Plasma and hepatic apoE isoproteins of nonhuman primates. Differences in apoE among humans, apes, and New and Old World monkeys

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Abstract We have used two-dimensional polyacrylamide gel electrophoresis (PAGE) to study the plasma and hepatic apoE isoproteins of nonhuman primates and have compared them with their human counterparts. We have found that apoE obtained from fresh monkey or ape plasma, as well as nascent apoE synthesized by perfused monkey livers, is composed of several isoproteins that resemble the homozygous (β) apoE phenotype observed in humans. The nonhuman primate plasma apoE pattern of 90 animals from nine different species consisted of a major isoprotein designated apoE3 and a few minor isoproteins. A group of acidic apoE isoproteins is eliminated after treatment with *C. perfringens* neuraminidase and has been designated sialo apoE ($apoE_s$). Nonhuman primate liver apoE isoproteins comigrate with their plasma apoE isoprotein counterparts on two-dimensional PAGE, but hepatic apoE is enriched in sialo apoE isoproteins when compared to plasma apoE. The apparent molecular weight of asialo and sialo apoE obtained from Old World monkeys and apes is identical to the molecular weight of the corresponding human isoproteins (E3 = 38K, E_s = 38.5–39.5K). However, the apparent molecular weight of apoE isoproteins obtained from New World monkeys is increased by approximately 0.5K (E3 = 38.5K, E_s = 39.0–40.0K) as compared to the molecular weight of human and Old World monkey and ape isoproteins. The isoelectric points of apoE3 obtained from Old World monkeys, New World monkeys, chimpanzees, and gibbons are 5.74, 5.76, 5.95, and 5.89, respectively. The entire New or Old World monkey, chimpanzee, and gibbon apoE pattern is shifted by approximately −2.0, −0.5, and −1.0 charges, respectively, relative to the pattern of the corresponding human E3/3 phenotype. The molecular weight difference in apoE observed among New and Old World monkeys, as well as the molecular weight and/or charge differences observed among monkey, ape, and human apoE are consistent with structural changes in the apoE gene which have occurred following the divergence of the different species. The observation of only the homozygous apoE phenotypes in all animals studied suggests that the common apoE genetic polymorphism recently described in humans may not be present in nonhuman primates. — **Zannis, V. I., R. J. Nicolosi, E. Jensen, J. L. Breslow, and K. C. Hayes.** Plasma and hepatic apoE isoproteins of nonhuman primates. Differences in apoE among humans, apes, and New and Old World monkeys. *J. Lipid Res.* 1985. **26:** 1421–1430.

Supplementary key words two-dimensional polyacrylamide gel electrophoresis • evolution of primate apoE

Apolipoprotein E was first identified in human VLDL (1) and has subsequently been found in various lipoprotein classes of all mammalian species studied (2, 3). Human apoE is a single polypeptide chain composed of 299 amino acids of known sequence (4). ApoE synthesis has been demonstrated in liver, kidney, adrenal gland, and reticuloendothelial cells (5–10). In addition, apoE mRNA has been found in all human and animal tissues studied (11–13). Newly synthesized apoE is found in VLDL and in nascent discoidal HDL particles (5, 6, 14–16). Recent studies have shown that apoE is an important determinant of lipoprotein catabolism in both extrahepatic (17–19) and hepatic tissues (20–24). Alterations in apoE concentration and its distribution between various lipoproteins during diet-induced hypercholesterolemia of human and experimental animals indicate a potential role for apoE in regulating plasma cholesterol metabolism (2, 3, 25–27). We have recently reported an extensive polymorphism of apoE in humans which results in part from genetic variability of the apoE allele in the human population and from a post-translational modification of an original apoE polypeptide with carbohydrate chains containing sialic acid (28–31). The purpose of the present study was to investigate the biochemical characteristics of plasma and hepatic apoE in nonhuman primates and search for inter- and intra-species variability in apoE.

EXPERIMENTAL PROCEDURES

Materials

Bovine serum albumin, ovalbumin, lysozyme, trypsin inhibitor, O-phenanthroline, phenylmethane sulfonyl

Abbreviations: PAGE, polyacrylamide gel electrophoresis; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; LDL, low density lipoproteins; apoE, apolipoprotein E; $apoE_s$, sialo apolipoprotein E.

fluoride, neuraminidase (*C. perfringens*), Tris, glycine, and glutamic pyruvic transaminase and glutamic oxaloacetic transaminase reagent kits were purchased from Sigma Chemical Company. Acid and alkaline phosphatase were purchased from Worthington Biochemical Corp. and phospholipase C was from Calbiochem. Ampholines pH 2.5–4.0 and 5–8 were obtained from LKB. Nonidet P-40 was purchased from Particle Data Laboratories, Ltd. Sodium dodecyl sulfate (SDS), manufactured by British Drughouse Chemical Ltd., was purchased through Gallard-Schleisinger. Acrylamide, bisacrylamide N,N,N',N',tetramethylenediamine, ammonium persulfate, Coomassie brilliant blue, bromophenol blue, and Biolites (ampholines, pH 4–6) were obtained from BioRad. Urea ultrapure grade was a product of Schwarz-Mann. [^{35}S]Methionine (300 Ci/mm) and Na^{125}I (17 Ci/mg) were obtained from New England Nuclear Corp. X-ray film, Cronex-4, was purchased from Du Pont. Eagle's minimum essential medium was purchased from Microbiological Associates.

