Characterization of guinea pig plasma lipoproteins: the appearance of new lipoproteins in response to dietary cholesterol

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Abstract Dietary cholesterol induces a hemolytic anemia in guinea pigs, accompanied by changes in the lipid composition of red cells and of plasma lipoproteins. This report presents a characterization of the lipoprotein species present in each main density class in both control and cholesterol-fed guinea pigs. Traces of a typical high density lipoprotein (HDL) were detected in control plasma. HDL from cholesterol-fed, anemic guinea pigs differed from control HDL in electron microscopic appearance and lipid and peptide composition. Long stacks of discs were observed in the electron microscope in addition to smaller, spherical particles characteristic of control HDL.

Low density lipoproteins (LDL) from cholesterol-fed, anemic guinea pigs had two main populations, which were separated by gel chromatography. One population appeared in the electron microscope as large transparent discs and contained mainly unesterified cholesterol and phospholipids in a 2:1 molar ratio. The other population resembled control LDL in size and composition except for its high unesterified cholesterol content. Dietary cholesterol also altered the composition and decreased the electrophoretic mobility of very low density lipoproteins. Gel electrophoretic and immunochemical evidence indicates that a peptide (mol wt 35,000) appears in lipoproteins from cholesterol-fed, anemic guinea pigs that is undetectable in those of controls.

Similarities between the cholesterol-induced lipoprotein abnormalities in guinea pigs and those reported in patients with obstructive jaundice, biliary cirrhosis, type III hyperlipoproteinemia, or familial lecithin:cholesterol acyltransferase deficiency are discussed.

Supplementary key words hemolytic anemia · electron microscopy · plasma lipids · hyperlipoproteinemia in humans

When guinea pigs are fed a diet containing 1% cholesterol, the plasma, red-cell, and liver cholesterol levels are elevated significantly above normal within 1 wk; after 10–12 wk on the diet, tissue lipids and cholesterol are increased to several times the normal levels, and a fatal hemolytic anemic usually develops (1, 2). About 20% of any group of guinea pigs fed the cholesterol-containing diet show smaller increases in tissue lipids and do not develop the anemia during this time interval.[1] We have shown in a previous paper (3) that cholesterol-fed, anemic guinea pigs have significant amounts of a high density lipoprotein (chol HDL) that was undetectable in plasma from control animals by the methods used. This HDL has a broad bimodal distribution of flotation rates in the analytical ultracentrifuge that is quite different from that of normal mammalian HDL.

We also reported changes in the composition and ultracentrifugal profile of LDL caused by dietary cholesterol (3). These changes suggested that LDL from cholesterol-fed, anemic guinea pigs (chol LDL) might contain several lipoprotein species similar to those recently dis-

Abbreviations: VLDL, plasma very low density lipoprotein, d < 1.006 g/ml; LDL, plasma low density lipoprotein, d 1.006–1.063 g/ml; HDL, plasma high density lipoprotein, d 1.063–1.21 g/ml; chol (HDL, LDL, VLDL) designates the lipoproteins defined above that were isolated from plasma of cholesterol-fed, anemic (chol) guinea pigs (chol plasma); resistant (HDL, LDL, VLDL) designates the lipoproteins defined above that were isolated from plasma of cholesterol-fed guinea pigs that did not become anemic after 17 wk of cholesterol feeding (resistant plasma); control (HDL, LDL, VLDL) designates the lipoproteins defined above that were isolated from plasma of guinea pigs fed the control diet (control plasma); SDS, sodium dodecyl sulfate; UC, unesterified cholesterol; CE, cholesteryl esters; TG, triglycerides; PL, phospholipids; LP, lipoproteins; anti-chol LP, rabbit antiserum against total lipoprotein fraction d < 1.21 g/ml from cholesterol-fed, anemic guinea pigs; anti-control LP, rabbit antiserum against total lipoprotein fraction d < 1.21 g/ml from control guinea pigs.

[1] Ostwald, R. Unpublished observations.

covered in human patients with obstructive jaundice, biliary cirrhosis, or familial plasma lecithin:cholesterol acyltransferase (LCAT) deficiency (4–8).

We are now reporting the results of further studies of the plasma lipoproteins of control guinea pigs, chol guinea pigs, and cholesterol-fed, nonanemic (resistant) guinea pigs. These studies were designed to answer the following questions. First, does the HDL of chol guinea pigs represent only an increase in an HDL present in trace amounts in control guinea pig plasma or does some or all of it appear de novo with cholesterol feeding? Second, is chol LDL heterogeneous; if so, what are the characteristics of each population and how are they related to those of the abnormal LDL in the human pathologies mentioned above? The results indicate the presence of traces of a typical mammalian HDL in control guinea pig plasma and the appearance of new lipoprotein species in response to dietary cholesterol.

METHODS

Animals

Young male albino guinea pigs (200–250 g, Simonsen, Gilroy, Calif.) were fed a semisynthetic diet with or without the addition of 1% cholesterol (9). They were autopsied when the red blood cell count of the cholesterol-fed guinea pigs dropped below 3.5×10^6 cells/mm^3, usually after 10–12 wk on the diet. The animals were fasted 15–17 hr and anesthetized with sodium pentobarbital (Diabutal, Diamond Laboratories, Inc., Des Moines, Iowa). The blood was taken by open chest heart puncture in the presence of EDTA (0.5 ml of 4% EDTA, disodium salt, per 20 ml of blood). Plasmas were isolated by centrifugation at 1300 *g* for 15 min at 4°C and were recentrifuged at 1500 *g*. Lipoprotein fractionation was started within 6 hr of blood collection.

Preparation of lipoproteins

Lipoproteins were isolated by standard flotation techniques (10). Plasma and lipoprotein fractions were adjusted to desired densities by the addition of NaCl–NaBr solutions containing 0.01% EDTA and were centrifuged at 40,000 rpm for 16–24 hr at 16°C (Beckman model L2-65B ultracentrifuge with type 40.3 rotor).

Fractionation into six density classes. We fractionated plasma into the following six density classes: VLDL, d < 1.006 g/ml; LDL, d 1.006–1.019 and 1.019–1.063 g/ml; HDL, d 1.063–1.090 and 1.090–1.21 g/ml; and infranate, d > 1.21 g/ml. HDL subfractions were refloated in salt solutions of densities of 1.090 and 1.21 g/ml, respectively.

Preparation of VLDL, LDL, and HDL for analytical purposes. Chylomicrons were removed by centrifugation at 12,000 *g* for 20 min at 4°C (Sorvall RC2-B centrifuge with SS-34 rotor) (11). VLDL was isolated at d 1.010 g/ml and refloated at d 1.006 g/ml. LDL was isolated at d 1.070 g/ml and refloated at d 1.050 g/ml (LDL of d 1.010–1.050 g/ml). HDL was isolated at d 1.21 g/ml and was sedimented at d 1.070 g/ml and refloated at d 1.21 g/ml for further purification (HDL of d 1.070–1.21 g/ml). Samples were stored under nitrogen in their salt solutions, at 4°C, and used within 2 months; gel filtration, lipid extractions, and agarose electrophoresis were always completed within the first month.

Gel filtration of lipoproteins

HDL and LDL (0.5 ml in their salt solutions) were layered on a 2% agarose gel column (Bio-Gel A 50m, 100–200 mesh, Bio-Rad Labs) (12). Column dimensions were 0.9 × 100 cm; bed height, 85 cm. The eluant was 0.2 M NaCl containing 0.01 M EDTA; flow rate was 5 ml/hr/cm^2 with a pressure head of 25 cm. The effluent was monitored for absorbance at 280 nm (ISCO model UA-2 UV analyzer), and 1.5-ml fractions were collected. Liproprotein fractions were concentrated by vacuum dialysis in 0.25-inch dialysis tubing (Will Scientific Inc.). Filtration and concentration were done at room temperature.

In addition to the above, HDL was also filtered through the following two columns: *1*) Sephadex G-200 (Pharmacia Fine Chemicals), 1 × 40 cm, eluted with pH 7.4 Tris buffer (13); and *2*) 8% agarose (Bio-Gel A 1.5m, 200–400 mesh, Bio-Rad Labs), 0.9 × 60 cm, eluted with 0.2 M NaCl containing 0.01 M EDTA.

Chemical analysis

Lipids of whole plasma and of isolated lipoproteins were extracted with chloroform–methanol (14), and the total lipid was determined gravimetrically. Lipids were separated into UC, CE, TG, and PL on thin-layer plates of washed silica gel H (Kensington Scientific Corp., Oakland, Calif.), developed with petroleum ether–ethyl ether–acetic acid 90:10:1 (15). Lipid bands were visible in UV light after the plates were sprayed with 0.4% dichlorofluorescein in methanol. UC and CE were eluted with hot methanol and were quantitated, after digitonide precipitation (16), by the method of Zlatkis, Zak, and Boyle (17). PL was eluted (9) and quantitated according to Bartlett (18); the factor 25 was used to convert phosphorus into phospholipid. In some cases phospholipid classes were separated by thin-layer chromatography before quantitation (19). TG was eluted, transmethylated, and quantitated by gas–liquid chromatography of the fatty acid methyl esters (Wilkens Aerograph 200) (9). Protein was determined by a modified procedure of Lowry et al. (20), with bovine serum albumin (BSA, Armour Pharmaceutical Co., Fr. V) used as a standard. A factor of 0.8 was used to convert BSA

protein into lipoprotein protein on the basis of Nessler nitrogen analyses of BSA, LDL, and HDL (21).

