Interaction of bovine serum high density lipoprotein with mixed vesicles of phosphatidylcholine and cholesterol

Ana Jonas

Department of Biochemistry, School of Basic Medical Sciences and School of Chemical Sciences, University of Illinois, Urbana, IL 61801

Abstract The interaction of sonicated, small vesicles of egg phosphatidylcholine and cholesterol (2:1, mol/mol) with bovine high density serum lipoproteins was examined in terms of lipid transfer between both types of particles and the resulting changes in lipoprotein structure. Saturation of high density lipoprotein preparations with vesicle lipids gave final lipoprotein particles with essentially unchanged protein content and composition, unchanged cholesteryl ester and nonpolar lipid content, but with markedly increased phospholipid content (59% increase by weight) and moderately increased cholesterol content (20% increase by weight). The lipoproteins enriched in lipid were relatively uniform, spherical particles, 110 ± 3.6 Å in diameter (6 Å larger than the original lipoproteins); they had a markedly decreased intrinsic protein fluorescence, a red-shifted fluorescence wavelength maximum, and more fluid lipid domains. These results indicate that the direct addition of excess lipids from membranes or other lipoproteins is a possible mechanism for lipid transfer to high density lipoproteins. Also they suggest a structural flexibility of high density lipoproteins that allows the addition of significant amounts of surface components.—**Jonas, A.** Interaction of bovine serum high density lipoprotein with mixed vesicles of phosphatidylcholine and cholesterol. *J. Lipid Res.* 1979. **20:** 817–824.

Supplementary key words lipid transfer · lipoprotein structure

A postulated function of high density serum lipoproteins (HDL) is the removal of cholesterol from peripheral tissues, followed by esterification by lecithin:cholesterol acyltransferase, and transport to the liver, the organ primarily responsible for the metabolism and excretion of this sterol (1). Recent work has shown that HDL or HDL apolipoproteins can remove lipids from cells in tissue culture. Stein et al. (2) demonstrated that HDL and complexes of its apolipoproteins with phospholipids removed cholesterol from smooth muscle cells, and Jackson et al. (3) reported that apolipoprotein A-I, the major protein component of HDL, adsorbed to and removed phospholipids from Landshutz ascites cells. Slutzky et al. (4) showed that mycoplasma membranes can take up cholesterol from HDL without any transfer of lipoprotein phospholipids or proteins to the cells. The most extensively investigated cells in terms of their lipid transfer and exchange with serum lipoproteins are the erythrocytes (see review by Bell, 5). Since erythrocyte membranes are at equilibrium with plasma, the interaction between the cells and lipoproteins appears to be a simple exchange of lipids; however, when the exchangeable lipid contents of one of the particles is changed, net lipid transfer can occur (6). Therefore, the interaction between HDL and cell membranes may result in lipid exchange or transfer in either direction depending on the relative affinities of the lipid for both particles and on lipid contents of the particles. In situations where net lipid transfer occurs it is very important to assess the structural changes that take place in the lipoprotein, since they may affect the function and metabolism of the lipoprotein. Tall and Small (7) and Tall et al. (8) reported that human HDL, in the presence of dimyristoylphosphatidylcholine liposomes, releases part of its apolipoprotein which then forms disc-like complexes with the synthetic phospholipid. The remaining HDL components apparently fuse into larger particles depleted of protein. In this report I describe the interaction of egg phosphatidylcholine (PC) vesicles containing 33 mol% cholesterol with bovine HDL, and demonstrate that the transfer of lipid from these vesicles occurs directly into preexisting HDL particles. The present observations may be relevant to the mode of lipid uptake by HDL from cell membranes (1–6) and from triglyceride-rich lipoproteins during lipolysis (9, 10).

Abbreviations: HDL, high density lipoprotein (d 1.063–1.170 g/ml); PC, phosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene, fluorescent lipophilic probe; apoA-I, apolipoprotein A-I, the major protein component of HDL; PL, phospholipid.

EXPERIMENTAL PROCEDURE

Materials

Crystalline cholesterol (99+%) and egg PC of type III-E were obtained from Sigma Chemical Company. The egg PC was purified by chromatography on a silicic acid column according to the procedure of Rouser et al. (11). Cholesterol was not purified further. The lipids were examined for purity by TLC on silica gel plates in the following solvent systems: petroleum ether–diethyl ether–acetic acid 90:10:1 (v/v) or hexanes–diethyl ether–acetic acid 70:30:1 (v/v) for cholesterol and chloroform–methanol–water 65:25:4 (v/v) for PC. Lipids on the plates were detected by spraying with 6 N H_2SO_4 and charring, or by exposure to I_2 vapors. The cholesterol and PC used in this study gave single spots by the TLC analysis. Very similar results were obtained on lipids extracted with chloroform–methanol 2:1 (v/v) after the sonication procedure or collected under the vesicle peak in the gel-filtration experiments. [^{14}C]Dimyristoylphosphatidylcholine was purchased from P. J. Cobert Associates.

Organic solvents of reagent grade were distilled prior to use. The buffer in these studies was 0.1 M Tris-HCl, pH 8.0, 0.001% EDTA, 10^{-3} M NaN_3; it was prepared with reagent grade materials and deionized water. The Sepharose CL-4B gel was obtained from Pharmacia Fine Chemicals. The lipophilic fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) was purchased from Aldrich Chemical Company, and was prepared as a 10^{-3} M stock solution in tetrahydrofuran.