Nonhuman primate selection and lipid determination

Monkey species were maintained at the New England Primate Center or the Department of Nutrition, Harvard School of Public Health. The monkeys used were not inbred and only some of the animals were first generation born in captivity. The chimpanzee and gibbon plasma specimens were provided by Dr. H. McClure of the Emory University, Yerkes Regional Primate Center, Atlanta, GA.

Blood was drawn from animals after a 12-hr fast into EDTA-containing tubes. For certain experiments, DTNB was added to a final concentration of 0.1 mM and the plasma was separated by centrifugation at 2,500 rpm for 20 min in a refrigerated centrifuge at 4°C.

Lipoprotein separation by density gradient ultracentrifugation

Density gradient ultracentrifugation was performed according to the method of Redgrave, Roberts, and West (32). Four ml of plasma was adjusted to a density of 1.21 g/ml with potassium bromide and overlayered sequentially with 3 ml each of d 1.063 g/ml and d 1.019 g/ml potassium bromide solutions followed by 2.9 ml of normal saline. The tubes were then centrifuged in a Beckman SW41 rotor at 38,000 rpm for 44 hr. Liver perfusate was concentrated to 4 ml and fractionated in a similar fashion. After centrifugation, ten 1-ml fractions were collected from the top of the tube and 0.3-ml aliquots from each fraction were dialyzed, lyophilized, and analyzed by one-dimensional SDS or two-dimensional polyacrylamide gel electrophoresis (33).

Liver perfusion

Squirrel monkeys were anesthetized with sodium pentobarbital (34 mg/kg body weight) following an 18-hr fast. Ten ml of blood was collected from the inferior vena cava and used for plasma lipoprotein fractionation. The bile duct, portal vein, and hepatic vein were cannulated and the liver was immediately perfused with Krebs-Ringer bicarbonate buffer containing 2% albumin as previously described (34). To assess hepatic production of apoE, the liver was then removed from the animal and perfused for 4 hr with 125 ml of methionine-free Eagle's minimum essential medium supplemented with 2 mM glutamine, 4% fatty acid-poor albumin, 20 mM glucose, penicillin (5 μg/100 ml), streptomycin (5 μg/100 ml), and 1 mCi of [^{35}S]methionine. The perfusate was recirculated and the perfusion pressure was determined by gravity with a hydrostatic pressure of 15 cm of water and a flow rate of 60 ml/min. During the perfusion, the medium was constantly oxygenated in a glass lung with a humidified phase of 95% O_2 and 5% CO_2. Liver viability was judged by gross appearance, perfusion flow rate, bile flow, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase as previously described (34). The total protein output of the perfused liver was 40.5 μg of protein/g of liver per hr. The perfusate was concentrated at 4°C in a Serva Speed-Vac Concentrator to 4 ml and used for lipoprotein fractionation.

Enzymatic treatments of plasma and hepatic apoE

Lipoprotein fractions containing plasma or hepatic apoE were dissolved in either 0.1 M sodium acetate (pH 5), 0.1 M Tris HCl (pH 8), or 0.1 M Tris HCl (pH 7.3) containing 0.05 M $CaCl_2$ at a protein concentration of 1 mg/ml. These fractions were then treated as follows. *a*) Fractions in sodium acetate buffer (0.5 ml) were incubated with either two units of neuraminidase (*C. perfringens*) at 37°C for 2 hr or 250 μg of acid phosphatase at 25°C for 1 hr; *b*) fractions in 0.1 M Tris HCl (pH 8) (0.5 ml) were incubated with two units of alkaline phosphatase at 25°C for 1 hr; and *c*) fractions in 0.1 M Tris HCl (pH 7.3) containing 0.05 M $CaCl_2$ (0.5 ml) were incubated with 40 μg of phospholipase C at 37°C for 2 hr. Control experiments were also performed where the above fractions were supplemented with a combination of protease inhibitors (35) consisting of 20 μg/ml trypsin inhibitor, 3×10^{-4} M phenylmethane sulfonyl chloride, 3×10^{-4} M O-phenanthroline, and 1×10^{-2} M EDTA. After incubation, the fractions were heated at 55°C for 10 min. The treated samples were dialyzed against water, lyophilized, dissolved in O'Farrell's lysis buffer (33), and analyzed by two-dimensional polyacrylamide gel electrophoresis.

Immunoprecipitation of apoE from plasma

An aliquot of 0.5 ml of fresh monkey plasma containing 0.1 mM DTNB was mixed with 60 μl of goat anti-human apoE. This mixture was adjusted to a final concentration of 10 mM potassium phosphate (pH 7.0). Following

immunoprecipitation at 4°C for 3 days, the suspension was centrifuged in a Beckman microfuge for 3 min. The precipitate was washed twice with ice-cold normal saline, once with water, and finally suspended in the O'Farrell lysis buffer and analyzed by two-dimensional polyacrylamide gel electrophoresis as described previously (28).

