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Apolipoprotein E synthesis in human kidney, adrenal gland, and liver

(lipoprotein/atherosclerosis/reverse cholesterol transport)

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ABSTRACT **Human tissues were incubated *in vitro* with radiolabeled amino acids to determine whether plasma apolipoproteins are synthesized in human kidney. Subsequently, tissue extracts were screened with antisera directed against apolipoprotein E (apo E), apolipoprotein B (apo B), apolipoprotein AI (apo AI), and bulk apoliproproteins of high density lipoprotein (HDL). Newly synthesized apo E, but not apo AI or apo B, was identified in kidney and adrenal cortex. Estimates of relative rates of apo E synthesis *in vitro* suggest that a substantial portion of adrenal and kidney protein synthesis is committed to apo E synthesis. The relative rate of apo E synthesis was 4–6 times greater in kidney cortex than in kidney medulla. Analysis of immunoreactive apo E showed that kidney and adrenal apo E species have the same electrophoretic mobility in NaDodSO$_4$/polyacrylamide gels as does plasma apo E. Further characterization by high resolution two-dimensional gel analysis indicated that the isoforms of newly synthesized kidney and adrenal apo E correspond to specific isoforms of plasma apo E. These findings suggest that apolipoproteins arising from peripheral tissues may play an important role in lipid transport and metabolism.**

Apolipoprotein E (apo E) is a major plasma apolipoprotein found in both very low density (VLDL) and high density lipoproteins (HDL). Recent studies suggest that apo E plays a central role in the transport and removal of cholesterol-laden lipoproteins from the circulation. Lipoproteins containing apo E can deliver cholesterol to cultured cells via interaction with cell surface apolipoprotein B (apo B), E receptors (1–4) and hepatic receptors for apo E appear to be responsible for the removal of cholesterol-laden HDL subfractions and chylomicron remnants from the circulation (5–7). Plasma apo E is found to be elevated in hyperlipidemic patients (8) and is a major component of cholesterol-rich lipoproteins that accumulate during cholesterol feeding in humans and experimental animals (9–12). Biochemical and genetic analyses have shown the existence of multiple apo E isoforms (13) arising from three common alleles and post-translational modifications (14). Type III hyperlipoproteinemia, a human disease associated with premature atherosclerosis (15), is strongly associated with homozygosity for a specific apo E allele (16).

Although the biochemical properties of apo E and other human apolipoproteins have been investigated extensively, little is known about lipoprotein biosynthesis or the mechanisms that regulate the synthesis of apolipoproteins. Studies with experimental animals have shown the liver and small intestine to be the major sources of plasma apolipoproteins (17–21). However, at least one apolipoprotein also is synthesized by the kidney in the chicken (22), and an apo E-like protein is secreted by cultured mouse macrophages (23). Studies with human tissues (24–27) or cell lines derived from liver tumors (28, 29) have confirmed the liver and small intestine as sites of plasma apolipoprotein synthesis in man. It is not known whether human tissues other than the liver and small intestine play a role in apolipoprotein biosynthesis.

The aim of the present study was to determine whether human kidney and adrenal tissues participate in apolipoprotein biosynthesis. Human liver, kidney, and adrenal tissues were incubated with radiolabeled amino acids *in vitro*, and tissue extracts were examined for newly synthesized apolipoproteins by immunoprecipitation with specific antisera. apo E synthesis was detected in both kidney and adrenal, indicating that human tissues other than the liver and small intestine synthesize at least one plasma apolipoprotein. These results raise the possibility that apolipoproteins arising from tissues other than liver and intestine may be important in lipid transport and metabolism.

