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Synthesis and Secretion of Apolipoprotein A-I by Chick Skin*

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Chick skin slices were incubated with [^{35}S]methionine and labeled apoA-I was immunoprecipitated from incubation medium and tissue homogenate. ApoA-I accounted for approximately 13 and 2.5% of radioactive medium and cell proteins, respectively. After ultracentrifugation of the medium, 55% of labeled apoA-I was found as a constituent of lipoproteins ($d < 1.210$ g/ml) and 45% in a lipid-poor form (1.210–1.260 g/ml). To ascertain whether this large proportion of lipid-poor apoA-I was due to a dissociation of this peptide from medium lipoproteins during ultracentrifugation, labeled incubation medium was applied to an anti-chick apoA-I immunoaffinity column. The material bound to the column was analyzed by nondenaturing polyacrylamide gradient gel electrophoresis and found to contain three subpopulations of lipoproteins with a particle size of 12, 11, and 9 nm, respectively. The radioactivity of these subpopulations accounted for 82% of total radioactive medium apoA-I. ApoA-I was localized by immunohistochemistry in the viable cells of the epidermis and in the stratum corneum. Rat skin slices were found to synthesize and secrete apoE but no apoA-I.

ApoA-I and apoE secreted by chick and rat skin, respectively, may play a role in the secretion of lipids from the differentiating keratinocytes and thus contribute to the formation of the hydrophobic barrier of the skin.

Plasma lipoproteins are macromolecules consisting of complexes of lipids and one or more specific proteins called apolipoproteins (1). These water-soluble macromolecules serve as transporters of lipids in the extracellular compartments among the various cells of the body (1). Although liver and small intestine represent the main sites of synthesis of lipids and apolipoproteins and secretion of lipoproteins (1), a measurable production of some apolipoprotein occurs also in a variety of peripheral tissues. In mammals, various cell types (macrophages, astrocytes, Kuppfer cells, smooth muscle cells) synthesize and secrete a specific apolipoprotein, that is, apolipoprotein E (apoE)[1] and various organs (such as brain, kidney, spleen, adrenals, ovary, etc.) contain apoE mRNA and produce this peptide (2–8). The synthesis of other apolipoproteins (A-I, B, A-IV, and C) in mammalian peripheral tissues is negligible or extremely low (2, 9). Since apoE is present in high concentrations in interstitial fluid, it has been suggested that apoE participates in the cholesterol redistribution from cells with an excess of cholesterol to those requiring cholesterol (see Ref. 2, for review).

Apolipoprotein synthesis in peripheral tissues has been documented also in avian species. In the chick, whose plasma lipoproteins do not appear to contain apoE (10, 11), several organs (such as brain, heart, skeletal muscle, kidney, spleen) synthesize apolipoprotein A-I (apoA-I) or contain apoA-I mRNA (12–19). Furthermore, an increased synthesis of apoA-I occurs at sites of peripheral nerve injury and regeneration (20, 21) and in the dystrophic muscle (15). In previous studies it was reported that a striking production of apoA-I occurs in skeletal muscle of the newborn chick (13–15, 18) presumably in relation to the depletion of lipids which takes place in muscle during the first week of postnatal life (11). In view of these observations it has been proposed that in chick peripheral tissues, apoA-I functions as a local lipid transporter as apoE does in mammalian peripheral tissues (17, 18, 20).

The skin is one of the peripheral organs that is most actively involved in the production and redistribution of lipids (22–24). In both mammals and birds lipids synthesized by viable epidermal cells are present intracellularly in both lipid droplets and membrane-enclosed lamellar granules (multigranular bodies). The latter are extruded from the uppermost viable cells into the extracellular space where they spread to form broad multilamellar sheets that constitute the water barrier (25–29). In view of these findings we assumed that the production and intracellular transport of lipids, the assembly of multigranular bodies, and the secretion of lipid material by chick skin might be associated with the local synthesis of some apolipoprotein (for example, apoA-I) and the formation and secretion of lipoprotein particles. While our study was in progress some reports documented that human keratinocytes and grafts of human epidermal keratinocytes secrete apoE (30–32). In the present study we demonstrate that chick skin synthesizes apoA-I that is secreted predominantly as a constituent of lipoprotein particles.

EXPERIMENTAL PROCEDURES

Animals—One-day-old chicks (Arbor Acres strain) obtained from a local supplier and 15-day-old male Sprague-Dawley rats were used. After killing, skin without hair was excised from the back and dissected free of fat and connective tissue.

Metabolic Labeling—Freshly excised skin (200 mg) pooled from three animals was rinsed in cold phosphate-buffered saline, cut into 2-mm slices, and incubated at 37 °C in 5 volumes (v/tissue weight) of methionine-free Dulbecco's modified Eagle's medium supplemented with 50 units/ml penicillin and 50 μg/ml streptomycin containing 600 μCi/ml of [^{35}S]methionine (800 Ci/mmol) (Amersham, United Kingdom) for 12 h (11). In some experiments chick skin slices were

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[1] The abbreviations used are: apo, apolipoprotein; SDS, sodium dodecyl sulfate; HSS, high speed supernatant; HDL, high density lipoprotein.

incubated in Dulbecco's modified Eagle's medium containing 50 units/ml penicillin and 50 μg/ml streptomycin but no [^{35}S]methionine. Medium was harvested, adjusted to contain 0.2 mg of phenylmethylsulfonyl fluoride/ml, centrifuged twice at 12,000 × *g* for 5 min, and dialyzed for 48 h at 4 °C against 0.9% NaCl or 10 mM NH_4HCO_3, containing 1 mM Na_2EDTA, 1 mM methionine, and 100 kallikrein units/ml of Trasylol (33). Tissue slices were washed with cold phosphate-buffered saline containing 1 mM unlabeled methionine. The slices were homogenized in 15 volumes (v/tissue weight) of 20 mM Na-phosphate, pH 7.4, 150 mM NaCl, 5 mM Na_2EDTA, 2% Triton X-100, 200 μg/ml of phenylmethylsulfonyl fluoride, and centrifuged for 1 h at 226,000 × *g* at 4 °C to prepare the high speed supernatant, (HSS) (33).