Electron microscopy

The lipoprotein fractions were dialyzed against pH 7.2 ammonium acetate buffer (ammonium acetate, 9.31 g/l; ammonium carbonate, 0.23 g/l; EDTA, 5 mg/l). Samples were prepared by a negative staining technique with a final concentration of 1% sodium phosphotungstate (6). Samples to be examined after shadowing were fixed in 1% buffered osmium tetroxide before shadow casting with platinum–carbon at a 30° angle (22). All samples were examined in a Philips EM 300 electron microscope at magnifications of 35,000 and 90,000.

Electrophoresis

Lipoprotein samples (5–30 times their plasma concentration) were dialyzed against either isotonic saline or acetate buffer before electrophoresis. Ether-extracted samples were extracted 5–6 times with about 4 vol of freshly redistilled ether.

Agarose gel electrophoresis. Lipoprotein samples were electrophoresed in either 0.6% or 1.2% agarose gels (Bio-Rad Labs) with or without 1% BSA in 0.05 M barbital buffer at pH 8.6 (23). Gels were stained either for lipid, with oil red O (Beckman), or for protein with amido black (C.I. 20470; Matheson Coleman & Bell).

Starch block electrophoresis was performed by the method of Quarfordt, Levy, and Fredrickson (24).

Polyacrylamide gel electrophoresis in the presence of SDS. Lipoprotein samples and standard proteins of known molecular weight were incubated overnight at 37°C in solutions containing 1% SDS (Eastman, recrystallized from absolute ethanol) and 1% mercaptoethanol (Matheson Coleman & Bell). 10% polyacrylamide gels with 0.1% SDS and 0.62% methylenebisacrylamide (Bio-Rad Labs) were prepared according to Weber and Osborn (25). Electrophoresis was conducted at 8 ma/tube for 4 hr in 0.1 M phosphate buffer, pH 7.2, containing 0.1% SDS. Gels were stained for protein with either 0.5% amido black in 7% acetic acid or alcoholic amido black (1% amido black in methanol–20% acetic acid 1:1). From the proteins of known molecular weight, a calibration curve was obtained for each run by plotting log molecular weight vs. distance of migration of each protein (25).

Polyacrylamide gel electrophoresis in the presence of Triton X-100. Lipoprotein samples were preincubated 2–3 hr in 1% Triton X-100 (polyoxyethyleneoctylphenol, Rohm and Haas) and electrophoresed in 7.5% acrylamide gels containing 0.5% Triton X-100 for 1.5 hr at 2.5 ma/tube (26, 27). Gels were fixed for 1 hr in 12.5% mercuric chloride in methanol–20% acetic acid 1:1 and stained for protein with 0.5% amido black in 7% acetic acid.

Immunological methods

Antigens against lipoproteins of both control and chol guinea pigs were produced in one rabbit for each group. Rabbits were injected subcutaneously, in the back, 3 times at 3–5-wk intervals with a total lipoprotein fraction ($d < 1.21$ g/ml) isolated from one to three guinea pigs. 1–4 mg of protein in complete Freund's adjuvant (Difco) was injected each time; 7–10 days after each injection, blood was obtained from the ear of the rabbit, and the plasma was frozen. Immunodiffusion and immunoelectrophoresis in 1% agarose gels were carried out by standard procedures (28, 29), and samples were stained for protein with amido black. Plasmas or lipoprotein fractions from about 20 guinea pigs were reacted against these two antisera.

RESULTS

Chemical composition of guinea pig lipoproteins

Fig. 1 shows the amounts and chemical compositions of plasma lipoprotein classes from three control and five chol guinea pigs. Recovery of the chloroform–methanol-extractable material in the five lipoprotein fractions plus the infranate accounted for 90% of the total extractable material in whole plasma.

VLDL. In fasted guinea pigs, control VLDL carried 30% of the total lipoprotein lipid, while chol VLDL carried only 4%. Absolute amounts of VLDL were quite variable among both control guinea pigs (27.8 ± 14.5 mg LP/100 ml plasma[2]) and chol guinea pigs (15.4 ± 6.4 mg LP/100 ml plasma). Dietary cholesterol introduced significant changes in the composition of VLDL (Fig. 1). The percentage of TG decreased significantly, while percentages of UC and CE increased ($P < 0.01$). In terms of moles, most of the cholesterol present in control VLDL was unesterified, while chol VLDL contained equimolar amounts of CE and UC. The large increase of CE in chol VLDL compensated for the decrease in TG, so that the sum of the least polar components (CE + TG) was equivalent to that found in control VLDL—about 70% of the lipoprotein, by weight.

LDL. In control guinea pig plasma there were only traces of lipoproteins with hydrated densities between 1.006 and 1.019 g/ml, while in chol plasma the concentration of lipoproteins in that density interval was 18.1 ± 8.0 mg LP/100 ml plasma.

Most guinea pig LDL had hydrated densities in the interval d 1.019–1.063 g/ml. In control guinea pigs this LDL carried 58% of the total lipoprotein lipid and in

[2] Data are means ± SD from analysis of five to seven guinea pigs, except control LDL, which are data from three guinea pigs.

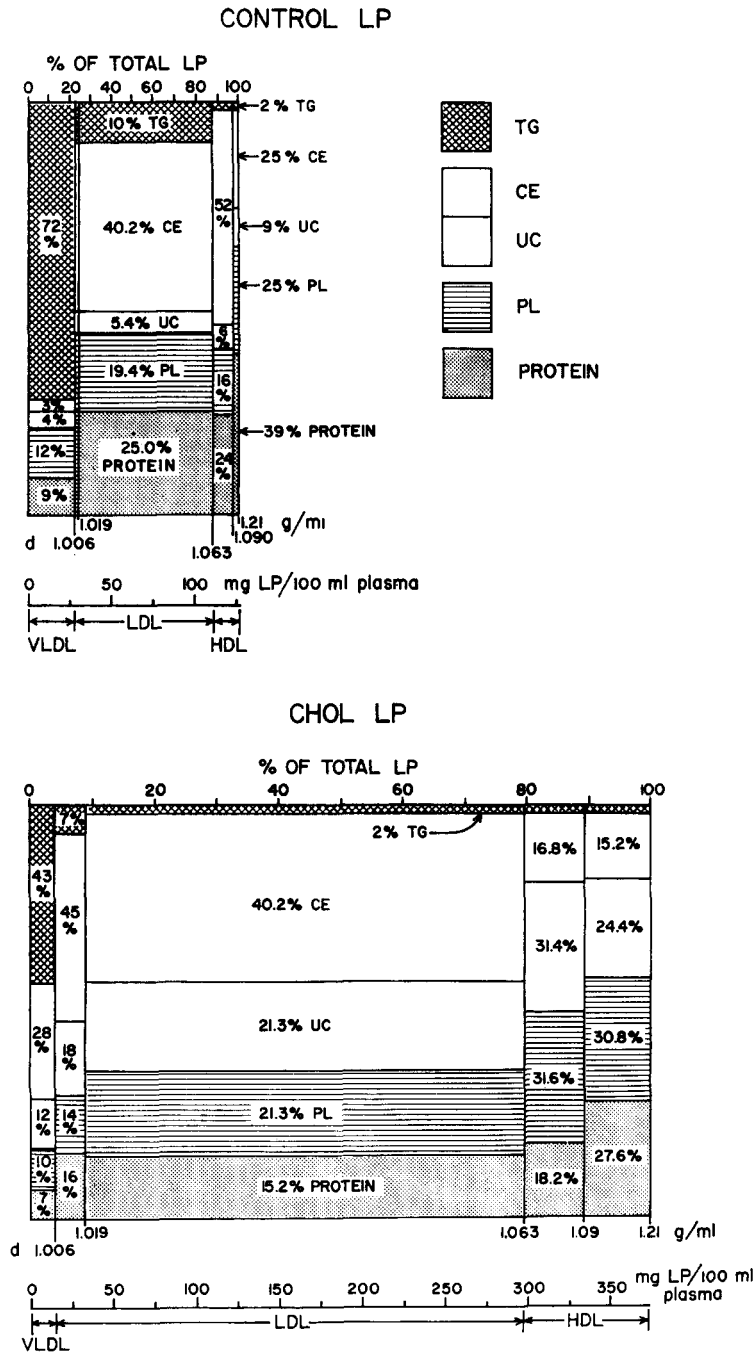

FIG. 1. Chemical compositions of five plasma lipoprotein classes of control and chol guinea pigs: VLDL d < 1.006 g/ml; LDL d 1.006–1.019 g/ml and d 1.019–1.063 g/ml; HDL d 1.063–1.090 g/ml and d 1.090–1.21 g/ml. Data are means of analyses of five chol and three control guinea pigs. Column width shows the LP concentration (mg LP/100 ml plasma) in each density fraction. (Total control LP = 137.8 ± 26.8 mg/100 ml; total chol LP = 372.2 ± 14.2 mg/100 ml.) Numbers in areas indicate wt % composition of each lipoprotein. (Standard deviations of the data were less than 15% of the means for chol HDL and chol LDL constituents and were 3–30% of the means for VLDL and control LDL constituents.) % TG for HDL and LDL are data from earlier experiments (3). Mg CE = 1.67 × mg cholesterol. To convert percentages of UC, CE, and PL to mole ratios: moles CE/moles UC = 0.6 × %CE/%UC; moles UC/moles PL = 2 × %UC/%PL. Recovery of lipids averaged 80–90% of total lipid; data were corrected to 100% recovery of lipid.

chol plasma, 72%. Three times as much LDL was present in chol plasma (265 ± 31 mg LP/100 ml plasma[2]) as in control plasma (83 ± 31 mg LP/100 ml plasma). There was, however, only about twice as much protein in chol LDL as in control LDL. The resulting decrease in the percentage of protein ($P < 0.05$) and the large increase in UC ($P < 0.005$) suggested that chol LDL might be heterogeneous. We therefore subfractionated LDL by gel filtration (see below).

