Molecular characterization and differential mRNA tissue distribution of rabbit apolipoprotein D

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Abstract We report for the first time the quantification of relative apolipoprotein D (apoD) mRNA concentrations in a wide selection of organs and a detailed characterization of the rabbit protein. ApoD cDNA clones were isolated from a rabbit testis cDNA library by screening with a human apoD cDNA-derived RNA probe. The 912 nucleotide sequence of rabbit apoD cDNA contains a unique reading frame coding for a protein sharing 80% homology with human apoD. The two sequences have two potential asparagine-linked glycosylation sites at the same positions, almost superimposable hydrophobicity plot, and the antigenic proteins show similar charge polymorphism, M_r, and lipoprotein distribution. This high degree of similarity shows that the rabbit system can be used as a model for apoD studies. Moreover, the two consensus sequences of the hydrophobic ligand carrier (α_2-microglobulin) family present in human apoD are also found in the rabbit protein and these sequences coincide with the most conserved regions. The distribution of apoD mRNA among rabbit organs was determined by Northern blot and quantitative dot blot analysis. The highest levels of mRNA were found in spleen, adrenal glands, lungs, brain, testis, and kidneys. Moderate or low concentrations were detected in all the other organs tested including liver and small intestine. Thus, our results show that the apoD gene is expressed mainly in peripheral organs, with levels as high as 59-fold that of the liver, unlike other apolipoproteins. We suggest that apoD exerts its main function locally in peripheral organs.—**Provost, P. R., P. K. Weech, N. M. Tremblay, Y. L. Marcel, and E. Rassart.** Molecular characterization and differential mRNA tissue distribution of rabbit apolipoprotein D. *J. Lipid Res.* 1990. 31: 2057-2065.

Supplementary key words α_2-microglobulin family

Human apolipoprotein D (apoD) is a glycoprotein of apparent M_r 29,000 that was isolated and partially characterized by McConathy and Alaupovic (1, 2), and subsequently by Drayna et al. (3), Weech et al. (4, 5), and Albers et al. (6). In the plasma it is distributed mainly in the high density lipoproteins (HDL) with minor amounts in LDL, VLDL, and the plasma protein fraction (5 and references therein). It is associated with lecithin: cholesterol acyltransferase (LCAT) (6-10) and it may stabilize the enzymatic activity (11), or transport the substrates or products of the reaction.

The human apoD cDNA and gene have been cloned and sequenced (3, 12). The deduced protein sequence was found to share 25% of amino acid homology with retinol-binding protein (RBP) (12), and to contain the two consensus sequences common to all members of the ligand carrier family (13-18), and a pattern of exons common to genes of this α_2-microglobulin family (12). Drayna et al. (3) reported that the human apoD gene is expressed not only in the liver and intestine, which are the two major sites of synthesis of the other apolipoproteins, but also in the adrenal glands, pancreas, kidneys, and placenta.

So far, apart from man, only the baboon has been reported to have apoD (19). We have measured and compared the salient characteristics of rabbit and human apoD and its mRNA, and we report here for the first time a systematic and quantitative comparison of the apoD mRNA levels in organs. This shows that the major concentrations were in spleen, adrenal gland, lung, brain, testis, and kidney, with only minor concentrations in liver and intestine. This suggests that apoD should perform a local role in tissues that is likely to be novel and different from that of the major apolipoproteins.

Abbreviations: apoD, apolipoprotein D; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; VHDL, very high density lipoproteins; LCAT, lecithin: cholesterol acyltransferase; RBP, retinol-binding protein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IEF, isoelectric focusing; SSC, sodium saline citrate; apoA-I, apolipoprotein A-I; bp, base pair; kbp, kilobase pair; apoE, apolipoprotein E.

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MATERIALS AND METHODS

Materials

Restriction enzymes were purchased from Pharmacia LKB and New England Biolabs. T7-RNA-polymerase, Klenow fragment, Moloney (MuLV) reverse transcriptase, *Eco*RI adaptors, T7-DNA-polymerase sequencing kit, and Sephacryl S-400 Spun Columns were from Pharmacia LKB. [α-^{32}P]CTP, [α-^{32}P]TTP, [α-^{32}P]dATP, [α-^{35}S]dATP, ^{125}I, and cDNA synthesis kits were purchased from Amersham, whereas lambda-gt-10-arms-*Eco*RI-CIP and Gigapack Plus packaging extracts were from Stratagene.

Isolation of lipoproteins

Lipoproteins were isolated by a modification (5, 20) of the density gradient ultracentrifugation technique of Terpstra, Woodward, and Sanchez-Muniz (21) prestaining the sample with Sudan Black in dimethyl sulfoxide. Sequential fractions of 0.5 ml were pipetted from the top of each gradient for analysis, and pooled afterwards for further study. Protein was measured using bovine serum albumin as standard (22, 23).