Immunoprecipitation and Gel Electrophoresis—Aliquots of radiolabeled media and HSS were analyzed for chick apoA-I and albumin, and rat apoA-I and apoE by immunoprecipitation (11, 34). In the case of chick apoA-I, the incubation medium and HSS were precipitated with nonimmune rabbit IgG before the addition of the specific anti-apoA-I IgG. Immunoprecipitates and total proteins of the incubation medium and HSS were analyzed by SDS-polyacrylamide gradient (5–20%) gel electrophoresis (11, 34). Gels were treated for fluorography and exposed to Hyperfilm-MP (Amersham) at −80 °C. Densitometric scanning of fluorograms was performed using a XL-Ultroscan Laser Densitometer (LKB, Sweden). In some experiments the bands corresponding to radioactive apoA-I were cut from the dried gels and counted by liquid scintillation counting (33).

Separation of Lipoproteins from the Incubation Medium and Chick Plasma—Labeled incubation medium of chick skin slices was dialyzed (as specified above) and subjected to density gradient ultracentrifugation (10, 11). After centrifugation, aliquots of 500 (fraction 1) or 400 μl (fractions 2–24) were collected and precipitated with trichloroacetic acid for radioactivity counting. ^{35}S-Labeled apoA-I was immunoprecipitated from the pooled fractions of the density gradient corresponding to HDL peak (11). Lipoproteins were separated from unlabeled incubation medium and chick plasma using the same ultracentrifugation procedure. The protein concentration of each density fraction was measured by the method of Lowry *et al.* (35). Lipoprotein density fractions isolated from both labeled and unlabeled medium were analyzed by SDS-polyacrylamide gradient (5–20%) gel electrophoresis (11). Gels were stained with Coomassie Blue and processed for fluorography. Pooled HDL fractions (1.070–1.150 g/ml) of unlabeled incubation medium and plasma were analyzed by nondenaturing polyacrylamide gradient gel electrophoresis (see below). HDL lipids were extracted, separated by thin layer chromatography, and measured colorimetrically (11).

To obtain the HDL apolipoproteins, an aliquot of chick plasma HDL was delipidated according to the procedure of Osborne (36). Total HDL apolipoproteins (>90% apoA-I) were dissolved in 10 mM Tris, 100 mM NaCl, 1 mM Na_2EDTA, pH 7.4, and analyzed by nondenaturing gel electrophoresis (see below).

Immunoaffinity Chromatography of Labeled Lipoproteins—^{35}S-Labeled incubation medium of chick skin slices was dialyzed against 150 mM NaCl, 15 mM Tris, pH 7.4, and applied to a column of anti-chick apoA-I IgG covalently linked to CNBr-activated Sepharose 4B (11). The anti-chick apoA-I IgG reacted only with apoA-I on an immunoblot of total chick plasma lipoproteins. The material bound to the immunoaffinity column was eluted with 1 M acetic acid, pH 3, and immediately neutralized with 2 M Tris solution (11). Both bound and unbound fractions were monitored for protein concentration (absorbance at 280 nm) and trichloroacetic acid-precipitable radioactivity. The bound fractions were pooled, concentrated by Centricon 10 microconcentrators (Amicon), and immediately analyzed by nondenaturing polyacrylamide gradient gel electrophoresis using Pharmacia precasted PAA 4/30 gels (37). Nondenaturing gels were stained with Coomassie G-250, processed for fluorography, and exposed to x-ray films (34). The densitometric scanning of the Coomassie-stained gel and the corresponding fluorogram were performed as specified above.

RNA Extraction and Northern Blot Hybridization—Total cellular RNA was extracted from pools of chick skin and livers (taken from 10 animals) by the guanidine HCl method as previously described (34). Northern blots of skin and liver RNA were prepared as described (18) following fractionation through 2.2 M formaldehyde, 1% agarose gel. Blotting membrane was prehybridized and hybridized at 42 °C in 50% formamide, 3 × SSC (1 × SSC = 154 mM NaCl, 15 mM Na citrate, pH 7), 50 mM Tris-HCl, pH 7.5, 5 mM Na_2EDTA, 1 × Denhardt's solution, 0.2 mg/ml denatured herring sperm DNA, 0.5% SDS, and 10% dextrane sulfate containing 0.8 × 10^6 cpm/ml of chick apoA-I ^{32}P cDNA probe (18). Washing was carried out at room temperature in 2 × SSC, 0.1% SDS and 0.1 × SSC, 0.1% SDS prior to autoradiography.

Histochemistry and Immunohistochemistry—Fragments of skin were fixed in 4% paraformaldehyde in phosphate-buffered saline and embedded in paraffin. Tissue sections (5-μm thick) were incubated in either rabbit anti-chick apoA-I IgG or rabbit preimmune IgG for 24 h at 4 °C (11). Immunopositive areas were visualized by the peroxidase antiperoxidase system. Nuclei were counterstained with hematoxylin (11). Skin fragments were fixed also in 2.5% glutaraldehyde in Tyrode's solution and embedded in Spurr resin. Semi-thin sections (0.8 μm thick) were stained with 0.1% Toluidine Blue-O.