Preparations

Bovine HDL was prepared and analyzed for purity as described previously (12). Prior to use, the HDL preparations were heated at 58°C for 30 min in order to remove any traces of lecithin:cholesterol acyltransferase activity that may have been associated with the lipoproteins. HDL preparations that were not heated in this manner showed a measurable increase in cholesteryl ester content after incubation at 37°C for 10 hr. "Heated" and original lipoprotein preparations, passed through a Sepharose CL-4B column (2.0 × 90 cm) at 5°C, eluted at exactly the same position, had identical protein and lipid contents and compositions, and had the same physical properties, including intrinsic protein fluorescence, wavelength of maximum fluorescence, circular dichroism spectra (data not reported), fluidity of lipid domains, and sizes from electron micrographs. The native and "heated" HDL preparations were stable by the criteria listed above for at least 1 month when stored at 5°C.

Vesicles were prepared by the method of Huang (13) and Newman and Huang (14) except that shorter sonication times were employed. Typically 30.0 mg of egg PC, 7.4 mg of cholesterol (i.e., 2:1, mol PC/mol cholesterol), and approximately 10^6 cpm of ^{14}C-labeled PC were mixed in 3–5 ml of chloroform–methanol 2:1 (v/v), and were dried under N_2. The lipid mixture was suspended in 5.0 ml of buffer by vortexing and was then sonicated with a Heat Systems-Ultrasonics, Inc. sonifier, model W185, using a power setting of 5, for a total of 30 min under an atmosphere of N_2 and at temperatures around 2–5°C. Following centrifugation of the sonicated lipid at 18,000 rpm, at 5°C for 1 hr, the supernatant, containing essentially all of the lipid, was placed on a Sepharose CL-4B column (2.4 × 45 cm) at 5°C and was eluted with buffer. The column fractions of about 2.2 ml were assayed by scintillation counting in a Beckman LS-100 counter. This procedure gave approximately 80% small, Huang-type vesicles and 20% large vesicles or multilamellar liposomes, with a total lipid recovery of at least 80% from the column. The peak corresponding to the small vesicles was pooled, concentrated about 5-fold by Amicon-cone filtration, analyzed for PC and cholesterol (2:1, mol/mol), and stored at 5°C until use. Under these conditions, the vesicles were stable for over 2 weeks as judged by the elution pattern from the Sepharose CL-4B (2.4 × 45 cm) column.

Incubation and fractionation of HDL and vesicles. Incubations of HDL with vesicles were carried out under N_2 in a covered, shaking water bath (40 oscillations/min) at 37°C for up to 20 hr. HDL concentrations were of the order of 2–10 mg/ml (about 0.6–3 mg/ml of phospholipid (PC)) and the vesicle concentrations varied from about 0.05 to 5 mg/ml (0.04–4 mg/ml of PC)—covering ratios from 1/10 to 10/1 for vesicle PL to HDL PL by weight. After it was determined that saturation of HDL with vesicle lipids occurred at a weight ratio of vesicle PL/HDL PL of 1/2 (see Fig. 3), ratios of approximately 1/1 were normally used to prepare "equilibrated" HDL. The vesicle and HDL mixtures were fractionated after the selected incubation period (5 hr were sufficient for equilibration of the system) on a Sepharose CL-4B column (2.0 × 90 cm) at 5°C. The column fractions were assayed for protein and lipid content and, in some cases, for [^{14}C]dimyristoylphosphatidylcholine cpm. Equilibrated HDL was stable for about 2 weeks when stored at 5°C by the criteria listed above for "heated" and native HDL.

Chemical analyses. Protein was determined by the method of Lowry et al. (15) using bovine serum albumin as the standard. Total lipids were determined from dry weight after quantitative extraction by the

procedure of Folch, Lees, and Sloane Stanley (16). Phospholipids were determined by the method of Chen, Toribara, and Warner (17) and free and total cholesterol by the procedure of Sperry and Webb (18). The cholesterol determinations were confirmed by use of the enzymatic kits supplied by Beckman Instruments, Inc. and BioDynamics, a division of Boehringer-Mannheim.

Electron micrographs of native and equilibrated HDL preparations were obtained at instrument magnifications of 40,000–80,000 using a Siemens 102 electron microscope operating at 80 Kv. The negative staining procedure with sodium phosphotungstate at pH 7.4 was taken from Anderson et al. (19), except that lower lipoprotein concentrations were used, about 0.2 mg of total lipoprotein per ml.

Fluorescence methods

Intrinsic protein fluorescence spectra of HDL preparations were recorded with a Hitachi-Perkin-Elmer MPF3 spectrofluorometer. Diluted lipoprotein solutions of identical protein concentration and absorbance (absorbance at 280 nm ≅ 0.06) were excited at 280 nm and emission spectra were recorded from 290 to 400 nm using 6-nm slit widths. Temperature was regulated at 25°C. Relative fluorescence yields were determined from the areas under the uncorrected emission spectra.