HDL. Very low levels of HDL d 1.063–1.21 g/ml were present in control guinea pig plasma. The density fraction d 1.063–1.090 g/ml had a composition similar to that of LDL and a concentration of 12.5 mg LP/100 ml plasma. The HDL subfraction d 1.090–1.21 g/ml was present only in trace amounts (2.3 mg LP/100 ml plasma); its composition was similar to that of human and rat HDL (30, 31).

A 5–20-fold increase in the concentration of HDL occurred with cholesterol feeding and the resulting anemia. The concentrations of the two chol HDL subfractions were similar: chol HDL d 1.063–1.090 g/ml = 36.7 ± 7.3 mg LP/100 ml plasma;[2] chol HDL d 1.090–1.21 g/ml = 39.5 ± 8.3 mg LP/100 ml plasma. Both chol HDL subfractions had an unusually high UC content. Chol HDL d 1.063–1.090 g/ml had significantly less protein ($P < 0.005$) and more UC ($P < 0.05$) than chol HDL d 1.090–1.21 g/ml. The concentration of circulating HDL and the ratio of UC to protein in HDL were roughly proportional to the severity of the anemia (Fig. 2).

Infranate, d > 1.21 g/ml. The d > 1.21 g/ml infranate from chol plasma yielded 90 mg of chloroform–methanol-extractable material per 100 ml of plasma, slightly more than was found in control plasma infranate (75 mg/100 ml plasma). Free fatty acids and lysolecithin were the main lipids detected in these fractions, while only traces of UC, CE, and TG were present.

In addition to these lipids, there was much pigment in fresh infranates. We estimated that unstable pigments such as heme and bilirubin accounted for roughly two-thirds of the chloroform–methanol-extractable material in fresh infranates, because infranates which were extracted after 2 months of storage had only one-third as much chloroform–methanol-extractable material as fresh infranates and showed much less brown color.

Lipoproteins of resistant guinea pigs. Although about 80% of the guinea pigs became anemic after 10–12 wk on the cholesterol-containing diet, the remaining guinea pigs became anemic either much later or not at all. We isolated the lipoproteins from one such group of animals which had been on the cholesterol-containing diet for 17 wk. Of the four guinea pigs studied, two were not

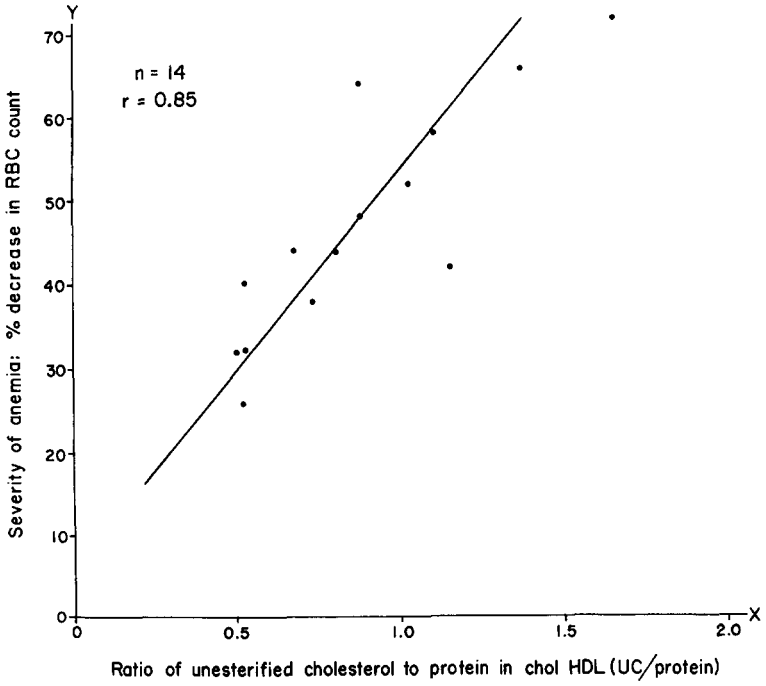

FIG. 2. Correlation between the severity of anemia and the ratio of unesterified cholesterol to protein in chol HDL. The severity of the anemia resulting from dietary cholesterol is expressed as the % decrease in red blood cell (RBC) count relative to control guinea pig RBC count; % decrease = (control guinea pig RBC count − chol guinea pig RBC count)/(control guinea pig RBC count) × 100. Control guinea pig RBC count was taken to be 5×10^6 cells/mm^3. The correlation ($r = 0.85$) was obtained by a linear regression analysis of the data (linear regression equation $y = 47.6x + 5.7$).

anemic (resistant guinea pigs) and two were slightly anemic, with blood counts of 3.1 and 3.4 × 10^6 cells/mm^3 (semiresistant guinea pigs). Lipoproteins of three control guinea pigs of the same age were analyzed at the same time; their compositions were very close to those given in Fig. 1 for control guinea pigs. Because of the small number of animals available in the resistant and semiresistant groups, it was not possible to determine mean lipoprotein compositions. However, some interesting trends were observed. Resistant VLDL had more CE (50%) and less TG (15%) than chol VLDL (Fig. 1), while the VLDL of the semiresistant guinea pigs had an intermediate composition (30–35% each of TG and CE). Resistant LDL had less UC (16%) and more CE (49%) than chol LDL (Fig. 1); the LDL of the semiresistant guinea pigs had approximately the same composition as chol LDL. Resistant HDL was present at a lower concentration than chol HDL but had a similar composition.

Appearance of guinea pig lipoproteins in the electron microscope

VLDL. Shadowed preparations revealed the presence of heterogeneous populations of large spherical particles 400–1300 Å in diameter in both control and chol VLDL samples (Fig 3, *A* and *B*). Some VLDL particles were also observed in the chol LDL fraction d 1.006–1.019 g/ml.

LDL. In negatively stained preparations (Fig. 3, *C* and *E*), control LDL d 1.019–1.063 g/ml appeared individually as spherical particles of homogeneous size (259 ± 27 Å)[3] and were only slightly deformed when in contact with other LDL. They showed no obvious substructure and formed linear or polygonal aggregates resembling those observed in human LDL preparations (6).

The appearance of LDL from chol guinea pigs was very different. Two populations of particles were present—particles similar in size to control LDL and large translucent discs. The smaller LDL (290 ± 37 Å)[3] appeared, however, as a fairly heterogeneous population of round or square particles often seen to aggregate in strings or in square arrays (Fig. 3*F*). The angular aspect of some isolated or aggregated LDL particles, although variable from sample to sample, was probably not an artifact of negative staining, since shadowed preparations also emphasized the square shape of some LDL particles (Fig. 3*G*). The large translucent discs had diameters of 800–1100 Å (957 ± 132 Å) (Fig. 3, *F* and *H*). Their appearance in negatively stained preparations resembled the structures observed in the LDL fraction of human patients with obstructive jaundice (4, 7) or with familial lecithin:cholesterol acyltransferase (LCAT) deficiency (6). The discs of LCAT-deficient patients are also about 1000 Å in diameter, while discs in obstructive jaundice plasma are 400–600 Å in diameter.

Some of the guinea pig chol LDL discs were seen filled with stain, suggesting that these discs might be vesicles (Figs. 3*H* and 4*C*). These vesicles aggregated to form overlapping images or, occasionally, stacks (Figs. 3*H* and 4*C*). Translucent vesicles of varying sizes were seen in the density range d 1.006–1.090 g/ml; most were found in the LDL density interval d 1.019–1.050 g/ml. They were present in different amounts in 10 different chol preparations isolated from cholesterol-fed guinea pigs with different degrees of anemia, and constituted 0–15% of total LDL particles. LDL from semiresistant guinea pigs had very few vesicles, and none were seen in resistant LDL.