Electrophoresis of proteins

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (24), isoelectric focusing (IEF) (25), and two-dimensional IEF-SDS-PAGE (5) were performed as described. Electrotransfer of proteins to nitrocellulose essentially followed the method of Towbin, Staehelin, and Gordon (26), and immunoreactions were performed using monoclonal antibodies 5G10 and 4E11 and ^{125}I-labeled anti-mouse IgG (4, 5).

cDNA cloning and hybridization procedure

Poly (A)$^+$ RNA was purified from total rabbit testis RNA by oligo (dT)-cellulose chromatography (27). After oligo (dT)-primed cDNA synthesis from 5 μg of poly (A)$^+$ rabbit testis RNA, *Eco*RI adaptors were ligated, and a cDNA library was constructed using lambda-gt-10-*Eco*RI-CIP-arms. This library contained 50% recombinant phage particles.

An RNA probe was synthesized with T7-RNA-polymerase (28), using human-apoD-cDNA subcloned in pGEM-1. This plasmid was constructed from the pAPOD6 clone provided by Dennis Drayna (Genentech, Inc.) (3). After hybridization with the ^{32}P-labeled RNA probe in 50% formamide, 50 mM sodium phosphate, pH 7.0, 800 mM NaCl, 1 mM EDTA, 2.5 × Denhardt's, 250 μg/ml sheared and boiled sperm DNA, 500 μg/ml boiled yeast tRNA at 42°C, the nitrocellulose filters were washed in 20 mM sodium phosphate, pH 7.0, 150 mM NaCl, 1 mM EDTA, and 0.1% SDS for 1 h at 50°C, and exposed to X-ray film. Identification and purification of positive clones and DNA extractions were performed as described (27). The insert of three clones was excised by *Eco*RI and subcloned in Bluescript KS$^+$ vector.

DNA sequence analysis

The apoD cDNA cloned into Bluescript was treated with different restriction endonucleases to create deletions. DNAs from the resulting clones and from the entire-cDNA-containing clone were isolated by miniprep (27). After alkaline denaturation, the DNAs were purified on Sephacryl S-400 Spun Columns and sequenced by the dideoxy chain termination method (29) using T7-DNA-polymerase and universal, reverse, and synthetic primers (Gene Assembler II, Pharmacia).

Northern blot analysis

Total RNA from various rabbit organs was extracted by the acid-guanidium thiocyanate-phenol-chloroform method (30), denatured by glyoxal, and then electrophoresed in 1.1% agarose gel and transferred to Nytran membrane. Filters were hybridized with the rabbit apoD cDNA probe (pAPOD-RAB-6) at 65°C as described (31). Blots were washed in SET-2 ×, 1 min at room temperature (SET-1 ×: 30 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA), followed by SET-2 × containing 0.2% SDS at 60°C for 45 min, followed by SET-1 × containing 0.2% SDS at 60°C for 45 min and finally, in SET-1 × for 1 min at room temperature. Membranes were exposed at − 70°C to Kodak Xar-5 X-OMAT film with a Cronex Lighting Plus intensifying screen (E.I. du Pont de Nemours & Co., Wilmington, DE).

Dot blot assay

Different amounts of total RNA extracts (from 0.1 to 5 μg) were denatured in formaldehyde (2.2 M formaldehyde/6 × SSC; 15 min at 65°C) and then spotted on wet Nytran membrane equilibrated in 6 × SSC (1 × SSC; 150 mM NaCl, 15 mM sodium citrate). Prehybridization, hybridization, and washing procedures were the same as for Northern blot. [^{32}P]poly(dT) probe, used for the quantification of poly (A)$^+$ RNA in extracts, was prepared as follows. Reaction was performed at 37°C for 1 h in 50 mM Tris, pH 8.3, 20 mM DTT, 0.6 mM $MnCl_2$, 60 mM NaCl, 20 ng oligo (dT)$_{12\text{-}18}$ (Pharmacia), 200 ng poly (rA) (Pharmacia), 50 μCi [α-^{32}P]TTP, 8 × 10^{-6}M TTP, and 15 units of reverse transcriptase (Moloney MuLV; Pharmacia) in a final volume of 100 μl. This probe was used under the same conditions as for the pAPOD-RAB-6 probe. After autoradiography, the membranes were divided and the radioactivity of each individual spot was measured by liquid scintillation counting (Ready Protein$^+$; Beckman). For each RNA extract, a slope was calculated from the linear portion of the given curve (cpm vs μg). The relative concentrations of poly (A)$^+$ RNA or apoD mRNA were thus estimated from the slope ratios.