Fluorescence polarization of the lipophilic 1,6-diphenyl-1,3,5-hexatriene (DPH) probe as a function of temperature was used as a measure of the fluidity of the lipid domains of the lipoprotein preparations. The theory and applications of this technique were previously described by Shinitzky and co-workers (20–22) and were applied to lipoproteins in our own laboratory (23, 24). The fluorescence polarization measurements were carried out with the SLM, Series 400 fluorescence polarization instrument, using 366-nm exciting light, 4-nm slits, and 3–74 Corning Glass cut-off filters in the path of the emitted light. DPH was added in a tetrahydrofuran solution by injecting 10 μl of this solution into 2.0 ml of stirred sample solution in a fluorescence cuvette. The molar ratio of fluorophore to lipids in the samples was around 1:400. Prior to measurement the solutions were equilibrated at room temperature for 1 hr and the tetrahydrofuran was removed by flushing the samples with N_2. Temperature was regulated with a circulating water bath and was measured with a Fluke 2100 A digital thermometer equipped with a copper/constantan probe that was immersed directly in the sample cell. Fluorescence lifetimes were measured in Dr. G. Weber's laboratory (University of Illinois, Urbana) using the cross-correlation phase fluorometer described by Spencer and Weber (25) with an improved electronics system from SLM Instruments. The conditions employed in the lifetime measurements were identical to those used in the polarization measurements.

RESULTS

The elution profiles of ^{14}C-labeled vesicles, native HDL, equilibrated HDL, and bovine apoA-I, from the preparative Sepharose CL-4B (2.0 × 90 cm) column, are shown in **Fig. 1.** Clearly, the separation of vesicles from HDL was essentially complete. Contamination of the top equilibrated HDL fractions by vesicle lipids was insignificant even when reaction mixtures containing a 3/1 vesicle PL/HDL PL weight ratio were fractionated. The bulk of free bovine apolipoprotein A-I eluted eight fractions later than equilibrated HDL; apolipoprotein A-I was sufficiently well resolved to be detectable as a separate peak or a shoulder if present in our HDL preparations. Equilibrated HDL eluted in a symmetrical, relatively narrow peak slightly ahead of native HDL, indicating a small difference in average Stokes radius for these two lipoprotein preparations.

Fig. 2 shows a series of elution profiles obtained on the same column for reaction mixtures of different vesicle PL to HDL PL weight ratios: 3/1, 1/1, and 0.2/1. The elution of [^{14}C]dimyristoylphosphatidyl-

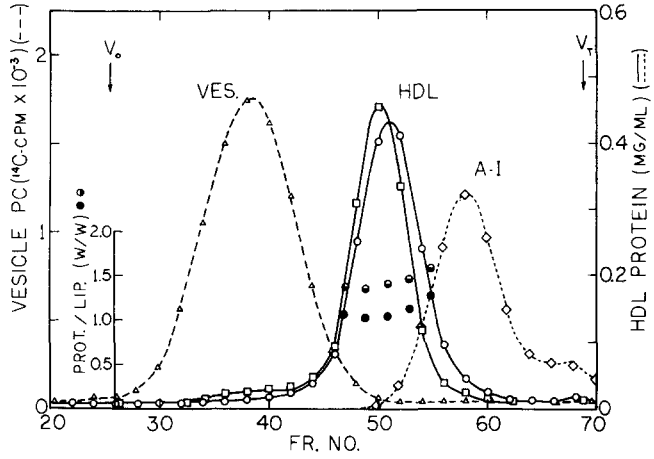

Fig. 1. Elution profiles of ^{14}C-labeled vesicles (△ —— △), native HDL (○ —— ○), equilibrated HDL (□ —— □), and bovine apolipoprotein A-I (◇ – – – ◇) from a Sepharose CL-4B column (2 × 90 cm). The column was equilibrated with 0.1 M Tris-HCl buffer, pH 8.0, 0.001% EDTA, 10^{-3} M NaN_3 and was operated at 5°C. V_0 and V_t denote excluded and total column volume, respectively; fraction volumes were 4.1 ml. Both HDL samples and the apolipoprotein A-I sample contained the same amount of protein, as determined by the method of Lowry et al. (15), which was also used to assay the protein content of column fractions. The weight ratios of protein to phospholipid (●), and protein to cholesteryl ester (◒) are shown across the native HDL elution profile.