MATERIALS AND METHODS

Preparation of Tissue Extracts. Tissue samples were obtained from surgical specimens excised because of renal adenocarcinoma (nephrectomy, liver biopsy) or adrenal adenoma (adrenalectomy). Except where indicated, only normal tissues at some distance from the tumor, as identified by the attending pathologist, were used for tissue incubations. Informed consent was obtained. Specimens were transported in iced Krebs–Ringer solution to the laboratory. Tissues available for study included two liver biopsies, five kidney samples, one adrenal cortex and one adrenal adenoma sample, two adipose tissue samples adherent to excised kidneys, and several samples of vascular tissues from the renal vein and artery. Tissue slices (20–30 mg) were incubated for 1.5 hr at 37°C in 0.1 ml of Krebs–Ringer bicarbonate solution containing 250 μCi of L-[4,5-^{3}H]leucine (60 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) or L-[^{35}S]methionine (1,083 Ci/mmol), 5 units of penicillin, and 5 μg of streptomycin under an atmosphere of 95% O_2/5% CO_2 (21, 22). Radioisotopes were from New England Nuclear. After incubation, tissue slices were washed, homogenized, and centrifuged to prepare a high speed supernatant exactly as described (21), except that a ground glass tissue grinder was used. If not analyzed immediately, supernatants were frozen in liquid N_2 and stored at −70°C.

Preparation of Antisera. Plasma low density lipoprotein (LDL) and HDL were prepared from normal fresh plasma by standard ultracentrifugal procedures by using KBr for density adjustment (30). Each fraction was washed twice by floatation

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Abbreviations: apo E, apo B, and apo AI, apolipoproteins E, B, and AI, respectively; HDL, LDL, and VLDL, high density, low density, and very low density lipoproteins, respectively.

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through the appropriate density and was dialyzed against 0.15 M NaCl/0.01 M sodium phosphate, pH 7/phenylmethylsulfonyl fluoride at 100 μg/ml. apo B was isolated from LDL by two cycles of chromatography on Sepharose 6B equilibrated with 1% $NaDodSO_4$/0.01 M sodium phosphate, pH 7/phenylmethylsulfonyl fluoride at 100 μg/ml (31). Analysis by $NaDodSO_4$/5% polyacrylamide gel electrophoresis showed a single apo B band with an apparent M_r of 350,000. HDL was adjusted to 1% $NaDodSO_4$ and chromatographed on Sepharose 6B as above. The column profile was analyzed by $NaDodSO_4$/10% polyacrylamide gel electrophoresis, and fractions corresponding to M_r <40,000 were pooled and used as antigen. New Zealand White rabbits were immunized with apo B or HDL apolipoproteins (1 mg) in Freund's complete adjuvant with booster injections at 4-week intervals. Rabbits were bled 9 days after the third injection. When tested by double-immunodiffusion, anti-apo B showed a single line of identity between apo B and LDL but no reactivity toward HDL or HDL apolipoproteins.

Anti-apo E was prepared and characterized as described (8). For the purification of apolipoprotein AI (apo AI), HDL was dialyzed against 0.01 M NH_4CO_3, lyophilized, and delipidated (32). apo HDL was dissolved in 6 M urea/0.01 M Tris·HCl, pH 8.0/0.01% NaN_3 and fractionated on DEAE-cellulose with a linear NaCl gradient extending from 20 to 100 mM. The apo AI peak showed a single band upon $NaDodSO_4$/polyacrylamide and urea gel electrophoresis. Antiserum directed against apo AI was raised in New Zealand White rabbits as described for anti-apo E (8). Characterization of anti-apo AI by double-diffusion analysis yielded a single precipitation line against whole human plasma, HDL, and apo AI and no reactivity with LDL and VLDL.