One-dimensional SDS polyacrylamide slab gel electrophoresis

Electrophoresis was performed on a vertical gel apparatus as previously described (29). The separating gel (11.7% acrylamide and 0.32% bisacrylamide) and the stacking gel (4.4% acrylamide and 0.12% bisacrylamide) were prepared according to the method of Davis (36).

Two-dimensional polyacrylamide gel electrophoresis

The method used was similar to that described by O'Farrell (33) with the following modifications. The isoelectric focusing tubes were 15 cm in length with an inner diameter of 3 mm. The ampholines used were 1.4% (pH 5–8), 0.45% (pH 4–6), and 0.16% (pH 2.5–4). The samples were loaded on the first dimension without prerunning, and focusing was carried out for a total of 9,500 volt hours with the equilibration time between the two dimensions limited to 15 min.

For the second dimension, the focused cylindrical gels were placed on slab gels identical to those described above for SDS slab gel electrophoresis. After electrophoresis, the gels were fixed in 50% methanol–10% acetic acid for 1 hr and stained in a solution of 0.25% Coomassie brilliant blue, 50% methanol, and 10% acetic acid for 1 hr. Destaining was accomplished by alternating exposure of the gel for 1-hr periods to either 200 ml of fixing solution or 400 ml of water until the proper stain background was achieved. Two such exchanges were usually sufficient.

Isoelectric points of apoE isoproteins and quantitation of the relative concentration of the asialo and sialo apoE isoproteins

The isoelectric points of apoE isoproteins were determined by previously described procedures (29). The relative concentrations of the plasma apoE isoproteins were estimated from the intensity of the Coomassie brilliant blue dye eluted from the E and E_s spots of the polyacrylamide gels (37). The protein spots were cut from the two-dimensional gels, extracted overnight with 25% pyridine, and the absorbance at 605 nm was determined. The volume of the solvent used for extract was estimated to give an absorbance at 605 nm in the range of 0.05 to 0.25 optical density units. Four sets of two-dimensional gels (containing three gels each) were used for this analysis. The results of all four sets were then averaged. Control experiments in which known amounts of purified human apoE were eluted from acrylamide gels under these conditions showed linearity of absorbance at 605 nm versus protein concentration. The relative concentrations of the [^{35}S]methionine-labeled hepatic apoE and $apoE_s$ were estimated as follows. The acrylamide pieces containing the ^{35}S-labeled protein spots from four different experiments were cut from the two-dimensional gel, placed separately in 1 ml of 30% H_2O_2, and incubated at 56°C for 15 hr. An aliquot of 2 ml of the resulting solution was mixed in a vial with Instagel solution fluid and counted in a Beckman LS8100 scintillation counter. These four analyses were averaged.

RESULTS

SDS polyacrylamide gel electrophoresis of plasma lipoproteins separated by density gradient ultracentrifugation

Lipoproteins were separated from plasma in a single spin by density ultracentrifugation as explained in the Experimental Procedures. Following centrifugation, fractions of 1 ml were collected from the top of the tube. Aliquots of 0.3 ml from each fraction were dialyzed, lyophilized, and analyzed by SDS-PAGE. This type of analysis showed the apoprotein profiles of the various lipoprotein fractions. In **Fig. 1, A and B,** the lipoprotein fractions are contained in lanes c to l. The density of these fractions (in g/ml), determined from the refractive index of the solution, was as follows: c = 1.002, d = 1.005, e = 1.011, f = 1.019, g = 1.031, h = 1.042, i = 1.068, j = 1.096, k = 1.125, and l = 1.17. Thus, the fractions in lanes c and d are VLDL, the fractions in lanes e and f are IDL, the fractions in lanes g and h are LDL, and the fractions in lanes i through l are HDL.

Lipoprotein fractions obtained from the plasma of squirrel and rhesus monkeys are shown in Fig. 1, A and B, respectively. These figures show that squirrel apoE has a higher and rhesus apoE the same apparent molecular weight as human apoE. Additional experiments (not shown) revealed that the apoE of four other New World monkey species (cebus, spider monkey, owl monkey, and marmoset) had the same molecular weight as squirrel monkey apoE; whereas one other Old World monkey species (cynomolgus) had the same molecular weight as rhesus apoE. All these experiments also showed that in monkeys fed regular chow diets the majority of apoE was distributed in the HDL and LDL regions. Finally, this analysis showed that monkey apoE occasionally appears as two protein bands of different molecular weight designated E_a (lower mol. wt.) and E_b (higher mol. wt.).

