Secretion of apoB- and apoA-I-containing lipoproteins by chick kidney

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Abstract Previous studies showed that chick kidney is a site of synthesis of apolipoprotein (apo) B(B-100) and A-I. Aims of the present study were: *a*) to compare apoB and apoA-I production in chick kidney and liver; *b*) to investigate whether kidney apolipoproteins were secreted as constituents of lipoproteins; and *c*) to define the cellular sites of renal apolipoprotein synthesis. Kidney and liver slices taken from the same animals were incubated with ^{35}S-labeled amino acids and radioactive apoB and apoA-I were immunoprecipitated from cell homogenate and incubation medium. The percentage of total protein radioactivity incorporated into cell plus medium apoB and apoA-I was 0.23 ± 0.08 and 0.19 ± 0.11 in kidney and 0.38 ± 0.05 and 0.38 ± 0.07 in liver, respectively ($P < 0.05$ kidney vs. liver). ^{35}S-labeled medium lipoproteins were separated by density gradient ultracentrifugation and three major classes corresponding to VLDL + IDL, LDL, and HDL were identified. Most of the apoB secreted by the liver was found in VLDL, IDL, and LDL whereas kidney apoB was found in VLDL, LDL and "light" HDL (d 1.070–1.130 g/ml). In both hepatic and renal lipoproteins apoA-I was found not only in HDL but also in the other lipoproteins. Immunohistochemical analysis of kidney sections showed that apoB and apoA-I were present almost exclusively in the epithelial cells of proximal and distal convoluted tubules. Thus apoB and apoA-I synthesized by the epithelial cells of the proximal and distal convoluted tubules of chick kidneys are secreted as constituents of lipoprotein particles floating within the density range of plasma lipoproteins. These observations suggest that in the chick, the kidneys may contribute to the plasma lipoprotein pool.—**Tarugi, P., G. Ballarini, B. Pinotti, A. Franchini, E. Ottaviani, and S. Calandra.** Secretion of apoB- and apoA-I-containing lipoproteins by chick kidney. *J. Lipid Res.* 1988. **39:** 731–743.

Supplementary key words chick kidney • apolipoprotein synthesis • lipoprotein secretion • tubular epithelial cells • immunohistochemistry

Chick plasma lipoproteins contain a single form of apolipoprotein B corresponding in apparent molecular mass to mammalian apolipoprotein B-100 (1–3). This apolipoprotein is the main constituent peptide of plasma VLDL, IDL, and LDL and of the portomicrons, the intestinal lipoproteins of the chick (2, 3). Little is known about the amino acid sequence of chick apolipoprotein B-100 (apoB) as only 10% of the cDNA has been sequenced so far (4). As in mammals, liver and intestine are the major sites of apoB production in the chick (5). Hepatic apoB synthesis is regulated by estrogens; in the laying hen and in the estrogen-stimulated rooster, plasma VLDL and apoB are strikingly elevated as the result of increased hepatic apoB synthesis and VLDL secretion (6–8). In sharp contrast, intestinal apoB synthesis and lipoprotein secretion do not appear to be regulated by estrogens (4). A striking difference between the mammalian species and the chick is that in the latter apoB is also synthesized by the kidney (4, 5). As for the intestine, apoB mRNA and apoB synthesis in chick kidney exhibit an estrogen-independent mode of expression (9). Although it has been known for a long time that chick kidney synthesizes apoB, it is not known whether renal apoB is secreted as a constituent of lipoprotein particles that may contribute to the plasma pool of apoB-containing lipoproteins.

ApoA-I is the most abundant apolipoprotein present in chick plasma, where it is found not only in HDL but also in VLDL, IDL, and LDL (2, 10). Although in the adult chick, liver and intestine represent the major sites of apoA-I synthesis and secretion, measurable amounts of apoA-I mRNA and apoA-I synthesis have been found in a variety of peripheral tissues including the kidney (11–15). As for apoB, little is known about the secretion of apoA-I-containing lipoproteins by chick kidney.

The present study addresses the following questions:

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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a) whether apoB and apoA-I production by chick kidney is comparable to that of the liver; *b*) whether the kidney secretes lipoproteins containing apoB and apoA-I; and *c*) which are the cellular sites of apoB and apoA-I synthesis in the kidney.

MATERIALS AND METHODS

Materials

Pro-mix (l-[^{35}S]methionine and l-[^{35}S]cysteine > 37 TBq/mm), [^{3}H]oleic acid (370 GBq/mmol), and Hyperfilm-MP X-ray films were obtained from Amersham (U.K.). Solvable and Formula 989 were obtained from DuPont de Nemours (Dreieich, Germany). Protein A-Sepharose was obtained from Pharmacia (Uppsala, Sweden) and silica gel G plates for thin-layer chromatography were purchased from Merck (Darmstadt, Germany). Molecular weight protein standards and nitrocellulose membranes were obtained from Bio-Rad (Richmond, CA). Polybrene, leupeptin, aprotinin, soy bean trypsin inhibitor, lima bean trypsin inhibitor, and glutathione were obtained from Sigma (St. Louis, MO). Ketalar (ketamine) was obtained from Parke-Davis (Milano, Italy).

Animals

Male chicks (Warren strain) from the same batch of eggs were obtained from a local poultry supplier. Chicks were fed a standard diet (25% soya proteins, 4.5% lipids, and 5.5% fibers) ad libitum until the time of killing. The standard diet contained 0.045% (w/w) total sterols and 0.005% (w/w) cholesterol. Chicks were killed 21 days after hatching.

Plasma lipoproteins

Chicks were anesthetized by peritoneal injection of Ketalar (5 mg/100 g body weight). Blood was collected by cardiac puncture using K_3 EDTA as anticoagulant. Plasma lipoproteins were separated from pooled plasma by density gradient ultracentrifugation (16). Apolipoproteins of lipoprotein density fractions were separated by a linear 5–20% gradient SDS–polyacrylamide gel electrophoresis (10).

Synthesis of apolipoproteins in vitro

Kidney and liver taken from the same animals were used. After exanguination tissues were perfused with cold 0.154 m NaCl, removed, carefully washed in cold 0.154 m NaCl, and cut into slices (3–4 mg per slice, 0.5–1 mm thick) (16). Tissue slices (250 mg) taken from individual animals (four animals in each experiment) were incubated in duplicate for 3 h at 40°C under an atmosphere of 95% O_2–5% CO_2, in 3 ml of Krebs-Ringer bicarbonate buffer containing: 60 μCi/ml of [^{35}S] methionine/[^{35}S]cysteine, 50 units/ml of penicillin, 50 μg/ml of streptomycin, 100 kallikrein inhibitor units/ml of aprotinin, and a mixture of unlabeled amino acids (with the exception of methionine and cysteine), each at a final concentration of 0.04 mm. At the end of the incubations, media were collected and supplemented with leupeptin (1 mm), polybrene (25 μg/ml), Na_2EDTA (0.1 mm), soy bean trypsin inhibitor (20 μg/ml), lima bean trypsin inhibitor (20 μg/ml), glutathione (0.02%), phenylmethylsulfonyl fluoride (PMSF) (0.2 mg/ml) and aprotinin (5 μg/ml). This material was exhaustively dialyzed against 10 mm NH_4HCO_3, 1 mm Na_2EDTA, Trasylol (100 kallikrein inhibitor units/ml), 1 mm methionine, and 1 mm cysteine and lyophilized. Tissue slices were washed with ice-cold Krebs-Ringer bicarbonate buffer, 1 mm unlabeled methionine and cysteine, and Trasylol (20 kallikrein inhibitor units/ml) and homogenized in 15 volumes (v/tissue weight) of 20 mm Na-phosphate buffer, pH 7, 150 mm NaCl, 5 mm Na_2EDTA, 2% Triton X-100, and 200 μg/ml PMSF. This material was centrifuged at 226,000 *g* for 1 h at 4°C to prepare the high speed supernatant (HSS) (17). Aliquots of incubation media and high speed supernatants (HSS) were immunoprecipitated by anti-chick apoB, anti-chick apoA-I, and anti-chick albumin rabbit IgG or non-immune rabbit IgG (16). The apoB immunocomplexes were dissolved in 100 mm Tris-HCl, pH 7.4, 20% glycerol, 10% SDS, and 5% 2-mercaptoethanol, heated at 100°C for 5 min, and applied to a linear 5–10% gradient SDS-polyacrylamide gel. The apoA-I and albumin immunocomplexes were dissolved in 2% SDS, 3.5% 2-mercaptoethanol, 50 mm Tris-HCl, pH 7, heated at 95°C for 3 min, and applied to a linear 5–20% gradient SDS-polyacrylamide gel (16). Gels were stained with Coomassie Blue R-250, destained, processed for fluorography, and exposed to Hyperfilm-MP X-ray films at −80°C. Radioactive bands corresponding to apoB, apoA-I, and albumin were excised from the gel and soaked in 0.5 ml of water for 15 min. After the addition of 0.5 ml of Solvable, samples were shaken for 3 h at 50°C and counted in scintillation fluid (Formula 989) according to manufacturer's instructions.