HDL. Two types of particles were present in control HDL samples. Spherical particles with a mean diameter similar to that of control LDL (215 ± 20 Å)[3] and smaller particles (97 ± 20 Å) were seen in control HDL d 1.090–1.21 g/ml (Fig. 3*I*). Only the former were present in control HDL of d 1.063–1.090 g/ml (Fig. 3*J*).

The appearance of chol HDL was very different from that of control HDL (Fig. 3, *L* and *M*). Long stacks of disc-shaped particles were seen, 51 ± 4 Å in width,[3] 252 ± 11 Å in diameter, with a periodicity of 68 ± 2 Å. Unaggregated particles 120–240 Å in diameter, characteristic of control HDL, were also observed. A rough correlation existed between the degree of anemia and the presence of many long stacks of uniformly sized discs; resistant HDL showed only a few short stacks of irregular discs. Long stacks were sometimes seen aligned with other stacks such that adjacent discs fused into parallel layers (Fig. 3*L*). Some discs also showed a substructural detail, a fine dark line separating the disc into two discs 25 Å wide (Fig. 3*L*, arrow). These observations suggest that the chol HDL discs might be lipid bilayers.

Most of the long stacks were found in chol HDL d 1.090–1.21 g/ml; in chol HDL d 1.063–1.090 g/ml, there were short stacks of discs with diameters greater than 250 Å that resembled chol LDL vesicles in their translucent appearance and overlapping pattern of stacking. HDL stacks have been reported before only in the plasma of patients with LCAT deficiency, whose HDL discs are somewhat smaller, 150–200 Å in diameter, with a periodicity of 50–55 Å (6).

Gel chromatographic behavior of guinea pig lipoproteins

LDL. In order to characterize the two types of chol LDL seen in the electron microscope (Figs. 3*H* and 4*A*), we filtered chol LDL d 1.010–1.050 g/ml through

[3] These are means of 30 measurements ± SD, made on the same electron microscope plate. Measurements on other plates gave similar values.

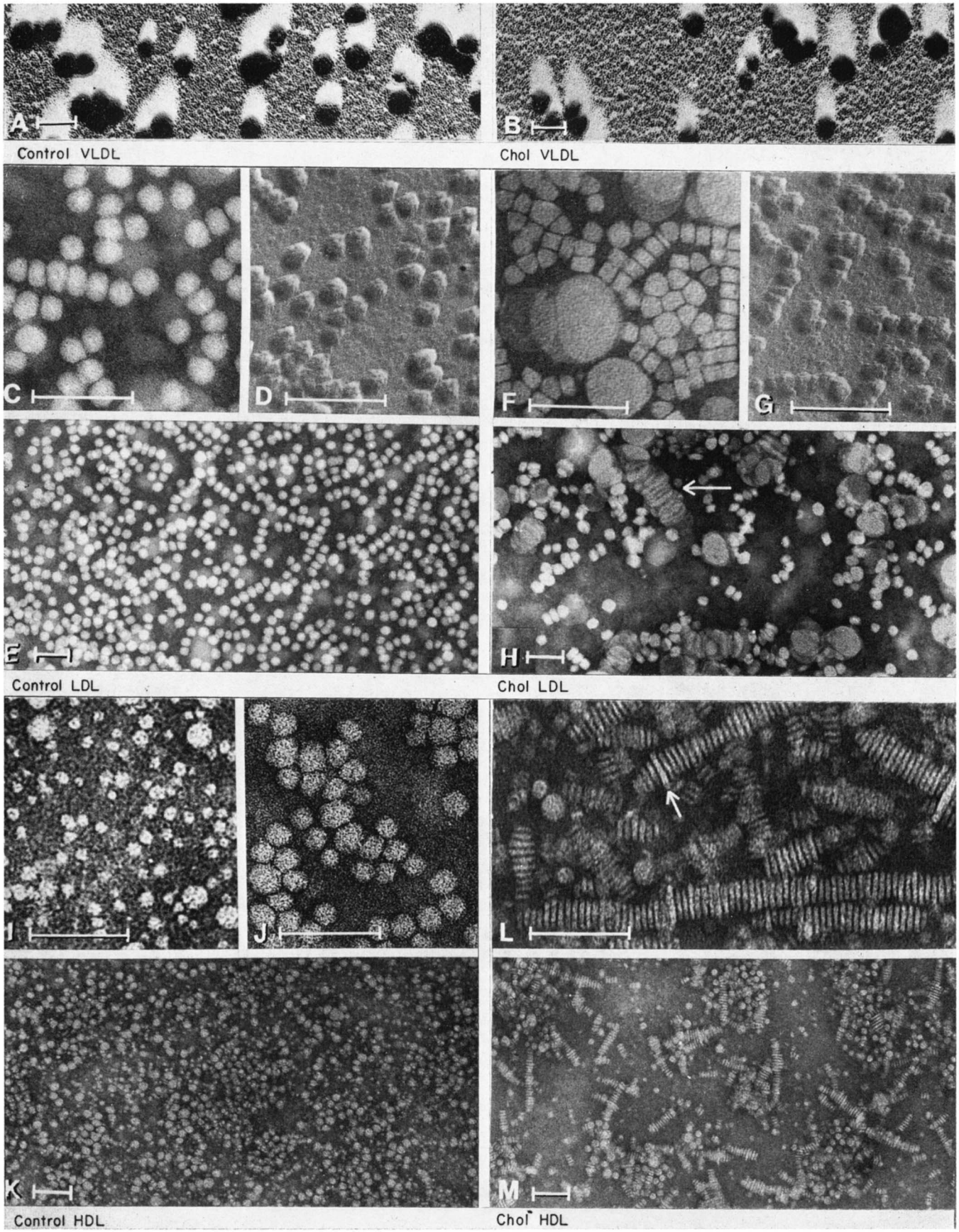

FIG. 3. Electron micrographs typical of control and chol guinea pig lipoproteins. Bars represent 1000 Å. *A*, *B*, *D*, and *G*, shadowed with Pd–Pt at a 30° angle. All others, negatively stained with 1% sodium phosphotungstate. *A*, *B*, *E*, *H*, *K*, and *M*, × 70,000; *C*, *D*, *F*, *G*, *I*, *J*, and *L*, × 180,000. LDL samples (*C–H*), d 1.010–1.050 g/ml. HDL samples *K*, *L*, and *M*, d 1.070–1.21 g/ml; *I*, d 1.090–1.21 g/ml; and *J*, d 1.063–1.090 g/ml. Arrow in *H* (chol LDL) designates large flattened vesicles forming overlapping images. Arrow in *L* (chol HDL) designates line that splits disc longitudinally.

