Lipoprotein[a] is the major apoB-containing lipoprotein in the plasma of a hibernator, the hedgehog (*Erinaceus europaeus*)

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Abstract We have undertaken studies aimed at elucidating the interrelationships existing between the seasonal modifications in endocrine status (already demonstrated by Saboureau, M., and J. Boissin. 1978. *C.R. Acad. Sci. (Paris)* **286D:** 1479–1482) and plasma lipoprotein metabolism in the male hedgehog. During the course of these studies, we discovered that a lipoprotein comparable to human Lp[a] was a prominent component of the plasma lipoprotein spectrum in the hedgehog. This lipoprotein was present in the 1.040–1.100 g/ml density range (approximately), exhibited preβ mobility upon agarose gel electrophoresis, and its Stokes diameter was 275 Å. Its apolipoprotein moiety consisted of two proteins with molecular weights and amino acid compositions similar to those of human apoB-100 and apo[a], respectively. These two apolipoproteins were present in hedgehog Lp[a] as a complex that could be dissociated using dithiothreitol and whose stoichiometry could be 1:1. Lp[a] polymorphism due to size heterogeneity of apo[a] appeared to be present in the hedgehog as in man. The chemical composition of hedgehog Lp[a], obtained from animals bled during spring and summer, differed from that of its human counterpart in that the proportion of triglycerides was approximately three times higher in the hedgehog particle (13% vs. 4%), to the detriment of cholesteryl esters. Dissociation of the apoB:apo[a] complex has allowed us to obtain Lp[a] devoid of its specific polypeptide (Lp[a–]), a particle that retained the characteristics of Lp[a] as regards its lipid composition but whose Stokes diameter decreased by 30 to 40 Å. The plasma concentration of LDL particles, defined as lipoproteins containing apoB-100 as their sole apolipoprotein constituent, was considerably lower than that of Lp[a]. These findings suggest that the hedgehog could be a unique animal model for studies regarding Lp[a] metabolism. **—Laplaud, P. M., L. Beaubatie, S. C. Rall, Jr., G. Luc, and M. Saboureau.** Lipoprotein[a] is the major apoB-containing lipoprotein in the plasma of a hibernator, the hedgehog (*Erinaceus europaeus*). *J. Lipid Res.* 1988. **29:** 1157–1170.

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The hedgehog is a hibernator in which we have demonstrated seasonal variations of large amplitude in both thyroid and testis activities (1). During the past few years, reports from our laboratory have shown the interest in studies dealing with endocrine regulation of the metabolism of plasma lipoproteins in an animal species exhibiting similar seasonal variations, the European badger (present status of our research on the badger is reported in ref. 2). We have therefore undertaken lipoprotein studies in the hedgehog to investigate whether this animal would provide supplementary information in this area. We have reported the hydrodynamic and chemical characteristics of hedgehog plasma lipoproteins during spring (3). We demonstrated the presence of a complex lipoprotein spectrum in this animal, characterized by *1*) a low concentration of VLDL, *2*) a continuity between the respective distributions of LDL and HDL, together with an overlap of different lipoprotein components as demonstrated by the simultaneous presence of apolipoproteins with migration characteristics similar to those of human apoB and apoA-I in both the 1.006–1.063 g/ml and 1.063–1.21 g/ml density intervals, and *3*) the presence of unusual VHDL (d 1.178–1.259 g/ml) responsible for the

Abbreviations: VLDL, very low density lipoproteins, d < 1.006 g/ml, unless otherwise defined; IDL, intermediate density lipoproteins, d 1.006–1.019 g/ml; LDL, low density lipoproteins, d 1.006–1.063 g/ml, unless otherwise defined; HDL, high density lipoproteins, d 1.063–1.21 g/ml, unless otherwise defined; VHDL, very high density lipoproteins, density as defined; apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; DTT, dithiothreitol.

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transport of up to 15% of plasma cholesterol, in addition to phospholipids.

We have continued our investigations regarding different aspects of the plasma lipids and lipoproteins in the hedgehog. Thus, alongside a circannual study of the seasonal variations of these parameters (Laplaud, P. M., et al., unpublished results), we have attempted to perform a characterization of the major circulating apolipoproteins in this species. As reported in the following pages, this latter study has led us to the discovery that lipoproteins containing a polypeptide comparable to human apo[a] are prominent components of the plasma lipoprotein spectrum in the hedgehog.

In humans, elevated plasma levels of Lp[a] are considered a major risk factor for atherosclerosis (4–12) while, at the same time, the metabolism of this lipoprotein remains largely unknown. Therefore, the demonstration that Lp[a] is present as a quantitatively important component of the plasma lipoprotein spectrum in the hedgehog suggests that this species could be a valuable animal model for further studies regarding Lp[a] metabolism and especially its endocrine regulation.

MATERIALS AND METHODS

Animals and diets

Male adult hedgehogs were caught in the fields surrounding the Centre d'Etudes Biologiques des Animaux Sauvages, located in western central France. They were kept individually in 6 m^2 parks under natural conditions of light, temperature, and rainfall. The animals were fed daily with a mixture of crushed chicken meat and commercial food for dogs (Canina Duquesne-Purina) containing the following proportions by weight of the major constituents: protein, 20%; animal fat, 6%; carbohydrate, 5%; vitamin A, 15,000 I.U./kg; vitamin D-3, 1,500 I.U./kg. Water was provided ad libitum.

Blood specimens

All the experiments reported in the present study were performed on blood samples obtained between April and mid-September from active animals. In each case, blood was taken from hedgehogs that had been fasted overnight for approximately 18 hr. The animals were slightly anesthetized with Fluothane (ICI Pharma) in oxygen, and blood was withdrawn by heart puncture. In the first series of experiments, in which only sequential and/or density gradient ultracentrifugations of lipoproteins were performed, followed by electrophoretic characterization of their apolipoprotein content, blood was collected on EDTA (final concentration 1 mM). In subsequent experiments leading to purification and characterization of Lp[a] and apo[a], blood was collected on EDTA at the same concentration, but sodium azide (0.02%) and a kallikrein inactivator (Aprotinin, Sigma, 10 units/ml) were immediately added to plasma. In every series of experiments, all manipulations were performed as quickly as possible.

Chemical analysis

The enzymatic techniques used for measurement of the concentrations of the different classes of lipids, both in plasma and in lipoprotein fractions, have been described elsewhere (13). The methodology of Lowry et al. (14) was employed for the assay of protein concentrations, using bovine serum albumin as standard. The sialic acid content of hedgehog Lp[a] was estimated according to Warren (15).

Ultracentrifugation of hedgehog plasma lipoproteins

Sequential isolation of d < 1.006 g/ml and d 1.006–1.100 g/ml lipoproteins was performed using established procedures (16), adapted to the MSE 8 × 14 ml aluminum fixed-angle rotor. All centrifugations reported in this paper were carried out in MSE Superspeed 65 or Prepspin 50 ultracentrifuges (MSE Scientific Instruments, Crawley, UK). Density gradient ultracentrifugation was performed using the methodology of Chapman et al. (17), adapted to the MSE 6 × 14 ml titanium swing-out rotor as described in one of our preceding reports (18). Following ultracentrifugation, lipoprotein fractions were dialyzed in Spectrapor tubing (Spectrum Medical Industries, Los Angeles, CA, exclusion limit 6,000–8,000) for 3 × 12 hr at 4°C against a solution containing 0.15 M NaCl, EDTA (1 mM), and sodium azide (0.02%).