RESULTS

Comparison of rabbit and human apoD proteins

To select an animal model for further study we compared the immunoreaction of plasma HDL protein from 12 different

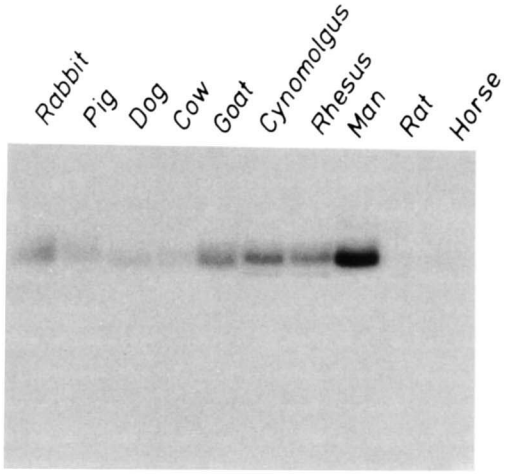

Fig. 1. Presence of the apoD epitope 5G10 in various species. Ten μg of each denatured HDL fraction was electrophoresed on SDS-PAGE, transferred to a nitrocellulose membrane, and reacted with antibody 5G10, followed by autoradiography. The samples were from rabbit, pig, dog, cow, goat, cynomolgus, rhesus, human, rat, and horse.

species with that of the human after SDS-PAGE and electrotransfer to nitrocellulose. Monoclonal antibody anti-human apoD 4E11 reacted with human HDL only, but monoclonal antibody 5G10 reacted with the reduced proteins of rabbit, pig, dog, cow, goat, and cynomolgus and rhesus monkeys (**Fig. 1**), and sheep. Rabbit HDL protein gave a strong immunoreactive band on SDS-PAGE at M_r 29,000 like the human, as already described by Camato et al. (5). The horse reacted very weakly and no reaction was seen with cat, guinea pig (not shown), or rat. The relative lack of reaction could be due to a difference in distribution of apoD in these species compared with humans. To assess that question, we tested rat and horse HDL, VHDL, and plasma proteins with antibody 5G10 after reduction and IEF. No convincing reaction was seen, not even with 40 μl of the plasma protein fraction (data not shown).

Given the antibody 5G10 cross-reactive protein in the rabbit HDL, we compared the distribution of this antigen and apoA-I (the major HDL apolipoprotein) in lipoproteins and proteins of rabbit and human plasma (**Fig. 2**). Protein peaks (determined by the Lowry et al. (22) assay) were found at the expected densities of HDL in the two species, and in the rabbit, the apoD distribution corresponded well with that of apoA-I as detected by anti-human apoA-I (Fig. 2). The apoD antigen 5G10 was found principally in the dense HDL (1.078–1.211 g/ml) of the rabbit and human, with much less in the VLDL and LDL (Fig. 2), thus exhibiting a similar distribution.

Using the HDL pooled from the experiment in Fig. 2, the charge polymorphisms of rabbit and human 5G10 antigens were compared by IEF and two-dimensional IEF-SDS-PAGE. Eight isomorphs could be detected in the rabbit (data not shown) with the same pI values (pH 4.66–5.19) and M_r as in the human (5).

Isolation of rabbit apoD cDNA

By screening 1 × 10^6 phage plaques from the oligo (dT)-primed rabbit testis cDNA library with human apoD RNA probe (derived from pAPOD6 clone (3)), 129 positive clones were obtained. Thirty positive clones were purified and the corresponding DNA inserts were analyzed by *Eco*RI digestions and agarose gel electrophoresis.

The *Eco*RI inserts from three potentially full-length clones (APOD-RAB-1, 6 and 42) were subcloned in the Bluescript KS$^+$ plasmid vector.

Characterization and sequence analysis of rabbit apoD cDNA

Restriction enzyme analysis of pAPOD-RAB-1, 6 and 42 revealed one restriction site each for *Nar*I, *Eco*RV, and *Sau*I in the three clones, giving the same restriction map (data not shown). Around 200 nucleotides at both ends of the