Two-dimensional analysis of plasma and hepatic apoE forms. Proposed nomenclature and relative abundance of apoE isoproteins

Plasma or hepatic lipoprotein fractions containing apoE were analyzed by two-dimensional gel electrophore-

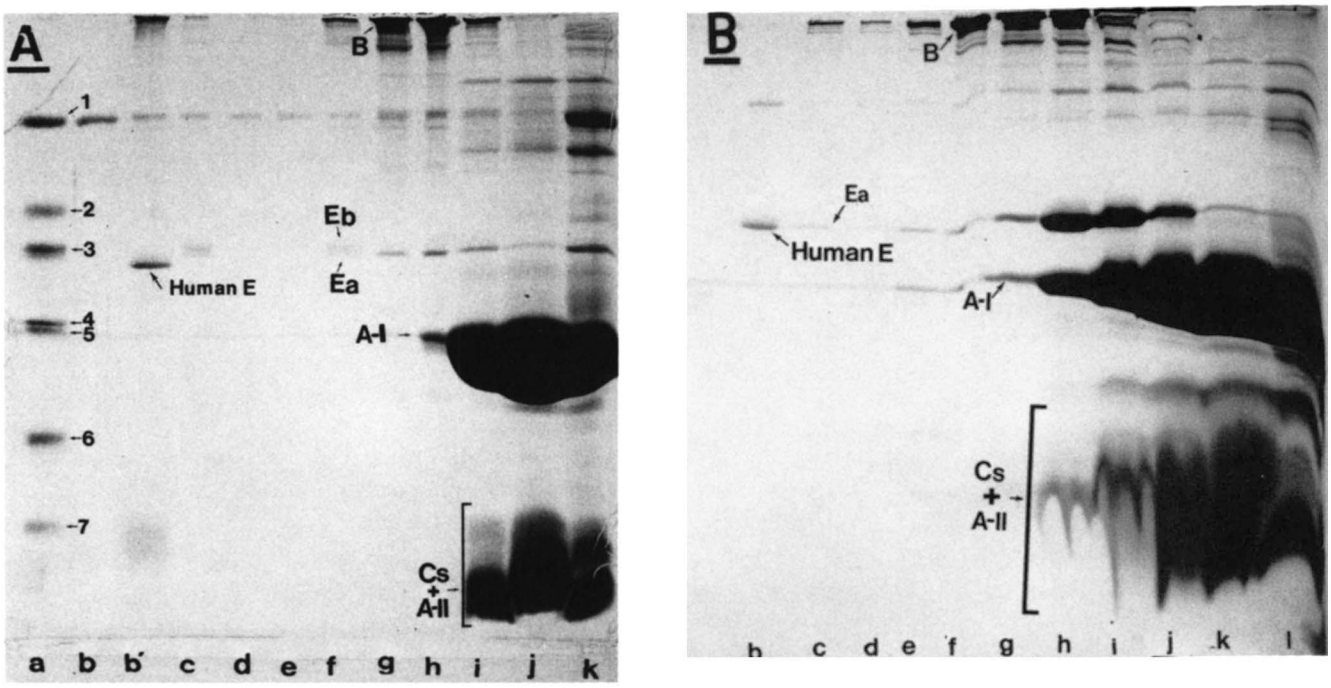

Fig. 1. Analyses of monkey plasma lipoproteins by density gradient ultracentrifugation and SDS-PAGE as explained in the Experimental Procedures. Panel A, SDS-PAGE of fresh squirrel monkey plasma. Lane a contains the following molecular weight markers: 1) bovine serum albumin, 68K; 2) ovalbumin, 43K; 3) aldolase, 40K; 4) human apoA-I, 28K; 5) chymotrypsinogen, 25.7K; 6) trypsin inhibitor, 19K; and 7) egg white lysozyme, 14.3K. Lane b contains 1 μg of bovine serum albumin. Lane b' contains normal human VLDL. The lipoprotein fractions are in lanes c through k. Panel B, SDS-PAGE electrophoresis of fresh rhesus monkey plasma. Lane b contains 1 μg of bovine serum albumin and normal human VLDL. A-I, A-II, E, and C's indicate apoA-I, apoA-II, apoE, and apoC-II plus apoC-III. In this and all subsequent figures the letters A, B, etc., which indicate the panels, are underlined.

sis and radioautography. This analysis showed that the apoE band(s) seen after one-dimensional SDS gel electrophoresis of plasma or hepatic forms of apoE can be resolved into several isoproteins (**Fig. 2, A–C**). In accordance with a recently proposed uniform nomenclature system for human apoE (31), the major asialo monkey apoE isoprotein has been designated apoE3.

A subgroup of apoE isoproteins, which are more acidic and of slightly higher molecular weight than the major apoE isoproteins, is eliminated by treatment with *C. perfringens* neuraminidase, whereas the major isoproteins apoE3 and two minor components designated $apoE2_a$ and $apoE2_b$ are not affected by the treatment (Fig. 2D). This finding suggests that this group of apoE isoproteins resulted from post-translational modification of apoE with carbohydrate chains containing sialic acid and has been collectively designated $apoE_s$. The elimination of the acidic and higher molecular weight apoE isoproteins by neuraminidase treatment was not affected by the presence of a combination of protease inhibitors in the reaction mixture (35). This observation strongly suggests that the disappearance of the sialo apoE isoproteins after treatment with neuraminidase was not the result of preferential destruction of the higher molecular weight acidic isoproteins by protease impurities that might have been present in the neuraminidase preparation. In further experiments, treatment of apoE with acid phosphatase, alkaline phosphatase, phospholipase C, or delipidation in chloroform–ether 2:1 did not affect the apoE isoprotein pattern. Quantitation of the relative concentration of apoE of squirrel monkey showed that the sialo apoE isoproteins ($apoE_s$) in the perfusate comprise 34 ± 8% of the total hepatic apoE; similar quantitation of the plasma sialo apoE isoproteins showed that they comprise 11 ± 6% of the total squirrel monkey plasma apoE. The proposed nomenclature of monkey apoE isoproteins would allow description of putative rare variant monkey apoE isoproteins similar to those seen in humans (31). For example, a more acidic variant apoE isoprotein would be designated apoE2 and would be distinguishable from $apoE2_a$. A more basic variant apoE isoprotein would be designated apoE4, etc.