Isolation of hedgehog Lp[a]

Analysis of density gradient subfractions showed that the distribution of particles with characteristics (i.e., Stokes diameter and M_r of the principal apolipoprotein component) corresponding to those of typical LDL did not extend to densities higher than 1.055 g/ml (see Results, Density gradient ultracentrifugation). Therefore, isolation of Lp[a] was conducted as follows. Note that all NaCl solutions used for ultracentrifugation in this series of manipulations contained the kallikrein inactivator Aprotinin (10 units/ml) in addition to EDTA and sodium azide at the concentrations reported above. Aliquots (6 ml) from hedgehog plasma pools were first brought to a density of 1.055 g/ml by addition of KBr, then placed in ultracentrifuge tubes (14 ml nominal capacity, suitable for the MSE 8 × 14 ml aluminum fixed-angle rotor), layered with 2 ml NaCl, d 1.055 g/ml, and centrifuged for 24 hr at 100,000 g_{avg} at 17°C. The top 3 ml was discarded and the bottom fraction was collected, placed in similar new centrifuge tubes and mixed with NaCl, d 1.190 g/ml, so as to obtain a final density of 1.100 g/ml. Such samples were subsequently ultracentrifuged for 26 hr at 100,000 g_{avg} at

17°C. The top fraction (1.5 ml) was then collected and dialyzed for 3 × 12 hr at 4°C against NaCl 0.15 M, pH 7.0. This fraction contained a mixture of Lp[a] and HDL-like particles (see Results, Density gradient ultracentrifugation). After concentration to a suitable volume using an Amicon 8 MC microultrafiltration system fitted with XM-50 ultrafiltration membranes, exclusion limit 50,000 (Amicon, Lexington, MA), these two types of lipoprotein particles were separated by means of gel filtration chromatography on a column of Sepharose CL-4B (12 × 1000 mm), operated at 4°C. Elution was performed using NaCl 0.15 M, pH 7.0, at a rate of 12 ml/hr, and 2-ml fractions were collected. Elution was monitored at 280 nm using an LKB Uvicord detector and recorder (LKB Instruments, Bromma, Sweden). This type of chromatography led to an incomplete separation of the two lipoprotein populations, as the trace on the recorder did not return to baseline between the two peaks. Therefore, the fractions corresponding to the first peak were pooled and rechromatographed in the same system. The resulting peak was approximately Gaussian. Fractions corresponding to the trailing edge of this peak were discarded, and the remainder of the peak was shown to contain pure Lp[a] (see Results, Characterization of Lp[a]).

Purification of apo[a]

This was performed according to a modification of the methodology of Armstrong, Walli, and Seidel (19). Briefly, native Lp[a] was brought to a concentration of ca. 1 mg/ml total apoprotein in 20 mM Tris (pH 7.4), 0.15 M NaCl, 1 mM EDTA, and 0.02% NaN_3. Dithiothreitol was freshly added to a final concentration of 10 mM and the solution was incubated at 37°C for 3 hr. The solution was then adjusted to 1.100 g/ml with solid KBr and centrifuged in the MSE 6 × 14 ml titanium swing-out rotor at 150,000 g_{avg} for 24 hr at 10°C. After completion of centrifugation, apo[a] was a pellet that had sedimented at the base of the tube. It was solubilized in 20 mM Tris (pH 8.0), 1 mM EDTA, 10 mM DTT, containing 25 g/l SDS. Reduced Lp[a] (Lp[a-]) was recovered in the top fraction and dialyzed against 0.15 M NaCl as reported above (see Ultracentrifugation of hedgehog plasma lipoproteins) before subsequent electrophoretic and chemical analysis.

Double immunodiffusion

Double immunodiffusion was carried out by the technique of Ouchterlony (20) in 1% agarose. Hedgehog Lp[a] and human reference standard to Lp[a] (Immuno AG, Wien, Austria) were reacted with antiserum against human Lp[a] (Immuno AG). Slides were stained for protein using Amido black.

Electrophoretic methods

Agarose gel electrophoresis was performed using the Ciba Corning (Palo Alto, CA) universal films and buffers. Following electrophoretic migration, films were processed according to the manufacturer's instructions and stained using Oil Red O. Polyacrylamide gel electrophoresis of plasma lipoproteins and of lipoprotein fractions was performed using commercially available polyacrylamide gel slabs (Lipofilm, Sebia, Issy-les-Moulineaux, France). Continuous-gradient slab-gel electrophoresis was performed on a Pharmacia electrophoresis apparatus GE-2/4 loaded with gradient gels PAA 2/16 (Pharmacia Fine Chemicals, Uppsala, Sweden), according to the conditions of Nichols, Krauss, and Musliner (21). For particle size calibration, we used both a series of molecular weight markers ranging in hydrated diameters from 71 to 170 Å (High Molecular Weight electrophoresis calibration kit, Pharmacia Fine Chemicals), and a solution of latex particles (Dow Chemical, 380 Å diameter). The Stokes diameters of the particles were calculated using the Stokes-Einstein equation as described by Anderson et al. (22).

Prior to electrophoretic examination of the content of their apolipoprotein moiety, lipoproteins were delipidated with ethanol–diethylether 3:1 (v/v) as described by Brown, Levy, and Fredrickson (23); the apoprotein residue was dried under N_2. The molecular weights of apolipoproteins were estimated by electrophoresis in SDS-polyacrylamide gels of either 10% monomer concentration as described by Weber and Osborn (24), or 3% monomer concentration according to the modification of Weisgraber et al. (25) of the methodology of Stephens (26). When desired, reduction of apolipoprotein complexes was obtained prior to electrophoresis by incubation of the sample for 1 hr at 37°C in the presence of 10 mM DTT. Calibration curves for estimation of molecular weights were constructed using Low and High Molecular Weight electrophoresis calibration kits from Pharmacia Fine Chemicals. Staining of the gels was performed using either Coomassie brilliant blue R-250 for 10% monomer gels or the technique of Karlson et al. (27) for 3% monomer gels.

Isolation of the apo[a]–$apoB_H$ complex from 1.006–1.100 g/ml lipoproteins was performed using preparative electrophoresis according to Stephens (26). Details of our procedures have been previously published (28).

Immunoblotting was performed, after electrophoresis of the lipoproteins of density gradient subfractions, on polyacrylamide gradient gels PAA 2/16 as described above, except that 30 to 40 μg protein from each subfraction was electrophoresed. Lipoprotein transfer onto nitrocellulose sheets (Bio-Rad, Richmond, CA) was then carried out overnight at 20 V and 8°C using the transfer buffer Tris–glycine, pH 8.3, as described in ref. 29, except that methanol was omitted. The paper was blocked by incubation for 1 hr at 40°C with a 3% (w/v) solution of bovine serum albumin in a buffer containing 0.15 M NaCl and 0.01 M Tris-HCl, pH 7.3 (buffer A). The nitrocellulose sheet was then incubated with the first antibody in the presence of 3% bovine serum albumin and

10% pig serum in the same buffer at room temperature for 3 hr. Antiserum against human apolipoprotein[a] was prepared in sheep, using purified apo[a] which was a gift from Dr. V. W. Armstrong (Göttingen University, West Germany). This antiserum was used at a dilution of 1/20. After three washings (10 min each) of the nitrocellulose paper in buffer A, the sheet was incubated with the second antibody, conjugated to horseradish peroxidase (Dakopatts a/s, Glostrup, Denmark). The blot was developed in the chloronaphthol-peroxide substrate (30) until bands were visible.