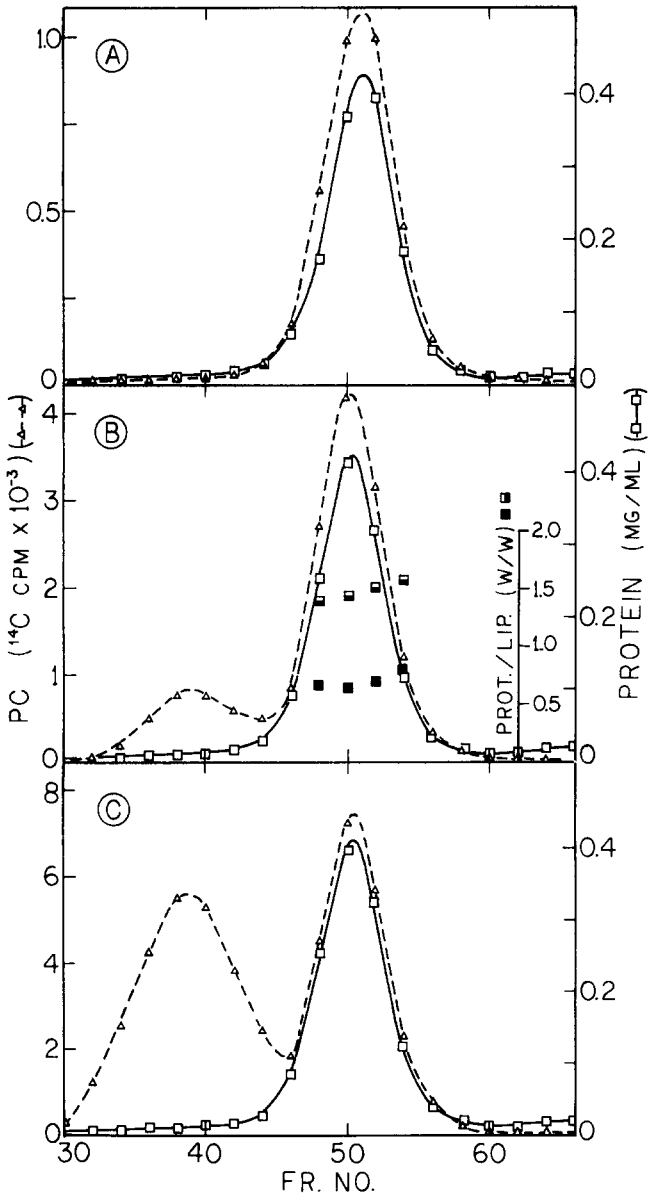

Fig. 2. Elution profiles of reaction mixtures of HDL equilibrated with increasing amounts of egg PC:cholesterol vesicles (2:1, mol/mol), from a Sepharose CL-4B column (2 × 90 cm). The experimental conditions were the same as those given in the legend to Fig. 1. Panel *A*, 0.2/1 vesicle PL/HDL PL weight ratio; panel *B*, 1/1 vesicle PL/HDL PL weight ratio, and panel *C*, 3/1 vesicle PL/HDL PL weight ratio in the equilibration mixtures. The weight ratios of protein to phospholipid (■), and protein to cholesteryl ester (⬓) are shown across the equilibrated HDL elution peak in panel *B*.

choline corresponded to vesicle and to HDL phospholipids; however, it cannot be regarded as a quantitative measure of phospholipid transfer into HDL, because radiolabel transfer and exchange processes are superimposed. Protein elution was only associated with the HDL peaks. Over 95% of the HDL protein applied to the column was recovered in column fractions 42–58 in all cases. For lipid and protein analysis we used the top HDL fractions (no. 50 or 51) plus one fraction on the leading side and two fractions on the trailing side. The results of the analyses, averaged over the four fractions, are presented in **Fig. 3** as concentrations of protein or phospholipid in equilibrated HDL vs. the vesicle PL/HDL PL weight ratio in the incubation mixture. The protein concentration in the HDL fractions remained constant regardless of the proportion of vesicles present in the mixture, indicating that there was no detectable transfer of protein from HDL to the vesicles under the conditions of our experiments. A net increase in the phospholipid content of the HDL was apparent in all the samples. The maximum phospholipid level in HDL was attained at a vesicle PL to HDL PL weight ratio of 1/2. This content of phospholipid remained essentially constant at higher vesicle proportions, suggesting the formation of stable HDL particles saturated with phospholipid.

Subsequent experiments were performed with HDL preparations equilibrated with vesicles in a 1/1 weight ratio of vesicle PL to HDL PL. Incubation times were limited to 6 hr since transfer of lipid appeared complete after 5 hr. The chemical composition of equilibrated HDL (pooled fractions 48–54) relative to the chemical composition of the original HDL (pooled fractions 49–55) is summarized in **Table 1,** together with the percent changes in the components. The contents of protein, cholesteryl ester, and "other" nonpolar lipids remained essentially constant during equilibration. Significant changes occurred in the content of total lipid (30% increase) and in phospholipids (59% increase). The increase in free cholesterol was modest (20%) and variable from preparation to preparation of HDL; some HDL preparations even lost cholesterol to the vesicles. The total mass increase of the HDL upon equilibration with vesicles was 19%. The proportion of excess phospholipid and free cholesterol in equilibrated HDL was very different from

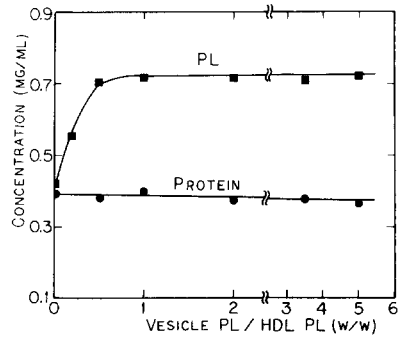

Fig. 3. Changes in the content of protein and phospholipid in HDL equilibrated with vesicles of egg PC:cholesterol (2:1, mol/mol), as a function of the vesicle PL/HDL PL weight ratio. The reaction mixtures were separated as shown in Fig. 2 and protein and phospholipids were determined on fractions 49 through 53.