Fig. 2, A–C also serves to compare the plasma and hepatic apoE isoproteins of nonhuman primates. For this comparison perfusate fractions containing apoE obtained by density gradient ultracentrifugation were mixed with plasma lipoprotein fractions containing apoE and analyzed by two-dimensional gel electrophoresis. The gel stained for protein obtained from the analysis shows the position of the carrier plasma apoE (Fig. 2A). The radioautogram of the gel shows the positions of the newly synthesized [^{35}S]methionine-labeled apoE (Fig. 2B). A

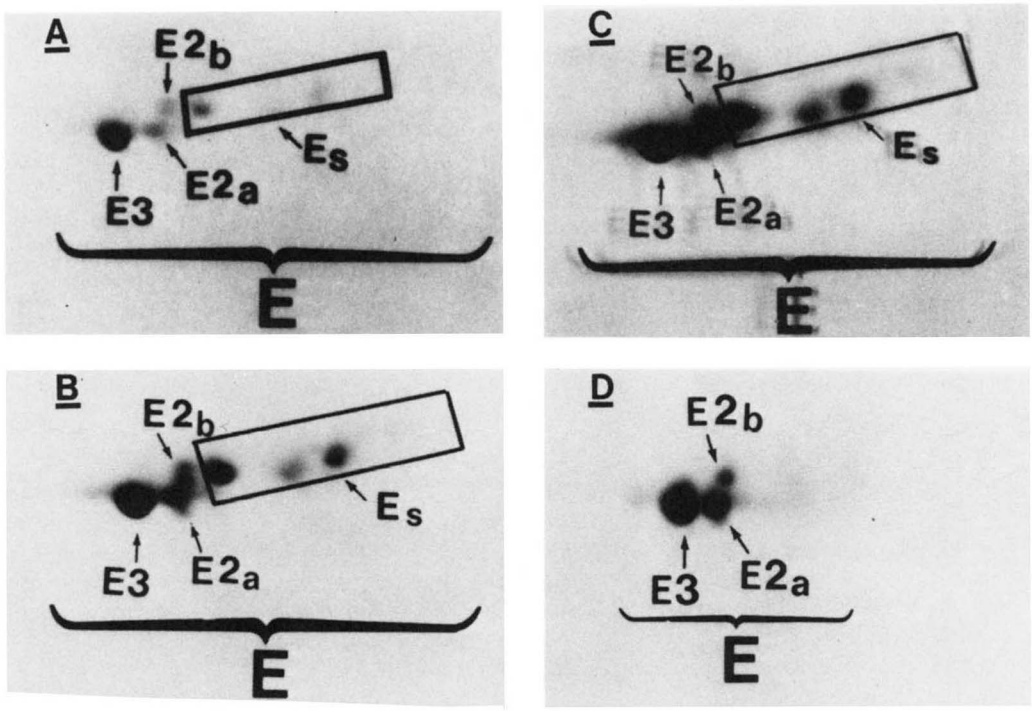

Fig. 2. Comparison of plasma and hepatic apoE isoproteins. A 50-μg aliquot of pooled plasma apoE-containing lipoprotein fractions (d 1.019–1.042 g/ml) obtained from squirrel monkeys was mixed with 150 μl of pooled apoE-containing fractions (d 1.031–1.096 g/ml) obtained from the perfused liver of the same monkey. The mixture was then subjected to two-dimensional gel electrophoresis and radioautography. Panel A is the gel stained for protein and shows the position of the carrier plasma apoE isoproteins. (In control experiments, apoE could not be detected by staining for protein when 150 μl of the concentrated perfusate was analyzed alone.) Panel B is the radioautogram of the gel in panel A and shows the position of the [^{35}S]methionine-labeled hepatic apoE isoproteins. Panel C shows the result of superimposing Fig. 2B upon Fig. 2A for comparison of the plasma and hepatic apoE isoproteins. Only the area of the gel or radioautogram in the vicinity of apoE is shown. Note the difference in $apoE_s$ between plasma (Panel 2A) and perfusate (Panel 2B). Panel D shows a radioautogram of hepatic apoE isoproteins from squirrel monkey shown in Fig. 2B following treatment with *C. perfringens* neuraminidase. Note that the neuroaminidase treatment eliminated the sialo apoE isoproteins ($apoE_s$). Mixing experiments (not shown) revealed that the positions of isoproteins apoE3, $apoE2_a$, and $apoE2_b$ are not affected by neuraminidase treatment. In this and all subsequent figures, the cathode is on the left and the anode is on the right.

comparison of the plasma with the hepatic apoproteins was obtained by superimposing the radioautogram of Panel A on the gel of Panel B (Fig. 2C). This comparison revealed homology of plasma and hepatic apoE isoproteins of squirrel monkey provided that the lipoprotein fractions were collected and stored in DTNB.