The amount of ^{35}S-labeled amino acids incorporated into total cell and medium proteins was measured by trichloroacetic acid precipitation (17). Preliminary studies showed that the incorporation of [^{35}S]methionine/[^{35}S]cysteine into cellular proteins by kidney and liver slices reached a plateau at 3 h and remained substantially stable up to 5 h. The radioactivity incorpo-

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rated into medium proteins increased linearly from 1 to 4 h and then tended to plateau.

Isolation of ^{35}S-labeled lipoproteins secreted in vitro

In some experiments, incubation media of kidney and liver slices that had been incubated in the presence of 400 μCi/ml of [^{35}S]methionine/[^{35}S]cysteine for 3 h as specified above were dialyzed against 0.154 m NaCl, 10 mm Na_2EDTA, 1 mm methionine, 1 mm cysteine, and Trasylol (100 kallikrein inhibitor units/ml) and then subjected to density gradient ultracentrifugation to separate ^{35}S-labeled lipoproteins (16). After centrifugation, aliquots of 500 μl (fraction 1) and 400 μl (fractions 2–24) were collected and their protein radioactivity was measured after precipitation with 10% trichloroacetic acid. Aliquots of each density fraction were applied to a linear 5–20% gradient SDS-polyacrylamide gels for the separation of ^{35}S-labeled apolipoproteins (16). Gels were stained with Coomassie Blue R-250, destained, processed for fluorography, and exposed to Hyperfilm-MP X-ray films at −80°C.

In one experiment, radioactive incubation media of kidney slices were pooled and subjected to sequential ultracentrifugation to separate the following lipoprotein fractions: VLDL (d < 1.006 g/ml), IDL + LDL (d < 1.006–1.063 g/ml), and HDL (d < 1.063–1.210 g/ml). These lipoprotein fractions as well as the lipoprotein infranate (d > 1.210 g/ml) were exhaustively dialyzed against 0.154 m NaCl, 10 mm Na_2EDTA, 1 mm methionine, 1 mm cysteine, and Trasylol (100 kallikrein inhibitor units/ml). An aliquot of each lipoprotein class was immunoprecipitated with anti-chick apoB rabbit IgG (as specified above). The apoB immunocomplexes were dissolved as specified above and applied to a linear 5–10% gradient SDS-polyacrylamide gels for the separation of ^{35}S-labeled apoB. The gels were processed for fluorography and exposed to X-ray films as specified above.

Isolation of ^{3}H-labeled lipoproteins secreted in vitro

In one experiment, aliquots of kidney and liver slices (~60 mg) taken from four animals were pooled and duplicate samples were pulse-labeled for 1 h in 3 ml of Krebs-Ringer bicarbonate buffer (containing a mixture of all unlabeled amino acids, each at a final concentration of 0.04 mm), in the presence of 70 μCi/ml of [^{3}H]oleic acid–albumin complex (18). At the end of the pulse period, the slices were washed in Krebs-Ringer bicarbonate buffer (see above) and then chased for 3 h in the same buffer (18). At the end of the chase period, incubation media from duplicate samples were collected, pooled, and subjected to density gradient ultracentrifugation to isolate medium lipoproteins. Each lipoprotein fraction was extracted in chloroform–methanol 2:1 (v/v) (19) and lipids were separated by thin-layer chromatography (18). The spots corresponding to ^{3}H-labeled cholesteryl esters were scraped and counted.

Immunohistochemistry

Several fragments of kidney and liver taken from three animals were immediately fixed in Bouin's solution and embedded in paraffin. To allow a uniform fixation and avoid artifacts in immunostaining, small tissue blocks were used. Tissue sections (7-μm thick) were washed in methanol containing 0.3% H_2O_2 for 15 min at room temperature to inhibit endogenous peroxidase (10). Sections were incubated overnight in either anti-chick apoB rabbit IgG or anti-chick apoA-I rabbit IgG at 4°C. Immunopositive areas were visualized by immunoperoxidase technique using avidin–biotin peroxidase complex. Nuclei were counterstained with hematoxylin (10, 15). For control experiments, tissue sections were incubated with IgG isolated from non-immune rabbit serum (10).

Statistical analysis

Student's *t* test was used for statistical analysis.

RESULTS

^{35}S-labeled amino acids incorporation into cell and medium apolipoproteins

The radioactivity incorporated into total cell proteins was 1.59 ± 0.13 and 1.07 ± 0.17 × 10^8 cpm/g in kidney and liver slices, respectively ($P < 0.01$); the radioactivity incorporated into medium proteins was 0.08 ± 0.01 and 0.38 ± 0.15 × 10^8 cpm/g in kidney and liver slices, respectively ($P < 0.01$).

^{35}S-labeled apoB immunoprecipitated from cell and medium of the two organs appeared as a single band comigrating with chick plasma apoB-100 in SDS-PAGE (data not shown) (3). The counting of apoB bands excised from SDS-gels revealed that the radioactivity incorporated into cell and medium apoB, when expressed per gram of tissue, was similar in the two organs (**Table 1**). However, if this parameter is calculated taking into account the total organ weight, the radioactivity of hepatic apoB was found to be approximately 3-fold that of kidney apoB (Table 1). The radioactivity incorporated into apoA-I immunoprecipitated from cell and medium (when expressed per gram of tissue) was lower in the kidney than in the liver, but this difference was significant only in the case of medium apoA-I (Table 1). However, if this parameter is

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TABLE 1. ^{35}S-labeled amino acid incorporation into cell and medium apolipoproteins by chick kidney and liver

Tissue	ApoB Radioactivity		ApoA-I Radioactivity	
	Cell	Medium	Cell	Medium
		cpm × 10^3/g of tissue		
Kidney	325.65 ± 123.90	54.18 ± 11.65	283.84 ± 164.49	28.67 ± 6.65
Liver	498.67 ± 144.12	63.09 ± 27.91	492.13 ± 133.37	58.62 ± 18.01
	NS	NS	NS	$P < 0.02$
		cpm × 10^3/organ weight		
Kidney	689.21 ± 229.63	116.70 ± 27.63	599.95 ± 359.03	61.61 ± 14.91
Liver	2264.69 ± 764.26	287.32 ± 132.21	2225.86 ± 688.87	266.26 ± 91.53
	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.01$

