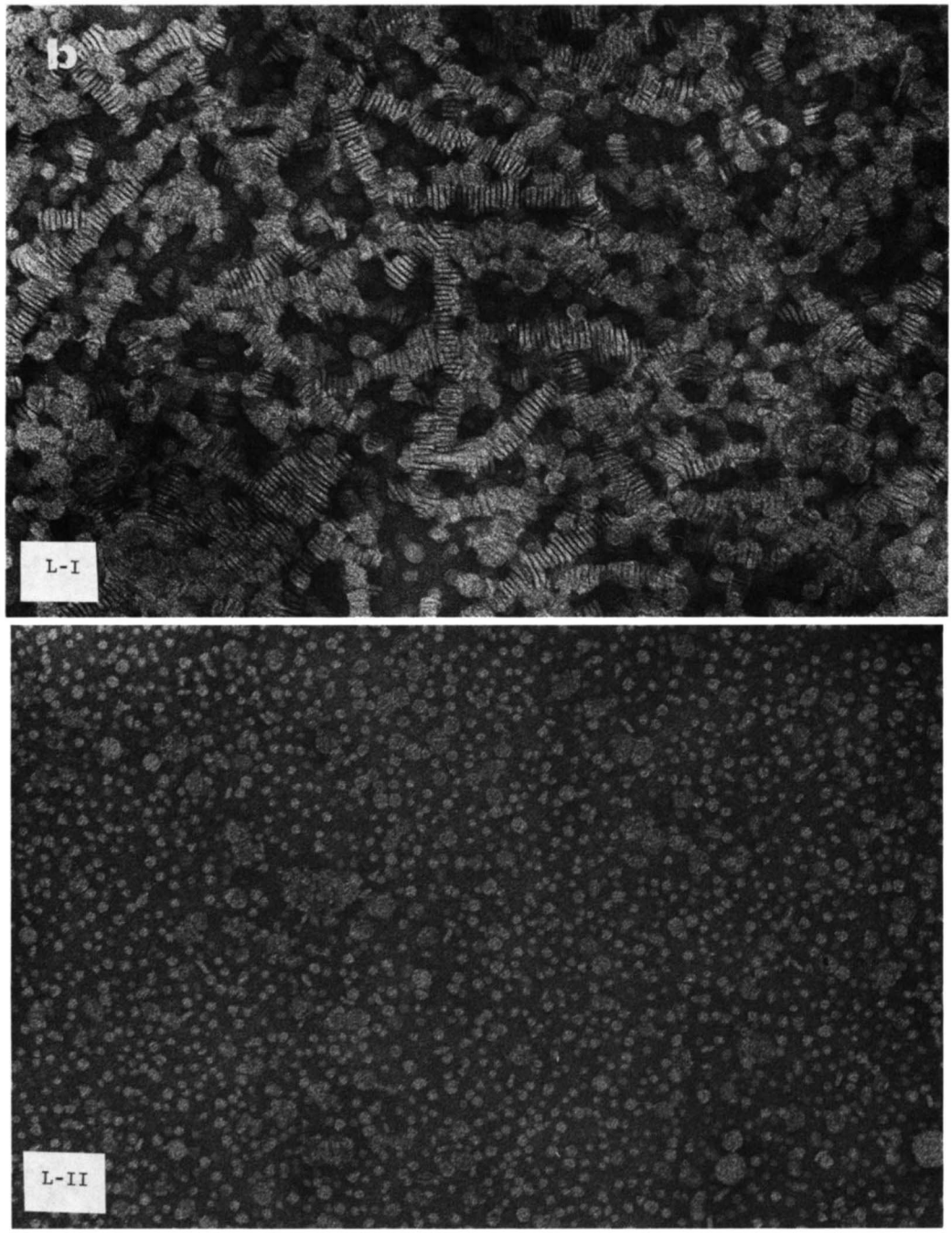


Fig. 4. Negatively stained plasma (a, P-I and P-II) and interstitial fluid (b, L-I and L-II) HDL subfractions obtained from a cholesterol-fed dog.

average, approximately 15% of the particles in this fraction are discoid (as determined by counting 500 particles on several micrographs). Both L-II and P-II appear to be uniformly spherical.