RESULTS

In Vitro Synthesis and Secretion of apoA-I by Chick Skin—To study the synthesis of apoA-I, skin slices taken from 1-day-old chicks were incubated in the presence of [^{35}S]methionine in short-term organ culture. We chose newborn animals since their skin is thin, almost free of fat, and easy to remove from the subcutaneous tissue. Histological examination of several skin sections showed the presence of epidermis, consisting of the stratum corneum, three layers of viable epithelial cells, and the dermis containing collagen fibrils, capillaries, and occasionally, few areas of isolated muscle fibers (data not shown) (28). ^{35}S-Labeled apoA-I was immunoprecipitated from the medium and the HSS of skin slice homogenates (Fig. 1, *lanes 4–7*). ApoA-I was clearly detectable in the fluorogram of total medium and HSS proteins separated by SDS-polyacrylamide gel electrophoresis (Fig. 1, *lanes 1–3*). Densitometric scanning of the fluorogram revealed that apoA-I accounted for approximately 13% of total medium proteins and

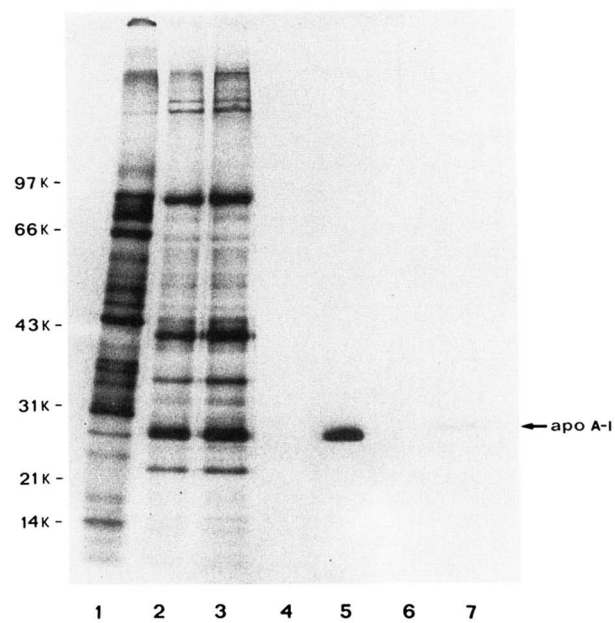


FIG. 1. **Biosynthesis and secretion of apoA-I by chick skin.** Skin slices of 1-day-old chicks were incubated in short-term organ culture with [^{35}S]methionine. Total cellular proteins (4 × 10^5 cpm) (*lane 1*) and total medium proteins (3 × 10^5 cpm and 5 × 10^5 cpm, *lanes 2* and *3*, respectively) were fractionated by SDS-polyacrylamide gradient (5–20%) gel electrophoresis and detected by fluorography. Equal amounts of total protein radioactivity were immunoprecipitated with anti-chick apoA-I IgG from the incubation medium (*lane 5*) and the 226,000 × *g* supernatant of skin homogenates (HSS) (*lane 7*). Immunoprecipitation of the medium and HSS with preimmune IgG is shown in *lanes 4* and *6*, respectively. Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis. The gel was processed for fluorography and exposed to x-ray film for 48 h at −80 °C. The *arrow* indicates the mobility of chick plasma apoA-I. *Lines* and *numbers* on the side indicate the mobility and the molecular mass of protein markers.

2.5% of total HSS proteins. These results were confirmed by direct counting of radioactive apoA-I eluted from the SDS gels (Table I).

To ascertain whether the synthesis and secretion of apoA-I by chick skin reflected a more general capacity of producing other plasma proteins, ^{35}S-labeled medium and HSS were immunoprecipitated with anti-chick albumin IgG. No labeled albumin was detectable either in the cells or in the medium, even after a prolonged exposure of the gel to x-ray film (data not shown).

To investigate whether apoA-I was synthesized also by the skin of a mammalian species, the *in vitro* production of apoA-I and apoE was investigated in 15-day-old rats. The immunoprecipitation of radioactive medium with specific immune IgGs revealed that rat skin secreted apoE but no apoA-I (Fig. 2).

Isolation of ApoA-I containing Lipoproteins from the Incubation Medium—The medium of chick skin slices that had been incubated with [^{35}S]methionine was subjected to density gradient ultracentrifugation to investigate whether labeled apoA-I was secreted as a component of lipoprotein particles. The profile of the density gradient of the labeled incubation medium shows a peak of trichloroacetic acid-precipitable radioactivity in the 1.070–1.150 g/ml density interval, corresponding to the typical density range of chick plasma HDL (Fig. 3B). After immunoprecipitation with anti-chick apoA-I IgG, the pooled fractions of this peak were found to contain a peptide comigrating with chick plasma apoA-I in SDS-polyacrylamide gel electrophoresis (Fig. 3, *inset*). The peak of radioactive high density lipoproteins shown in Fig. 3*B* was superimposable to that obtained after density gradient ultracentrifugation of unlabeled incubation medium (Fig. 3*A*).