Purity of the apo[a] preparations

A possible contamination of our apo[a] preparations by hedgehog apoB was evaluated by means of dot blotting under the same conditions as those reported above for immunoblotting. This technique could detect concentrations of apoB as small as 2.5 ng/μl.

Amino acid analysis

Amino acid analyses were carried out on a Beckman 121 MB analyzer (Beckman Instruments, Fullerton, CA) after hydrolysis of samples in 6 N HCl for 20 hr at 110°C in sealed, evacuated tubes.

RESULTS

Plasma lipids

Total and esterified cholesterol, triglycerides, and phospholipids were measured individually in the plasmas of our animals on each series of samplings. Results were in the same ranges of values as those already reported (3), i.e., 120–200 mg/dl for total cholesterol, 20–60 mg/dl for triglycerides, and 180–260 mg/dl for phospholipids.

Density distribution of high molecular weight apolipoproteins

Density gradient ultracentrifugation on five different plasma pools led to results entirely consistent, as far as the density distribution of lipoproteins was concerned, with data obtained previously in this laboratory on hedgehog plasma samples collected during spring (3) (**Fig. 1**). Electrophoresis of apolipoproteins in polyacrylamide-SDS gels with 10% and 3% monomer concentration was subsequently performed on all fractions in which sufficient lipoprotein material was present for such an analysis to be performed, i.e., the top fraction of the gradient (d < 1.013 g/ml) and from d 1.027 g/ml to 1.162 g/ml. In such fractions, results obtained in 10% monomer gels first showed that apolipoproteins with M_r > 250,000 were present up to and including that with d 1.087–1.100 g/ml (data not shown). Electrophoresis in 3% monomer SDS gels in the absence of reducing agents provided evidence for a distribution of high M_r apolipoprotein components which modified largely according to density (Fig. 1). Indeed, in the top fraction (d < 1.013 g/ml), these high M_r proteins were represented by one prominent component with M_r in the 500,000–550,000 range and thus comparable to that found for human apoB-100 using similar electrophoretic techniques. By analogy with the nomenclature proposed for the rat B proteins (31), we denote this apolipoprotein $apoB_H$. In addition, traces of a supplementary smaller protein, with M_r ca. 280,000 ($apoB_L$) were consistently observed. The relationship of the M_r of this latter protein to that of $apoB_H$ was approximately 50:100. This type of analysis was repeated five times. In each case, results were entirely comparable, especially with regard to the absence of any variable supplementary bands suggesting partial proteolytic degradation of native proteins. Therefore, it seems reasonable to consider that $apoB_L$ in the hedgehog is homologous to human intestinal apoB-48 as described by Kane, Hardman, and Paulus (32). Finally, in the lower part of the gels, an intensely stained band representative of a peptide with M_r ca. 36,000–40,000 was observed in all samples examined. Data from 10% monomer SDS gels, more suitable for the determination of M_r in the 10,000–100,000 range, confirmed the presence of such a protein, which exhibited an M_r of 39,000–41,000 in this latter system (not shown). It is of note that such an M_r is comparable to that found for human apoE (39,000) when using SDS gels (33). Authentic VLDL were also prepared by ultracentrifugal flotation at a density of 1.006 g/ml. Results obtained when examining such lipoprotein samples in the same electrophoretic systems were strictly identical to those reported above (data not shown).

The use of 3% monomer gels allowed further resolution of the different high M_r apolipoprotein components whose presence was detected in the density range 1.027–1.100 g/ml (Fig. 1). These high M_r proteins had similar general characteristics in all series of fractions examined. However, some differences regarding the number of components detected in the gels were noted according to the sample under investigation (see below). Thus, in the successive fractions corresponding to the 1.027–1.055 g/ml density interval, two different bands representative of proteins with respective M_r values of 850,000–970,000 and 520,000–570,000 approximately were detectable when electrophoresis was performed in the absence of reducing agents. However, the relative intensities of staining of these two bands were modified according to the fraction considered. Indeed, with increasing density, the slower migrating band became more prominent while the faster one was progressively fainter, being present as a trace component only in the d 1.046–1.055 g/ml fraction. Then, from d 1.055 g/ml to 1.100 g/ml, only the protein with higher M_r was observable. This apparent M_r typically decreased by 40,000 to 80,000 in the fractions where the highest concentration of the corresponding protein was