Kidney and liver slices taken from the same animals were incubated for 3 h in the presence of [^{35}S]methionine/[^{35}S]cysteine as specified in the Methods. ^{35}S-labeled apoB and apoA-I were immunoprecipitated from cell homogenates (high speed supernatant) and incubation media and separated by 5–10% (apoB) or 5–20% (apoA-I) linear gradient SDS-PAGE. ApoB and apoA-I bands were excised from the gels and the amount of incorporated label was determined by scintillation counting. Each value represents the mean ± standard deviation for data obtained from four animals. Statistical analysis was performed using Student's *t* test; NS, not significant.

calculated taking into account the total organ weight, the radioactivity of hepatic apoA-I (cell and medium) was found to be approximately 4-fold that of kidney apoA-I (Table 1). As the incorporation of ^{35}S-labeled amino acids into total cell and medium proteins was different in the two organs, the radioactivity incorporated into apoB and apoA-I was normalized by calculating the results relative to the radioactivity incorporated into total protein (17). **Table 2** shows that the percentage of total protein radioactivity incorporated into cell apoB and apoA-I was lower in the kidney than in the liver, whereas that incorporated into medium apoB and apoA-I was higher in the kidney than in the liver (4- and 2.3-fold, respectively). The total apoB production (cell plus medium ^{35}S-labeled apoB normalized for total protein production) was 0.23 ± 0.08 in the kidney and 0.38 ± 0.05 in the liver ($P < 0.05$). The total apoA-I production (cell plus medium ^{35}S-labeled apoA-I normalized for total protein production) was 0.19 ± 0.11 in the kidney and 0.38 ± 0.07 in the liver ($P < 0.05$).

TABLE 2. Percent of total protein radioactivity incorporated into apoB and apoA-I by kidney and liver slices

Tissue	ApoB		ApoA-I	
	Cell	Medium	Cell	Medium
Kidney	0.25 ± 0.03	0.71 ± 0.23	0.18 ± 0.10	0.38 ± 0.14
Liver	0.46 ± 0.06	0.16 ± 0.03	0.46 ± 0.12	0.16 ± 0.02
	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.05$

The radioactivity incorporated into cell and medium apoB and apoA-I (see Table 1) is given as the percentage of total ^{35}S-labeled cell and medium proteins, respectively. Each value represents the mean ± standard deviation for data obtained from four animals. Statistical analysis was performed using Student's *t* test.

To investigate whether kidney slices produced another plasma protein that is known to be specifically synthesized and secreted by the liver, ^{35}S-labeled albumin was immunoprecipitated from cell and medium. No radioactive albumin was detected in kidney cell and media (data not shown).

Isolation of ^{35}S-labeled medium lipoproteins

To investigate whether the apolipoprotein production by the kidney was associated with the secretion of lipoprotein particles, we performed additional experiments where kidney and liver slices were incubated in the presence of a larger amount of ^{35}S-labeled amino acids. ^{35}S-labeled lipoproteins secreted into the incubation media were separated by density gradient ultracentrifugation. The density profile of hepatic lipoproteins indicated the presence of three major classes of lipoproteins corresponding to VLDL + IDL (fraction 1, d < 1.019 g/ml), LDL (fractions 2–9, d 1.022–1.062 g/ml), and HDL (fractions 10–18, d 1.073–1.210 g/ml) (**Fig. 1A**). The profile of renal lipoproteins was similar to that of hepatic lipoproteins apart from the fractions corresponding to "light" HDL (fractions 10–14) which formed a shoulder rather than a well-defined peak (**Fig. 1B**). To allow a more accurate identification of medium lipoproteins, kidney and liver slices were pulse-labeled with [^{3}H]oleic acid for 1 h and then chased for 3 h. At the end of the chase period medium lipoproteins were isolated by density gradient ultracentrifugation. Lipoprotein lipids were extracted and ^{3}H-labeled cholesteryl esters were separated by thin-layer chromatography. **Figure 2** shows the density profile of ^{3}H-labeled cholesteryl esters in medium lipoproteins. This profile allows a better identification of the peaks corresponding to the major lipoprotein classes.

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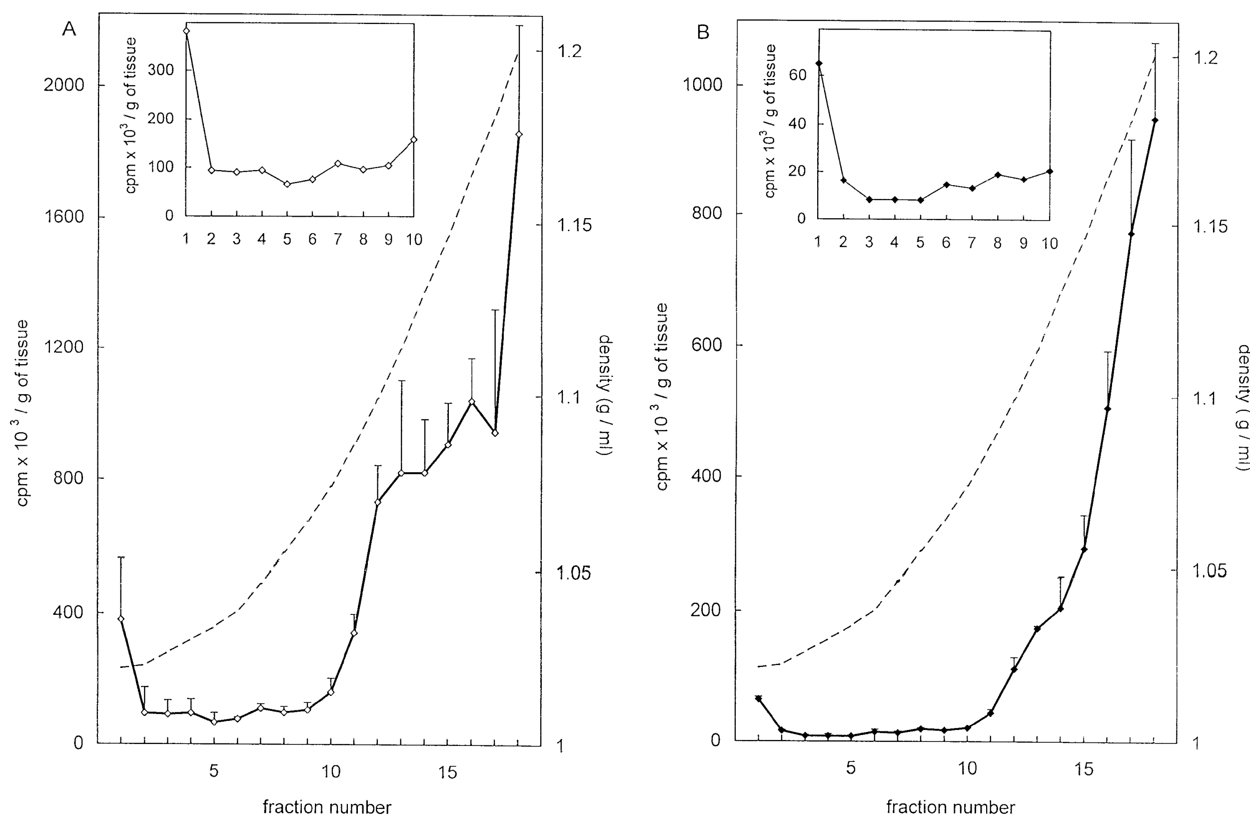

Fig. 1. Density profile of ^{35}S-labeled lipoproteins secreted into the medium by liver (A) and kidney (B) slices. Kidney and liver slices taken from four animals were incubated in duplicate in the presence of [^{35}S]methionine/[^{35}S]cysteine for 3 h. At the end of incubation, the media of duplicate samples were pooled and subjected to density gradient ultracentrifugation: VLDL + IDL, fraction 1; LDL, fractions 2–9; HDL, fractions 10–18. The protein radioactivity of each fraction was measured after trichloroacetic acid precipitation. Each value represents the mean ± standard deviation for data obtained from four animals. The inset shows a magnification of the density profile of fractions 1–10.