Immunoprecipitation and Gel Electrophoresis. Radiolabeled tissue supernatants were analyzed with the double-antibody procedure as described (22) by employing one of the primary antisera described above and goat anti-rabbit gamma globulin as second antibody. The primary antibody was used in excess to ensure quantitative immunoprecipitation. Washed immunoprecipitates and tissue supernatants were solubilized in 0.01 M Tris·HCl, pH 6.5/2% $NaDodSO_4$/2% 2-mercaptoethanol/0.001 M EDTA/4% glycerol, boiled for 5 min, and electrophoresed on $NaDodSO_4$/polyacrylamide slab gels containing 5% or 10% acrylamide (gel system A; ref. 31) with the buffer system of Laemmli (33). Radioactive proteins were visualized by fluorography (34). To determine the relative rates of apo E synthesis, ^{35}S-labeled tissue supernatant (100,000–300,000 cpm) was immunoprecipitated, and the washed immunoprecipitate was electrophoresed in parallel with dilutions of the tissue supernatant. Immunoprecipitation with preimmune rabbit serum (normal serum) as the primary antibody was carried out to correct for nonspecific immunoprecipitation (21, 22). After fluorographic exposure of the gel, the gel lanes on the x-ray film were scanned with a Joyce–Loebl microdensitometer. Densitometry was carried out with a single wedge that provided <50% full scale deflection for the immunoprecipitated apo E peak and ≈50% full scale deflection for the largest of the peaks in the profile of total supernatant proteins. The apo E peak and the profile of total proteins were cut from the tracings and weighed. After correction for nonspecific immunoprecipitation (<5% of the apo E peak weight), the relative rate of apo E synthesis was calculated as (immunoprecipitated apo E peak weight)/(weight of total supernatant protein profile) × 100. Multiple film exposures were carried out to ensure that the signals were within the linear response range of the film, and the linear range of the fluorographic procedure was established by analyzing dilutions of the immunoprecipitated apo E.

Two-Dimensional Gel Analysis. High resolution two-dimensional gel analysis was carried out as described by O'Farrell (35) with minor modifications (36). Immunoprecipitates and apolipoprotein samples were solubilized in 0.05 M N-(cyclohexylamino)ethanesulfonic acid, pH 9.5/2% $NaDodSO_4$/1% dithiothreitol/10% glycerol/0.005 M phenylmethylsulfonyl fluoride and boiled for 5 min prior to loading onto 12.5 × 0.25 cm isoelectric focusing tube gels prepared and prefocused as described (35). Isoelectric focusing was at 350 V for 15 hr, followed by 800 V for 1 hr. Gels were equilibrated for 30 min in 5 ml of 0.0625 M Tris·HCl, pH 6.8/2.3% $NaDodSO_4$/5% 2-mercaptoethanol/10% glycerol prior to electrophoresis on a second dimension $NaDodSO_4$/10% polyacrylamide slab gel as described above. The pH gradient in the isoelectric focusing dimension was measured in four replicate gels as described (35).

RESULTS

Synthesis of apo E in Kidney, Adrenal, and Liver. In preliminary experiments, liver tissue was incubated *in vitro* with radiolabeled amino acid, and the tissue supernatant was reacted with anti-HDL and anti-apo E. Analyses of the immunoprecipitates by $NaDodSO_4$/10% polyacrylamide gel electrophoresis showed that anti-HDL specifically precipitated two newly synthesized proteins that migrated with purified apo E and apo AI (data not shown). Anti-apo E precipitated only the protein that migrated with apo E at an apparent M_r of ≈38,000. These procedures were used subsequently to determine whether the synthesis of apo E, apo AI, or other HDL apolipoproteins could be detected in kidney tissue. Kidney specimens were routinely separated into cortex and medulla prior to *in vitro* incubation with radiolabeled amino acids as described.

Fig. 1 shows the electrophoretic profiles of [^{35}S]methionine-labeled supernatant proteins from kidney medulla (lane 1) and cortex (lane 2). Reaction of anti-HDL with medulla (lane 5) and cortex (lane 7) supernatants precipitated a protein that comigrated with plasma apo E when analyzed by $NaDodSO_4$/10%