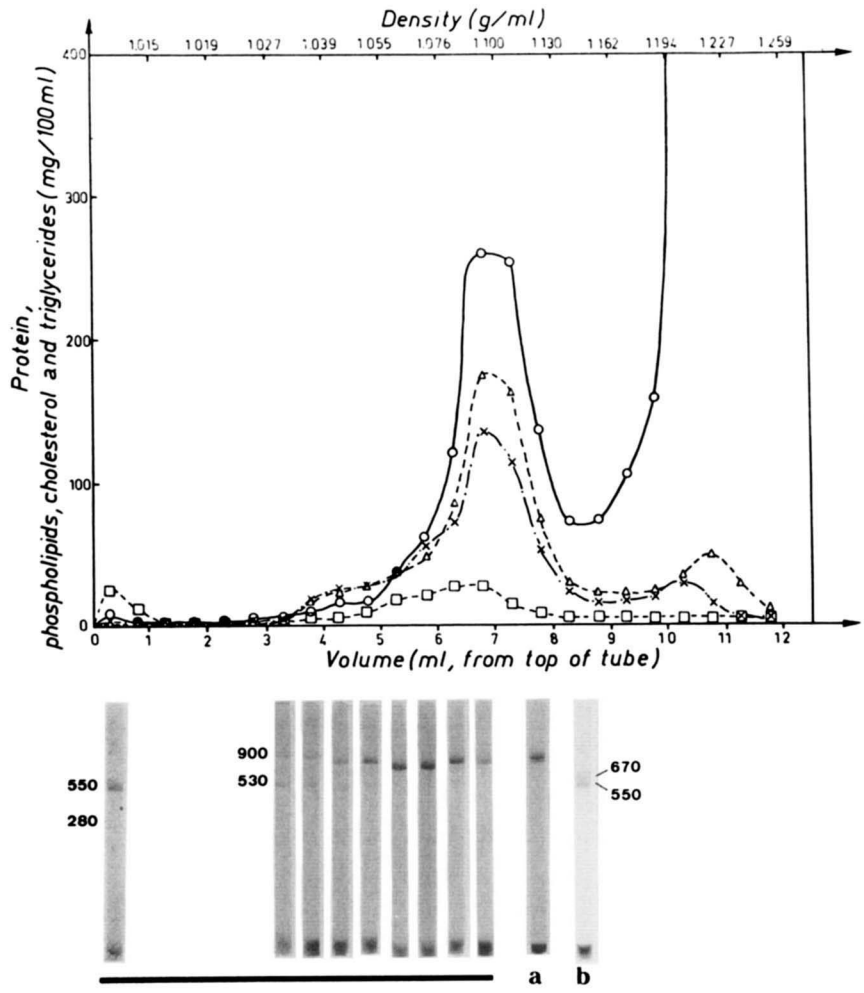

Fig. 1. Density distribution of the high molecular weight apolipoproteins of hedgehog plasma lipoproteins. Upper diagram: chemical analysis of a representative density spectrum of plasma lipoproteins. (—○—) Refers to protein, (-·-X-·-) to cholesterol, (--△--) to phospholipids, and (--□--) to triglycerides. Lower part of the figure: SDS-polyacrylamide gels with 3% monomer concentration. Each of the underlined gels is representative of the general high M_r apolipoprotein distribution, examined under nonreducing conditions, in the corresponding density fraction. On the right, gel a is representative of the pattern of higher density fractions (d 1.055–1.100 g/ml) in two of the five sample pools examined (see Results). Gel b is representative of the results of electrophoresis of the apolipoprotein content of fractions with d in the 1.055–1.100 g/ml range, and originating from three out of four plasma pools examined, under reducing conditions. Approximative M_r (kDa) are indicated; 70–100 μg protein was applied to each gel; gels were stained with Coomassie brilliant blue R-250.

observed (i.e., between d 1.055 and 1.076 g/ml), relative to that measured in adjacent fractions with lower or higher density. However, as is known to be the case with other large apolipoproteins such as human apoB-100 (32), this decrease in M_r could be artifactual, being solely a result of the increased amount of the protein with M_r 850,000–970,000 present in the fractions where the lowest apparent M_r values were observed. In addition, it is of note that, in three out of five plasma pools analyzed using this electrophoretic technique, the component with higher M_r presented as a unique band. In contrast, in the other two samples, this same component presented as a doublet (see Fig. 1, gel a). The upper portion of these 3% gels consistently showed the presence of a discrete band of very high M_r (1,500,000 to 2,000,000). This band could be representative of aggregates, due to the relatively heavy loading of our gels (70–100 μg).

The use of DTT induced radical modification of the pattern of high M_r apolipoproteins. Indeed, addition of such a reducing agent to the sample led to a complete disappearance of the band(s) representative of apolipoproteins with M_r 850,000–970,000. Thus, under these conditions, the pattern of high M_r apolipoproteins throughout the density range considered consisted exclusively of band(s) in the M_r 520,000–700,000 region. However, in lower density subfractions (i.e., d 1.027–1.039 g/ml), only

one band with M_r 520,000–570,000 was detectable in all samples examined. By contrast, in fractions of higher density (d 1.039–1.100 g/ml), the pattern observed consisted of either one or two bands according to the particular sample considered. Indeed, in three out of four pools of plasmas examined, two bands were detectable under these experimental conditions (see Fig. 1, gel b); the band representative of the protein with lower M_r (ca. 530,000–570,000) stained more intensely than the other (M_r ca. 640,000–700,000). In the last series of analyses, only one strongly staining and wide band was seen, with M_r ca. 550,000 (not shown).

In addition, at the lower end of the gels, an intensely stained band with $M_r < 40{,}000$ was consistently noted (Fig. 1), and was unaffected by the presence or absence of reducing agents. Results from SDS gels with 10% monomer concentration clearly showed that a peptide with M_r 28,000 (approximately) was a prominent component of the protein moiety of lipoproteins in this density region (data not shown). It thus seems most likely that this band is representative of an apoA-I equivalent whose presence was already suggested in our earlier report (3).

The observation that an apolipoprotein component with M_r almost twice that of apoB-100 was prominent over most of the d 1.027–1.100 g/ml range led us to examine the lipoprotein content of this density interval using gradient gel electrophoresis. **Fig. 2** shows typical results from such experiments, and thus provides evidence for the presence of different lipoprotein populations. To begin with, a molecular species of very large size was observable from d 1.039 to 1.100 g/ml, albeit as a trace component in the subfraction with lower density (1.039–1.046 g/ml). The apparent diameter of this lipoprotein varied slightly, decreasing from approximately 290 Å in the fraction with d 1.046–1.055 g/ml to a minimum of approximately 280 Å in the d 1.065–1.087 g/ml interval, and then increasing again to 283–285 Å in the fraction with d 1.087–1.100 g/ml. It should be noted that *1*) only in the fraction with d 1.065–1.076 g/ml did this particular lipoprotein species appear as a doublet; *2*) according to the particular experiment considered, trace amounts of this lipoprotein component were either observable or absent in the adjacent subfraction with higher density, i.e., 1.100–1.115 g/ml; and *3*) in fractions with density in the 1.046—1.065 g/ml range, a supplementary band representative of particles with a Stokes diameter of 310 Å, approximately, was observed as a trace component. A second type of lipoprotein particle, of smaller size, was noted as a faintly stained band from d 1.027 to 1.039 g/ml. Its apparent diameter was in the 250–260 Å range. Finally, a spectrum of strongly staining lipoproteins, whose apparent diameter decreased with increasing density from 200–212 Å to 90–110 Å, approximately, was observable throughout the density range submitted to examination.

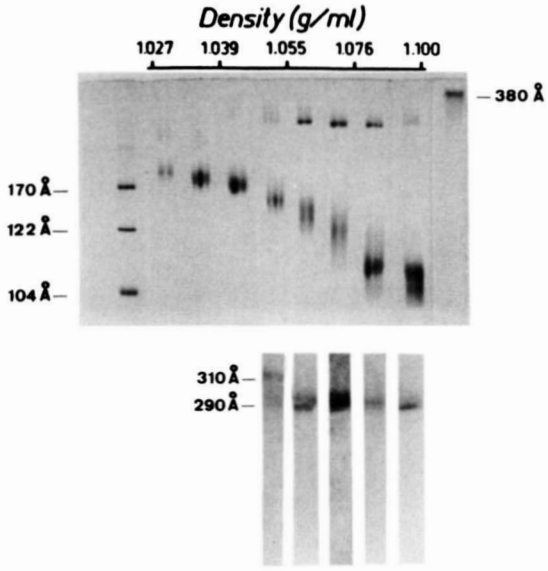

Fig. 2. Polyacrylamide gradient gel electrophoresis and immunoblotting of the lipoprotein content of density gradient subfractions from hedgehog plasma. Upper part of the figure: electrophoresis performed according to the conditions of Nichols et al. (21). The gel slabs contained a 2–16% monomer gradient (from top to bottom). From left to right: set of marker proteins (from top to bottom, Stokes diameters: thyroglobulin, 170 Å; ferritin, 122 Å; catalase, 104 Å); density gradient subfractions with respective densities indicated above the photograph (15 μg protein was layered in each lane); and latex particles, with diameter 380 Å. Gels were stained with Coomassie brilliant blue R-250. Lower part of the figure: immunoblotting using a polyclonal antibody to human apo[a] and performed on corresponding fractions in the upper photograph.

Immunoblotting using polyclonal antibodies against human apo[a] was performed following gradient gel electrophoresis. Results (Fig. 2) were consistent with the fact that the band representative of a lipoprotein with diameter in the 280–290 Å range and observed essentially in the d 1.046–1.100 g/ml region corresponded to Lp[a], as the protein content of this band consistently reacted with anti-apo[a]. It is of note that, in the subfraction with d 1.046–1.055 g/ml, the lipoprotein component with diameter 310 Å, approximately, which had been observed on gradient gel electrophoresis, reacted equally with anti-apo[a] antibodies. This suggested that it could be made of aggregating Lp[a] particles. By contrast, the lipoprotein species with diameter 250–260 Å and observed in fractions with lower density did not react and could therefore be considered as an LDL particle.