Figure 3 and **Figure 4** show the distribution of ^{35}S-labeled apolipoproteins in hepatic and renal lipoproteins. Most of the radioactive apoB (the band comigrating with plasma apoB-100) secreted by liver slices was present in VLDL, IDL, and LDL (fractions 1–9 of the density gradient shown in Fig. 1A) (Fig. 3). ^{35}S-labeled apo-A-I secreted by liver slices was found not only in HDL (fractions 10–18 of the density gradient) but also in the other lipoprotein classes (Fig. 3). No radioactive apoB and apoA-I were detectable in fractions 19–24 (d > 1.210 g/ml) of the density gradient (Fig. 3). Figure 4 shows the fluorogram of the SDS-PAGE of radioactive apolipoproteins present in kidney lipoproteins. A band comigrating with plasma apoB-100 was detectable in all lipoprotein fractions and appeared also to be present in the fractions of density higher than 1.210 g/ml (see below). Radioactive apoA-I secreted by kidney slices was present mostly in fractions 1–3 of the density gradient shown in Fig. 1B (d < 1.026 g/ml) and in "light" HDL (fractions 10–14 of the density gradient shown in Fig. 1B). In order to define whether the band migrating in the high molecular weight region of the gel shown in Fig. 4 was apoB, medium lipoproteins secreted by kidney slices were separated by sequential ultracentrifugation at the following densities: d < 1.006 g/ml (VLDL), d 1.006–1.063 g/ml (IDL + LDL), and d 1.063–1.210 g/ml (HDL) and immunoprecipitated with an anti-chick apoB polyclonal antibody. **Figure 5** shows that apoB was present in VLDL, IDL + LDL, and HDL secreted by kidney slices but was not detectable in the lipoprotein infranate (d > 1.210 g/ml). In order to compare the distribution of apoB and apoA-I in hepatic and renal lipoproteins with that of plasma lipoproteins, pooled plasma taken from 21-day-old chicks was subjected to density gradient ultracentrifugation (**Fig. 6**). The apolipoprotein distribution of plasma lipoproteins is shown in **Fig. 7**. ApoB-100 was detectable in VLDL-IDL-LDL (d < 1.063 g/ml, corresponding to fractions 1–9 of Fig. 6) and was present in trace amounts in fractions 10–11 (d 1.070–1.092 g/ml) of Fig. 6. ApoA-I was

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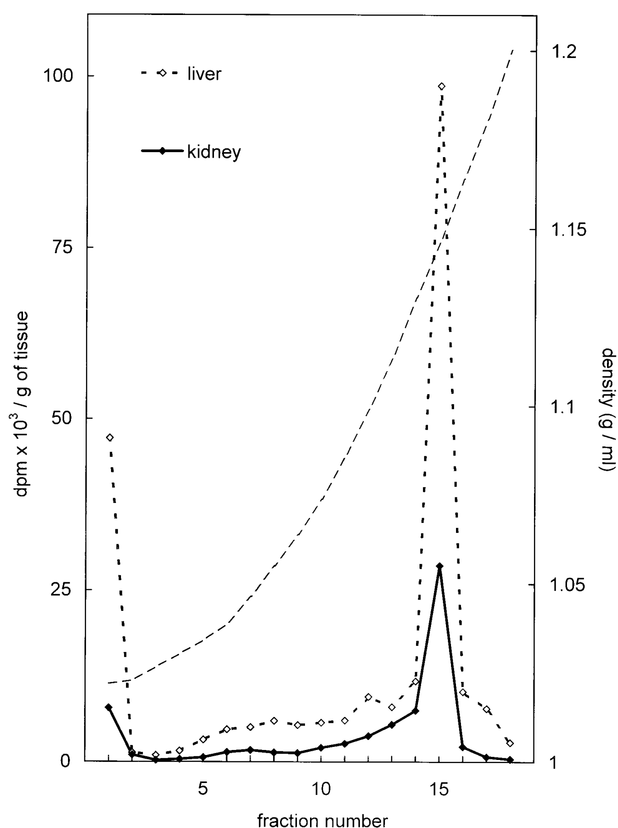

Fig. 2. Density profile of ^{3}H-labeled lipoproteins secreted into the medium by liver and kidney slices. Aliquots of kidney and liver slices (≈60 mg), taken from four animals were pooled and duplicate samples were incubated in the presence of [^{3}H]oleic acid for 1 h and chased for 3 h. At the end of incubation, the media were pooled and subjected to density gradient ultracentrifugation. Each lipoprotein fraction was extracted and ^{3}H-labeled cholesteryl esters were separated by thin-layer chromatography. The spots corresponding to cholesteryl esters were scraped and counted. VLDL + IDL, fraction 1; LDL, fractions 2–9; HDL, fractions 10–18.

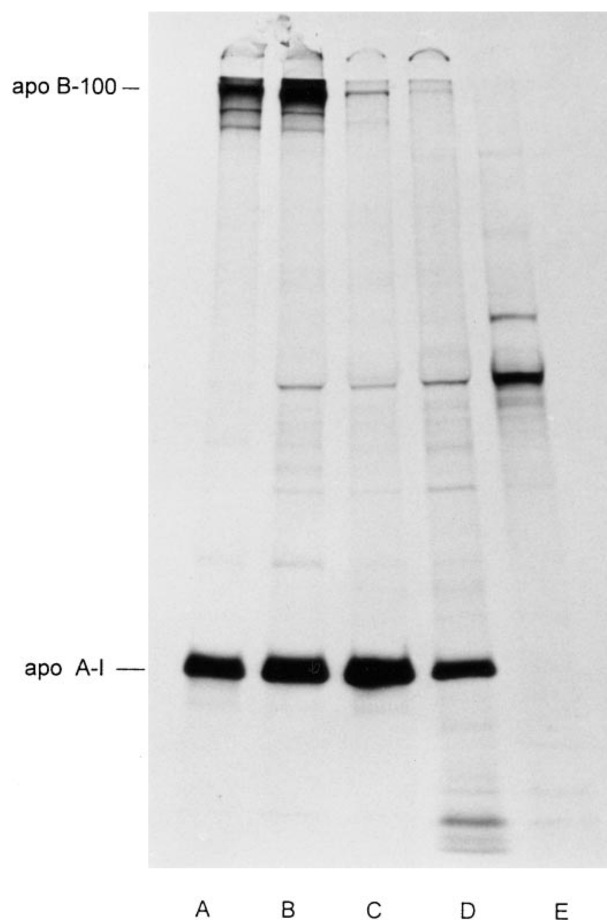

Fig. 3. Fluorogram of ^{35}S-labeled apolipoproteins of lipoprotein fractions isolated from the incubation media of liver slices. ^{35}S-labeled lipoprotein fractions isolated by density gradient ultracentrifugation (as shown in Fig. 1A) were pooled and analyzed on a 5–20% linear gradient SDS-PAGE. Approximately 70 × 10^3 cpm of protein radioactivity was applied to each lane. Lane A: fractions 1–3; lane B: fractions 4–9; lane C: fractions 10–14; lane D: fractions 15–18; lane E: fractions 19–24 (d > 1.210 g/ml), not included in Fig. 1A. The migration of chick plasma apoB-100 and apoA-I is shown on the left.

present in all density fractions, especially in fractions 10–16 corresponding to the HDL peak.