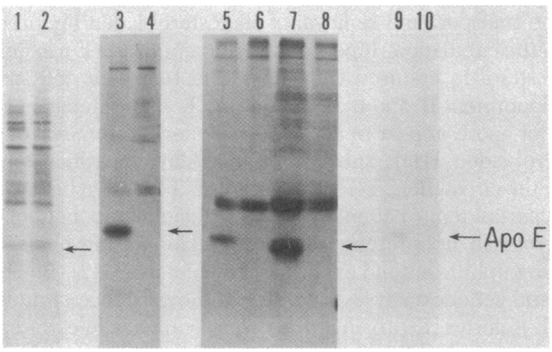

FIG. 1. Synthesis of apo E by kidney tissue. A kidney specimen was separated into medulla and cortex and incubated *in vitro* with [^3H]leucine or [^{35}S]methionine as described. Tissue extracts and immunoprecipitates were analyzed by $NaDodSO_4$/10% polyacrylamide gel electrophoresis, followed by fluorography. [^{35}S]Methionine-labeled medulla and cortex proteins are shown in lanes 1 and 2, respectively. Immunoprecipitates were formed with anti-HDL serum from [^{35}S]methionine-labeled medulla (lane 5) and cortex (lane 7) and with normal rabbit serum from medulla (lane 6) and cortex (lane 8). Immunoprecipitates were formed with anti-apo E from [^{35}S]methionine-labeled cortex (lane 3) and [^3H]leucine-labeled cortex (lane 9). Corresponding control immunoprecipitates with normal rabbit serum for [^{35}S]methionine-labeled cortex (lane 4) and [^3H]leucine-labeled cortex are shown. The immunoprecipitates displayed in lanes 3–8 were formed with 10 times the total supernatant protein radioactivity displayed in lanes 1 and 2. The arrows indicate the electrophoretic mobility of purified plasma apo E on the four slab gels illustrated.

polyacrylamide gel electrophoresis. Radiolabeled apo E was not precipitated when normal rabbit serum was used as primary antiserum (lanes 6 and 8), indicating that the apo E band is specific to the anti-HDL serum. The identity of the newly synthesized kidney apo E was confirmed by reaction with antiserum specific to plasma apo E (8). As shown in Fig. 1, the apo E band was precipitated by anti-apo E (lane 3) but not by normal serum (lane 4). Anti-apo E also precipitated specifically the radiolabeled apo E band from kidney medulla (data not shown). Newly synthesized apo E also was seen when tissues were incubated with [^{3}H]leucine (lane 9) as well as [^{35}S]methionine, indicating that the detection of apo E synthesis is independent of the precursor amino acid and the specific radioisotope. Incorporation of radioactivity into kidney protein was abolished when tissue slices were incubated with 250 μM cycloheximide for 10 min prior to the addition of radiolabeled amino acid.

Analysis of tissue samples from four patients showed that newly synthesized apo E was more prominent in kidney cortex than in medulla. When equal amounts of protein radioactivity were precipitated with anti-apo E, densitometric comparisons showed that the newly synthesized apo E band was 4–6 times greater in cortex than in medulla. Estimates of the relative rate of apo E synthesis in kidney cortex samples from three patients were made by densitometry of the fluorographs. apo E synthesis represented 0.15%, 0.21%, and 0.3% of precursor incorporation into total supernatant protein in these samples.

Adrenal tissue also showed a significant synthesis of apo E. Fig. 2 shows a $NaDodSO_4$/10% polyacrylamide gel profile of [^{35}S]methionine-labeled proteins from the zona fasciculata region of the adrenal cortex (lane 1). A prominent newly synthesized protein with the mobility of apo E was seen in the tissue supernatant (lane 1), and this protein was precipitated by anti-apo E (lane 2) but not by normal rabbit serum (lane 3). Newly synthesized apo E also was seen after incubating an adrenal cortex sample with [^{3}H]leucine (lane 4). Densitometric measurement of apo E radioactivity indicated that adrenal apo E represented 1.2% of [^{35}S]methionine incorporation into total supernatant protein. Tissue samples from the adrenal tumor also were found to synthesize apo E, although to a far lesser extent than the apparently normal tissue.

Reaction of radiolabeled kidney supernatants with anti-apo AI or anti-apo B failed to detect these apolipoproteins when the immunoprecipitates were examined by electrophoresis and fluorography as above. However, the synthesis of both apo AI and apo B was readily detected in liver tissue. This result suggests that kidney tissue does not synthesize these apolipoproteins or the synthesis is below the detection limits of the procedure employed (≈0.05% of tissue protein synthesis). apo AI synthesis also was not detected in adrenal tissue. Analysis of adipose tissue, renal artery, and renal vein also failed to detect the synthesis of apo E, apo B, or apo AI. However, these tissues exhibited low rates of incorporation of radiolabeled amino acid into protein, thereby greatly decreasing the sensitivity of the analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography.