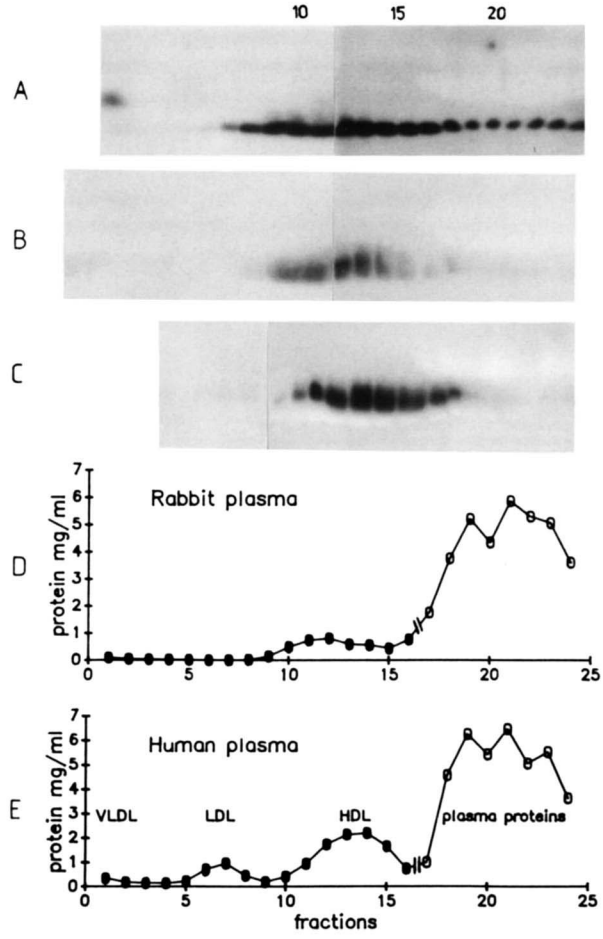

Fig. 2. Distribution of the anti-apoD 5G10 antigen among rabbit plasma lipoprotein density classes. The autoradiographs (panels A, B, C) show binding of antibodies 5G6 (anti-apoA-I; panel A) and 5G10 (anti-apoD; panel B) to rabbit plasma lipoprotein fractions, and 5G10 binding to human plasma fractions (panel C) after SDS-PAGE and Western blotting. Panels D and E show the distribution of protein among the same fractions from rabbit and human plasma, respectively. The ordinate for fractions 17–24 is compressed to 0.1 of that for fractions 1–16.

three corresponding inserts were sequenced (data not shown) and the results showed no substitution or mutations of any kind except for a varying poly (A) chain length. However, in two clones (pAPOD-RAB-1 and 42), we failed to find the *Eco*RI adaptor sequence at the 5' end of the insert, indicating the presence of a natural *Eco*RI site at the 5' end of the cDNA and that we had lost a small cDNA portion during the subcloning. The third clone (pAPOD-RAB-6), which is smaller in length, was not elongated as far as that *Eco*RI site and thus contained the adaptor sequence. Using *Nar*I, *Eco*RV, and *Sau*I enzymes and restriction sites in the polylinker, deletions were introduced in pAPOD-RAB-6. This allowed us to sequence both strands of the complete cDNA (**Fig. 3**) with the universal and the reverse primers except for the small region upstream to the 5' *Eco*RI site (position 13) which was sequenced using a synthetic DNA primer and lambda-APOD-RAB-35 as matrix.

Fig 3. shows that the rabbit apoD cDNA nucleotide sequence is composed of 912 residues, followed by a poly (dA) tail. From the nucleotide sequence a unique reading frame was found and the amino acid sequence was deduced. The apoD-mRNA 5' untranslated region has a minimum length of 85 bases and contains a perfect inverted repeat of 6 bases at the 5' end. The 3' untranslated region is 260 bases long and contains the polyadenylation signal hexanucleotide (AATAAA) from positions 879 to 884. By analogy to the human sequence (3), we have designated glutamine as the first residue of the mature protein. The predicted leader sequence contains 21 amino acid residues extending from nucleotide 86 to 148. The mature protein, composed of 168 amino acid residues, is encoded by nucleotides 149 to 652.

Comparison of inferred amino acid sequences between mature rabbit and human apoD, presented in **Fig. 4,** shows that the human residue 169 (the carboxyl terminus) is missing in the rabbit apoD, which ends at residue 168 as a consequence of a stop codon in the rabbit nucleotide sequence (TAG) instead of the serine codon (TCG) in the human cDNA. No other amino acid deletions or insertions can be observed when comparing the human and rabbit sequences. Rabbit and human mature apoD share an overall amino acid sequence homology of 80%. The nucleotide sequence of both mRNA, including the 5' and 3' untranslated regions, share an overall homology of 77%, which reaches 83% for the coding sequences. The two potential asparagine-linked glycosylation sites found in the human apoD are conserved at the same positions in the rabbit protein (+ 45 and + 78). Four of the five cysteine residues of the human protein are also conserved in the rabbit sequence (+ 8, + 41, + 114, + 165). The human Cys116 is replaced by a threonine residue in the rabbit protein.