ApoB and apoA-I localization in chick kidney

Immunohistochemical studies were carried out to identify the cell types responsible for apoB and apoA-I production in chick kidney. An intense immunopositive reaction for apoB was found in the tubular epithelial cells (**Fig. 8**). The staining was evenly distributed in the cytoplasm and was mostly confined to the pyramidal cells of the proximal convoluted tubules (characterized by the presence of a luminal brush border) (Fig. 8, panel A) and the cuboid cells of the distal convoluted tubules (Fig. 8, panel B). No immunoreactivity was found in the columnar cells lining the collecting tubules (Fig. 8, panel B).

Figure 9 shows that immunoreactive apoA-I was present in the epithelial cells of some proximal and distal convoluted tubules (Fig. 9, panel A); some tubules contained both positive and negative cells (Fig. 9, panel B). In addition, a light immunostaining was observed at the basal membrane of tubular cells (Fig. 9, panel B). A similar feature was found in several sections of kidneys taken from different animals. No staining for apoB and apoA-I was observed in the glomeruli and small renal vessels. No immuno-positive staining was found in kidney sections incubated with non-immune rabbit IgG (**Fig. 10**). The immunohistochemistry of the liver revealed that apoB and apoA-I were evenly distributed in the cytoplasm of all hepatocytes (data not shown).

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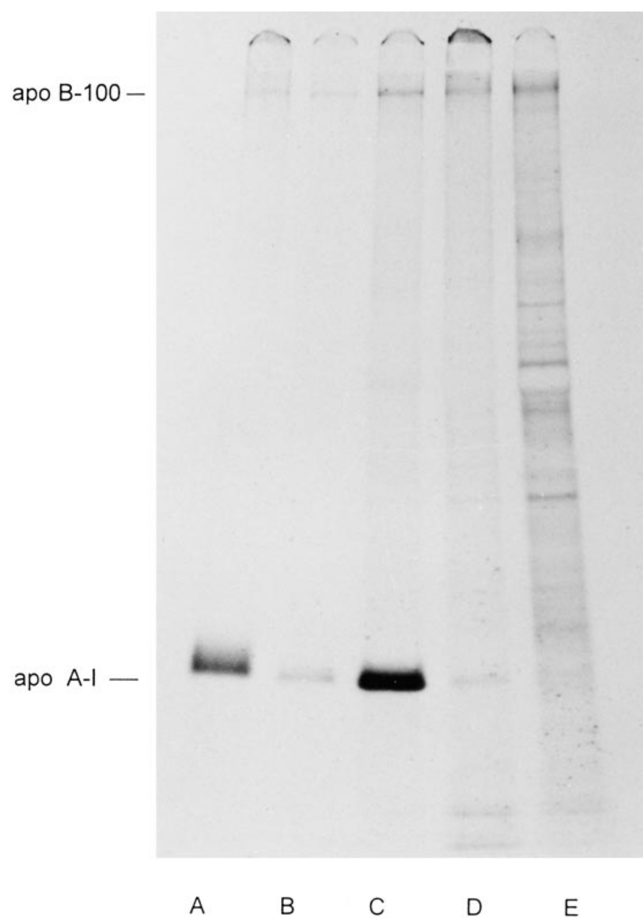

Fig. 4. Fluorogram of ^{35}S-labeled apolipoproteins of lipoprotein fractions isolated from the incubation media of kidney slices. ^{35}S-labeled lipoprotein fractions isolated by density gradient ultracentrifugation (as shown in Fig. 1B) were pooled and analyzed on a 5–20% linear gradient SDS-PAGE. Approximately 30–40 × 10^3 cpm of protein radioactivity was applied to each lane. Lane A: fractions 1–3; lane B: fractions 4–9; lane C: fractions 10–14; lane D: fractions 15–18; lane E: fractions 19–24 (d > 1.210 g/ml), not included in Fig. 1B. The migration of chick plasma apoB-100 and apoA-I is shown on the left.

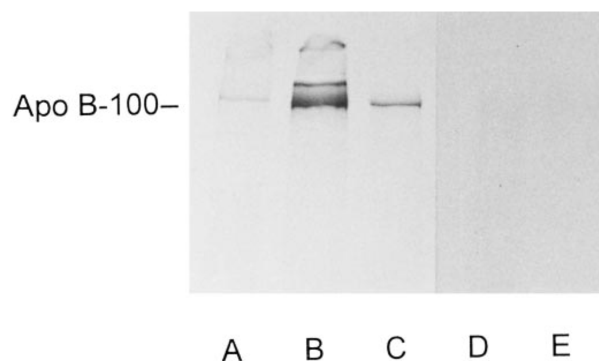

Fig. 5. Fluorogram of ^{35}S-labeled apolipoprotein B immunoprecipitated from lipoproteins isolated from the incubation media of kidney slices. ^{35}S-labeled lipoproteins were isolated from the incubation media by sequential ultracentrifugation: VLDL, d < 1.006 g/ml; IDL + LDL, d 1.006–1.063 g/ml; HDL, d 1.063–1.210 g/ml; lipoprotein infranate, d > 1.210 g/ml. Aliquots of each lipoprotein class, corresponding to 50 × 10^3 cpm of protein radioactivity, were immunoprecipitated with anti-chick apoB rabbit IgG and analyzed on a linear gradient 5–10% SDS-PAGE. Lane A, VLDL; lane B, IDL + LDL; lane C, HDL; lane D, lipoprotein infranate (d > 1.210 g/ml); lane E, a specific immunoprecipitation. The migration of chick plasma apoB-100 is shown on the left.

DISCUSSION

In the first set of experiments we confirmed, in a different chick strain, the results of previous observations that apoB and apoA-I are synthesized by chick kidney (4–5, 9, 11–12, 14). We found that kidney apoB production is 60% of that of the liver, a value that is comparable to that found in a previous report (5). In absolute terms, the total amount of apoB produced by the kidney, when expressed per organ basis, was approximately 30% of that of the liver. This result is in agreement with a previous study of apoB mRNA measurements indicating that kidney apoB mRNA (when expressed per organ basis) was approximately 15% of its liver counterpart (4). Kidney apoA-I production is 48% lower than that of the liver, in good agreement with an early report by Blue et al (11). In absolute terms, the total amount of apoA-I produced by the kidney, when expressed per organ basis, was approximately 25% of that of the liver. This result is difficult to reconcile with that of Rajavashisth et al. (12) who found that the content of apoA-I mRNA (when expressed per organ basis) in chick kidney was only 3% of that found in the liver. We do not have a simple explanation for this discrepancy. It is conceivable that the strain difference or the age of the animals (21-day-old chicks used in this study vs. adult animals) (12) may account for this difference.