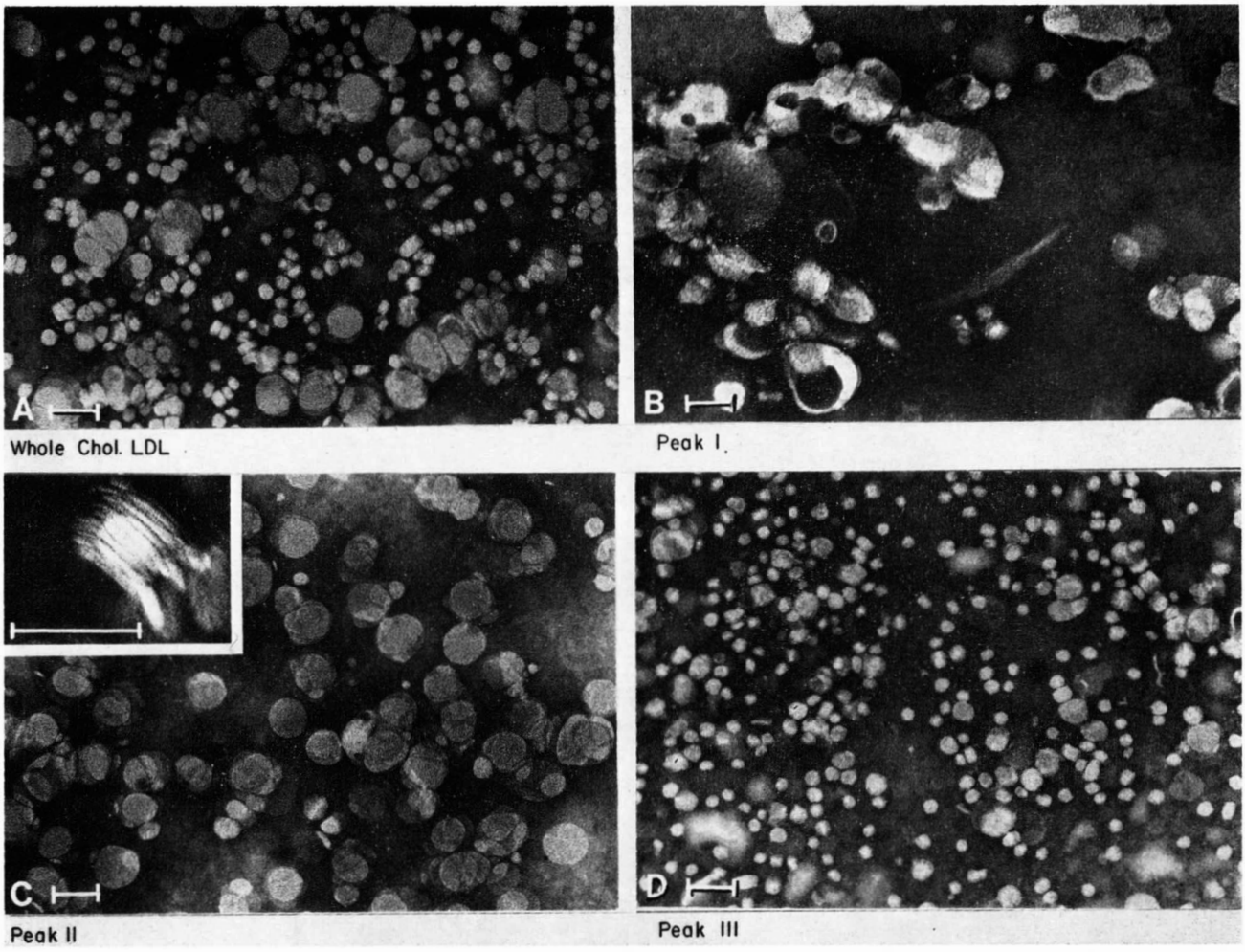

FIG. 4. Electron micrographs typical of chol LDL d 1.010–1.050 g/ml and of its subfractions separated by gel chromatography (see Fig. 5). Bars represent 1000 Å. Samples were negatively stained with 1% sodium phosphotungstate. × 70,000 except insert in *C*, × 180,000, showing aggregation of flattened vesicles into stacks.

columns containing 2% agarose gel. This density interval was chosen to eliminate the contamination by HDL at densities of 1.050–1.063 g/ml.

As shown in Fig. 5*A*, chol LDL yielded three protein-containing peaks. The void volume peak (peak I) had a high absorbance at 280 nm, mainly because of its turbidity. It consisted of large aggregates, as shown in Fig. 4*B;* myelin figures and aggregates of the large LDL vesicles were clearly visible in that fraction. A slightly turbid second peak (peak II) consisted of nonaggregated vesicles and a few contaminating, small LDL particles (Fig. 4*C*). Smaller LDL particles eluted last, forming the protein-rich peak III; a few vesicles were still present in that fraction (Fig. 4*D*). Rechromatography of peak I yielded a void volume peak as well as some peak II material (Fig. 5*C*). Rechromatography of peak III separated some material eluting with the column void volume from the small LDL particles (Fig. 5*B*). The protein profile of control LDL d 1.010–1.050 g/ml after filtration on the same agarose column showed one peak only, which was identical with peak III of chol LDL (Fig. 5*D*).

Table 1 presents the chemical composition of control LDL, of chol LDL, and of peaks I–III obtained by gel filtration of chol LDL. Peaks I, II, and III accounted for 20%, 40%, and 40% of the total chol LDL lipids, respectively. Peaks I and II had similar lipid compositions, as was expected from the predominance of vesicles in both. These vesicles were characterized by a low protein content (5% by weight) and an extremely high UC content. The high molar ratio of UC to PL (approximately 2:1) in these large, liposome-like structures is also noteworthy. Phosphatidylcholine and sphingomyelin in approximately equimolar amounts accounted for most of the PL. The composition of the smaller LDL particles (peak III) was found to be intermediate between the composition of control LDL and chol LDL in all respects. The major difference between the composi-

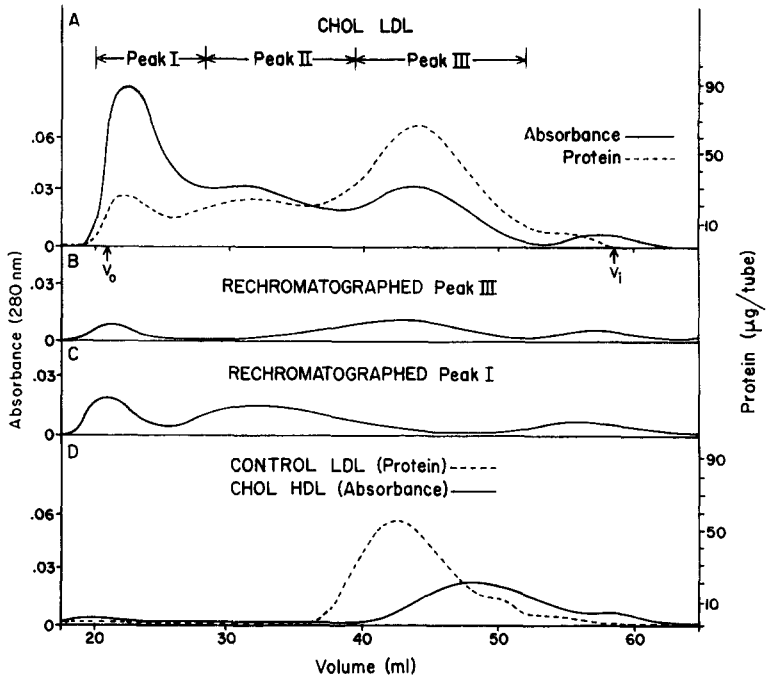

FIG. 5. Gel chromatography of guinea pig LDL and HDL on 2% agarose (A 50m). Arrows indicate the column void volume (V_o) and inner volume (V_i). Eluant was monitored by absorbance at 280 nm (——) and Lowry protein determination (– – –) on each 1.5-ml fraction. *A*, chol LDL d 1.010–1.050 g/ml, showing which fractions were pooled. Chol LDL isolated from 2.5 ml of plasma was applied to the column. The small UV-absorbing peak at V_i contained the NaBr in which the LDL was applied to the column. *B*, rechromatography of peak III. *C*, rechromatography of peak I. *D*, control LDL d 1.010–1.050 g/ml (– – – –) isolated from 3.5 ml of plasma and chol HDL d 1.07–1.21 g/ml (——) isolated from 2 ml of plasma.

tion of chol LDL peak III and control LDL was that the molar ratio of UC to PL was still almost 2:1 in peak III while it was less than 1:1 in control LDL. The elution profile of resistant LDL d 1.010–1.050 g/ml (not shown here) consisted of a very small void volume peak and a large peak III, as expected from the electron microscopic results.

HDL. Chol HDL was completely excluded from Sephadex G-200 columns. It was slightly included in the agarose A 1.5m column (>1.5 × 10^6 mol wt excluded) and was eluted slightly after LDL on agarose A 50m (Fig. 5*D*). Control HDL behaved similarly to chol HDL on the 1.5m agarose column. No evidence for the presence of more than one species of HDL was obtained by UV absorbance and protein analysis. The chromatographic behavior of chol HDL suggested that it existed in solution as free subunits rather than as the large aggregates observed in the electron microscope.

Electrophoretic mobility on agarose gel

We have previously published agarose gel patterns of HDL and LDL from control and chol guinea pigs (3).

TABLE 1. Chemical composition of control LDL, chol LDL, and chol LDL subfractions isolated by gel chromatography[a]

Fraction	UC	CE	PL	TG	PC/S[b]	% Protein[c]
		µmoles lipid/mg protein ± SD			*molar ratio*	
Control LDL (5)[d]						
d 1.010–1.050 g/ml	0.8 ± 0.4	3.4 ± 1.0	1.4 ± 0.6	0.78 ± 0.31		22.1 ± 4.0
Whole chol LDL (3)						
d 1.010–1.050 g/ml	8.5 ± 0.5	4.3 ± 0.3	3.9 ± 0.3	0.24 ± 0.10	1.4	9.6 ± 0.6
Peak I (3)	15.8 ± 4.5	3.9 ± 0.5	8.6 ± 2.0	trace	1.3	4.9 ± 0.9
Peak II (3)	13.0 ± 3.4	2.6 ± 0.7	6.3 ± 1.5	0.14 ± 0.03		6.6 ± 1.5
Peak III (3)	3.9 ± 0.2	3.5 ± 0.2	2.3 ± 0.5	0.24 ± 0.06		12.9 ± 1.4

[a] See Fig. 5.
[b] PC, phosphatidylcholine, mol wt 800; S, sphingomyelin, mol wt 750.
[c] % protein = (g protein/g lipoprotein) × 100.
[d] The number of animals used in each case is shown in parentheses.

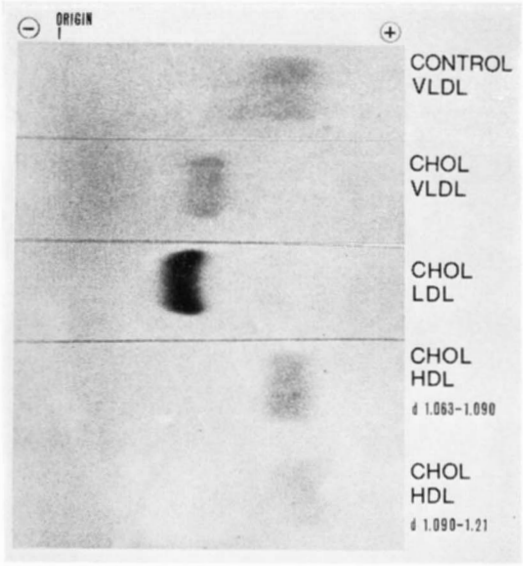

FIG. 6. Agarose gel electrophoresis of guinea pig lipoproteins in 0.6% agarose gel with 1% BSA (23), showing the decrease in VLDL mobility with dietary cholesterol and the difference in mobility of two density subfractions of chol HDL. Chol LDL, d 1.019–1.063 g/ml. The agarose sheet was stained for lipids with oil red O.