Comparison of plasma apoE obtained from humans and from nonhuman primates

Two-dimensional polyacrylamide gel electrophoresis was used to compare the plasma apoE isoproteins obtained from 90 animals representing nine different species of monkeys [including cebus (12), spider (10), owl (10), squirrel (12), marmoset (10), cynomolgus (12), and rhesus (14)] and apes [chimpanzees (5) and gibbons (5)]. This comparison was facilitated by the recent development of goat anti-human apoE antibodies which cross-react and immunoprecipitate from plasma monkey apoE (**Fig. 3A**). The immunoprecipitated apoE has the same features as apoE in VLDL prepared from plasma by density gradient ultracentrifugation (compare Fig. 2A with Fig. 3A). This analysis showed that all 90 animals studied had a homozygous apoE phenotype which has been designated E3/3. In order to compare the apoE of different species, aliquots of samples obtained from two or more species were mixed and analyzed by two-dimensional gel electrophoresis. In a typical experiment, an aliquot of unknown apoE was mixed with an aliquot containing a mixture of rhesus and/or human apoE and analyzed by two-dimensional gel electrophoresis. Fig. 3B shows a mixture of cebus and rhesus apoE and indicates that the molecular weight of cebus apoE is higher by approximately 0.5K than that of rhesus apoE. Similar experiments (Fig. 3, C and D and other data not shown) revealed that apoE of four other species of New World monkeys (squirrel, owl, spider, and marmoset) is superimposable on two-dimensional gels with the cebus apoE. Similarly, as shown in Fig. 3E, the apoE of cynomolgus, which is an Old World monkey, is superimposable on two-dimensional gels with rhesus apoE. The same figure shows that the molecular weights of the corresponding sialo apoE isoproteins are identical. This figure also shows that the monkey apoE pattern is shifted by approximately -3 charges relative to