In a second set of experiments we show, for the first time, that kidney apoB and apoA-I are secreted into the incubation medium as constituents of lipoprotein particles (Fig. 3 and 4). ApoB secreted by the kidney is incorporated partly in VLDL-LDL and partly in HDL, specifically the "light" HDL (floating in the density range 1.070–1.130 g/ml). This is in sharp contrast with the distribution of hepatic apoB which is found predominantly in VLDL and LDL fractions. The reason why renal apoB is incorporated to a large extent into lipoproteins floating in the HDL density range is not clear. It is conceivable that this finding reflects the relatively low amount of lipids available for the lipidation of newly synthesized apoB in kidney epithelial cells (as opposed to hepatocytes) as the availability of newly syn-

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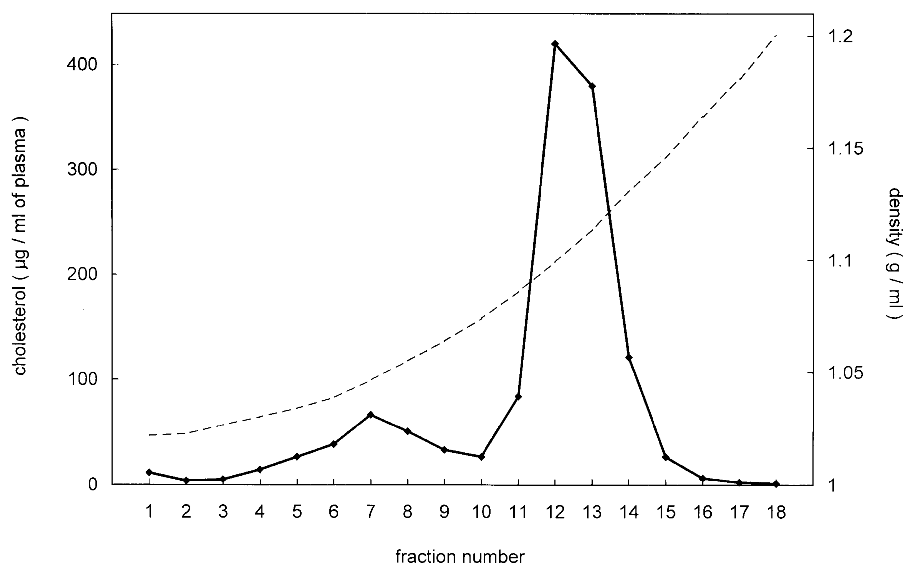

Fig. 6. Density profile of plasma lipoproteins of 21-day-old chicks. Plasma lipoproteins were separated by density gradient ultracentrifugation from pools of plasmas taken from four animals. VLDL + IDL, fraction 1; LDL, fractions 2–9; HDL, fractions 10–18. Lipoprotein concentration is given as lipoprotein cholesterol.

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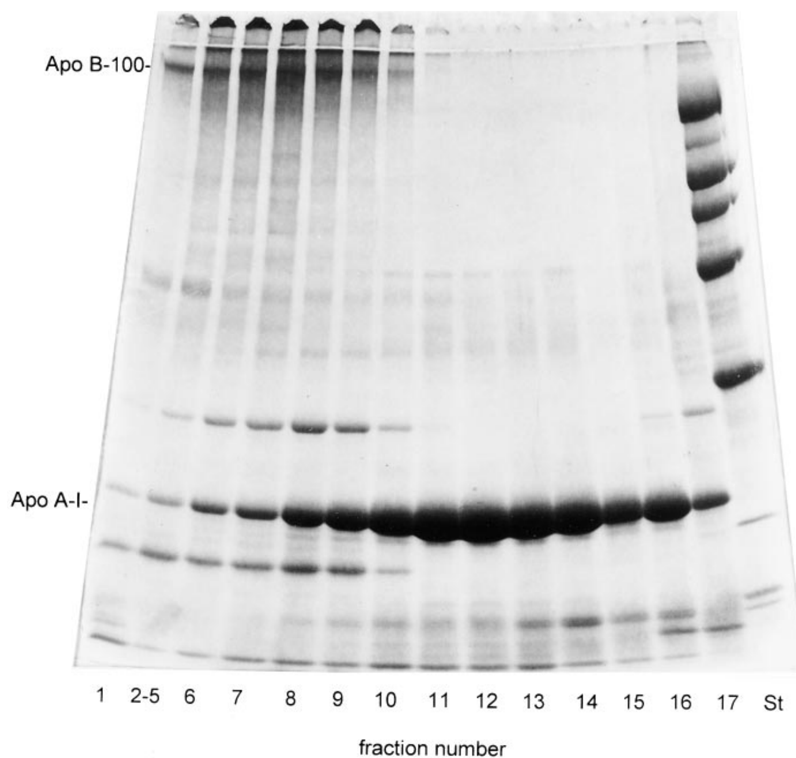

Fig. 7. Apolipoprotein composition of chick plasma lipoproteins. Aliquots (30–50 μg of lipoprotein-protein) of lipoprotein fractions isolated by density gradient ultracentrifugation were analyzed on a 5–20% linear gradient SDS-PAGE. The numbers below each lane indicate the lipoprotein fractions of the density gradient shown in Fig. 6. The migration of molecular weight standards is shown on the right (St).

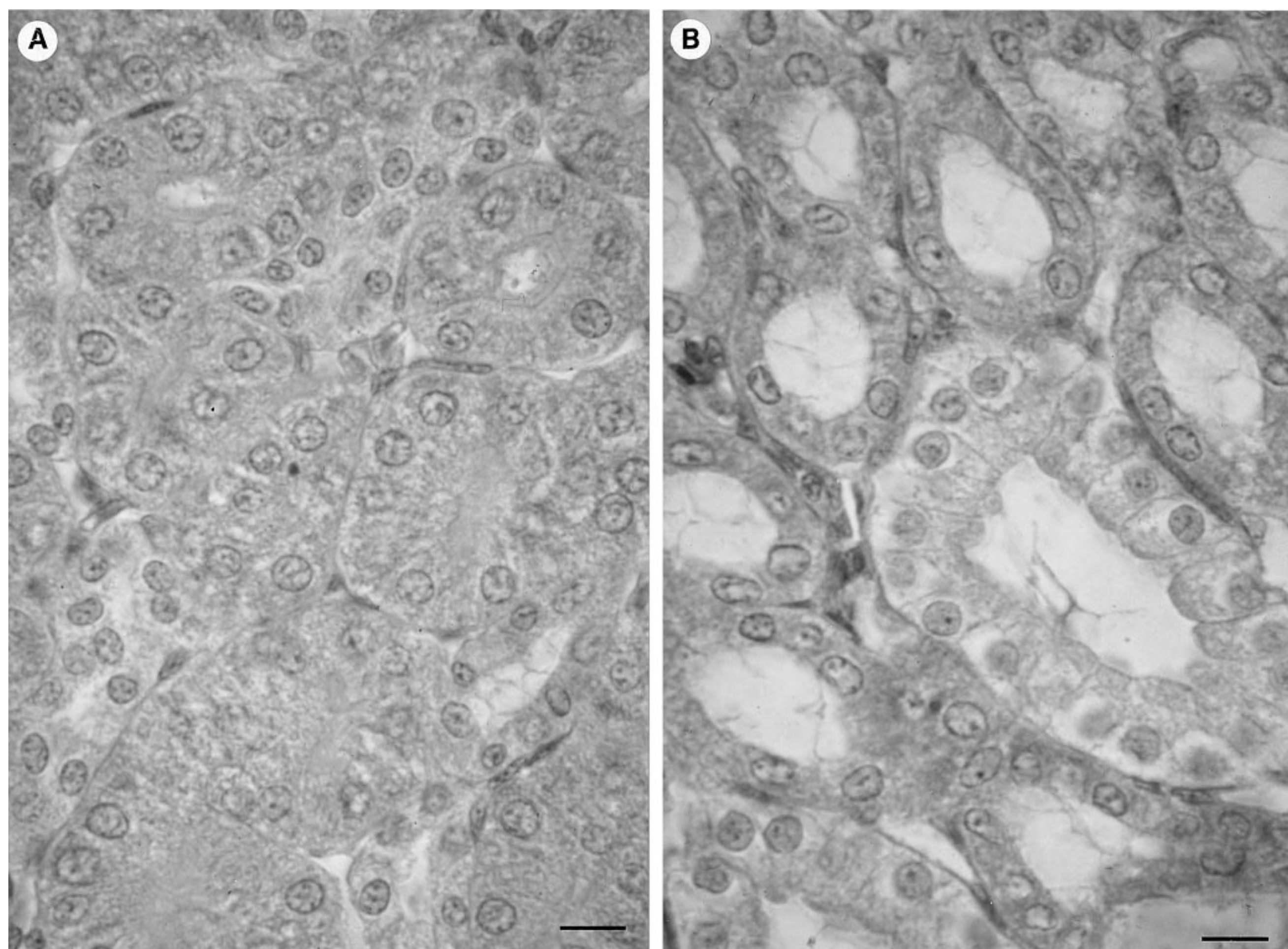

Fig. 8. Immunolocalization of apoB in chick kidney. Kidney sections of 21-day-old chicks were incubated with anti-chick apoB rabbit IgG. Immuno-positive areas were visualized by the avidin–biotin peroxidase complex. Nuclei were counterstained with hematoxylin. Panel A shows a section containing several immuno-positive proximal convoluted tubules (light brown color). Panel B shows some immuno-positive distal convoluted tubules and two immuno-negative collecting tubules. Bar = 10 μm.