TABLE I

[^{35}S]Methionine incorporation into cell and medium proteins

Skin slices of 1-day-old chicks were incubated in short-term organ culture with [^{35}S]methionine. Total protein radioactivity was measured after precipitation of medium and cell proteins with trichloroacetic acid. Medium and cell proteins were separated by SDS-polyacrylamide gradient (5–20%) gel electrophoresis followed by fluorography. The bands corresponding to apoA-I were cut from the gel and counted by liquid scintillation counting.

	^{35}S-Labeled	
	Total proteins	ApoA-I
	cpm × 10^3/mg wet wt	
Medium (experiment 1)	105.5	14.5
Medium (experiment 2)	108.3	14.6
Cell (experiment 1)	1403.4	39.3
Cell (experiment 2)	1710.5	44.5

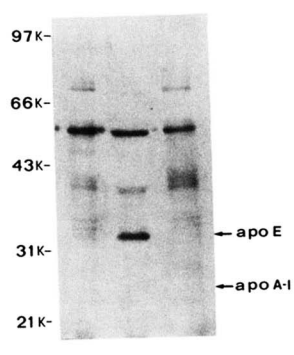


FIG. 2. **Biosynthesis and secretion of apoE by rat skin.** Skin slices of 15-day-old rats were incubated with [^{35}S]methionine in short-term organ culture. Labeled proteins present in the incubation medium were immunoprecipitated with anti-rat apoA-I IgG (*lane 1*), anti-rat apoE IgG (*lane 2*), preimmune IgG (*lane 3*), and analyzed as described in the legend of Fig. 1. *Arrows* indicate the migration of rat plasma apoE and apoA-I. *Lines* and *numbers* on the side indicate the migration and the molecular mass of protein markers.

To further study the density distribution of labeled apoA-I in the lipoproteins of the incubation medium, all fractions of the density gradient were analyzed by SDS-polyacrylamide gel electrophoresis followed by fluorography. Labeled apoA-I was detectable in all fractions of density less than 1.260 g/ml (Fig. 4, *lanes A–O*). After densitometric scanning of this fluorogram and taking into account the total protein radioactivity of each density fraction, we found the following distribution of labeled apoA-I: 10% in the fractions of density less than 1.075 g/ml; 32% in the density fractions 1.070–1.150 g/ml; 13% in the density fractions 1.150–1.210 g/ml; 45% in the density fractions 1.210–1.260 g/ml. The density distribution of labeled apoA-I was superimposable to that of apoA-I present in lipoproteins isolated from the unlabeled incubation medium by density gradient ultracentrifugation (data not shown). The fluorogram of Fig. 4 shows that the lipoproteins floating in the 1.100–1.180 g/ml density interval contained an additional radioactive band with a molecular mass of 52 kDa (Fig. 4, *lanes F–K*). No attempts were made to isolate and characterize this protein.

To investigate whether the large proportion of radioactive apoA-I found in the lipid-poor fractions of the density gradient (1.210–1.260 g/ml) (Fig. 4) was an artifact due to prolonged ultracentrifugation, medium lipoproteins were separated by alternative techniques. To isolate apoA-I containing material (*i.e.* apoA-I lipid complexes and apoA-I in lipid-poor form) the radioactive incubation medium was applied to an affinity column prepared with immune IgG monospecific for chick apoA-I. As shown in the inset of Fig. 5 the unbound fractions contained no apoA-I, whereas the fractions bound to the immunoaffinity column contained only radioactive apoA-I. The bound fractions also contained unlabeled apoA-I as detected in the Coomassie-stained SDS-polyacrylamide gel (data not shown). The apoA-I containing fractions were pooled and subjected to nondenaturing polyacrylamide gradient gel electrophoresis. The gel was stained with Coomassie Blue and treated for fluorography. In the Coomassie-stained gel the apoA-I containing material was found to consist of different amounts of three clearly defined subpopulations of lipoproteins (with a particle size of 12, 11, and 9 nm, respectively) and an ill defined component that appears as a pale smear in the lower part of the gel (Fig. 6, *lane 1*). The two most abundant subpopulations (with a particle size of 11 and 9 nm) were similar to those of HDL isolated by density gradient ultracentrifugation from 1 day-old-chick plasma or the unlabeled incubation medium of skin slices (Fig. 6, *lanes 2–3*). In the Coomassie-stained gel, apoA-I obtained from delipidated chick plasma HDL appears as a smear in the lower part of the gel (Fig. 6, *lane 4*). In the fluorogram the apoA-I containing material isolated from the incubation medium was found to contain three subpopulations of labeled lipoproteins with a particle size similar to that of the corresponding unlabeled material (Fig. 6, *lane 5*). The fluorogram also shows the presence of an additional band that appears as a smear in the lower part of the gel. Its mobility was partly superimposable to that of apoA-I obtained from delipidated plasma HDL (Fig. 6, *lanes 4* and *5*). Densitometric scanning of the fluorogram showed that the three main subpopulations of lipoproteins accounted for 83% of the total radioactivity incorporated into material containing apoA-I.