Characterization of Lp[a], Lp[a-], and of their constituent apolipoproteins

The above results clearly demonstrated that an Lp[a]-like particle was the predominant apoB-containing lipoprotein in hedgehog plasma. We therefore attempted to characterize it after chromatographic purification according to the procedure described in the Materials and Methods section. Thus, double immunodiffusion revealed partial

identity between hedgehog and human Lp[a] (**Fig. 3**). Indeed, the precipitation line formed by human Lp[a] exhibited a spur beyond the point of its closest proximity with that formed by the animal lipoprotein. We then submitted hedgehog Lp[a] to electrophoretic analysis in three different systems. **Fig. 4** compares the respective migrations, on agarose gel electrophoresis, of two different preparations of purified hedgehog Lp[a] with those of the different lipoproteins contained in hedgehog plasma and in the plasma of a normolipidemic human donor. This figure shows that isolated hedgehog Lp[a] consistently migrated as a single band (lanes 1 and 2); its mobility was, however, slightly higher than that of the preβ band seen in hedgehog whole plasma. Electrophoresis on Lipofilm slabs (**Fig. 5**) showed hedgehog Lp[a] (lane b) to migrate as a band with mobility similar to that of human LDL. It is of note that, in addition to this band, lane b shows the presence of two "bands," one at the position of the layer and the other at the limit between the two acrylamide gels constituting Lipofilm slabs, i.e., at the position where human and hedgehog VLDL migrate. This is a regularly observed phenomenon when electrophoresis of a lipoprotein fraction is carried out on this type of gel. We have already suggested that some of these "bands" could be representative of surplus stain not bound to lipoproteins (2). This is especially the case for the former "band," observable at the position of the layer, while the other, present at VLDL position, is merely a result of the change in the structure (concentration) of the gel at this particular place when photographed using a high contrast paper. This assumption is further supported by the fact that electrophoresis of the same lipoprotein material on agarose gel showed the presence of a single fraction (see Fig. 4). Finally, gradient gel electrophoresis on a 2–16% monomer gradient (**Fig. 6**) again resulted in a single band representative of a particle with a Stokes diameter of

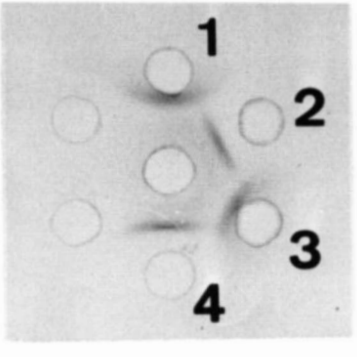

Fig. 3. Relationship of human and hedgehog Lp[a] as revealed by double immunodiffusion. Wells 1 and 3: reference standard for human Lp[a] (12 μg lipoprotein). Wells 2 and 4: two different preparations of purified hedgehog Lp[a] (50 μg lipoprotein protein). Central well: antiserum against human Lp[a] (25 μl). The slides were stained for protein using amido black.

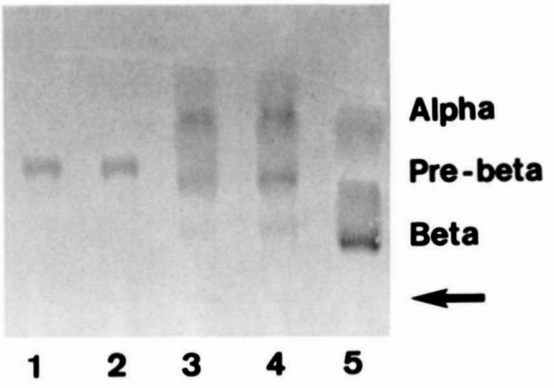

Fig. 4. Agarose gel electrophoresis, stained for lipids using Oil Red O. Arrow indicates the position of sample applications. Lanes 1 and 2: two different preparations of purified hedgehog Lp[a]. Lanes 3 and 4: two different hedgehog plasmas. Lane 5: plasma from a normolipidemic human donor, with respective positions of migration of the different lipoprotein fractions indicated.

275 ± 1 Å (mean ± SD, n = 3). Such a diameter was similar to that of the lipoproteins present in the d 1.039–1.100 g/ml range after density gradient ultracentrifugation which reacted on immunoblotting with an anti-apo[a] antiserum (see Fig. 2).

We subsequently performed chemical analysis of hedgehog Lp[a] and compared our results with those in several recent reports on human Lp[a] (19, 34, 35) (**Table 1**). We found that the composition of hedgehog Lp[a] differed from that of its human counterpart in several respects. With regard to the most hydrophobic lipids usually defined as core components, i.e., cholesteryl esters and triglycerides, the respective proportions of these two lipid classes were modified when compared to human Lp[a]. Indeed, the ratio of % triglycerides/% cholesteryl esters was approximately four times higher in the hedgehog (mean = 0.47) than in humans (0.10–0.13, according to the set of data considered). In contrast, the sum of the percentages of the core components was remarkably similar in the two species, 40.5 (mean) in the hedgehog and 39.2–42.1 in humans.

Differences between our animals and man were equally of note when considering the more hydrophilic surface components, i.e., phospholipids and apolipoproteins. An evaluation of the ratio of % protein/% phospholipids showed that it was higher in the hedgehog (mean = 1.86) than noted in man by Gaubatz et al. (34) or Kostner (9), i.e., 1.11 and 1.17, respectively, although Armstrong et al. (19) reported a much closer value (1.63). Again, the sum of the percentages of protein and phospholipids was similar in the hedgehog and in humans, 49.8 (mean) and 48.2–49.9, respectively.

In man, it is well known that the sialic acid content of Lp[a] is higher than that of LDL. We therefore measured the concentration of sialic acid in two different prepara-

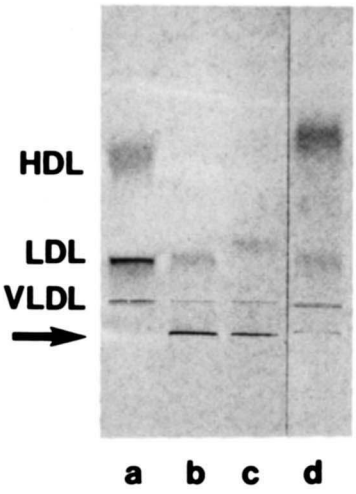

Fig. 5. Polyacrylamide gel electrophoresis on commercially available slabs (Sebia Lipofilm). Arrow indicates the position of layers. Prestaining was performed using Sudan Black. From left to right: lane a, normal human plasma, with VLDL, LDL, and HDL indicating the respective positions of migration of these different lipoproteins; lane b, Lp[a] isolated from hedgehog d 1.055–1.100 g/ml lipoproteins; lane c, Lp[a-] prepared from hedgehog Lp[a] by ultracentrifugal flotation following reduction of Lp[a] by DTT; and lane d, a representative hedgehog plasma.

tions of hedgehog Lp[a]; results were 33.2 and 34.3 μg sialic acid/mg protein, respectively. It is however of note that existing results regarding the sialic acid/protein ratio in human Lp[a] are at considerable variance according to the particular Lp[a] preparation and the analytical procedure in use (see references 35 and 36, and the comparative study of analytical methods by Onodera, Hirano, and Hayashi (37)).