Note, however, that despite the apparent morphological similarity between P-II and L-II, size distribution analysis reveals significant differences (**Fig. 5**). P-II is a narrowly distributed population with a mean diameter of 100 ± 2 Å (mean ± SEM). The size distribution of L-II is significantly skewed toward a larger mean diameter of 120 ± 2 Å. Similar differences also exist between the size distribution of L-I and P-I. Both have a wide range of sizes, but L-I has a greater mean diameter of 250 ± 4 Å. This size represents the length of the discs, while the mean diameter of P-I (160 ± 3 Å) is that of the spherical particles (a vast majority of the total).



The chemical composition of the plasma and interstitial fluid HDL subfractions of cholesterol-fed and control dogs is shown in **Table 2**. Two very important features become evident: *1*) both interstitial fluid HDL subfractions from cholesterol-fed dogs and the L-I fraction of control dogs are significantly enriched in unesterified cholesterol when compared to equivalent plasma HDL subfractions; and *2*) fraction I HDL (both P-I and L-I) have a significantly higher cholesterol-to-protein ratio than the corresponding fractions II. In addition, in cholesterol-fed dogs, even the apparently spherical interstitial fluid HDL subfraction has an elevated cholesterol-to-protein ratio and an unusually high content of unesterified cholesterol. The chemical composition of the discoid HDL subfraction of interstitial fluid (L-I) correlates well with its physical appearance (i.e., very high unesterified choles-

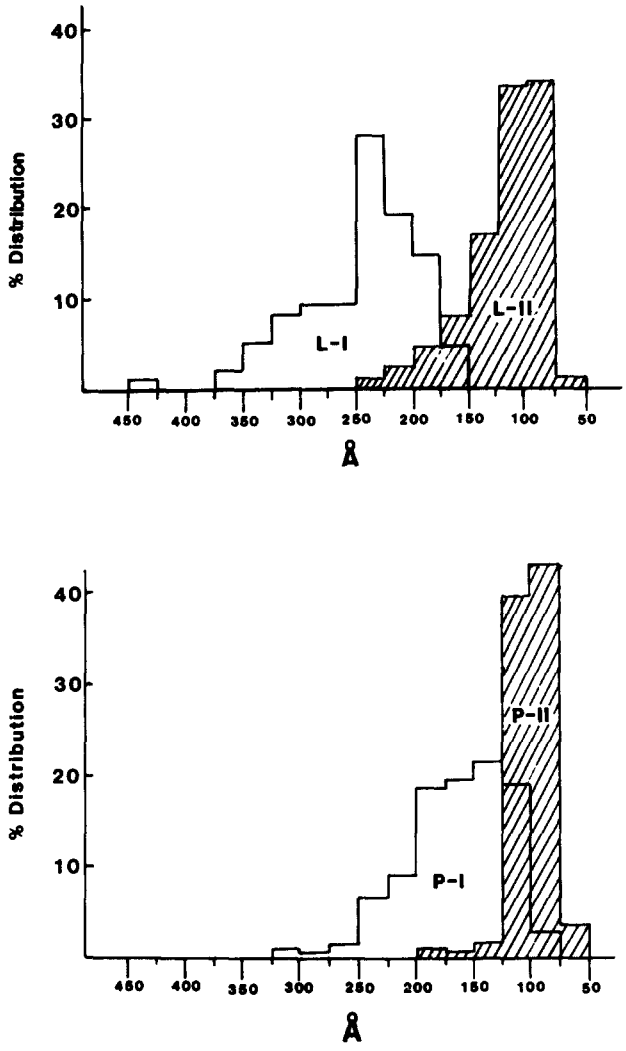


Fig. 5. Particle size distribution (obtained from electron micrographs) of plasma (P-I and P-II) and interstitial fluid (L-I and L-II) HDL subfractions obtained from a cholesterol-fed dog. 500 particles were measured for each distribution pattern.

terol and phospholipid content).