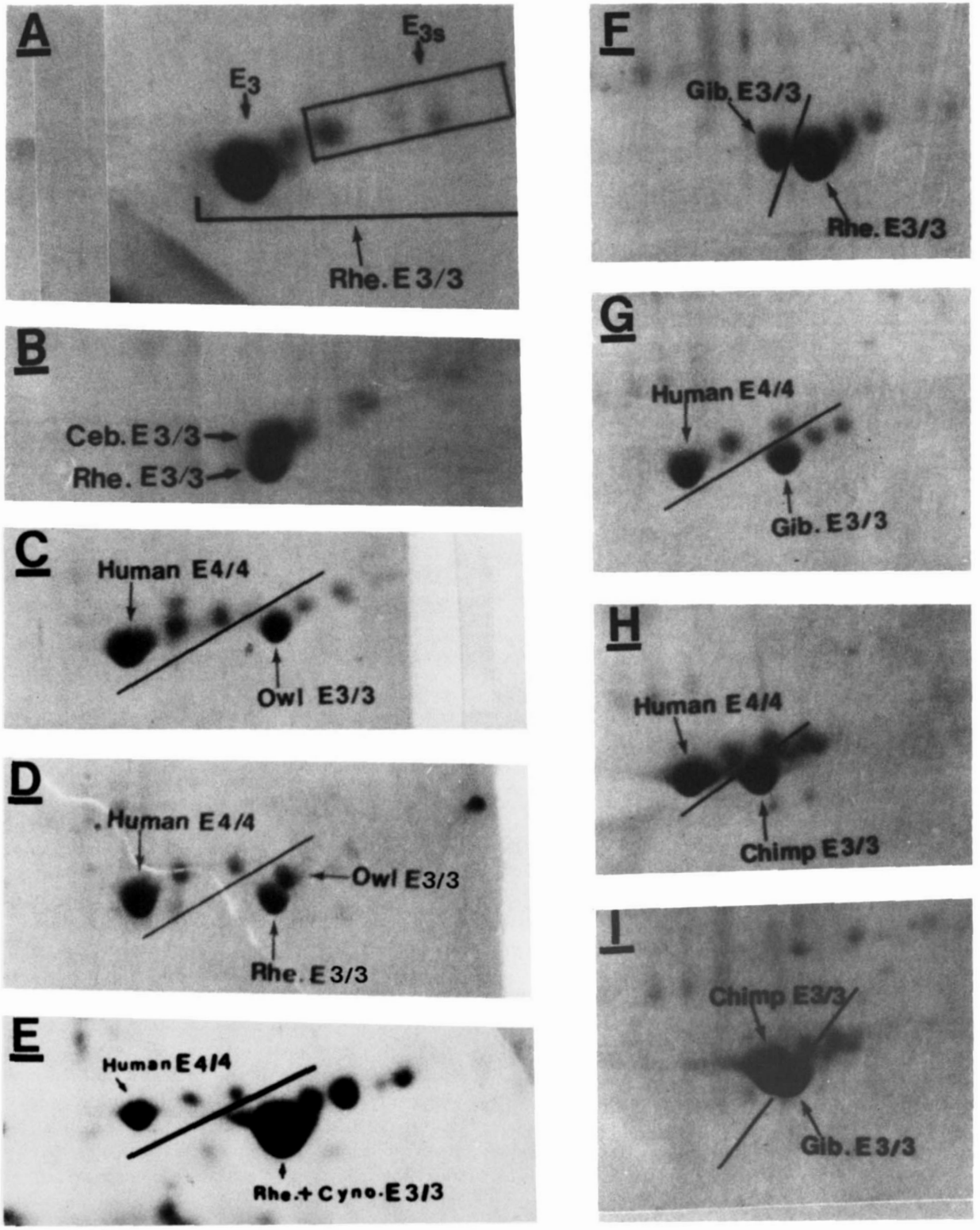

Fig. 3. A comparison of the plasma apoE patterns of humans, New World and Old World monkeys, and apes is shown. Panel A, two-dimensional gel electrophoresis pattern of 20 μg of rhesus monkey apoE immunoprecipitate from plasma with goat anti-human apoE. Panel B, two-dimensional gel electrophoresis patterns of 5 μg of cebus and 5 μg of rhesus apoE. Panel C, mixture of 20 μg of human (E4/4 phenotype) and 10 μg of owl monkey apoE. Panel D, mixture of 15 μg of human (E4/4 phenotype), 10 μg of rhesus, and 5 μg of owl monkey apoE. Note the increased molecular weight of apoE of New World monkeys (cebus, owl monkey) as compared to the apoE of Old World monkeys (rhesus) and of humans. The human apoE4 differs by +3 charges from the New and Old World monkey apoE3. Panel E, two-dimensional gel electrophoresis pattern of 8 μg of human (E4/4

the pattern of human E4/4 phenotype. Fig. 3F shows that rhesus and gibbon apoE have the same molecular weight; however, the gibbon apoE3 pattern is shifted by +1 charges relative to the rhesus apoE pattern. Fig. 3, G and H, compare the human to the gibbon and chimpanzee apoE and Fig. 3I compares the gibbon to the chimpanzee apoE. These figures show that the molecular weights of human and ape apoE are the same; however, the human apoE4/4 pattern is shifted by +2 and +1.5 charges relative to the gibbon and chimpanzee apoE3/3 pattern. The gibbon apoE pattern is shifted by −0.5 charges relative to the chimpanzee apoE pattern. The molecular weight and charge differences of primate apoE are shown diagramatically in **Fig. 4.**

DISCUSSION

ApoE, first identified in 1973, has attracted considerable research interest in recent years. Numerous studies have established that apoE plays an important role in lipoprotein catabolism by extrahepatic tissues (17–19). In addition, it has been shown that apoE mediates the hepatic uptake and catabolism of chylomicron remnants and other apoE-containing lipoprotein particles and through this mechanism might play an important role in the regulation of hepatic cholesterol biosynthesis (20–24). Extrahepatic tissues catabolize apoE-containing lipoproteins by the LDL (apoB/E) receptor (17–19). Hepatic tissues catabolize apoE-containing lipoproteins by the LDL (apoB/E) receptor as well as by the chylomicron remnant (apoE) receptor (24, 38). A role for apoE in lipoprotein metabolism has also been suggested by various studies with animals, in which cholesterol feeding has resulted in the accumulation in plasma of lipoprotein particles designated HDL_c, βVLDL, and IDL, which are not normally present in large amounts in plasma (2, 3, 25–27). All of these lipoproteins are enriched in cholesteryl ester and apoE and have a high affinity for the fibroblast LDL (apoB/E) receptor and the hepatic chylomicron remnant (apoE) receptor (17–24).

We have recently shown that the complex array of isoproteins seen in humans results both from genetic variation and post-translational modification of human apoE (28–31). Family studies have shown that the genetic polymorphism of apoE results from three common apoE alleles (ϵ4, ϵ3, and ϵ2) at a single genetic locus. The three apoE alleles generate six common apoE phenotypes designated E4/4, E3/3, E2/2, E3/2, and E4/2 (28–31). One of these phenotypes, E2/2, is found in 90% of the patients with type III hyperlipoproteinemia (type III HLP) (39, 40). The previous work of other laboratories and our own studies all indicate that apoE is intimately involved in the regulation of plasma lipoprotein levels and thus may play a role in atherogenesis. Since an important animal model for the study of atherosclerosis has been the nonhuman primate (41–44), the purpose of this investigation has been to describe the monkey apoE isoproteins and relate these findings to those of human apoE. SDS polyacrylamide gel electrophoresis of plasma lipoproteins separated by density gradient ultracentrifugation showed that the majority of plasma apoE of monkeys fed a chow diet resides in the LDL and HDL. In addition, it revealed the presence of a second apoE band designated E_b. Two-dimensional gel electrophoresis showed that the plasma and hepatic apoE pattern resembles that of human subjects with the homozygous (β) apoE phenotype. We have shown that several minor monkey apoE isoproteins are removed by treatment with *C. perfringens* neuraminidase, suggesting that these isoproteins result from post-translational modification of apoE with carbohydrate chains containing sialic acid. We have previously shown that similar sialo apoE isoproteins exist in humans (29), but in monkey plasma the relative sialo apoE isoprotein concentration is lower than that observed in human plasma (45). The finding that sialo apoE isoproteins are more abundant in nascent hepatic apoE from perfused monkey liver compared to plasma apoE suggests that the liver synthesizes and secretes sialo apoE which then is desialated in plasma. Increased sialation has also been observed in human apoE which is synthesized by liver organ cultures (8) and by human hepatoma cell lines (10). The physiological significance of sialo apoE isoproteins is not currently known. One possibility is that sialation may be required for apoE secretion; another is that sialation may prevent cells from recognizing, internalizing, and catabolizing apoE just after it has been secreted.