Fig. 4 also shows that the two consensus sequences of the ligand carrier family are particularly well conserved between both species (+ 16 to + 32 and + 98 to + 111) (13). The first reported consensus sequence is Asn-hyd-acidic-

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                                                                                    -21
                                                                                    MET ALA PRO THR LEU LEU LEU LEU
 TCCACGTCGGATGAATTCGTGGACATCACTCTCTTCTTGGTGAAAAAAAAAAGCTCCAGGTTCCGGTGCAGCCGCCGGCCCCCAG  ATG GCG CCC ACG CTG CTG CTG CTG
                    20                  40                  60                  80                      100
               -10                                   +1                                      10
 LEU PRO ALA LEU ALA GLY LEU ILE SER VAL ALA GLN GLY GLN ALA PHE HIS LEU GLY ARG CYS PRO THR PRO PRO VAL GLN GLU ASN PHE
 CTC CCG GCG CTG GCC GGC CTG ATC TCC GTG GCG CAG GGG CAG GCG TTC CAC CTG GGC AGG TGC CCC ACC CCT CCC GTG CAG GAG AAC TTC
                120                         140                         160                         180
           20                                    30                                      40
 ASP VAL HIS LYS TYR LEU GLY ARG TRP TYR GLU ILE GLU LYS ILE PRO VAL SER PHE GLU LYS GLY ASN CYS ILE GLN ALA ASN TYR SER
 GAC GTG CAC AAG TAT CTC GGA CGA TGG TAC GAA ATT GAG AAG ATC CCG GTG AGC TTT GAG AAA GGA AAC TGC ATC CAG GCC AAC TAC TCA
200                          220                        240                         260                         280
           50                                    60                                      70
 LEU MET GLU ASN GLY ASN ILE LYS VAL LEU ASN GLN GLU LEU ARG PRO ASP GLY THR VAL ASN GLN ILE GLU GLY GLN ALA THR GLN SER
 CTA ATG GAG AAC GGA AAC ATC AAA GTG CTG AAT CAG GAG TTG AGA CCT GAT GGA ACT GTG AAT CAA ATT GAA GGC CAA GCC ACC CAG AGC
                300                         320                         340                         360
           80                                    90                                      100
 ASN LEU THR GLU PRO ALA LYS LEU GLY VAL LYS PHE PHE GLN LEU MET PRO THR ALA PRO TYR TRP VAL LEU ALA THR ASP TYR GLU ASN
 AAC CTC ACG GAG CCC GCC AAG TTG GGA GTG AAG TTT TTC CAG TTG ATG CCA ACG GCC CCG TAC TGG GTC CTG GCC ACC GAC TAT GAG AAC
380                          400                        420                         440                         460
           110                                   120                                     130
 TYR ALA LEU VAL TYR SER CYS THR THR ILE ILE TRP LEU PHE HIS MET ASP HIS VAL TRP ILE LEU GLY ARG ASN ARG TYR LEU PRO PRO
 TAC GCA CTC GTG TAC TCC TGT ACC ACA ATC ATC TGG CTT TTT CAC ATG GAT CAT GTT TGG ATC TTG GGA AGA AAC CGT TAT CTC CCT CCT
                480                         500                         520                         540
           140                                   150                                     160
 GLU THR VAL THR TYR LEU LYS ASP ILE LEU THR ALA ASN ASN ILE ASP ILE GLU LYS MET THR VAL THR ASP GLN VAL ASN CYS PRO GLU
 GAA ACA GTG ACC TAT CTG AAG GAT ATC CTG ACT GCC AAT AAC ATC GAC ATT GAG AAG ATG ACT GTC ACA GAT CAG GTG AAC TGC CCC GAG
560                          580                        600                         620                         640

 PHE *** *** ***
 TTC TAG TAA TGA  GGCTCAAAGGGGAGGCTGCACCTGCTCCATGTCACTTCTCCTGCTTCGCTTTCCCCTGCCCCACTCTACCCTTCCTAAGGACAGACCACCCGGCCAGACAAAC
                660                  680                  700                  720                  740                  760

 ATGTGTGAATACCAGAAGGGAGGCTTAACGGCAATGGAGCCAATGAAGGGCAGTTGAAGGAAACTTGGCCCCAACACTTAGCCGTGCCCCACTCTGCTGTCTTGCTGGCCTCCTAATAAA
                780                  800                  820                  840                  860                  880

 CGTGTTGCTGACCTGCTGTGCTCACAGT
                900
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Fig. 3. Sequence of rabbit apoD cDNA. The complete amino acid sequence inferred for the rabbit apoD protein is shown above the nucleotide sequence. The negative numbers refer to the presumed leader prepeptide, whereas positive numbers refer to the mature protein. Stop codons are denoted by asterisks. The inverted repeats at the 5' end and the polyadenylation signal hexanucleotide are marked, respectively, by arrows and a line above the nucleotide sequence.