The apolipoprotein composition of the HDL subfractions of cholesterol-fed dogs is shown in **Fig. 6**. Noteworthy is the enrichment in apoE and apoA-IV relative to apoA-I in L-I as well as P-I. In contrast, P-II and L-II contain almost exclusively apoA-I, with a small amount of apoA-IV in the case of L-II. These results indicate that the apoE found in the interstitial fluid HDL, and to a lesser extent in plasma HDL, is restricted to fraction I HDL. The apolipoprotein composition of the HDL subfractions from control dogs is similar (not shown).

Fraction I of plasma and interstitial fluid HDL of cholesterol-fed dogs can be further subfractionated by heparin-Sepharose affinity chromatography into apoE-enriched, apoA-IV- and apoA-I-containing, and apoE-deficient, apoA-IV- and apoA-I-containing particles (**Fig. 7**). Using interstitial fluid HDL, both of these subfractions appear to be of discoidal shape and contain large amounts of unesterified cholesterol and phospholipid, consistent with their physical appearance (results not shown). These results suggest that the association of apoE with discoid particles is frequent but not necessary. Lack of sufficient material in P-I and L-I of control plasma or interstitial fluid precluded a similar analysis.

DISCUSSION

We previously reported the heterogeneity of peripheral lymph HDL with respect to size (10). We have also shown that dog interstitial fluid HDL differs in apolipoprotein and lipid composition from plasma HDL (10). Specifically, interstitial fluid HDL contained two (in control) to four times (in cholesterol-fed) the amount of unesterified cholesterol, at the expense of cholesteryl esters, and it also contained an increased proportion of phospholipid and a decreased mass of protein (10). Such extensive differences between plasma and interstitial fluid HDL imply extensive modification of filtered plasma HDL by peripheral tissues or, possibly, de novo peripheral HDL assembly from components filtered from plasma and/or synthesized by peripheral cells. In the present study, we characterized two morphologically distinct HDL populations present in interstitial fluid of dogs and compared them to their equivalent plasma subfractions.

Our results clearly demonstrate that interstitial fluid HDL (d 1.05–1.21 g/ml) can be subfractionated on the basis of size into two populations: a smaller, spherical

TABLE 2. Chemical composition (% weight) of control and cholesterol-fed dog interstitial fluid and plasma HDL subfractions I and II

	Control				Cholesterol-Fed			
	Plasma		Interstitial Fluid		Plasma		Interstitial Fluid	
	I	II	I	II	I	II	I	II
Protein	24 ± 2	42 ± 1	26 ± 3	43 ± 2	20 ± 2	41 ± 1	21 ± 1	36 ± 3
Unesterified cholesterol	14 ± 1	5 ± 0.2	15 ± 1	6 ± 1	16 ± 2	5 ± 0.5	28 ± 1	15 ± 3
Cholesteryl ester	20 ± 1	14 ± 1	15 ± 1	13 ± 1	17 ± 6	14 ± 2	6 ± 2	12 ± 2
Phospholipid	43 ± 5	39 ± 1	44 ± 1	38 ± 2	47 ± 3	40 ± 2	45 ± 1	37 ± 1
Unesterified cholesterol/protein	0.58	0.12	0.58	0.14	0.80	0.12	1.33	0.42
% Unesterified cholesterol	42	25	50	32	48	26	82	56