TABLE 1. Chemical composition of native and equilibrated HDL[a]

Component	HDL		Eq. HDL		Change
	mg/ml	*wt.%*	*mg/ml*	*wt.%*	*%*
Protein	5.42	30	5.14	24	−5.2
Total lipids	12.7	70	16.6	76	+30.4
Phospholipids	5.73	32	9.12	42	+59.1
Cholesteryl esters	5.04	28	5.35	25	+6.1
Cholesterol	0.57	3.1	0.68	3.2	+20.0
Other[b]	1.34	7.4	1.33	6.6	−0.5

[a] The results are the average of two preparations, each determined in duplicate. The experimental errors were of the order of ±5%, and the variability between the two preparations was of the same magnitude (i.e., ±5%).

[b] "Other" lipids are the difference between the total lipids determined by the Folch lipid extraction procedure (16) and the three classes of lipids determined colorimetrically.

the ratio of these two lipids in the original vesicles, suggesting a preferential uptake of PC by HDL.

Since there may be several modes of lipid transfer from vesicles into particles of the size of HDL, we set out to characterize the properties of the isolated, equilibrated HDL preparations relative to the native HDL.

Morphological information on the heterogeneity and size differences of native HDL relative to equilibrated HDL was obtained from negatively stained electron micrographs (**Fig. 4**). Both preparations show fairly uniform spherical particles, frequently packed in hexagonal arrays. The average center-to-center diameter was measured manually on rows of five or more particles. For the native HDL particles, the average diameter was 104 ± 4.0 (SD) Å and for the equilibrated HDL it was 110 ± 3.6 (SD) Å. The 6 Å difference in the diameter of the native and equilibrated HDL is of the order of 1.5 standard deviations for a sample size of 40 and is, therefore, statistically significant. Close inspection of nine electron micrographs, each containing an average of 3300 equilibrated HDL particles, revealed only 0.1% of particles that had the appearance of discs or vesicular structures.

Intrinsic fluorescence spectra obtained for identical protein concentrations of native and equilibrated HDL indicated a considerable decrease in the quantum yield of the protein fluorescence of the latter (39% decrease). The wavelength of maximum fluorescence shifted from 326 nm for the native HDL to 329 nm for the equilibrated preparation. These results are summarized in **Table 2,** together with the results from the fluorescence polarization experiments using DPH

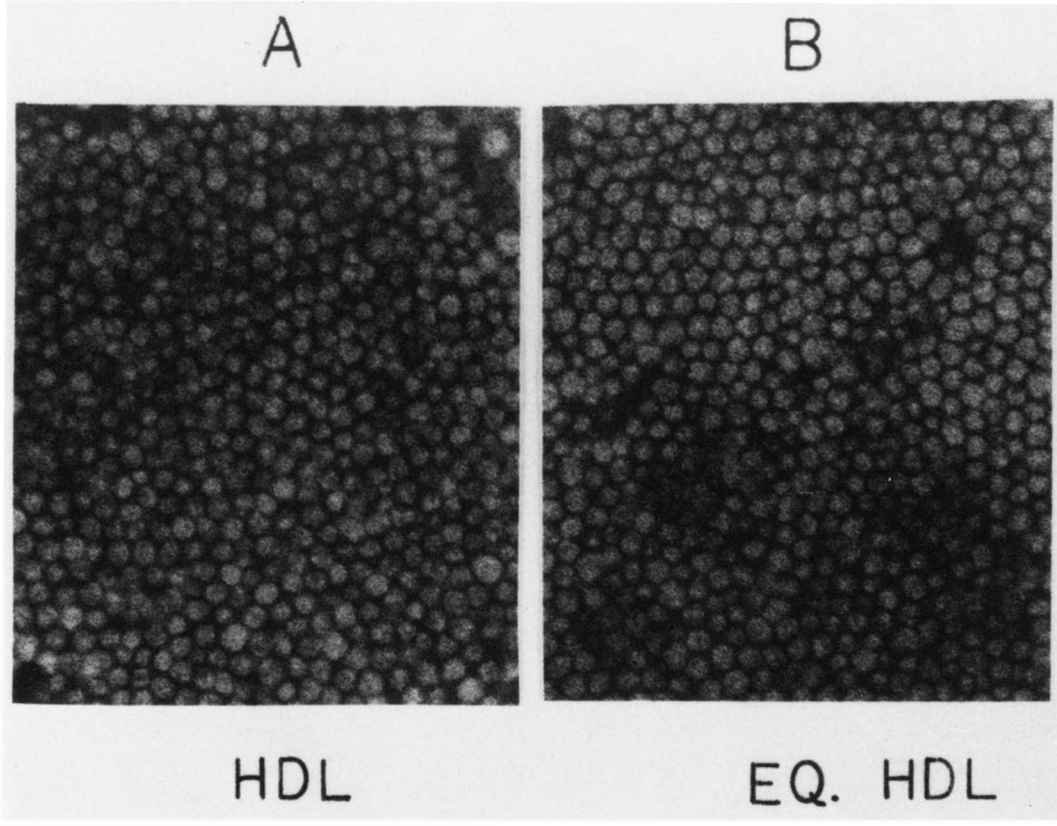

Fig. 4. Electron micrographs of negatively stained native HDL (*A*) and equilibrated HDL (*B*). The total magnification is 300,000-fold and the average center-to-center distances for the particles are 104 ± 4.0 Å for native HDL and 110 ± 3.6 Å for equilibrated HDL.