In contrast to previous findings with humans, where we observed both homozygous (β) and heterozygous (α) apoE phenotypes (28–31), the present study showed that 90 nonhuman primates originating from nine different species have only one type of homozygous apoE pheno-

phenotype), 15 μg of rhesus, and 15 μg of cynomolgus apoE. Note the similarity in apoE of the two Old World monkey species and their charge differences from human apoE. Panel F, mixture of 25 μg of rhesus and 15 μg of gibbon apoE. The gibbon apoE3 differs by +1 charges from the rhesus apoE3. Panel G, mixture of 20 μg of human (E4/4 phenotype) and 15 μg of gibbon apoE. The human apoE4 differs by +2 charges from the gibbon apoE3. Panel H, mixture of 15 μg of human (E4/4 phenotype) and 15 μg of chimpanzee apoE. The human apoE4 differs by +1.5 charges from the chimpanzee apoE3. Panel I, mixture of 15 μg of gibbon and 15 μg of chimpanzee apoE. The gibbon apoE differs by −0.5 charges from the chimpanzee apoE3.

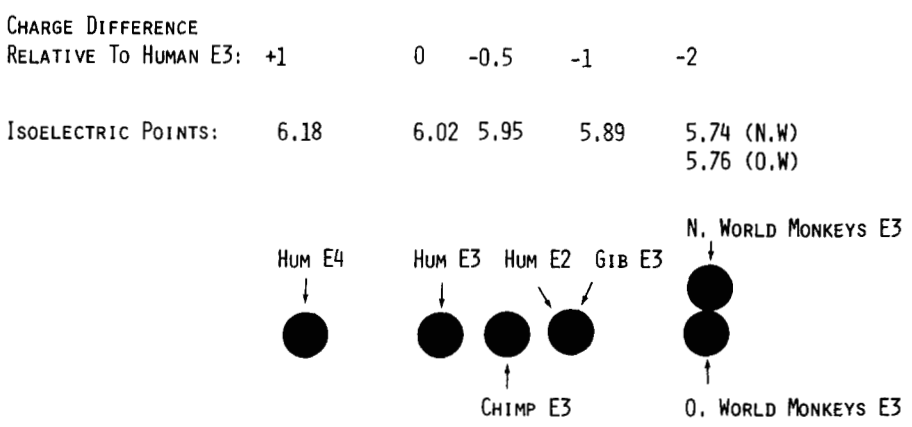

Fig. 4. Schematic presentation and isoelectric points of the major plasma apoE isoproteins of primates.

type. This phenotype, in accordance with the human apoE nomenclature (31), has been designated as monkey (i.e., rhesus, cebus, etc.) E3/3 phenotype. This finding suggests that the common genetic variation in the apoE polypeptide seen in humans (29, 30) may not be present in nonhuman primates, but does not preclude the existence of rare apoE variants or other genetic variants not detectable by the techniques employed in the present study. We have also observed two minor components in the two-dimensional pattern designated apoE2_a and apoE2_b. The concentration of these components increases upon storage of plasma samples at 4°C in the absence of DTNB, whereas the concentration of the original asialo apoE3 isoprotein decreases (Zannis, V. I., and J. L. Breslow, unpublished results).

Two important findings of these studies are the small molecular weight differences observed in apoE of New and Old World monkeys as well as the molecular weight and/or charge differences between human, ape, and monkey apoE. These changes are indicative of structural apoE gene mutations that occurred during the evolution of the primates. Based on homologous DNA sequence evolution, it has been estimated that the New and Old World monkeys and apes diverged from humans 36, 22, and 4 million years ago, respectively (46, 47). It has been proposed recently that the most frequent apoE allele (E3) gave rise to the less frequent (E2 and E4) as well as to rare apoE alleles (48). The similarity of the ape apoE phenotypes with the less frequent apoE phenotype E2/2 raises the possibility that E2 may be the ancestral gene; evolutionary pressure due, for example, to change in diet may have forced a rapid mutation of the human E2 to E3 and E4 alleles. In the near future, it will be possible to use existing molecular probes (49) to study the structural changes of apoE genes that led to the different forms of apoE observed in the present study. It will also be important to compare the in vitro and in vivo catabolism, as well as the affinity of human and monkey apoE to lipoprotein receptors. Such studies will establish the structural and functional similarities as well as the overall evolution of primate apoE.

This study was supported by NIH grants HL23792, HL26812, HL33952, USDA #8000479, and RR00168 from the Division of Research Resources, and a grant, #83-963, from the American Heart Association. Jan L. Breslow and Vassilis I. Zannis are Established Investigators of the American Heart Association.

Manuscript received 12 December 1983.

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