Lipid Composition of Medium HDL—HDL (1.070–1.150 g/ml) isolated from the unlabeled incubation medium of skin

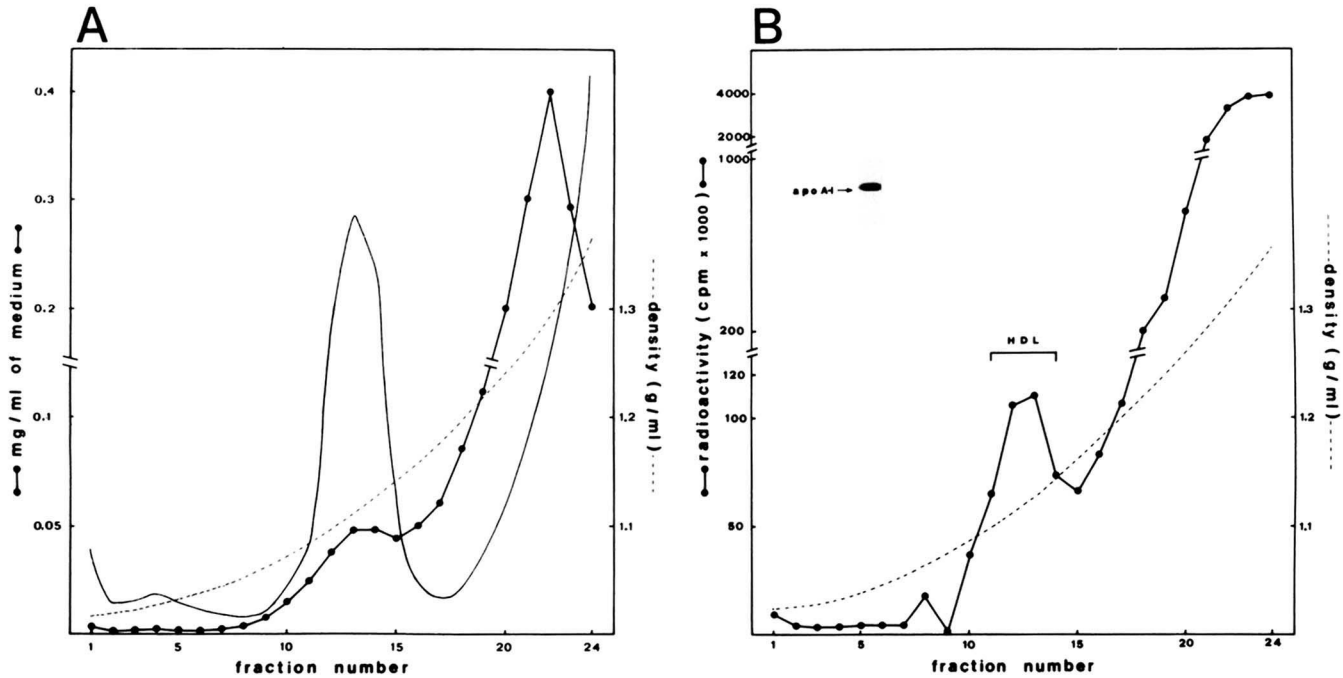
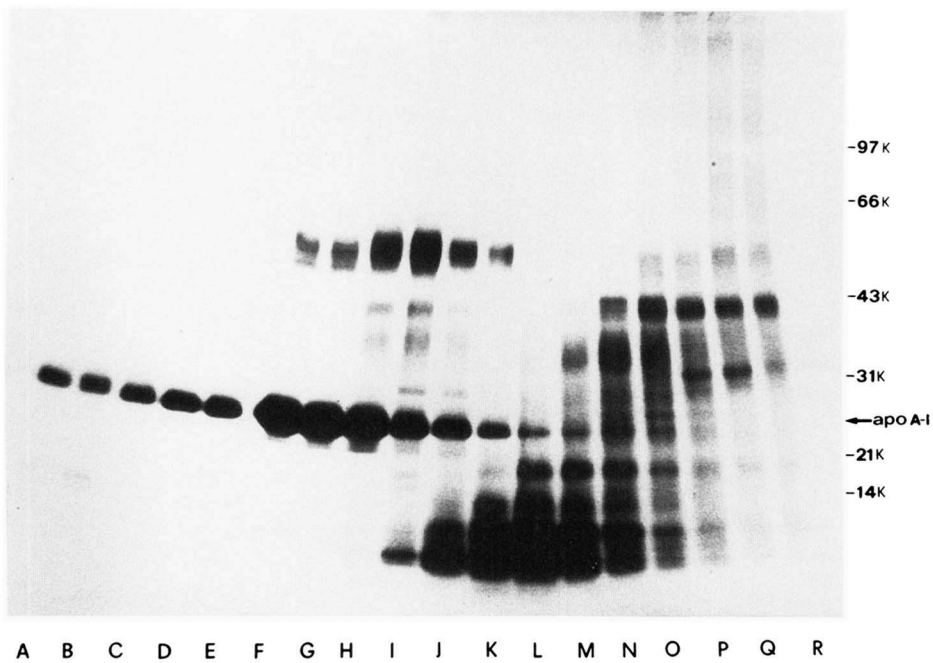


FIG. 3. **Density gradient profile of lipoproteins isolated from the incubation medium of skin slices.** Skin slices of 1-day-old chicks were incubated either in the absence or presence of [^{35}S]methionine in short-term organ culture. The incubation medium was subjected to density gradient ultracentrifugation to separate lipoproteins. *A*, the density distribution of unlabeled lipoproteins present in the incubation medium (●) and the density profile of plasma lipoproteins of 1-day-old chicks (——). *B*, the density distribution of protein radioactivity after the precipitation of each fraction with trichloroacetic acid. Fractions 11–15, corresponding to the HDL peak (1.070–1.150 g/ml) were pooled, dialyzed, and immunoprecipitated with anti-chick apoA-I IgG. The immunoprecipitate was analyzed by SDS-polyacrylamide gradient (5–20%) gel electrophoresis and fluorography (*inset*).