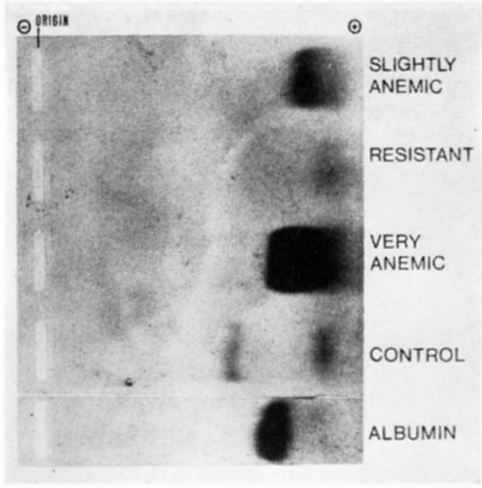

FIG. 7. Agarose gel electrophoresis of guinea pig HDL d 1.070–1.21 g/ml and guinea pig albumin in 1.2% agarose gel (23). The top three HDL samples, from cholesterol-fed guinea pigs, came from equal volumes of plasma, showing the increase in HDL that occurred as anemia progressed. The agarose sheet was stained for protein with amido black.

In the present study we investigated the electrophoretic mobility of several lipoprotein subfractions.

VLDL. Control VLDL exhibited fast pre-β to α mobilities, with occasional trailing to β mobility (Fig. 6). VLDL isolated from chol plasma had markedly slower mobilities (β or slow pre-β). Starch-block electrophoresis patterns showed a similar difference in the mobilities of control and chol VLDL.[4]

LDL. As reported earlier, control and chol LDL had identical electrophoretic mobilities (3). In some samples of chol LDL d 1.019–1.063 g/ml there was a minor component with α mobility that was never observed when chol LDL d 1.010–1.050 g/ml was electrophoresed.

HDL. Control HDL d 1.070–1.21 g/ml had two components, a fast β band with a high affinity for lipid stain and a fast α band staining more strongly for protein than for lipid (Fig. 7). Chol HDL showed some variation in the α mobility, depending on the hydrated density and degree of anemia. Chol HDL d 1.063–1.090 g/ml had a slower mobility than chol HDL d 1.090–1.21 g/ml (Fig. 6). HDL d 1.070–1.21 g/ml isolated from resistant guinea pigs had a faint fast α component corresponding to the fast component observed in control HDL (Fig. 7). As cholesterol feeding led to a more severe anemia, and consequently to higher levels of HDL and an increased UC:protein ratio, the mobility of the main HDL component decreased (Fig. 7). The HDL concentration began to increase within 1 or 2 wk after addition of cholesterol to the diet, as demonstrated by a faint α band in the agarose gel pattern of whole plasma that was not seen in control plasma.[4] This α band was neither a pre-β nor a β band.

Polyacrylamide gel electrophoresis

We incubated lipoproteins with SDS, an ionic detergent, and Triton X-100, a nonionic detergent, in order to dissociate them and identify the peptides characteristic of each density fraction.

SDS gels. Fig. 8 shows the patterns obtained for control and chol HDL and LDL on 10% acrylamide gels containing 0.1% SDS. Chol HDL showed two main bands: band I (mol wt $\sim$ 35,000), which sometimes appeared as a doublet, and band II (mol wt $\sim$ 25,000). In addition, there were several faint bands of mol wt $>$ 50,000, which might have been aggregates of the smaller peptides, since the strongest of these minor bands had mol wt $\sim$ 70,000. A striking pattern emerged from the comparison of control HDL and HDL of severely anemic, slightly anemic, and resistant cholesterol-fed guinea pigs (Fig. 8). In control HDL only band II was present, besides the faint high molecular weight bands; there was also protein which did not enter the gel, perhaps due to LDL contamination. In resistant HDL, band II was also predominant, with a trace of band I. In chol HDL from slightly anemic guinea pigs (Fig. 8*A*), bands I and II were about equal in intensity. As the anemia progressed (Fig. 8, *B* and *C*) the amount of band I increased relative to band II.

Most of the protein of LDL and VLDL did not enter the SDS gel, and the patterns obtained with these lipo-

[4] Sardet, C., and H. Hansma. Unpublished observations.

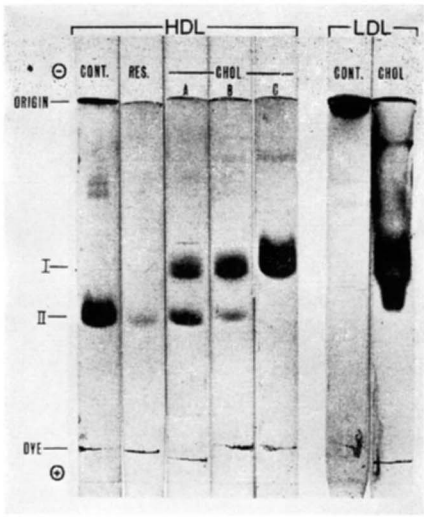

FIG. 8. Polyacrylamide gel disc electrophoresis of isolated guinea pig lipoproteins in SDS gels (pH 7.2, 0.1% SDS, 10% acrylamide). HDL d 1.07–1.21, showing change in peptide pattern as the anemia develops. CONT., control HDL; RES., resistant HDL; A–C, chol HDL from increasingly anemic guinea pigs. HDL isolated from the following plasma volumes were applied: CONT., 2.5 ml; RES., 0.3 ml; chol A, 0.2 ml; chol B, 0.1 ml; and chol C, 0.07 ml. Gels were stained for protein with amido black in 7% acetic acid. (Gels stained in alcoholic amido black [1% amido black in 20% acetic acid–methanol 1:1] showed the same bands, thus there was no loss of small peptides with the aqueous amido black stain.)

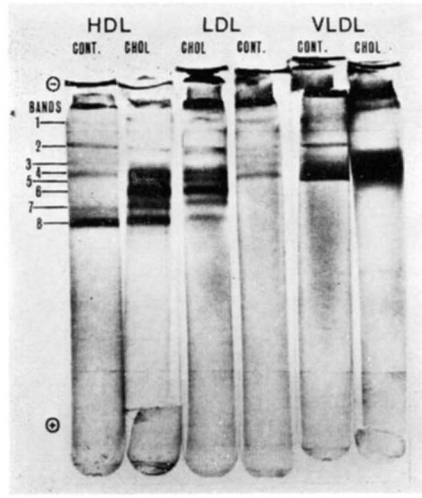

FIG. 9. Polyacrylamide gel disc electrophoresis of isolated guinea pig lipoproteins in Triton gels (pH 8.3, 0.5% Triton, 7.5% acrylamide). CONT., control. HDL, d 1.07–1.21 g/ml; LDL, d 1.01–1.05 g/ml; VLDL, d < 1.006 g/ml. Gels were fixed and stained for protein with amido black.

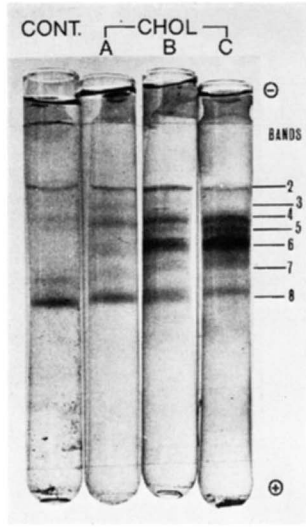

FIG. 10. Polyacrylamide gel disc electrophoresis of isolated guinea pig HDL (d 1.07–1.21 g/ml) in Triton gels (pH 8.3, 0.5% Triton, 7.5% acrylamide). CONT., control HDL; A–C are chol HDL from increasingly anemic guinea pigs. Samples are same as in Fig. 8. HDL isolated from the following plasma volumes were applied: control, 1.5 ml; chol A, 0.2 ml; chol B, 0.2 ml; and chol C, 0.07 ml. Gels were fixed and stained for protein with amido black.

proteins were smeared (Fig. 8). This made it difficult to identify the VLDL and LDL peptides that did enter the gel. Chol LDL showed both bands I and II, while control LDL showed little or none of either band. Of the chol LDL subfractions from gel filtration, peak III had most of its protein at the origin of the acrylamide gel, in contrast to peak I, where most protein entered the gel; both peaks had a faint band I (not shown).

Triton gels. Fig. 9 shows the patterns obtained for control and chol VLDL, LDL, and HDL on 7.5% acrylamide gels with 0.5% Triton X-100. In this system, at least eight bands were observed. Chol HDL and chol LDL had all eight bands, while control HDL and control LDL always had fewer bands.