We then attempted to purify hedgehog apo[a], using our modification of the ultracentrifugal method of Armstrong et al. (19) after reduction of Lp[a]. However, it should be noted that results presented below were obtained from a preparation of Lp[a] originating from a pool of plasma from ten hedgehogs. The resulting apo[a] was first analyzed using electrophoresis in 3% monomer SDS gels (**Fig. 7**). The content of this sample was shown to be heterogeneous, exhibiting two major bands with respective M_r values of approximately 660,000 and 730,000. In addition, two supplementary components were present in trace amounts, one with M_r 845,000 (approximately) and the other with M_r in the 530,000 region and thus comparable to that of hedgehog apoB_H (see above). However, this same preparation was tested by dot blotting for contamination by apoB, and no contamination could be detected down to an apoB/apo[a] concentration ratio of 0.25/100.

Table 2 shows the results of amino acid analysis of hedgehog apo[a] and compares them with different sets of data for human apo[a] (35, 36, 38). It is of note that these latter sets of data are at some variance with each other. Apart from resulting from experimental imprecision, such differences most probably reflect inter-individual variability. This is especially evident with regard to results obtained by Gaubatz et al. (36). Indeed, these latter authors related the differences that they observed between the respective content of lysine, proline, leucine, and phenylalanine of apo[a] in their two donors to the differing number and proportion of apo[a] isoforms present in each of them. On the whole however, the composition of hedgehog apo[a] as determined in the present experiments was very similar to that reported for its human counterpart; indeed, the animal protein values differed from the mean of the human apo[a] values presented in Table 2 by more than 1 mol per 100 mol only in the case of threonine (−1.2 mol per 100 mol) and alanine (−1.4 mol per 100 mol). As far as results from our SDS gel electrophoretic analyses demonstrate that apo[a] heterogeneity exists in the hedgehog, the amino acid composition reported here is representative of a mixture of different apo[a] isoforms whose respective proportions in each individual are unknown.

Reduced Lp[a] lipoprotein (Lp[a-]) was recovered in the top fraction of ultracentrifugation tubes used for preparation of apo[a]. On polyacrylamide slab gels (Fig. 5, lane c), Lp[a-] was shown to migrate farther than Lp[a]. With regard to the two supplementary "bands" appearing in this lane, a comment similar to that previously made with regard to electrophoresis of Lp[a] on the same type of gel can be repeated. On gradient gel electrophoresis (Fig. 6), our successive preparations of Lp[a-] consistently

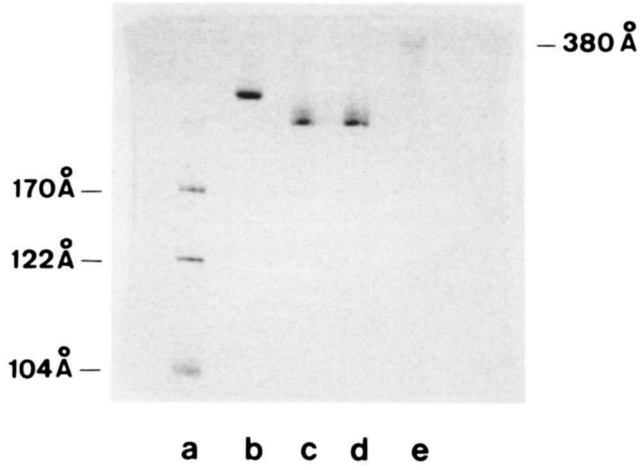

Fig. 6. Polyacrylamide gradient gel electrophoresis of hedgehog Lp[a] and Lp[a-] on a 2–16% monomer gradient, according to the conditions of Nichols et al. (21). From left to right: lane a, set of marker proteins (from top to bottom, Stokes diameters: thyroglobulin, 170 Å; ferritin, 122 Å; catalase, 104 Å); lane b, hedgehog Lp[a]; lanes c and d, two samples of hedgehog Lp[a-], originating from two different preparations; and lane e, latex particles, with diameter 380 Å.

TABLE 1. Chemical composition of hedgehog and human Lp[a] and Lp[a-]

Fraction	Lp[a]		Lp[a-]	
	Hedgehog[a] (d 1.055–1.100 g/ml)	Human (d 1.085 g/ml)	Hedgehog[b]	Human
	%	%	%	%
Cholesteryl esters	27.6 ± 1.5	35.5[c]	30.3	41.7 ± 1.3[d]
Cholesterol	9.7 ± 0.8	12.6	10.4	8.3 ± 1.4
Triglycerides	12.9 ± 1.2	3.7	16.8	5.7 ± 1.4
Phospholipids	17.4 ± 0.2	22.2	20.8	19.6 ± 1.9
Proteins	32.4 ± 1.3	26.0	21.7	24.5 ± 1.1
Sialic acid (μg/mg protein)	33.8[e]	66		

[a] Means ± SD of three different preparations, obtained from hedgehogs bled successively in June, August, and September.
[b] Mean of two different preparations, obtained from hedgehogs bled successively in June and August.
[c] Data from Kostner (9). See other compositional data in refs. 19 and 34.
[d] Data from Armstrong et al. (19) (means ± SD). See other compositional data in ref. 38.
[e] Mean of the results obtained from two different preparations, obtained respectively from hedgehogs bled in June and September. Each preparation was analyzed in duplicate.

showed the presence of two bands, the one migrating farther in the gel being consistently much more intensely stained than the other. The Stokes diameters of the particles constituting these two bands were 260 ± 2 Å and 250 ± 3 Å, respectively (means ± SD, n = 3), and were thus comparable to those of the LDL particles observed in the 1.027–1.039 g/ml density range (see above).

The chemical composition of Lp[a-] was determined and compared to that reported for its human counterpart (19, 38). As expected, the main difference between the compositions of Lp[a] and Lp[a-] was a large decrease in protein content (respective means: Lp[a], 32.4%; Lp[a-], 21.7%). Concerning lipid composition, the value of the ratio % triglycerides/% cholesteryl esters was two to four times higher in the hedgehog (mean, 0.55) than in humans (0.14 to 0.28, according to the set of data considered) (19, 38). This is similar to what was observed for Lp[a]. The % protein/% phospholipid ratio was higher (mean, 1.04) than reported by Fless, Zum Mallen, and Scanu (38) (i.e., 0.71 and 0.82) but lower than the results from Armstrong et al. (19) (i.e., 1.25). Again, the respective sums of the percentages of the core and surface components in hedgehog Lp[a-] were similar to those reported for human Lp[a-] (i.e., 47.1 (hedgehog) vs. 44.7–47.4 (man), and 42.5 (hedgehog) vs. 42.8–44.1 (man), respectively).

Electrophoresis in 3% monomer SDS gels (Fig. 7) showed that apoLp[a-] presented as a major band with M_r in the 550,000 region. In addition, we have examined apoLp[a-] in 10% monomer SDS gels to search for the possible presence of lower M_r apolipoproteins; however, none of these protein components could be detected upon visual examination of the gels (data not shown).