FIG. 4. **^{35}S-Labeled apolipoprotein A-I distribution in lipoproteins isolated from the incubation medium.** Skin slices of 1-day-old chicks were incubated in the presence of [^{35}S] methionine in short-term organ culture. The incubation medium was subjected to density gradient ultracentrifugation as shown in Fig. 3. Aliquots of the density fractions were analyzed by SDS-polyacrylamide gradient (5–20%) gel electrophoresis followed by fluorography. 20 × 10^3 cpm of protein radioactivity were applied to *lanes A–E*; 80–100 × 10^3 cpm of protein radioactivity were applied to *lanes F–R*. *Lane A*, gradient fractions 1–3; *lane B*, gradient fractions 4–6; *lane C*, gradient fractions 7–9. Individual gradient fractions 10–24 were applied to *lanes D–R*. The *arrow* indicates the mobility of chick plasma apoA-I. *Lines* and *numbers* on the side indicate the mobility and the molecular mass of protein markers.



slices by density gradient ultracentrifugation (Fig. 3*A*) had the following relative lipid composition (mean ± S.D. of three experiments): free cholesterol, 26 ± 1.2%; cholesteryl esters, 25.7 ± 2.3%; phospholipids, 46.9 ± 3.3%; triacylglycerols, 1.3 ± 0.1%. The relative lipid composition of HDL (1.070–1.150 g/ml) isolated from 1-day-old chick plasma was the following: free cholesterol, 15.2 ± 1.3%; cholesteryl esters, 44.2 ± 1.2%; phospholipids, 39.3 ± 0.8%; triacylglycerols, 1.2 ± 0.4%.

ApoA-I mRNA in Chick Skin—RNA extracted from chick skin (pooled from 10 newborn chicks) was assayed for the presence of apoA-I mRNA by using a full-length cDNA probe for chick apoA-I. Fig. 7 shows that a substantial amount of apoA-I mRNA was found in chick skin. The size of skin apoA-I mRNA (~1 kilobase) was identical to that of liver apoA-I mRNA. The counting of the bands corresponding to skin and liver apoA-I mRNA indicated that skin apoA-I mRNA was approximately 6–9% of that found in the liver.

ApoA-I Localization in Chick Skin—Immunohistochemical studies were carried out to identify the cellular and extracellular localization of apoA-I in chick skin. As shown in Fig.

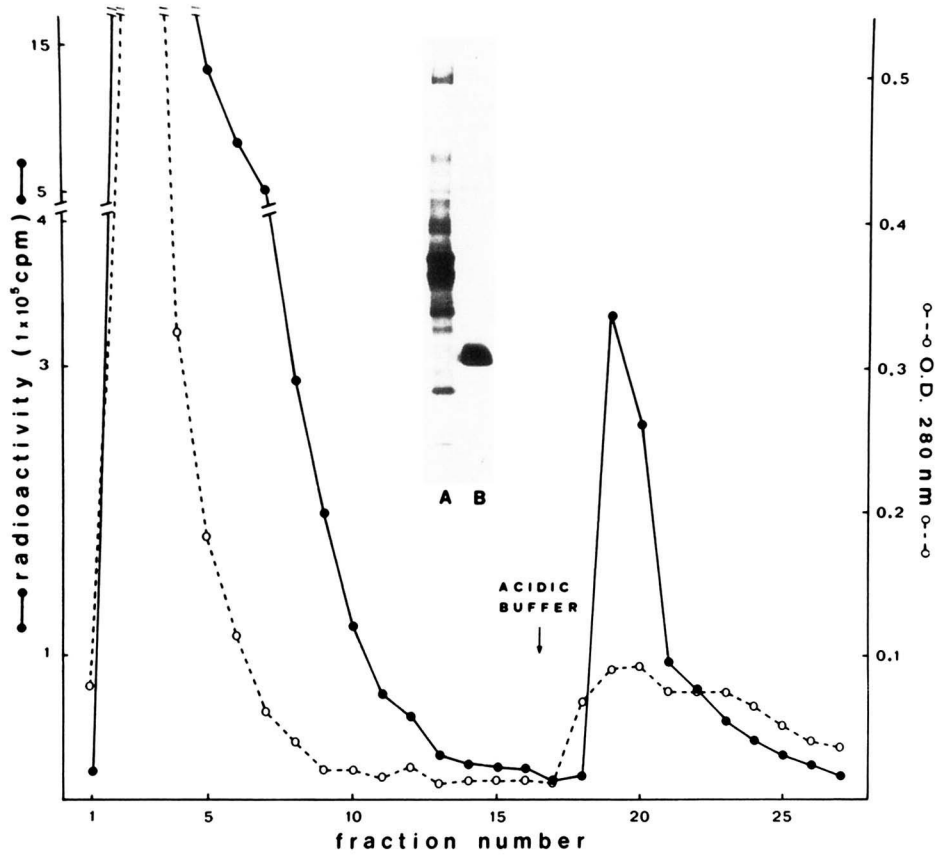


FIG. 5. **Immunoaffinity chromatography of labeled incubation medium.** Skin slices of 1-day-old chicks were incubated in the presence of [^{35}S] methionine in short-term organ culture. The incubation medium was applied to a column of anti-chick apoA-I IgG covalently linked to CNBr-activated Sepharose 4B. Fractions eluted from the column were monitored for trichloroacetic acid-precipitable radioactivity and absorbance at 280 nm. Unbound (1–16) and bound (17–27) fractions were pooled and fractionated by SDS-polyacrylamide gradient (5–20%) gel electrophoresis followed by fluorography, as shown in the *inset. Lane A,* unbound fraction; *lane B,* bound fraction.