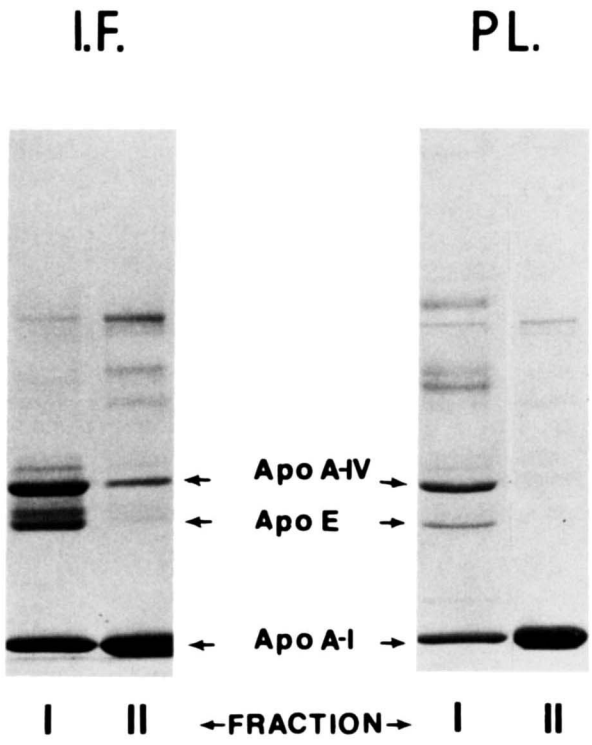


Fig. 6. SDS-PAGE of interstitial fluid and plasma HDL subfractions obtained from a cholesterol-fed dog.

HDL with a mean diameter of 120 Å (L-II) and a larger, predominantly discoid HDL with a mean diameter of 250 Å (L-I). There is little overlap between these two populations. The proportion of the larger, discoid HDL is very small in plasma or interstitial fluid of control dogs and in the plasma of cholesterol-fed dogs. Most of the material in P-I is, in fact, larger, spherical HDL_1 (in control dogs) or HDL_c (in cholesterol-fed dogs). The proportion of discs within the larger HDL fraction is greatly increased in the interstitial fluid of cholesterol-fed dogs (L-I) and represents close to 100% of the particles in fraction I of interstitial fluid HDL. Thus, discs represent approximately 50% of the total HDL mass in the interstitial fluid of cholesterol-fed dogs. Such a disproportionate increase in discs in the interstitial fluid and not in the plasma is inconsistent with the concept that interstitial fluid HDL is simply a filtrate of plasma HDL. Instead, two possibilities exist: *1*) a preferential filtration (or active transport process) of the larger, discoid HDL from plasma (an unlikely possibility, in our view); or *2*) peripheral HDL assembly from filtered plasma and peripherally derived components. Based on the known protein mass in each HDL subfraction and on our estimation of the proportion of discs in each fraction using electron microscopy, it appears that in control and cholesterol-fed dogs interstitial fluid contains a concentration of discs approximately fourfold higher than that of plasma. This may be an underestimate because the interstitial fluid compartment volume is significantly larger than that of the plasma compartment. The higher concentration of discs in the interstitial fluid strongly suggests peripheral assembly of these particles.

The increase in size of the smaller, spherical HDL of interstitial fluid in cholesterol-fed dogs suggests that an interaction (and subsequent modification) occurs between filtered plasma HDL and peripheral cells. There is a significant enrichment in unesterified cholesterol, resulting in a tripling of the unesterified cholesterol-to-protein ratio and a threefold increase in the unesterified cholesterol content when compared to small plasma HDL. The excessive unesterified cholesterol may be accommodated within the surface of the HDL sphere or within the neutral lipid core or both. It is tempting to speculate that this unesterified cholesterol enrichment is derived from peripheral cells through their interaction with HDL, representing an initial or early event in the process of reverse cholesterol transport.

Enrichment in unesterified cholesterol in the smaller, spherical HDL, possibly through a receptor-mediated interaction with peripheral cells (22), may be only one of many alternative ways peripheral tissues dispose of excessive cholesterol. The appearance of a large number of discoid HDL, composed of 28% unesterified cholesterol,