Isoforms of apo E. High resolution two-dimensional gel analysis of plasma apo E from the VLDL of pooled plasma from hypertriglyceridemic patients (8) gave the protein staining pattern shown in Fig. 3 *A* and *B*. In terms of the recently adopted nomenclature (37), the isoform pattern closely resembles that reported by Zannis and Breslow (14) for the mixture of E 4/4, E 3/3, and E 2/2 phenotypes. Note that isoform E_2 representing the ε2 allele was present in relatively higher amounts than determined from the calculated gene frequencies of 11%, 72%, and 17% for the ε4, ε3, and ε2 alleles, respectively, in the general population (14). However, this was expected because the apo E was isolated from a hypertriglyceridemic population that included a disproportionate number of type III patients exhibiting markedly elevated levels of plasma apo E and homozygosity for the ε2 allele (8, 14, 38, 39). The isoform E_s of slightly greater molecular weight (Fig. 3 *A* and *B*) is reported to represent sialated forms of apo E (14). The isoforms E_4, E_3, E_2, and E_s had pI values of 6.06, 5.91, 5.78, and 5.69, respectively.

To compare newly synthesized tissue apo E to the plasma apo E isoforms, plasma apo E was added to an immunoprecipitate containing [^{35}S]methionine-labeled adrenal apo E prior to analysis by two-dimensional gel electrophoresis. The gel from the

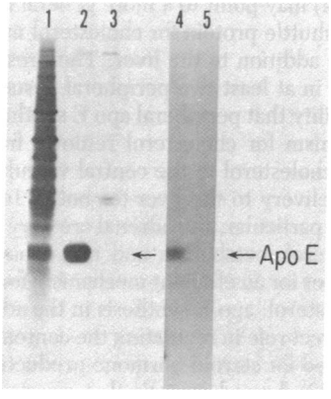

FIG. 2. Synthesis of apo E by adrenal cortex. After *in vitro* incubation of adrenal tissue with [^{35}S]methionine as described, a sample of tissue extract was analyzed directly (lane 1) or immunoprecipitated with anti-apo E (lane 2) or normal rabbit serum (lane 3) prior to analysis by $NaDodSO_4$/10% polyacrylamide gel electrophoresis and fluorography. The immunoprecipitates displayed in lanes 2 and 3 were formed from 2 times the total supernatant protein radioactivity displayed in lane 1. Another sample of adrenal cortex was incubated with [^{3}H]leucine *in vitro* prior to immunoprecipitation of the tissue extract with anti-apo E (lane 4) and normal rabbit serum (lane 5). The arrows indicate the electrophoretic mobility of purified plasma apo E on the two slab gels illustrated.