The pattern of bands changed in the sequence of control HDL, resistant HDL, and chol HDL from increasingly anemic guinea pigs (Fig. 10), as was the case for SDS gels. Band 8 was the strongest band in control HDL, while band 6 was absent even in overloaded gels. Resistant HDL also had little or no band 6 (not shown), and the intensity of band 6 increased relative to that of band 8 as the anemia became more severe (Fig. 10, *A–C*).

Most of the LDL and VLDL protein remained at the origin, but some peptides entered the separating gel. In contrast to control HDL, control LDL had only bands 1–5, while chol LDL had all the bands present in chol HDL (Fig. 9). Control VLDL had a pattern similar to that of control LDL. Chol VLDL had poorly defined bands in the region of control VLDL and occasionally several faster bands. Chol LDL peak III from the agarose gel column showed only band 2, while peak II also showed a faster band (band 7 or 8).

The results from both Triton and SDS polyacrylamide gel electrophoresis suggest that cholesterol feeding is accompanied by the appearance of a new peptide in guinea pig HDL (band 6 or band I). However, there are other possible interpretations. This new band could be a

new combination of control HDL peptides with each other or with bound lipid or carbohydrate. In Triton gels, where separations are based mainly on charge, aggregated peptides could account for some of the eight bands. In SDS gels, where separations are based on molecular weight, aggregates of band II would not produce band I. However, it is possible that band I contains the peptide of band II plus tightly bound lipid (or carbohydrate). It will be necessary to run polyacrylamide gel electrophoresis of completely delipidated HDL to eliminate this possibility; there is, however, considerable evidence that our incubation conditions were sufficient to dissociate the peptides and lipids. Shore and Shore (26) reported that human lipoproteins and their delipidated peptides have identical patterns on Triton gels; Helenius and Simons (32) have shown that SDS delipidates human LDL completely at an SDS-to-LDL ratio only slightly higher than ours. The patterns we have obtained on Triton gels with ether-extracted lipoproteins were identical with those of the unextracted lipoproteins.[4] We have also run chol HDL on gels with 1% SDS (above the critical micellar concentration) according to the method of Fairbanks, Steck, and Wallach (33); the same two bands were obtained under these conditions.[4]

Immunochemistry

Isolated lipoproteins and whole plasma from control and chol guinea pigs were reacted against antibodies to the total lipoprotein fraction (d < 1.21 g/ml) from control guinea pigs (anti-control LP) and chol guinea pigs (anti-chol LP) by means of immunodiffusion and immunoelectrophoresis techniques. From these results, we have tentatively established the number of lipoprotein species (immunologic determinants) in each density class and also the immunochemical relationships among lipoprotein species.

VLDL. Chol and control VLDL had one main immunological determinant which appeared to be identical with the main LDL determinant. In some VLDL samples there was also a minor determinant which was identical or partially identical with an HDL determinant.

LDL. Control and chol LDL shared the same main immunological determinant. On immunodiffusion titer plates (29), both control and chol LDL exhibited no more than two determinants when reacted against anti-control LP; but chol LDL showed as many as three determinants when reacted against anti-chol LP, while control LDL always showed fewer determinants (not shown). Immunoelectrophoresis shows that chol LDL reacted differently against anti-control LP and anti-chol LP, since an additional diffuse arc close to the antibody trough appears only with the anti-chol LP (Fig. 11). On immunodiffusion plates, this minor arc was identical or partially identical with a major arc of chol HDL.

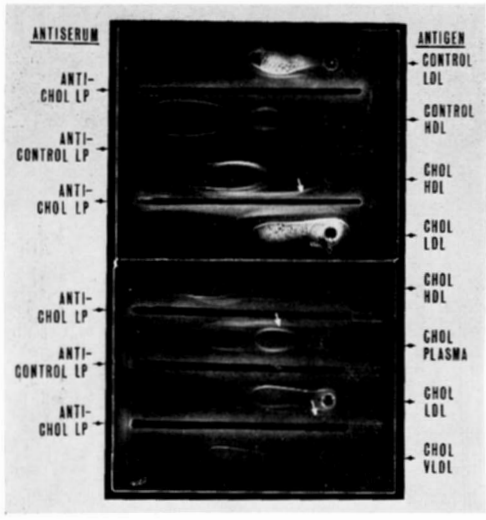

FIG. 11. Immunoelectrophoresis of guinea pig lipoproteins. Antibody troughs contain antisera to guinea pig whole lipoproteins (d < 1.21 g/ml). Antigen wells contain guinea pig whole plasma or isolated lipoproteins: VLDL, d < 1.006 g/ml; LDL, d 1.01–1.05 g/ml; HDL, d 1.07–1.21 g/ml. The two chol HDL and LDL were isolated from different guinea pigs. The arrows indicate the new arc present only when chol lipoproteins were reacted against anti-chol LP. Slides were stained for protein with amido black.

HDL. On immunodiffusion plates, control HDL (d 1.063–1.21 g/ml) had one determinant nonidentical and another determinant identical or partially identical with control LDL. These two determinants were clearly observed in immunoelectrophoresis: control HDL d 1.070–1.21 g/ml had an arc with β mobility and an arc with fast α mobility (Fig. 11), while control HDL d 1.063–1.090 g/ml had only the β arc and control HDL d 1.09–1.21 g/ml had mainly the fast α arc.

Chol HDL d 1.070–1.21 g/ml usually showed three immunological determinants when reacted against anti-chol LP. One minor determinant found in some chol HDL (and all control HDL) samples was identical with LDL; the major determinants were nonidentical with the main LDL determinant but were identical or partially identical with the other control HDL determinant(s). By immunoelectrophoresis chol HDL reacted differently against anti-chol LP and anti-control LP (Fig. 11). In addition to the two main determinants seen with either antibody, there were two more identical or partially identical determinants seen only with the anti-chol LP antibody; these additional determinants had a slow α and a β electrophoretic mobility.

The immunoelectrophoretic results obtained with several chol HDL samples from increasingly anemic guinea pigs paralleled those found with agarose gel electrophoresis: the average mobility of the determinants decreased from fast α to slow α as the anemia increased, because new components appeared which had β and slow α mobilities.

These results support the concept that the plasma of control guinea pigs contains an HDL protein distinct from the LDL protein; these proteins overlap in the intermediate density region (d $\sim$ 1.050–1.090 g/ml). The results also support the hypothesis that a new peptide appears in the lipoproteins of guinea pigs in response to dietary cholesterol and the resulting anemia; this peptide was undetectable by immunoelectrophoresis in control guinea pig lipoproteins. It will be necessary to repeat these experiments with isolated peptides (and to produce antisera in additional rabbits) in order to establish conclusively the existence of a new peptide. Ether extraction of chol HDL and LDL did not alter their immunodiffusion patterns with anti-control LP.[4] Also, if the new arcs in chol HDL and chol LDL (Fig. 11) represent only the control peptides and lipids in new combination, then there should be antibodies that react with these new lipoproteins in anti-control LP. Since this was not the case, we believe that the protein components of the chol LP are either not present in control LP or are present in even smaller traces than the other control HDL peptide(s). Levy and Fredrickson have shown variation in antibody response among rabbits (34). They found, however, that all antisera which reacted against HDL lipoprotein also reacted against partially or totally delipidated HDL; i.e., changing the HDL lipids did not prevent the antibody reaction.

DISCUSSION

The results reported above indicate that the compositional changes of guinea pig lipoproteins in response to dietary cholesterol reflected the appearance of new lipoprotein species. As the severity of the cholesterol-induced anemia increased, there was a gradual increase in these new lipoprotein species, as shown by the appearance of increasing numbers of abnormal HDL and LDL particles in electron micrographs and by the increasing load of UC carried in each lipoprotein. The presence of these new species seemed to be related to the appearance of a major new peptide undetectable in control guinea pig lipoproteins.[5]

HDL

In the present study we have shown that control guinea pig plasma contains an HDL species distinct from LDL with regard to size, chemical composition, electrophoretic mobility, and peptide composition. Its characteristics were similar to HDL from other mammalian species (30, 31), but its concentration was so much lower that we and other investigators had previously been unable to detect it (3, 35, 36). In addition to this typical mammalian HDL species with hydrated densities of 1.090–1.21 g/ml, lipoproteins were present with hydrated densities of $>$ 1.063 g/ml which appeared to belong to the LDL family as shown by their electrophoretic mobility, immunochemical properties, chemical composition, and size.

As reported earlier, dietary cholesterol leading to anemia dramatically increased the concentration of HDL (3). An increase in α-lipoprotein corresponding to HDL (3) was observed after guinea pigs had been on the cholesterol diet only 1 or 2 wk, and the HDL concentration continued to rise as the animals became anemic. The progressive increase in the amount of HDL was associated with the loading of this lipoprotein with UC (the ratio of UC to protein increased), with a slower electrophoretic mobility, and with the appearance of a major new peptide undetectable in control HDL. The changes in amounts and composition of chol HDL were accompanied by equally spectacular changes in their electron microscopic appearance. Long stacks of parallel discs were observed in increasing number as the anemia became more severe. These stacks were probably an artifact of the negative stain drying process, since the gel chromatographic behavior of chol HDL was inconsistent with the existence of large aggregates of discs.