TABLE 2. Properties of native and equilibrated HDL

Lipoprotein	Diameter Mean ± SD	Intrinsic Protein Fluorescence: Relative Fl.	Intrinsic Protein Fluorescence: Wavelength Mx. Fl.	Fluorescence Polariz. of DPH (25°C): Fl. Lifetime	Fluorescence Polariz. of DPH (25°C): Rotational Relax. Time
	Å	%	*nm*	*nsec*	*nsec*[a]
Native HDL	104 ± 4.0	100	326	8.3	97
Eq. HDL	110 ± 3.6	61	329	8.4	66

[a] The rotational relaxation time of DPH in the lipid environment was calculated from fluorescence polarization and lifetime data according to Perrin's equation, as described in previous work (20–24). The rotational relaxation time is inversely related to the fluidity of the probe environment.

to probe the lipid domains of HDL. Fluorescence polarization of DPH as a function of temperature in native HDL and equilibrated HDL is shown in **Fig. 5.** Addition of vesicle lipids to HDL increases the mobility of the DPH probe considerably, indicating a more fluid lipid environment for the probe. In the temperature range from 5–50°C there are no lipid phase transitions as reported by this fluorescence polarization method.

DISCUSSION

The results presented in this work indicate that bovine HDL preparations containing on the average 30% protein and 70% lipid can take up lipids from vesicles of egg PC and cholesterol (2:1 mol/mol) to give HDL particles with a content of 24% protein and 76% lipid by weight. The amounts of protein, cholesteryl esters, and "other" nonpolar lipids remain essentially constant in the HDL particles, whereas the content of phospholipids increases markedly (59% increase). Free cholesterol increased to a modest extent; but in a few HDL preparations not reported in this work, it actually was lost.[1] In any case, the incorporation of PC and cholesterol into HDL does not follow the proportions of these two lipids in the original vesicles; PC is incorporated preferentially. At this point we do not have an explanation for this experimental observation.

Recently Tall and Small (7) and Tall et al. (8) described the interaction of human HDL with dimyristoylphosphatidylcholine liposomes. These authors interpreted their results in terms of the formation of two types of particles: disc-like particles containing apolipoprotein A-I dissociated from HDL plus the synthetic phospholipid, and fused particles from the remaining components of HDL. Nichols et al. (26) investigated the interaction of sonicated vesicles of dimyristoylphosphatidylcholine with a subfraction of human HDL, HDL_{2b}. They also detected disc-like particle formation but attributed the spherical particles to dimyristoylphosphatidylcholine uptake by HDL_{2b}.

Similar experiments by Scherphof et al. (27) and by Chobanian, Tall, and Brecher (28) using mammalian plasma or HDL fractions in conjunction with PC liposomes or vesicles also indicated the incorporation of phospholipid into HDL-like particles; however, these authors did not investigate the morphology nor the composition of the resulting particles. The mechanism of lipid incorporation from mixed vesicles of egg PC and cholesterol into bovine HDL particles, described here, appears to be quite different from that proposed by Tall et al. (8).

Gel-filtration of the equilibrated HDL indicates a slight increase in Stokes radius relative to native HDL, and the narrowed elution peak suggests a similar, if not decreased, heterogeneity in particle sizes (see Fig. 1). Chemical composition across the elution peaks (Figs. 1 and 2) indicates a slight increase in protein content relative to lipid towards the trailing edge; however, the degree of heterogeneity suggested by the chemical composition of the peak fractions is very similar for native HDL and for equilibrated HDL. Furthermore, the added PC is uniformly distributed across the elution peak of equilibrated HDL, sug-

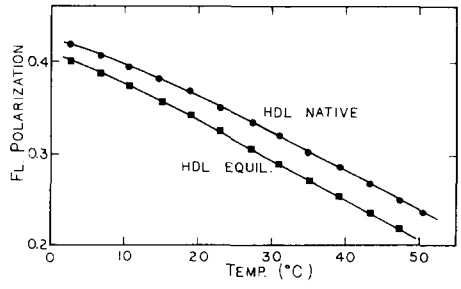

Fig. 5. Fluorescence polarization of DPH dissolved in the lipid domains of native HDL (● —— ●) and equilibrated HDL (■ —— ■) as a function of temperature. At 25°C the equilibrated HDL lipids are about 1.5 times more fluid than the native HDL lipids in terms of the rotational relaxation time of the fluorescence probe (see Table 2).

[1] Jonas, A. Unpublished results, 1978.