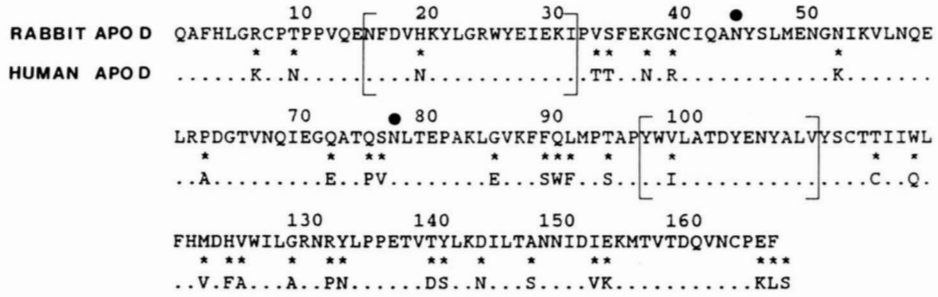

Fig. 4. Comparison of mature rabbit and human apoD. The top line is the cDNA-derived sequence of the mature rabbit apoD protein. The bottom line shows the human mature apoD amino acid sequence (3). Dots on the bottom line indicate the human residues that are identical to the rabbit sequence. Asterisks between the two lines highlight the amino acid substitutions. The two potential asparagine-linked glycosylation sites (Asn-X-Ser/Thr) are marked by dots above the rabbit sequence. The two boxes indicate the two consensus sequences of the ligand carrier superfamily.

hyd-X-basic-hyd-X-Gly-X-Trp-aro-X-hyd-hyd-hyd-hyd, where acidic is Asp or Glu, basic is Arg or Lys, hyd is a hydrophobic residue (Ala, Ile, Leu, Met, Phe, Tyr, Val or Trp), aro is aromatic (Phe or Tyr), and X is any residue. The second consensus sequence is aro-X-hyd-hyd-X-Thr-Asp-Tyr-Asp-X-aro-hyd-hyd-hyd (13). Mutations at position + 20 and + 100 that fall, respectively, in the first and in the second consensus sequence are not in contradiction with the consensus reported by Pevsner et al. (13). However, residues + 30, + 31, and + 106 in proteins of the two species do not fit in the consensus sequences.

Rabbit apoD gene expression

To determine the rabbit apoD mRNA distribution among the tissues, we extracted RNA from fifteen rabbit organs. After denaturation by glyoxal, total RNA was submitted

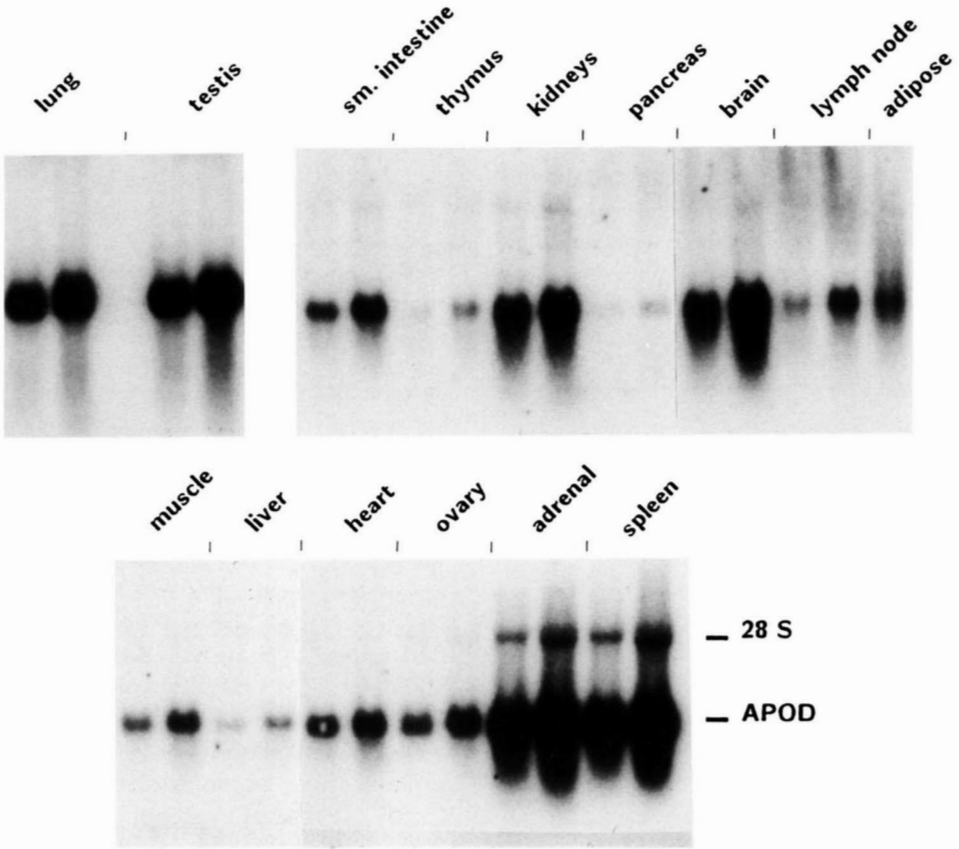

Fig. 5. Northern blot analysis of rabbit apoD in various organs. Ten and 20 μg of total RNA extracted from each rabbit organ was loaded on an agarose gel, electrophoresed, and transferred to Nytran membranes. The membranes were hybridized with ^{32}P-labeled rabbit apoD cDNA, washed, and autoradiographed. For adipose tissue, a single loading of 19 μg was performed.