These discs may be segments of lipid bilayers, since their width (50–55 Å), periodicity, and substructure were similar to those of bilayers observed in negatively stained PL–UC dispersions (37). We also observed that chol HDL discs had a tendency to fuse with adjacent discs; this behavior would be expected from lipid bilayers. We do not know if the new peptide (35,000 mol wt) that was detected in chol HDL plays an important role in the structure of these discs, but both the new peptide and stacks of discs were observed to increase as the anemia became more severe.

Stacks of discs strikingly similar to those of chol guinea pig HDL have been observed in negatively stained preparations of HDL from patients with LCAT deficiency (6). Chol guinea pig HDL discs are larger (250 Å diameter, 70 Å periodicity) than those of LCAT-deficient patients (150–200 Å diameter, 50–55 Å periodicity), but both HDL contain an abnormally high level of UC, a characteristic observed only in these two cases (3, 5). From the ultrastructural findings and the reconstitution of stacks by sonication of human HDL peptides with PL, Forte et al. (6) hypothesized that the discs were made up of a rosette of smaller globular units 50 Å in diameter. It is possible that the chol guinea pig HDL discs have a similar structure, although the longitudinal line seen separating some of the discs makes this possibility less

[5] The term "new peptide" is used on the basis of evidence discussed under "Polyacrylamide gel electrophoresis" and "Immunochemistry" sections (Results), although absolute proof awaits the separation of the peptides by ion exchange chromatography.

likely. Stacked-disc structures in negatively stained preparations have also been reported in studies of the red cell membrane (38) and bile salt–UC–PL micelles but not in dispersions of UC and PL alone (39).

The main peptide present in HDL of severely anemic guinea pigs was undetectable in control HDL.[5] The relative amount of this peptide appeared to be positively correlated with the degree of anemia, the level of UC in chol HDL, the number and length of stacks, and the decrease in electrophoretic mobility. At present we can only suggest several hypotheses for its origin: (*a*) Cholesterol-fed, anemic guinea pigs have a very fatty and histologically abnormal liver (40), which may release abnormal peptides or lipoproteins. Liver damage in rabbits has been associated with the presence in plasma of tissue macromolecules normally undetectable by immunochemical methods (41). (*b*) This new peptide might be present in control guinea pig plasma not associated with lipids and be either included in or adsorbed onto lipid-rich particles in chol guinea pig plasma. For example, albumin is a major protein constituent of the vesicles observed in obstructive jaundice (4, 7), and we have found traces of albumin in some chol LDL.[4] (*c*) It is possible that this new peptide is a product of the red cell hemolysis which is occurring in the anemic guinea pigs. In human red cell ghosts there is a 35,000 mol wt peptide that can be selectively eluted with isotonic saline (33).

LDL

Previous work indicated that chol LDL might consist of several types of lipoprotein particles (3). The present study has demonstrated that anemic guinea pigs have two LDL populations: one is composed of particles comparable in size to control LDL particles, but higher in their UC content and square; the other is composed of large vesicles, which become more numerous as the anemia progresses and carry up to two-thirds of the UC of chol LDL. These vesicle-like particles have only 6% protein (including the new peptide) and little neutral lipid, but contain large amounts of UC and PL in a 2:1 molar ratio. Such a high ratio is very unusual; synthetic PL–UC bilayers are saturated with cholesterol at a ratio of 1 mole of UC to 1 mole of lecithin (42).

Particles similar to chol guinea pig LDL vesicles have been observed in the plasma of human patients with LCAT deficiency (5, 6), obstructive jaundice (4, 7), or biliary cirrhosis (8). From electron microscopy, X-ray diffraction, and phospholipase treatment of the large LDL particles of jaundice patients, it has been hypothesized that these particles are flattened vesicles with a lipid bilayer surface and an aqueous interior (4, 7). Since the large LDL of chol guinea pigs have a similar electron microscopic appearance and a high lipid content, they might also have a vesicular structure. In chemical composition and size, the large LDL of LCAT-deficient patients are very similar to chol LDL vesicles—both have diameters of 900–1200 Å and are composed mostly of UC and PL in a 2:1 molar ratio. In contrast, the vesicles of patients with obstructive jaundice and biliary cirrhosis are smaller (400–600 Å) and have 1:1 molar ratios of UC and PL. These smaller vesicles aggregate in long stacks resembling chol HDL stacks or in chains of overlapping discs as do chol LDL vesicles. In contrast to the large LDL of LCAT-deficient patients, chol LDL vesicles have the same CE-to-protein ratio as the small chol LDL particles (peak III); this might be due to contamination of the vesicle peaks with peak III particles or to adsorption or inclusion of small LDL onto or into the vesicles.

In addition to the two main chol LDL species described above, immunological and electrophoretic data indicated that a lipoprotein with α mobility was present as a minor component of chol LDL, mainly in the density interval d 1.050–1.063 g/ml. Lipoproteins with such characteristics (HDL_1) have been detected in several mammalian LDL (43). The presence of these lipoproteins might account for the fact that chol LDL appeared to possess all peptides found in HDL, whereas control LDL did not. The chol LDL vesicles also had the 35,000 mol wt peptide which we believe to be associated with the stacked discs observed in chol HDL.

VLDL

Chol VLDL had a lower plasma concentration, a slower electrophoretic mobility, and a different chemical composition than control VLDL. The electrophoretic mobility decreased almost to β mobility in both chol and resistant guinea pigs, while the CE content increased at the expense of TG. The sum of the percentages of TG and CE, the least polar lipids of VLDL, remained essentially unchanged. The chemical composition (UC-, CE-, and PL-to-protein ratios) and electrophoretic mobility of chol VLDL resembled those of the small chol LDL species (peak III) except for the TG content. In contrast, control guinea pig VLDL and LDL were entirely different entities resembling those of other mammals (30, 31).

Human patients with type III hyperlipoproteinemia (24) have an abnormal VLDL which resembles the VLDL of chol guinea pigs. These patients have both a normal VLDL with α_2 mobility and normal composition and an abnormal VLDL with β mobility and a composition similar to that of chol guinea pig VLDL. Differences in the apoprotein composition of the α_2 VLDL and β VLDL were found, which probably accounted for their different electrophoretic mobilities (24). The β mobility of VLDL in Tangier disease has also been attributed to changes in the composition of the apoprotein, namely, the absence of HDL apoproteins (44). Apoprotein differ-

ences might account for the different electrophoretic behavior of guinea pig chol and control VLDL, although our characterization of VLDL peptides is not sufficient to test this hypothesis.

In human type III hyperlipoproteinemia, both fasting and heparin injection increased β VLDL at the expense of α_2 VLDL. This led the authors to propose a precursor–product relationship between the two VLDL (24). Lipoprotein lipase activity is negligible in postheparin plasma of normal guinea pigs (36). In the presence of rat HDL, however, such plasma efficiently hydrolyzes artificial TG emulsions (36). If chol HDL can activate guinea pig lipoprotein lipase as rat HDL can, then this might account for the differences between chol and control VLDL, since lipase is hypothesized to convert α_2 VLDL into a β VLDL containing less TG (24).

Lipoproteins and dietary cholesterol

Dietary cholesterol has been reported to induce various types of changes in the plasma lipoproteins of some mammalian species (45–49). Lower density lipoproteins ($d < 1.063$ g/ml) or β-lipoproteins increase in rats, dogs, rabbits, baboons, and guinea pigs. Cholesterol-fed rabbits (48), which are also susceptible to anemia, had a decreased VLDL mobility similar to that of chol guinea pigs. Higher density lipoproteins or α-lipoproteins decrease with cholesterol feeding in rats and dogs, both of which are normally rich in HDL (45, 46). Alpha lipoproteins of baboons fed an atherogenic diet were slightly increased and, like chol guinea pig HDL, had an increased ratio of UC to PL (49).

At present, the mechanism for these various changes is not well understood. The changes in guinea pig lipoproteins in response to dietary cholesterol show that the chol lipoproteins transport more lipid per unit of protein than do the control lipoproteins. As a consequence, the mean lipoprotein hydrated density is lowered. The same may be true for the lipoproteins of rats, dogs, rabbits, and baboons, since all show an increase in LDL and some also show a decrease in HDL. It is clear, however, that lipoprotein changes cannot be described solely in terms of alterations in hydrated density and electrophoretic mobility in order to understand the molecular changes taking place. Recent trends in lipoprotein research emphasize the presence of peptide families forming lipoprotein species with overlapping hydrated densities (50). It is necessary to characterize the different lipoprotein species and their protein and lipid constituents in the normal and abnormal states in order to understand the macromolecular changes induced by diet or disease.

The unusual lipoprotein species described in this report resulted from the dietary cholesterol, possibly via its consequent liver and red-cell damage. These unusual lipoproteins show similarities to those found in human diseases (4–8, 24). Guinea pigs fed cholesterol might thus provide a good model system for studying the molecular basis of changes in lipoprotein patterns.

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