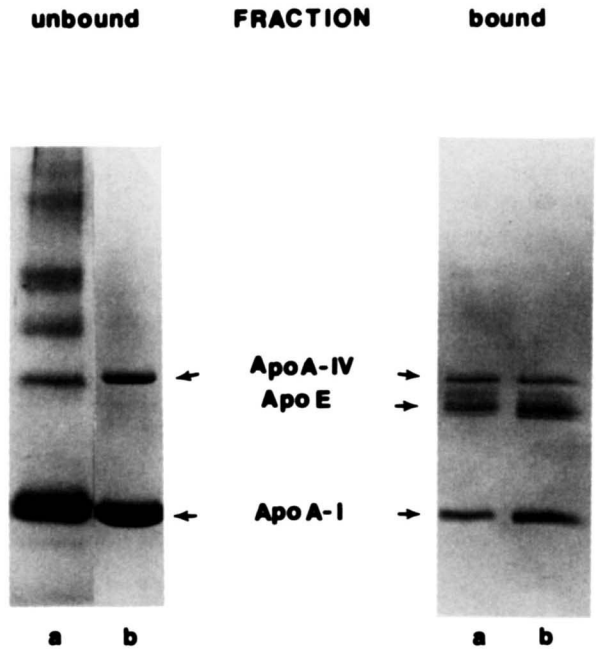


Fig. 7. Heparin-Sepharose affinity chromatography of interstitial fluid (a) or plasma (b) fraction I HDL (L-I or P-I) into apoE-rich (bound) and apoE-deficient (unbound) HDL. HDL obtained from a cholesterol-fed dog was used. The apoE-containing lipoproteins were eluted in a single step gradient by the running buffer (2 mM phosphate, pH 7.4, 0.01% EDTA, Na azide) containing 1 M NaCl.

45% phospholipid, and only 6% cholesteryl esters, may represent another major path in cholesterol excretion. The ability of a cholesterol-loaded macrophage to synthesize and secrete apoE, as well as excrete cholesterol, has been demonstrated by Basu, Goldstein, and Brown (23). A number of laboratories have since demonstrated the presence of apoE mRNA in a variety of tissues (24, 25). Therefore, it appears reasonable to suggest that newly secreted apoE, together with other apoproteins filtered from plasma and relatively lipid-deficient (namely apoA-IV, known to exist in such a state in dog plasma or interstitial fluid, Lefevre, M., and L. Dory, unpublished observations) may combine with plasma membrane cholesterol and phospholipid (destined for reverse cholesterol transport) to form the discoid particles.

The apoprotein composition of discoid HDL particles suggests that, whereas apoE is a major constituent, significant amounts of apoA-IV and apoA-I are also present. The use of heparin-Sepharose affinity chromatography suggested that at least some of the discs contain all three apolipoproteins. We have, however, presented evidence that there are some discoid particles containing no detectable apoE, but rich in apoA-IV and, to a lesser extent, apoA-I. Our observations suggest that apoA-IV may also play an important role in reverse cholesterol transport. If the assumption that mammalian peripheral tissues do not synthesize apoA-IV and apoA-I is correct, the assembly of discoid particles likely occurs in the interstitial space rather than within the cells. This hypothesis is supported by the recent demonstration that apoE and cholesterol secretion by macrophage were not linked and could be selectively inhibited (23). The use of immunoaffinity chromatography may yield HDL fractions homogeneous with respect to apoprotein composition and, thus, metabolic function. Our results clearly point to the importance of studies dealing with interstitial fluid lipoproteins and hint at some of the obtainable benefits in unraveling aspects of cholesterol flux between the liver and peripheral cells.

We would like to thank Ms. Linda Spiess and Ms. Lawrie Lehmann for their assistance in the preparation of this manuscript. This research was supported by an NIH Program Project Grant #HL 25596 at the Louisiana State University Medical Center in New Orleans.

Manuscript received 17 August 1984.