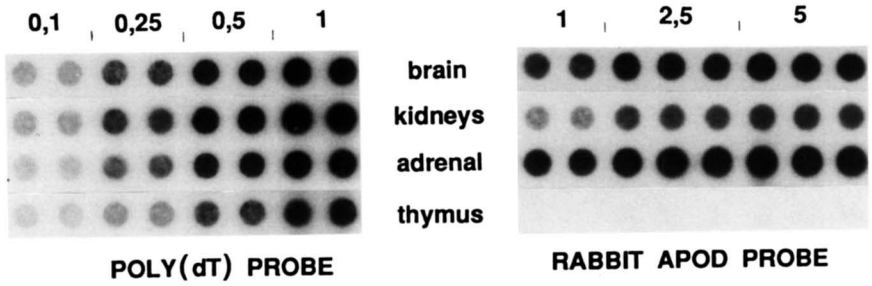

Fig. 6. Quantification of rabbit apoD mRNA in various organs. Varying microgram amounts (as indicated) of total RNA extracted from each rabbit organ were transferred by dot blot to Nytran membranes that were hybridized with ^{32}P-labeled poly (dT) (left panel) or ^{32}P-labeled rabbit apoD cDNA (right panel). The results are presented for brain, kidney, adrenal gland, and thymus. ApoD mRNAs were quantified by dot blot analysis as described in the Materials and Methods, and the results for all the tissues tested are summarized in Table 1.

to agarose gel electro horesis and Northern blot analysis. RNA bound to Nytran membrane was hybridized with rabbit apoD cDNA probe (pAPOD-RAB-6). ApoD mRNA was detected in all tested tissues as shown in **Fig. 5.** Total RNA from bone marrow was also tested and continued a low level of apoD mRNA comparable to that of the thymus (data not shown).

The rabbit apoD mRNA is longer than that reported for human (≈ 1 kb instead of ≈ 900 b). This difference is a consequence of a longer 3' non-coding region in the rabbit mRNA (260 bases compared with 182 bases in the human counterpart) as shown in Fig. 3.

The relative apoD mRNA concentration was clearly different from one organ to another as shown in Fig. 5. This tissue distribution was reproducible as shown by Northern blot analysis on four adult rabbits, including three male and one female, which gave almost the same relative intensity for apoD mRNA band from one organ to another (data not shown). In order to get precise measurements of the relative concentrations of apoD mRNA in the different organs studied, we performed dot blot assays. First, total RNA extracts were calibrated in terms of poly (A)$^+$ RNA concentration using a [^{32}P]poly (dT) probe as shown in **Fig. 6**. Second, increasing amounts of total RNA from the organs were spotted on Nytran membrane and hybridized with the rabbit apoD cDNA probe APOD-RAB-6 (Fig. 6) and radioactivity was measured by scintillation counting. A curve was drawn relating apoD mRNA cpm to the total RNA of each sample, and then the apoD mRNA abundance of each extract was corrected using the relative poly (A)$^+$ RNA concentration determined at the first step. The results are shown in **Table 1** and are expressed relative to the apoD mRNA concentration in the liver, which was arbitrarily given a value of one. The spleen, adrenal gland, lung, brain, testis, and kidney contained a high apoD mRNA concentration, 59- to 5-fold that of other organs such as the liver and small intestine.

TABLE 1. Quantification of apoD mRNA among rabbit organs

Organ	ApoD-mRNA Concentration (relative to liver)[a]
Spleen	59
Adrenal	17
Lung	15
Brain	14
Testis	8
Kidney	5
Heart	3.5
Small intestine	2
Bone marrow	1.5
Thymus	1
Pancreas	1
Skeletal muscle	1
Liver[a]	1
Lymph node	0.6

[a]The liver mRNA level of expression was arbitrarily given a value of 1.

DISCUSSION

The rabbit has several advantages as a model system for studying apoD metabolism. *1)* The rabbit apoD protein cross reacts well with the monoclonal antibody anti-human apoD 5G10 (Fig. 1) which reacts with an epitope composed of the polypeptide rather than the oligosaccharides of apoD and is preferentially expressed on the reduced, SDS-denatured protein (4). *2)* Rabbit and human apoD have the same M_r and charge polymorphism (not shown) indicating that post-translational modifications of apoD might be similar in both species. *3)* The distribution of apoD among the plasma lipoprotein density classes is almost identical in both species (Fig. 2). *4)* The apoD gene is expressed not only in the liver and intestine but also in other organs in both rabbit and man (Fig. 5). *5)* The primary structure of rabbit apoD is 80% homologous with that of human and the secondary and tertiary structures would be essentially identical, since: *a)* most of the amino acid substitutions are conservative (Fig. 4); *b)* the two potential glycosylation sites and four of the five cysteine residues of human apoD are conserved at the same positions in the rabbit (Fig. 4); and *c)* the hydrophobicity plots (32) are almost identical (not shown).