gesting the absence of an overlapping new class of particles. The electron micrographs of the equilibrated HDL show spherical, relatively uniform particles with an average center-to-center distance of 110 ± 3.6 Å, whereas the native particles are only 104 ± 4.0 Å (see Fig. 4). Based on these dimensions, the increase in volume from native HDL to the equilibrated HDL is 18%. Assuming that only egg PC contributes to the increase in mass of the particles ($\bar{v}$ = 0.988 ml/g) (13), the mass increase calculated from the observed volume increase is 18.2%, in very good agreement with the chemically determined value of 19% (see Table 1). Evidently phospholipids may be incorporated into HDL-like particles by at least two different mechanisms—the one described by Tall et al. (8) and the one observed in this work. The lipid transfer from vesicles into HDL that is reported here appears to be an uptake of vesicle lipid into preexisting HDL particles without a significant loss of lipoprotein components and without major changes in the morphology of the HDL. It should be kept in mind, however, that different experimental conditions can probably determine the different mechanisms of lipid transfer. Bovine HDL may not dissociate apolipoprotein A-I as readily as the human lipoprotein. Since the self-association of bovine apolipoprotein A-I is much stronger than that of the human apolipoprotein A-I in aqueous solution (29, 30), it is possible that the binding to the native particle is also stronger for bovine A-I. In addition, the formation of disc-like complexes from apolipoprotein A-I with phospholipids depends critically on the physical properties of the phospholipid dispersions (31) and on the presence of different levels of cholesterol (32). We demonstrated recently that disc-like complexes form readily from bovine apolipoprotein A-I and vesicles of dimyristoylphosphatidylcholine containing up to 32 mol% cholesterol, but that no interaction occurs at 37 mol% or higher cholesterol levels (32). The type of interaction is apparently dictated by the relative affinity of apolipoproteins for the lipid dispersions and for the lipoprotein particles, and by the ability of the apolipoproteins once associated with phospholipid dispersions to break down these structures into disc-like particles. Since we did not observe stable complexes of vesicles with protein or significant amounts of free protein, it appears that the apolipoprotein has a higher affinity for the lipoprotein in our system.

The addition of an average of 101 PC molecules and 8 cholesterol molecules per lipoprotein of an average molecular weight of 380,000 (12) contributes primarily to the surface of the particles. Since the core components do not change during PC and cholesterol addition, and presumably the surface components of the native HDL are closely packed, the increase in particle volume due to the added lipid is probably accomplished by moving the HDL phospholipids outward by about 3 Å. This may not be a major problem because the boundary between the phospholipid acyl chains and the core lipids in HDL is probably not well defined. The internal volume freed by the phospholipids that slide out may be compensated by the acyl chains of added PC and by cholesterol. The average increase in surface per lipoprotein is 4030 Å², sufficient to accommodate only about 65 close-packed PC molecules (assuming 62.7 Å²/phospholipid molecule) (33); therefore, some rearrangement of other surface components may be necessary. The intrinsic fluorescence spectrum of the equilibrated HDL does, in fact, indicate a structural or environmental change in the apolipoprotein components leading to a marked quenching and to a small red shift in the fluorescence emission. The red shift probably indicates a slight increase in the polarity of the tryptophanyl residue environments. Recently we showed that bovine, as well as human, HDL can take up significant amounts of free cholesterol (over 100 molecules) without major changes in their structures (34). Together with the results presented here, this suggests a significant flexibility in HDL structure to accommodate added PC and cholesterol.

The lipid domains of equilibrated HDL are significantly affected by the added lipids as indicated by the decreased fluorescence polarization of DPH (Fig. 5). The increased fluidity of the lipid domains of the lipoprotein must be due, in part, to the increased proportion of PC to cholesterol in the equilibrated particles. It has been shown that added cholesterol progressively decreases the fluidity of PC bilayers (20) and of human HDL (34). If the changes in protein structure involve some displacement of protein from contact with lipid, as the red fluorescence shift would suggest, then the increased fluidity of the lipids may be due, in addition, to the removal of some restrictions imposed by the protein components on the mobility of the lipids (24).

A. Jonas is an Established Investigator of the American Heart Association. Thanks are due to Ms. Susan Drengler for excellent technical assistance and to Mr. Carl Hall for the outstanding electron micrographs. This work was supported by grants from the National Institutes of Health (HL 16059) and the Illinois Heart Association (C-5).

Manuscript received 22 November 1978; accepted 10 April 1979.

REFERENCES

1. Glomset, J. A. 1972. Plasma lecithin:cholesterol acyltransferase. *In* Blood Lipids and Lipoproteins. G. J. Nelson, editor. Wiley-Interscience, New York, NY. 745–787.