thesized triglycerides and cholesteryl esters in the endoplasmic reticulum is a key factor regulating the assembly and secretion of VLDL, at least in the hepatocytes (20). In this context, apoB present in renal lipoproteins isolated in the LDL and HDL density range might be regarded as a constituent of primordial particles resulting from an incomplete intracellular lipidation of a full-length apoB-100. It is possible, however, that this unusual floating property of renal apoB-containing lipoproteins depends, at least in part, on the incubation conditions. The medium we used, although supplemented with amino acids, might have been devoid of other nutrients necessary to ensure optimal feeding conditions for kidney slices during a prolonged in vitro incubation.

Independent evidence of renal synthesis of apoB and apoA-I was obtained by immunohistochemistry which shows the localization of these apolipoproteins in the epithelial cells of most of the proximal and distal convoluted tubules. As immunostaining for apoB and apoA-I was not observed on the wall of glomerular and peritubular capillaries, it is most unlikely that the presence of these apolipoproteins in tubular cells reflects an intracellular retention after the uptake of plasma lipoproteins by the kidney. The finding that immuno-positive staining was confined to specific cell types is not surprising, as a similar distribution was observed for apoE in baboon kidney (21) and in the kidney of human apoE transgenic mice (22).

The large amount of apoB synthesized by chick kidney has no equivalent in the mammalian species. A very low level of expression of apoB was detected in rabbit (23), mouse (24), and human fetal and adult kidney (25). There are no reports, however, on the secretion

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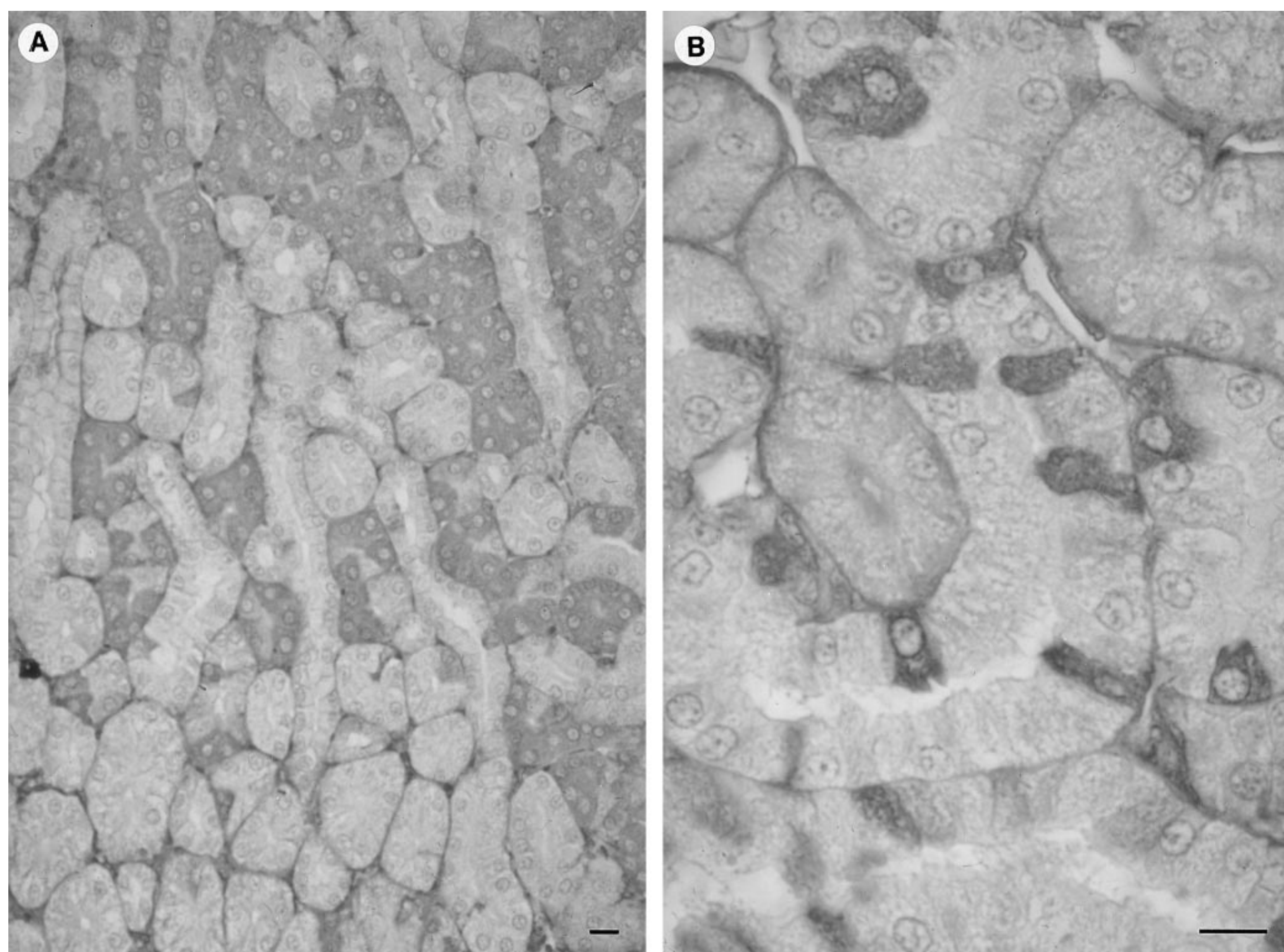

Fig. 9. Immunolocalization of apoA-I in chick kidney. Kidney sections of 21-day-old chicks were incubated with anti-chick apoA-I rabbit IgG. Immuno-positive areas were visualized by the avidin–biotin peroxidase complex. Nuclei were counterstained with hematoxylin. Panel A shows immuno-positive (brown color) and immuno-negative proximal and distal convoluted tubules. Panel B shows that immuno-negative tubules occasionally contain immuno-positive cells. Bar = 10 μm.

of apoB-containing lipoproteins by the mammalian kidney. The synthesis of apoA-I by chick kidney is not surprising, as this apolipoprotein is expressed in a variety of chick tissues (10–15). A survey of several tissues in some mammalian species indicated that the apoA-I gene expression in organs other than liver and intestine is very low or negligible (23). More specifically, no apoA-I mRNA expression was found in rabbit kidney (23), whereas minute amounts of kidney apoA-I mRNA were reported in rat and mouse (23, 26, 27) as well as in two nonhuman primates (28) and in human fetus (23). In some nonhuman primates metabolic labeling studies showed that kidney synthesizes low amounts of apoA-I in short term organ culture (28). However, despite these observations, a renal secretion of apoA-I has not been reported so far in mammals. In mammals, where the apoE gene is widely expressed, the kidney synthesizes substantial amounts of apoE (21, 29–33) but there are no data on the secretion of apoE-containing lipoproteins by the kidney. In human apoE transgenic mice, in which high levels of human apoE mRNA were present in the kidney but not in the liver, human apoE was found in plasma indicating that kidney is capable of secreting apoE possibly as a constituent of lipoprotein particles (22).