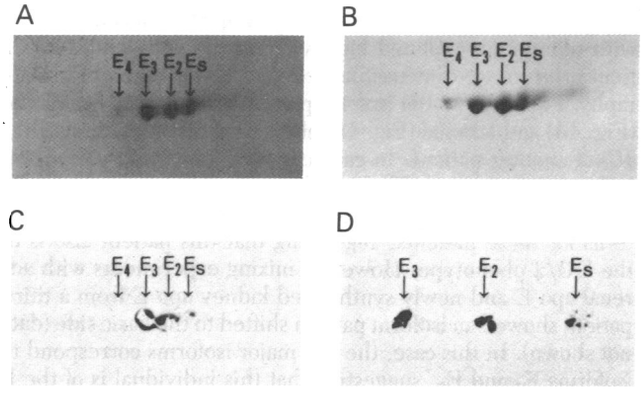

FIG. 3. Comparison of plasma apo E and newly synthesized adrenal apo E by two-dimensional gel analysis. (*A* and *B*) Coomassie blue staining patterns of 25 μg and 50 μg, respectively, of plasma apo E when examined by two-dimensional gel analysis. To determine the relationship between newly synthesized adrenal apo E and plasma apo E, plasma apo E was mixed with an anti-apo E immunoprecipitate from [^{35}S]methionine-labeled adrenal tissue and was analyzed by two-dimensional gel electrophoresis. (*D*) The spots corresponding to isoforms E_4, E_3, E_2, and E_s were excised from the gel, placed in individual vials, and processed for fluorography. (*C*) Fluorograph of the gel showing radioactivity around the rims of isoforms E_3, E_2, and E_s. The isoelectric focusing dimension is oriented with the basic end to the left. The second dimension $NaDodSO_4$/10% polyacrylamide gel was run from top to bottom.

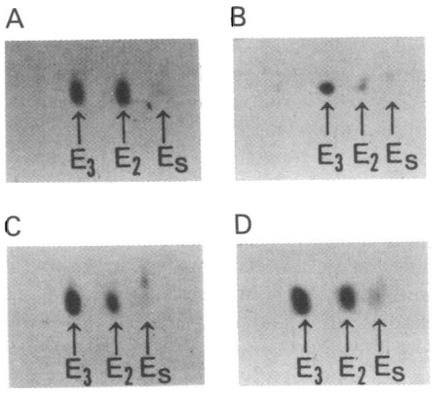

FIG. 4. Two-dimensional analysis of newly synthesized liver, kidney, and adrenal apo E. [^{35}S]Methionine-labeled liver (*A*) and adrenal cortex (*B*) extracts from one patient and a kidney cortex (*C*) extract from another patient were immunoprecipitated with anti-apo E, and the immunoprecipitates were analyzed by two-dimensional gel electrophoresis, followed by fluorography. (*D*) Analysis of a mixture of equal amounts of immunoprecipitate radioactivity of the samples in *B* and *C*. The isoelectric focusing dimension is oriented with the basic end to the left. The second dimension NaDodSO$_4$/10% polyacrylamide gel was run from top to bottom.

second dimension was stained with Coomassie blue to visualize the isoforms, and the major stained spots (E_4, E_3, E_2, and E_s) were excised from the gel. Subsequently, the remaining gel and the excised isoform spots were processed separately for fluorography. This procedure was adopted to eliminate the ambiguity that may occur due to gel shrinkage and loss of the Coomassie blue stain during preparation for fluorography. The fluorograph of the excised isoform spots (Fig. 3*D*) shows that the radioactivity is in isoforms E_3, E_2, and E_s, but not in E_4. Note that the fluorograph of the remaining gel (Fig. 3*C*) also shows radioactivity around the rims of the holes where isoforms E_3, E_2, and E_s were located but shows no radioactivity in the vicinity of the E_4 isoform. This result suggests that this patient (Fig. 3 *C* and *D*) is of the E 3/2 phenotype (14).

Further analysis of newly synthesized apo E was carried out by mixing adrenal ^{35}S-labeled apo E (^{35}S-apo E) from one patient with immunoprecipitated kidney ^{35}S-apo E from another patient prior to two-dimensional gel electrophoresis and fluorography. Fig. 4 shows the isoform pattern of ^{35}S-apo E from liver (Fig. 4*A*) and adrenal (Fig. 4*B*) of one patient and kidney (Fig. 4*C*) of another patient. In each case two major and one minor isoform were evident. The mixed sample of kidney and adrenal apo E (Fig. 4*D*) indicates apparent identity in the isoform patterns for these patients, suggesting that this patient also is of the E 3/2 phenotype. However, mixing experiments with adrenal apo E and newly synthesized kidney apo E from a third patient showed an isoform pattern shifted to the basic side (data not shown). In this case, the two major isoforms correspond to isoforms E_4 and E_3, suggesting that this individual is of the E 4/3 phenotype. Additional analyses of kidney ^{3}H-labeled apo E (^{3}H-apo E) from two other patients produced isoform patterns analogous to and superimposable on the pattern of adrenal apo E. In each case the pattern consisted of two major and one minor isoform. With longer fluorographic exposures than those shown (Figs. 3 and 4), a series of minor spots of progressively higher molecular weight also were seen to the acidic side of the isoelectric focusing dimension with either ^{3}H-apo E or ^{35}S-apo E (data not shown).