2. Stein, Y., M. C. Glangeaud, M. Fainaru, and O. Stein. 1975. The removal of cholesterol from aortic smooth muscle cells in culture and Landschutz ascites cells by fractions of human high-density apolipoprotein. *Biochim. Biophys. Acta.* **380:** 106–118.
3. Jackson, R. L., A. M. Gotto, O. Stein, and Y. Stein. 1975. A comparative study on the removal of cellular lipids from Landschutz ascites cells by human plasma apolipoproteins. *J. Biol. Chem.* **250:** 7204–7209.
4. Slutzky, G. M., S. Razin, I. Kahane, and S. Eisenberg. 1977. Cholesterol transfer from serum lipoproteins to mycoplasma membranes. *Biochemistry.* **16:** 5158–5163.
5. Bell, F. P. 1976. Lipoprotein lipid exchange in biological systems. *In* Low Density Lipoproteins. C. E. Day and R. S. Levy, editors. Plenum Press, New York, NY. 111–133.
6. Lange, Y., and J. S. D'Alessandro. 1977. Characterization of mechanisms for transfer of cholesterol between human erythrocytes and plasma. *Biochemistry.* **16:** 4339–4343.
7. Tall, A. R., and D. M. Small. 1977. Solubilization of phospholipid membranes by human plasma high density lipoproteins. *Nature.* **265:** 163–164.
8. Tall, A. R., V. Hogan, L. Askinazi, and D. M. Small. 1978. Interaction of plasma high density lipoproteins with dimyristoyllecithin multilamellar liposomes. *Biochemistry.* **17:** 322–326.
9. Blanchette-Mackie, E. J., and R. O. Scow. 1976. Retention of lipolytic products in chylomicrons incubated with lipoprotein lipase: electron microscopic study. *J. Lipid Res.* **17:** 57–67.
10. Patsch, J. R., A. M. Gotto, T. Olivecrona, and S. Eisenberg. 1978. Formation of high-density lipoprotein-2-like particles during lipolysis of very low-density lipoproteins in vitro. *Proc. Nat. Acad. Sci. USA* **75:** 4519–4523.
11. Rouser, G., G. Kritchevsky, D. Heller, and E. Lieber. 1963. Lipid composition of beef brain, beef liver and the sea anemone. *J. Am. Oil Chem. Soc.* **40:** 425–454.
12. Jonas, A. 1972. Physicochemical properties of bovine serum high density lipoprotein. *J. Biol. Chem.* **247:** 7767–7772.
13. Huang, C. 1969. Studies on phosphatidylcholine vesicles. Formation and physical characteristics. *Biochemistry.* **8:** 344–352.
14. Newman, G. C., and C. Huang. 1975. Structural studies on phosphatidylcholine–cholesterol mixed vesicles. *Biochemistry.* **14:** 3363–3370.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
16. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226:** 497–509.
17. Chen, P. S., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28:** 1756–1758.
18. Sperry, W. M., and M. Webb. 1950. A revision of the Schoenheimer–Sperry method for cholesterol determination. *J. Biol. Chem.* **187:** 97–106.
19. Anderson, D. W., A. V. Nichols, T. M. Forte, and F. T. Lindgren. 1977. Particle distribution of human serum high density lipoproteins. *Biochim. Biophys. Acta.* **493:** 55–68.
20. Cogan, U., M. Shinitzky, G. Weber, and T. Nishida. 1973. Microviscosity and order in the hydrocarbon region of phospholipid and phospholipid–cholesterol dispersions determined by fluorescence probes. *Biochemistry.* **12:** 521–528.
21. Shinitzky, M., and Y. Barenholz. 1974. Dynamics of the hydrocarbon layer in liposomes of lecithin and sphingomyelin containing dicetylphosphate. *J. Biol. Chem.* **249:** 2652–2657.
22. Shinitzky, M., and M. Inbar. 1976. Microviscosity parameters and protein mobility in biological membranes. *Biochim. Biophys. Acta.* **433:** 133–149.
23. Jonas, A., and R. Jung. 1975. Fluidity of the lipid phase of bovine serum high density lipoprotein from fluorescence polarization measurements. *Biochem. Biophys. Res. Commun.* **66:** 651–657.
24. Jonas, A. 1977. Microviscosity of lipid domains in human serum lipoproteins. *Biochim. Biophys. Acta.* **486:** 10–22.
25. Spencer, R. D., and G. Weber. 1969. Measurements of subnanosecond fluorescence lifetimes with a cross-correlation phase fluorometer. *Ann. N.Y. Acad. Sci.* **158:** 361–376.
26. Nichols, A. V., E. L. Gong, T. M. Forte, and P. J. Blanche. 1978. Interaction of plasma high density lipoprotein HDL_{2b} (d 1.063–1.100 g/ml) with single-bilayer liposomes of dimyristoylphosphatidylcholine. *Lipids.* **13:** 943–950.
27. Scherphof, G., F. Roerdink, M. Waite, and J. Parks. 1978. Disintegration of phosphatidylcholine liposomes in plasma as a result of interaction with high-density lipoproteins. *Biochim. Biophys. Acta.* **542:** 296–307.
28. Chobanian, J. V., A. R. Tall, and P. I. Brecher. 1979. Interaction between unilamellar egg yolk lecithin vesicles and human high density lipoprotein. *Biochemistry.* **18:** 180–187.
29. Jonas, A. 1975. Self-association of the major protein component of bovine serum high density lipoprotein. *Biochim. Biophys. Acta.* **393:** 471–482.
30. Vitello, L. B., and A. M. Scanu. 1976. Studies on human serum high density lipoproteins. Self-association of apolipoprotein A-I in aqueous solutions. *J. Biol. Chem.* **251:** 1131–1136.
31. Jonas, A., D. Krajnovich, and B. Patterson. 1977. Physical properties of isolated complexes of human and bovine A-I apolipoproteins with L-α-dimyristoylphosphatidylcholine. *J. Biol. Chem.* **252:** 2200–2205.
32. Jonas, A., and D. Krajnovich. 1978. Effect of cholesterol on the formation of micellar complexes between bovine A-I apolipoprotein and L-α-dimyristoylphosphatidylcholine. *J. Biol. Chem.* **253:** 5758–5763.
33. Shen, B. W., A. M. Scanu, and F. J. Kezdy. 1977. Structure of human serum lipoproteins inferred from compositional analysis. *Proc. Natl. Acad. Sci. USA.* **74:** 837–841.
34. Jonas, A., L. K. Hesterberg, and S. M. Drengler. 1978. Incorporation of excess cholesterol by high density serum lipoproteins. *Biochim. Biophys. Acta.* **528:** 47–57.