Taken together, our results raise several questions concerning three aspects: *1*) the mechanism underlying the secretion of apoB by the tubular cells; *2*) the physiological role of renal lipoprotein secretion in the chick; and *3*) the possible contribution of renal lipoproteins to the plasma lipoprotein pool. With regard to the first aspect it would be of interest to investigate whether kidney tubular cells contain the microsomal triglyceride transfer protein (MTP), a protein that in

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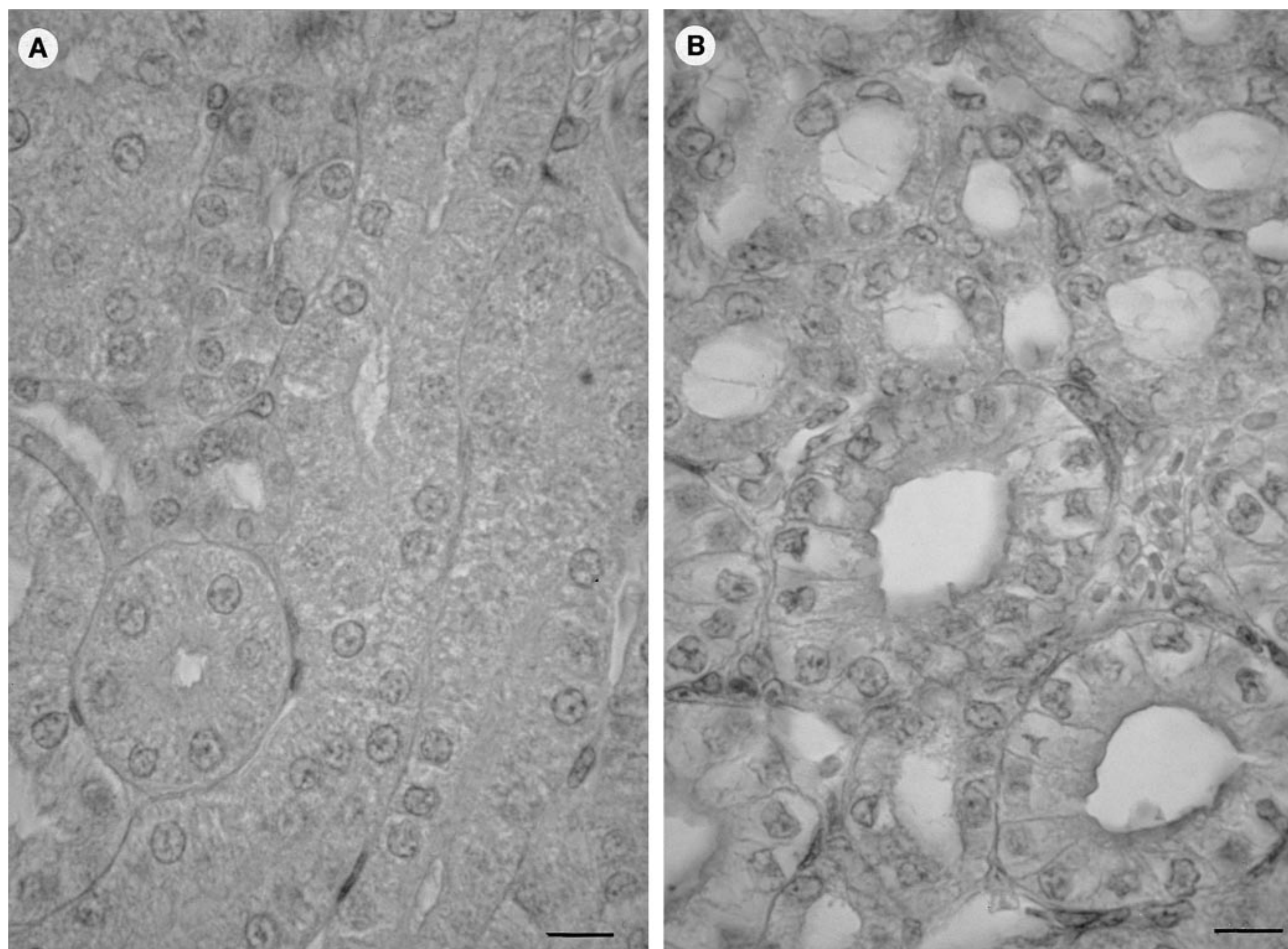

Fig. 10. Control sections of chick kidney taken from 21-day-old chicks. Several sections incubated with non-immune rabbit IgG showed no visible immuno-positive reaction products. Panel A shows several proximal convoluted tubules. Panel B shows some distal convoluted tubules and two collecting tubules. Bar = 10 μm.

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mammalian species is expressed exclusively in liver and intestine, where it plays an obligatory role in the assembly and secretion of apoB-containing lipoproteins (34). It has been recently reported that in non-hepatic and non-intestinal heterologous cell lines, the co-expression of MTP and apoB is sufficient for the assembly and secretion of apoB-containing lipoproteins (35–38). In view of our findings we are tempted to predict that chick kidney contains MTP at a level comparable to that of the liver.

The physiological role of lipoprotein secretion by chick kidney might be related to the presence of a renal portal circulation. Two sets of blood vessels provide the kidney with a dual blood supply: one arterial and one venous. The arterial blood is supplied by three renal arteries that ramify in the lobules to form the afferent and efferent arterioles of the glomeruli. The venous blood supply, called renal portal system, furnishes the kidney with venous blood drained from the limbs, the pelvis, and the abdominal viscera, including the terminal part of the small intestine. This afferent venous blood discharges into the capillary network surrounding the convoluted tubules (39). In view of this peculiar circulatory system, lipids absorbed by the terminal ileum may be transported, as intestinal lipoproteins (portomicrons), to the epithelial cells of the convoluted tubules where they may contribute to the intracellular lipid pool utilized for the assembly of lipoproteins.

The secretion of apoB and apoA-I by chick kidney that we documented in the present study raises the question as to whether in this species the kidney contributes to the regulation of plasma lipoprotein pool. The answer to this question requires in vivo experiments specifically designed to dissect the contribution of liver, intestine, and kidney to the plasma lipoprotein

pool. One experimental setting that may be suitable to ascertain whether renal apoB contributes to the plasma pool of apoB-containing lipoproteins is the estrogen-stimulated rooster. Estrogen treatment increases hepatic synthesis of apoB and induces the synthesis of a specific apolipoprotein, designated apoVLDL-II (8, 40). Both apolipoproteins, secreted as constituents of VLDL (1), are thought to be, to a large extent, on the same VLDL particles (1, 41, 42) and are not transferred to other plasma lipoproteins (43). Estrogen treatment has no effect on apoB synthesis nor does it induce apoVLDL-II synthesis in the kidney (4, 9). Under these circumstances one might assume that plasma VLDL of hepatic origin contain both apoB and apoVLDL-II, whereas plasma VLDL secreted by the kidney and, possibly by the intestine, would contain only apoB. Thus, in the estrogen-treated rooster, the measurement of the plasma level of apoB/apoVLDL-II-containing VLDL and that of VLDL containing exclusively apoB might provide an estimate of the contribution of the extrahepatic tissues (kidney and intestine) to the plasma pool of apoB-containing lipoproteins. If these measurements are performed in fasted animals (to eliminate the release of lipoproteins by the intestine) one might be able to assess the actual contribution of the kidney to the plasma pool of apoB-containing lipoproteins.

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