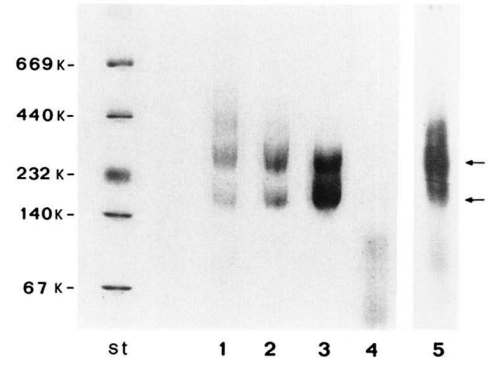


FIG. 6. **Nondenaturing polyacrylamide gel electrophoresis of apoA-I containing lipoproteins.** The apoA-I containing material isolated by immunoaffinity chromatography from the incubation medium of skin slices incubated with [^{35}S]methionine (Fig. 5) was analyzed by nondenaturing gradient (4–30%) polyacrylamide gel electrophoresis (*lane 1*). The electrophoretic separation of HDL (1.070–1.150 g/ml) isolated from the unlabeled incubation medium and chick plasma by density gradient ultracentrifugation is shown in *lanes 2* and *3*, respectively. *Lane 4* shows the migration of chick apoA-I obtained after delipidation of plasma HDL. The gel was stained with Coomassie Blue G-250 and treated for fluorography. The fluorogram shown in *lane 5* corresponds to *lane 1*. 10–20 μg of protein were applied to *lanes 1–4*. 8×10^4 cpm were applied to *lane 5*. The *arrows* indicate the two subpopulations of plasma HDL (1.070–1.150 g/ml). *Lines* and *numbers* on the side indicate the mobility and molecular mass of protein markers (thyroglobulin, 669 kDa; apoferritin, 440 kDa; catalase, 232 kDa; lactate dehydrogenase, 140 kDa; bovine albumin, 67 kDa). The hydrated Stokes diameters were 17, 12.2, 10.4, 8.2, and 7.5 nm, respectively.

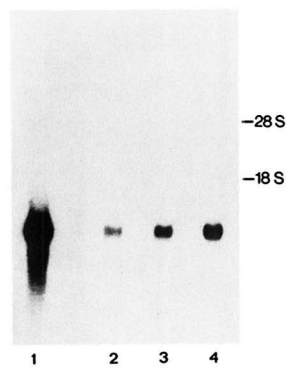


FIG. 7. **Northern blot analysis of chick skin apoA-I mRNA.** Total RNA extracted from pooled skins and livers taken from 1-day-old chicks was denatured, electrophoresed in 1% agarose gel, blotted onto a nylon membrane, and hybridized with ^{32}P-labeled chick apoA-I cDNA as described under "Experimental Procedures." *Lane 1,* 20 μg of total RNA from 1-day-old chick liver; *lanes 2–4,* 10, 20, and 30 μg of total RNA from 1-day-old chick skin.

8*A*, an intense immunopositive reaction was found in the viable epithelial cells and in the stratum corneum of the epidermis. In the dermis we could detect a very faint positivity that was spread among the collagen fibers. No immunopositive areas were found in the few muscle fibers of the dermis. No stained areas were seen in the sections incubated with rabbit preimmune IgG (Fig. 8*B*).

DISCUSSION

The present study shows that the skin of the newborn chick produces apoA-I *in vitro*. This statement is supported by the following observations: (*a*) skin slices incorporate [^{35}S]methionine into apoA-I and secrete it into the incubation medium; (*b*) the presence of apoA-I can be demonstrated in the keratinocytes by immunohistochemistry; (*c*) a measurable amount of apoA-I mRNA is present in the skin. In view of these results the skin can be included among the peripheral tissues of the chick that are capable of producing apoA-I (12–21). It

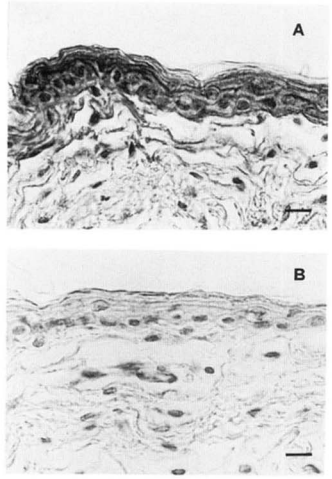


FIG. 8. **Immunolocalization of apoA-I in chick skin.** Skin sections of 1-day-old chicks were incubated either with anti-chick apoA-I rabbit IgG (*A*) or preimmune rabbit IgG (*B*). Immunopositive (*dark*) areas were visualized by the peroxidase-antiperoxidase system. Nuclei were counterstained with hematoxylin. *Bar*, 10 μm.

has emerged from previous studies that the total protein synthetic effort devoted to the production of apoA-I by the peripheral tissues of the chick varies considerably, according to the type of tissue and the age of development. For example, around the time of hatching the relative rate of apoA-I synthesis by breast muscle is over 4% of total protein synthesis (13–15). By contrast, at the same developmental age, the relative rate of synthesis of apoA-I by other muscles, such as the cardiac muscle, is much lower (13). Under our experimental conditions newly synthesized apoA-I accounted for 13 and 2.5% of medium and cellular proteins, respectively. This large production of apoA-I by chick skin is also supported by the observation that measurable amounts of unlabeled apoA-I accumulate in the incubation medium of skin slices during a short-term organ culture (Figs. 3 and 6). Thus the skin of the chick appears to be one of the most active apoA-I producing tissues, at least in the early stages of postnatal development.