Amino acid composition of hedgehog apoLp[a-] was determined and compared with that of hedgehog apoB (Table 2). The latter was obtained by isolating the protein with M_r 520,000–570,000 from d 1.006–1.100 g/ml lipoproteins, using preparative SDS-polyacrylamide gel electrophoresis. For purposes of comparison, Table 2 also shows data from Kane et al. (32) (composition of human apoB-100). It is thus evident that the respective amino acid compositions of the three proteins are very close to each other; indeed, differences greater than 1 mol per 100 mol were confined to glutamic acid (1.8) and leucine (1.4), between hedgehog $apoB_H$ and hedgehog apoLp[a-], and to glutamic acid (1.8) and isoleucine (1.6) between hedgehog $apoB_H$ and human apoB-100.

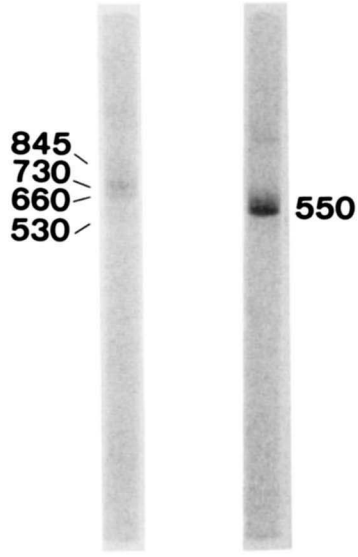

Fig. 7. Polyacrylamide gel electrophoresis of hedgehog apo[a] (left) and apoLp[a-] (right) in 3% monomer gels. Both samples originated from pools of plasma (see Results); 70–100 μg protein was applied to each gel; approximate M_r values are indicated in kDa.

TABLE 2. Amino acid composition of apolipoproteins B

Amino Acid	Hedgehog ApoB from d 1.006–1.100 g/ml Lipoproteins[b]	Hedgehog ApoLp[a-][b]	Human ApoB-100 Isolated from LDL[c]
Lys	7.4	7.2	8.0
His	2.6	2.6	2.6
Arg	3.6	3.9	3.4
Asp	10.9	10.7	10.7
Thr	6.4	6.3	6.6
Ser	8.6	7.9	8.6
Glu	13.4	11.6	11.6
Pro	4.1	4.1	3.8
Gly	5.4	6.0	4.7
Ala	6.9	6.1	6.1
Val	5.6	5.9	5.6
Met	1.5	1.0	1.6
Ile	4.4	5.4	6.0
Leu	11.6	13.0	11.8
Tyr	3.2	3.4	3.4
Phe	4.4	4.7	5.1
Cys	N.D.	N.D.	0.4

[a] Values are expressed as mol per 100 mol. Each sample of hedgehog apolipoprotein was analyzed in duplicate or in triplicate; N.D., not determined.
[b] Mean of two preparations.
[c] Data from Kane et al. (32).
[d] This mean has been calculated from results reported in refs. 35, 36, and 38.
[e] Calculated from data presented in this table.

Finally, in man, the question of the respective proportions of apo[a] and apoB-100 in Lp[a] particles is not fully elucidated at present (see Discussion). We have addressed this question in the hedgehog by isolating from d 1.006–1.100 g/ml lipoproteins, using preparative SDS-polyacrylamide gel electrophoresis, the protein component with M_r 850,000–970,000 that dissociated into $apoB_H$ and apo[a] upon reduction by DTT. Table 2 presents the amino acid composition of this complex and compares it with that of a hypothetical apo[a]:apoB complex of 50:50 stoichiometry. This latter composition was calculated by combining experimental results obtained for hedgehog apo[a] and $apoB_H$. Thus, the two sets of values were found to be very close to each other, no single difference for any amino acid being in excess to 0.8 mol per 100 mol.

DISCUSSION

The present experiments have clearly shown that Lp[a] is a major component of the plasma lipoprotein spectrum in the hedgehog. To our knowledge, the presence of such a lipoprotein has until now only been demonstrated in humans and in a variety of nonhuman primates (7, 39–41). From a phylogenetic point of view, however, the hedgehog, a member of the order Insectivora, is an animal that appeared comparatively early in the evolutionary process. Our demonstration that Lp[a] exists in such an ancient animal species provides evidence for the fact that this lipoprotein has been present during most of the evolution of terrestrial animals. With this prospect in mind, studies designed to demonstrate the presence of Lp[a] in various different animal species obviously appear of interest.

Our present results regarding Lp[a] in the hedgehog can be discussed from both a structural and a metabolic viewpoint. With regard to the former, it is evident that hedgehog Lp[a] shares several important physicochemical characteristics with its human counterpart. Thus, the density range in which most Lp[a] particles are found in the hedgehog is largely similar to that in man, i.e., 1.050–1.100 g/ml. The respective sizes of the animal and human particles fall equally in the same range. Indeed, using gradient gel electrophoresis, we demonstrated a Stokes diameter for hedgehog Lp[a] in the 280–290 Å region while, as early as 1970, Simons et al. (42) reported a diameter of 255 Å for human Lp[a], using electron microscopy. This latter value is generally in agreement with reports by other investigators (9, 43). Taking into account the distinct physicochemical environments of lipoprotein particles when studied by each methodology and the criteria employed for size calibration in each case, the respective diameters of the human and hedgehog Lp[a] particles may not be significantly different.

The chemical composition of hedgehog Lp[a] differed from that of the corresponding human lipoprotein in that its triglyceride content was approximately threefold higher, at the expense of cholesteryl esters. However, this applies to Lp[a] originating from pools of plasma obtained in spring and summer and may not be valid during other periods of the year. In the European badger, another animal species exhibiting marked seasonal variations of thyroid and testis activities, we recently demon-

and [a] from hedgehog and human plasma lipoproteins[a]

Hedgehog Apo[a] from 1.055–1.100 g/ml Lipoproteins	Human Apo[a]: Mean of Recent Results in the Literature[d]	Hedgehog Apo[a]-ApoB Complex Isolated from 1.006–1.100 g/ml Lipoproteins[b]	Composition of a Hedgehog Apo[a]-ApoB 50:50 Complex[c]
2.4	2.1	5.0	4.9
3.4	2.9	2.8	3.0
5.6	5.8	4.7	4.6
9.9	8.9	10.1	10.4
9.2	10.4	7.5	7.8
8.6	7.9	8.6	8.6
13.4	13.2	13.2	13.4
10.5	10.3	6.9	7.3
8.8	8.1	7.0	7.1
6.0	7.4	6.0	6.5
7.4	6.6	6.5	6.5
1.0	1.9	1.3	1.3
2.5	2.4	4.2	3.5
4.9	5.2	9.1	8.3
5.4	5.7	4.1	4.3
1.0	1.4	3.0	2.7
N.D.	N.D.	N.D.	N.D.

strated that the respective lipid compositions of lipoprotein subfractions belonging to the d 1.006–1.063 g/ml interval and separated by heparin-Sepharose affinity chromatography undergo significant modification according to the time of the year (2). More precisely, we suggested in this same report that seasonal variations in the plasma level of male sex hormones in the badger could influence the synthesis of apoB,E receptors and the activity of lipolytic enzymes (especially hepatic lipase), thus acting on both the concentration and the triglyceride content of some low density lipoprotein particles. Bearing in mind the considerable seasonal variation of testis activity in the hedgehog (1), a similar phenomenon could exist in this latter species and thus contribute to seasonal modifications in the chemical composition of Lp[a].