The exact role of apoD in lipid metabolism remains to be determined. However, the transport of a small hydrophobic molecule could be the major function of the protein by virtue of primary structure homology with the ligand carrier family (12, 13). In fact, some of these homologous proteins are known to transport small hydrophobic molecules such as retinol (retinol-binding protein (33, 34)) and biliverdin IX (*Manduca sexta* insecticyanin, (35)). Moreover, some members are known to transport more than one kind of ligand molecule. For example, odorant-binding protein (13) can bind and transport a variety of odorants. Our results in the rabbit (Fig. 4) show that the two consensus motifs of the ligand carrier family coincide with two regions of apoD that were the most conserved between rabbit and human. The consensus sequences of both human and rabbit apoD show three amino acid differences when compared with the published consensus sequences (13), but these do not seem to be significant since the three amino acid positions appear to be variable in at least one member of the family (13). Moreover, since α_2-microglobulin is the only family member with exact matches with both consensus sequences (13), it could be worthwhile to refine the consensus analysis.

The rabbit apoD system is essentially identical to that of the human as discussed earlier. Thus, one would expect that the organs producing high or low apoD mRNA levels would be the same in humans as those identified here in the rabbit. Our analysis of mRNA showed that the rabbit apoD gene is expressed in kidneys, liver, small intestine, adrenal glands, and pancreas as was reported for the human gene (3) (Fig. 5). We have examined additional organs and found high levels of mRNA in the rabbit spleen, lung, brain, testis, heart, adipose tissue, and ovary, and low amounts in bone marrow, thymus, skeletal muscle, and lymph node (Fig 5). This is the first report of the widespread expression of this gene.

Drayna et al. (3) suggested that apoD could transport the substrates or products of LCAT reaction (12) because apoD was found to stabilize the enzymatic activity of LCAT in vitro (11) and is a member of the ligand carrier family (12). The nature of the molecule(s) transported by apoD remains to be discovered, but unesterified cholesterol, lysophospholipids, and fatty acids are candidates as they are necessary to all organs and especially those in which the apoD gene is expressed. Excess concentrations of any of these lipids might destabilize cell membranes.

One hypothesis suggests that unesterified cholesterol can transfer to HDL by simple diffusion from the cell surface (36). In this situation apoD would not be essential for transport out of the cell and into the circulation. However, there may be cells that are not exposed to HDL from the circulation, and in that situation apoD could play a local paracrine role in the redistribution of its ligand among neighboring cells, or between cells and HDL in the circulation. ApoD, like apoE, is synthesized by many organs and its level is increased in nerve injury (37–39), so it may have an essential role in physiology and pathology. This role could be somewhat different in each organ.

Although apoE and apoD are both widely expressed in various organs (3, 40, 41, and the present studies), their respective tissue distributions are clearly different. For the mouse (40) and the baboon (41), the major apoE mRNA-producing organ is the liver. In addition, in both species, the apoE mRNA concentration found in peripheral organs was about 10% of that of the liver (40, 41), except for the baboon adrenal gland, which contained 60% relative to the major organ (41). In Table 1, our results show that apoD mRNA concentration in rabbit peripheral organs reaches a peak that is 59-fold higher (spleen) than that of the liver. Moreover, by estimation of the total amount of apoD mRNA in the organs (concentration × weight of the organ), the liver does not appear to be the major apoD mRNA-producing organ since lungs, brain, spleen, and kidneys would produce respectively, 1.9-, 1.5-, 1.0-, and 0.9- fold of the total amount synthesized in the liver (deduced from Table 1). Thus, the apoD gene is the only apolipoprotein gene that is not expressed mainly in the liver, the intestine, or both, but rather in peripheral organs. Based on this finding, we suggest that apoD exerts its main function locally in peripheral organs rather than in circulation.

The organs in which apoD mRNA is most concentrated are the adrenal gland, lung, brain, testis, and spleen (Table 1). Lipid metabolism is essential to the function of each of these organs, respectively, in steroid hormone biosynthesis, surfactant biosynthesis, myelination of neurones, steroid hormone biosynthesis, and growth of spermatozoa, and destruction of senescent erythrocytes. Except for the adrenal and spleen, each of these organs has a compartment that may not be in direct contact with HDL in the extracellular fluid. However, the difference between these organs and others lies in the concentration of apoD mRNA, rather than its presence or absence.

This report of the characterization of rabbit apoD protein and identification of high and low apoD mRNA-producing organs opens the way for identification of the cell type(s) that synthesize apoD in vivo, and measurement of up- and down-regulation both in vivo and in cell culture.

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