In order to investigate whether apoA-I produced by the skin is present in the incubation medium in a lipid-poor form or as a constituent of lipoprotein particles, we used two different methodological approaches (*i.e.* density gradient ultracentrifugation and immunoaffinity chromatography followed by nondenaturing polyacrylamide gradient gel electrophoresis). When the radioactive incubation medium was subjected to density gradient ultracentrifugation, 55% of labeled apoA-I was found to be bound to lipids (as a constituent of lipoproteins of density less than 1.210 g/ml) and 45% in a lipid-poor form (material floating mostly in the 1.210–1.260 g/ml density interval). The bulk of labeled lipid-bound apoA-I was present in HDL-like particles floating in the density range typical of chick plasma HDL (1.070–1.150 g/ml). The observation that a large amount of newly synthesized apoA-I was found in the medium in a lipid-poor form was not surprising. It has been previously reported in other tissues (12) or cell systems (38) that approximately 50% of radioactive (as well as unlabeled) apoA-I was present in the lipid-poor fractions ($d > 1.210$ g/ml) of the incubation medium. These results can be due either to the fact that apoA-I is secreted largely in a lipid-deficient form or to artifacts produced by the dissociation of apoA-I from nascent lipoproteins during ultracentrifugation. In order to overcome the artifacts of ultracentrifugation, we isolated the apoA-I containing material present in the medium by immunoaffinity chromatography and analyzed it in nondenaturing gel electrophoresis. When this material (that included lipid-bound and lipid-poor apoA-I) was subjected to nondenaturing gel electrophoresis, we found that most of the radioactivity was present in two subpopulations of macromolecules whose particle size was similar to that of the two subpopulations of HDL (1.070–1.150 g/ml) isolated from plasma or the unlabeled incubation medium of skin slices. The low radioactivity found in the lower part of the nondenaturing gel where delipidated apoA-I is expected to migrate, indicates that apoA-I is present in the incubation medium of skin slices predominantly as a constituent of lipoprotein particles. The discrepancy between this result and that obtained after the separation of medium lipoproteins by ultracentrifugation can be explained by assuming a dissociation of apoA-I from lipoproteins caused by prolonged ultracentrifugation.

The lipid composition of HDL (1.070–1.150 g/ml) isolated from the incubation medium of skin slices was different from that of plasma HDL (1070–1.150 g/ml). Medium HDL were enriched in phospholipids and contained equimolar amounts of free and esterified cholesterol, whereas in plasma HDL the content of free cholesterol was one-third that of cholesteryl esters. This difference in lipid composition suggests that the unlabeled HDL isolated from the incubation medium of skin slices do not derive from plasma HDL but are most likely produced by the skin. The high relative content of cholesteryl esters in HDL secreted by the skin might be due to the presence of lecithin-cholesterol acyltransferase in keratinocytes. A recent report indicates that, at least in rhesus monkey, lecithin-cholesterol acyltransferase mRNA is abundant in the basal cell layer of the epidermis (39). The lipid composition of skin HDL differs substantially from that of the whole chick epidermis that contains much more triacylglycerols and less cholesterol and phospholipids (40). This difference in lipid composition suggests that HDL produced by the skin are responsible for the removal of a defined intracellular pool of lipids of which cholesterol and phospholipids are the main constituents.

In chick skin, apoA-I may be involved in the process of assembly and release of lipid material from epidermal cells. During differentiation of chick epidermis from the basal cells to keratinocytes, lipid droplets, as well as multigranular bodies, appear in the cytoplasm (25–28). Multigranular bodies are ovoidal lipid-rich corpuscles surrounded by a trilaminar membrane which contain one or several stacks of oval discs. It is assumed that when the cells enter the horny stage the content of multigranular bodies is extruded into the intercellular space where it rearranges into broad sheets running in parallel to the surface of the horny cells (25–29). The morphological observations and the biochemical studies are consistent with the idea that the discs of multigranular bodies are lamellar structures similar to flattened phospholipid liposomes (28–29). During differentiation, apoA-I synthesized by the epithelial cells, by binding phospholipids and nonpolar lipids, would facilitate the intracellular transport of lipids, their assembly in the multigranular bodies and the extracellular release of lipids (such as cholesterol, cholesteryl esters, and triacylglycerols) contained in the lipid droplets. It is not surprising therefore to find apoA-I within the keratinocytes and in the lamellar sheets of the stratum corneum as shown by immunohistochemical studies. In this context apoA-I can be regarded as an important constituent of the highly impermeable barrier that protects the body from water loss and penetration of material from the environment.

Our study suggests that there are similarities of functions between apoA-I synthesized by chick skin and apoE synthesized by mammalian skin. ApoE is normally made by human

skin and it has been suggested that keratinocyte-derived apoE participates in local lipid transport associated with the formation of the lipid-rich lamellar layers of the stratum corneum (30–32). In the present study we show that also in the rat, an animal species capable of producing both apoA-I and apoE, only the latter is synthesized and secreted by the skin. In the chick, in which the apoE gene is not expressed, apoA-I produced by the skin appears to replace apoE as the major lipid carrier protein involved in the local transport of lipids.

Our observations therefore lend further support to the general idea that apoA-I might be the avian counterpart to mammalian apoE and the two proteins may perform functions common to both species not only in the local transfer of lipids in peripheral tissues (17–21) but, most likely, also in the reverse cholesterol transport.

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