The amino acid composition of hedgehog apo[a] was very similar to that determined for the corresponding human protein, which is interesting when considering the phylogenetic remoteness between the two species. However, one important aspect of Lp[a] characterization, which has not been fully dealt with in this study, is Lp[a] and apo[a] heterogeneity. In humans, Lp[a] heterogeneity was first reported by Harvie and Schultz (44), and in recent years several important studies have shed more light onto this question. Indeed, in the plasma of normolipidemic donors, Fless, Rolih, and Scanu (45) demonstrated Lp[a] heterogeneity and the presence of three different apo[a] species, with sizes respectively larger than, equivalent to, and smaller than that of apoB. For their part, Bersot et al. (35) fed normolipidemic volunteers a high fat diet; this led to the formation of d $<$ 1.006 g/ml lipoproteins with high binding affinity for the macrophage receptor for β-VLDL, and containing apo[a]$_v$, a high M_r form of apo[a]. Gaubatz et al. (36) reported the existence of four apo[a] isoforms between two donors. Finally, a family study recently performed by Utermann et al. (43) allowed these authors to categorize their patients into a series of seven phenotypes, according to the mobility of apo[a] relative to that of apoB-100, and into the respective double-band phenotypes. These results were found compatible with the concept that apo[a] phenotypes are controlled by a series of autosomal alleles at a single locus.

During the course of the present study, our main goal has been to provide unequivocal evidence for the presence of Lp[a] as a prominent lipoprotein in hedgehog plasma and to report the principal characteristics of this particle. Owing to the small amount of plasma obtainable from each animal at each series of samplings, we have found it necessary to work on pools made up of ten to twelve plasma samples. Therefore we have not been able to provide definitive data regarding hedgehog Lp[a] and apo[a] heterogeneity in single individuals, although results from our experiments suggest that such a heterogeneity may exist.

To our knowledge, no definitive information is available at present regarding the molar ratio of apoB and apo[a] in human Lp[a]. Research on this subject has been hampered for many years by the artifactual presence of albumin and apolipoproteins other than apoB and the [a] polypeptide in Lp[a] preparations (9). At present, as noted by Gaubatz et al. who have recently discussed this

question at length (36), results from different laboratories regarding human Lp[a] suggest an apoB:apo[a] molar ratio of 2:1 in this particle. However, in the light of their own experiments, these authors do not exclude a 1:1 ratio between the two proteins, an hypothesis consistent with some of our results in the hedgehog.

Such a ratio is compatible both with results from the amino acid analysis of the $apoB_H$-apo[a] complex isolated from d 1.006–1.100 g/ml lipoproteins and with the M_r of this complex in 3% monomer SDS gels when compared to those of the two bands observed after its reduction. In contrast, two series of experimental findings could argue against a 1:1 molar ratio of the two apolipoproteins present in hedgehog Lp[a]. First, when we examined (in 3% monomer SDS gels) the DTT-reduced apolipoprotein moiety of Lp[a] contained in density gradient fractions, some samples showed the presence of two bands attributable to $apoB_H$ and apo[a], respectively (see Fig. 1). In this case, however, the band representative of the protein with higher M_r (i.e., apo[a]) stained with lower intensity than the other. However, it is known that apo[a] binds Coomassie brilliant blue with much less efficiency than apoB in human Lp[a], probably due to apo[a]-linked carbohydrate interfering in the dye-binding process (36). Furthermore, results regarding the percentage of protein in hedgehog Lp[a-], compared to that in the corresponding Lp[a] sample, showed a decrease of approximately one third. Clearly then, further studies are needed, both in humans and in the hedgehog, to determine precisely the stoichiometry of the apoB:apo[a] complex in Lp[a] particles.

With regard to Lp[a] metabolism, our findings suggest that the hedgehog could prove a highly informative animal model. In humans, the plasma concentration of Lp[a], although largely variable (from traces to approximately 100 mg/100 ml, see refs 9 and 46), is always lower than that of LDL particles, the latter being defined as lipoproteins whose principal or exclusive apolipoprotein component is apoB-100. From data reported in the present study, we are not able to deduce precise values for the respective plasma concentrations in the hedgehog of LDL (defined as above) and of Lp[a]. However, a comparison of the respective intensities of staining of the different lipoprotein bands seen upon gradient gel electrophoresis of density gradient subfractions (see Fig. 2) provides clear evidence that the major apoB-containing lipoprotein in hedgehog plasma is Lp[a]. Therefore, studies regarding the consequences of such a situation on the general metabolism of lipids in the hedgehog will obviously be of great interest. Indeed, the well-known VLDL–IDL–LDL "metabolic cascade," if it is operative in the hedgehog, results only in a minimal plasma concentration of LDL (defined as above) while in contrast, much higher concentrations of Lp[a] are present. It thus remains to be determined whether or not this latter lipoprotein is, in the hedgehog, the equivalent of LDL in humans and many animal species for supplying cholesterol to peripheral tissues. If so, it will be necessary to delineate the pathway that hedgehog Lp[a] takes to reach the cell compartment, and especially whether or not specific high affinity membrane receptors are involved. In humans, conflicting results exist regarding the ability of Lp[a] to be eliminated from the plasma compartment using the apoB,E receptor pathway (19, 47–49). In the human species, it has been demonstrated that Lp[a] is not a metabolic product of other lipoproteins containing B-apolipoproteins (50), i.e., triglyceride-rich VLDL or chylomicrons. Current theories regarding human Lp[a] metabolism favor the hypothesis that this lipoprotein is biosynthesized in the liver and secreted in the form that is found in the circulation (9). On the other hand, at least during the period of the year when our experiments were performed, hedgehog Lp[a] appears much richer in glycerides than its human counterpart (approximately 12–14% vs. 3–5%), and exists in plasma at higher concentration than VLDL. Thus, if the site of synthesis of hedgehog Lp[a] is similar to that in man, i.e., the liver, then this particle is the prominent carrier for endogenous triglycerides in this animal. With this prospect in mind, it is important to note that the hedgehog, a typical hibernator, exhibits profound hypothermia and considerable modification of its metabolism during the cold months. Whether these seasonal changes result in modified plasma concentration of Lp[a] or in compositional changes in the lipid moiety of this lipoprotein particle, or both, should be the subject of future experimental work.

On the other hand, in man and other animal species studied to date, triglyceride-rich lipoproteins are catabolized under control of lipolytic enzymes (lipoprotein lipase and hepatic lipase) in the final stages of IDL to LDL conversion (51–53); these enzymes, and especially the latter, are regulated by thyroid hormones (54). It remains to be determined whether these enzymes act on hedgehog Lp[a] and, if so, what influence the seasonal variations in thyroid activity demonstrated by one of us in the hedgehog (1) can have on Lp[a] catabolism.

Finally, in humans, an elevated plasma level of Lp[a] has been repeatedly related to a higher risk of coronary heart disease and the progression of atherosclerotic lesions [4–12]. Furthermore, Lp[a] has been detected by the immunofluorescence technique in the intima of such lesions (55). Therefore, studies on hedgehog arterial walls will be of equal interest in investigating what consequences, if any, the prominent role that Lp[a] seems to play in this animal has on tissues prone to atherosclerosis. Studies on these different aspects of hedgehog Lp[a] are now in progress in our laboratory.

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