DISCUSSION

This study demonstrates the synthesis of apo E by human kidney and adrenal tissue *in vitro*. The apo E made by these tissues is reactive with antiserum against HDL-apolipoproteins and with antiserum specific to plasma apo E. Analysis of immunoreactive apo E showed that newly synthesized adrenal and kidney apo E have the same electrophoretic mobility in NaDodSO$_4$/polyacrylamide gels as does plasma apo E or newly synthesized liver apo E. Further characterization by high resolution two-dimensional gel analysis indicated that the isoforms of kidney and adrenal apo E correspond exactly to specific isoforms of plasma apo E. Patients corresponding to the E 3/2 and E 4/3 phenotypes were identified by analysis of newly synthesized kidney and adrenal apo E. These results identify the newly synthesized, immunoreactive kidney and adrenal protein as apo E or a protein remarkably similar to apo E. Measurements of apo E synthesis *in vitro* showed that the relative rates of kidney and adrenal apo E synthesis represent 0.15–1.2% of total protein synthesis. This result indicates that apo E is a moderately abundant product of kidney and adrenal cells.

The liver and small intestine are considered to be the major sites of synthesis of all the plasma apolipoproteins (17–22, 24–27). Studies with the rat also suggest that the liver is the primary source of plasma apo E (20). The hepatic synthesis of human apo E has been confirmed with cell lines derived from liver tumors (28, 29) and with fetal tissues in organ culture (27). The present finding that kidney and adrenal tissues synthesize apo E indicates that human tissues other than the liver also synthesize this apolipoprotein. However, it is not known to what extent this extrahepatic apo E synthesis contributes to the pool of plasma apo E. Interestingly, neither apo AI nor apo B synthesis was detected in kidney or adrenal, although the synthesis of both apolipoproteins was readily detected in liver tissue. This suggests that the kidney and adrenal are not general sites for the synthesis of all or most plasma apolipoproteins, as is the case with liver and small intestine.

Recent studies suggest that one function of apo E is as a recognition signal for the hepatic removal of cholesterol-laden HDL subfractions and chylomicron remnants from the circulation (5–7). This activity of apo E may be important in the overall movement of cholesterol from peripheral tissues to the liver for metabolism and elimination. However, the ability of cultured cells to recognize and internalize apo E-containing lipoproteins (1–4) may point to a more general role for apo E as a transport or shuttle protein for cholesterol movement to and from tissues in addition to the liver. The present finding that apo E is made in at least two peripheral tissues raises the additional possibility that peripheral apo E synthesis is associated with a mechanism for cholesterol removal from cells or the movement of cholesterol to the central vascular compartment for eventual delivery to the liver (or both). Interestingly, the kidney and, in particular, the adrenal are very active tissues in overall cholesterol metabolism and might have greater need than most tissues for an efficient mechanism for the elimination of excess cholesterol. apo E synthesis in the adrenal also might play a more direct role in regulating the content of cellular cholesterol required for steroid hormone production.

Basu *et al.* (23) showed recently that mouse macrophages secrete an apo E-like protein in a fashion suggestive of a coupling to cellular cholesterol content. This result raised the possibility that macrophages synthesize apo E to facilitate the transport of cholesterol from scavenger cells to the liver. Macrophage apo E synthesis was estimated to represent 0.2–2% of the total cell protein synthesis, depending on the extent of cholesterol loading (23). In the present study the relative rates of kidney and adrenal apo E synthesis are of a similar magnitude, representing 0.15–1.2% of total tissue protein synthesis. The magnitude of apo E synthesis argues strongly that a minor population of scavenger cells cannot account for apo E synthesis in these tissues.

It is more likely that apo E synthesis occurs in many or all cell types in the kidney and adrenal. Additional studies will be required to determine whether apo E synthesis is a common property of many cell types and to establish the relationship between peripheral apo E synthesis and cellular cholesterol metabolism.

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