REFERENCES

1. Miller, G. J., and N. E. Miller. 1975. Plasma high-density lipoproteins and development of ischaemic heart disease. *Lancet.* **1:** 16–19.
2. Mjøs, O. D. 1977. Editorial. High density lipoproteins and coronary heart disease. *Scand. J. Clin. Lab. Invest.* **37:** 191–193.
3. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
4. Stein, O., and Y. Stein. 1973. The removal of cholesterol from Landschuts ascites cells by high-density apolipoproteins. *Biochim. Biophys. Acta.* **326:** 232–244.
5. Ho, Y. K., M. S. Brown, and J. L. Goldstein. 1980. Hydrolysis and excretion of cytoplasmic cholesteryl esters by macrophages: stimulation by high density lipoproteins and other agents. *J. Lipid Res.* **21:** 391–398.
6. Rothblat, G. H., and M. C. Phillips. 1982. Mechanism of cholesterol efflux from cells. Effects of acceptor structure and concentration. *J. Biol. Chem.* **257:** 4775–4782.
7. Oram, J. F., J. J. Albers, M. C. Cheung, and E. L. Bierman. 1981. The effects of subfractions of high density lipoproteins on cholesterol efflux from cultured fibroblasts. Regulation of low density lipoprotein receptor activity. *J. Biol. Chem.* **256:** 8348–8356.
8. Fielding, C. J., and P. E. Fielding. 1982. Cholesterol transport between cells and body fluids. *Med. Clin. N. Am.* **66:** 363–373.
9. Sloop, C. H., L. Dory, B. R. Krause, C. Castle, and P. S. Roheim. 1983. Lipoproteins and apolipoproteins in peripheral lymph of normal and cholesterol-fed dogs. *Atherosclerosis.* **49:** 9–21.
10. Sloop, C. H., L. Dory, R. Hamilton, B. R. Krause, and P. S. Roheim. 1983. Characterization of dog peripheral lymph lipoproteins: the presence of a disc-shaped "nascent" high density lipoprotein. *J. Lipid Res.* **24:** 1429–1440.
11. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat livers. *J. Clin. Invest.* **58:** 667–680.
12. Green, P. H. R., A. R. Tall, and R. M. Glickman. 1978. Rat intestine secretes discoid high density lipoprotein. *J. Clin. Invest.* **61:** 523–534.
13. Dory, L., C. H. Sloop, L. M. Boquet, R. L. Hamilton, and P. S. Roheim. 1983. Lecithin:cholesterol acyltransferase-mediated modification of discoidal peripheral lymph high density lipoproteins: possible mechanism of formation of cholesterol-induced high density lipoproteins (HDL_c) in cholesterol-fed dogs. *Proc. Natl. Acad. Sci. USA.* **80:** 3489–3493.
14. Mahley, R. W., K. H. Weisgraber, and T. Innerarity. 1974. Canine lipoproteins and atherosclerosis. II. Characterization of the plasma lipoproteins associated with atherogenic and nonatherogenic hyperlipidemia. *Circ. Res.* **35:** 722–733.
15. Mitamura, T. 1982. Separation of cholesterol-induced high density lipoproteins (HDL_c) by concanavalin A-Sepharose affinity chromatography. *J. Biochem.* **91:** 25–29.
16. Shelburne, F. A., and S. M. Quarfordt. 1977. The interaction of heparin with an apoprotein of human very low density lipoprotein. *J. Clin. Invest.* **60:** 944–950.
17. Lowry, O. H., M. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
18. Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20:** 470–475.
19. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
20. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature.* **227:** 680–685.
21. Hamilton, D. L., J. Goerke, L. S. S. Guo, M. C. Williams, and R. J. Havel. 1980. Unilamellar liposomes made with the French pressure cell: a simple preparative and semi-quantitative technique. *J. Lipid Res.* **21:** 981–992.

22. Biesbroeck, R., J. F. Oram, J. J. Albers, and E. L. Bierman. 1983. Specific high-affinity binding of high density lipoproteins to cultured human skin fibroblasts and arterial smooth muscle cells. *J. Clin. Invest.* **71:** 525–539.
23. Basu, S. K., J. L. Goldstein, and M. S. Brown. 1983. Independent pathways for secretion of cholesterol and apolipoprotein E by macrophages. *Science.* **219:** 871–873.
24. Williams, D. L., P. A. Dawson, and L. L. Rudel. 1983. Synthesis of apo A-I and apo E in nonhuman primate peripheral tissues. *Circulation.* **68:**III, 119.
25. Zannis, V. I., S. K. Karathanasis, G. Goldberg, F. S. Cole, G. Forbes, and J. L. Breslow. 1983. Synthesis of apo E, apo A-I, and apo C-III mRNA and protein by human cell cultures. *Circulation.* **68:**III, 291.