An 87-kilobase (kb) P1 bacteriophage clone (p649) spanning the mouse apolipoprotein (apoB) B gene was used to generate transgenic mice that express high levels of mouse apoB. Plasma levels of apoB, low density lipoprotein cholesterol, and low density lipoprotein triglycerides were increased, and high density lipoprotein cholesterol levels were decreased in the transgenic mice, compared with nontransgenic littermate controls. Although p649 contained 33 kb of 5'-flanking sequences and 11 kb of 3'-flanking sequences, the tissue pattern of transgene expression was different from that of the endogenous apoB gene. RNA slot blots and RNase protection analysis indicated that the transgene was expressed in the liver but not in the intestine, whereas the endogenous apoB gene was expressed in both tissues. To confirm the absence of transgene expression in the intestine, the mouse apoB transgenic mice were mated with the apoB knockout mice, and transgenic mice that were homozygous for the apoB knockout mutation were obtained. Because of the absence of transgene expression in the intestine, those mice lacked all intestinal apoB synthesis, resulting in a marked accumulation of fats within the intestinal villus enterocytes. The current studies, along with prior studies of human apoB transgenic animals, strongly suggest that the DNA sequence element(s) controlling intestinal expression of the apoB gene is located many kilobases from the structural gene.

Within the past two years, transgenic mice expressing high levels of human apolipoprotein B (apoB) \(^1\) have been generated, both by our group \((1)\) and by Callow and his co-workers \((2)\). These mice were generated with a \(P1\) bacteriophage clone that spanned the entire human apoB gene. The human apoB transgenic mice displayed several noteworthy features that posed questions for subsequent studies. First, the tissue pattern of transgene expression was decidedly abnormal. In multiple lines of mice, the human apoB transgene was expressed at high levels in the liver, but expression was undetectable in the intestine \((3, 4)\). This finding was surprising in view of the fact that the 80-kb transgene contained rather extensive flanking sequences 19 kb 5' from the transcriptional start site and 17.5 kb 3' from the translation termination codon. One possible explanation for absent transgene expression in the intestine was that the intestinal tissue-specific regulatory element was located so far from the structural gene that it simply was not contained within the 80-kb transgene. However, other potential explanations existed. One possibility was that the human intestinal element was located within the 80-kb fragment but that it was not recognized by the mouse transcriptional machinery. An obvious means of testing the latter possibility would be to analyze transgene expression patterns in mice generated with a \(P1\) clone spanning the mouse apoB gene.

A second issue that arose during the study of the human apoB transgenic mice was the finding of the high levels of triglyceride-rich low density lipoproteins (LDL) in the plasma of the mice \((1)\). Although we were initially tempted to ascribe the high levels of LDL to apoB overproduction by the liver, another potential explanation existed. Human apoB100 binds to the mouse LDL receptor with very low affinity, compared with mouse LDL \((5)\), so it is possible that the increased LDL levels could have been due, in large part, to defective clearance of the human LDL from the plasma. Indeed, this issue raised serious doubts as to whether transgenic expression of human apoB, with its defective binding to the mouse LDL receptor, could yield an accurate portrayal of the phenotype of apoB overproduction by the liver. An obvious way to obtain an accurate view of the phenotype of hepatic apoB overproduction was to develop and characterize transgenic mice that overexpress mouse apoB.

To address the issues posed by the human apoB transgenic mice, we obtained a \(P1\) clone spanning the mouse apoB gene and used that clone to develop transgenic mice that expressed high levels of mouse apoB.

**MATERIALS AND METHODS**

Generation of Mouse apoB Transgenic Mice—A \(P1\) bacteriophage library made from Mus musculus (strain RII) genomic DNA was screened for clones containing the mouse apoB gene (a service of Genome Systems, Inc., St. Louis, MO). Two pairs of polymerase chain reactions were used for screening. Oligonucleotides Mus5-5' (5' - GAGCTTTCTATTGTGGC-3') and Mus5-3' (5'-GATTCAGGAGCTTCTATGGGCGC-3') were used to amplify a 448-bp fragment located between –582 and –135 bp 5’ of the apoB transcriptional start site. Oligonucleotides Mus3-5' (5'-GATTCAGAGCCTTCTATGGGCGC-3') and Mus3-3' (5'-ATCATGGCGTCTGCTAGG-3') were used to amplify a 294-bp fragment starting 8 bp upstream from the apoB translation termination codon. A single clone, designated p649,
yielded positive polymerase chain reactions with both pairs of primers. The clone was mapped by standard restriction analysis and Southern blots, using both P1 vector probes (see probes A and B in Fig. 1) and mouse apoB gene probes (probes C and D).

To prepare p649 DNA for microinjection, plasmid DNA was isolated from Escherichia coli strain NS3529 (6) and cleaved with MluI. A 95-kb MluI fragment containing the entire 87-kb insert and 8-kb of P1 vector sequences (2200 bp 5' and 5800 bp 3') was purified from a pulsed-field agarose gel and dialyzed against a microinjection buffer (6). After adjusting the DNA concentration to 3 ng/µl, the DNA was microinjected into F2 murine zygotes (C57BL/6J × SJL), both at the Gladstone Institute of Cardiovascular Disease and at DNX Transgenics.

Identification of Transgenic Animals and Breeding—Transgenic animals were identified by Southern blot analysis of EcoRI-digested tail DNA using P1 vector probes A and B (see Fig. 1). Six transgenic founders were identified; two of the founders (6690 and 6691, both males) had increased levels of mouse apoB in the plasma, as judged by Western blots of agarose gels probed with a 125I-labeled rabbit antiserum to mouse apoB (1). Founders 6690 and 6691 were mated with C57BL/6J mice, and F1 transgenic offspring (genotype MBTg1/0) were identified by Southern blot analysis of tail DNA and by agarose gel electrophoresis of plasma samples. In initial experiments, founder animals 6690 and 6691 appeared to have a similar increase in the plasma apoB levels, compared with nontransgenic mice. We arbitrarily used the progeny from founder 6691 for most lipoprotein analyses since we initially obtained more F1 animals from that line. After obtaining additional F1 animals from line 6690, we studied those animals in greater detail. Those studies revealed that the F1 animals from line 6690 actually had 2-fold more plasma apoB than F1 animals from line 6691. Accordingly, some of the subsequent experiments used mice from transgenic line 6690. For all experiments, nontransgenic littermates were used as controls.

Founder 6691 was mated to mice heterozygous for an apoB knockout mutation (7) to generate mouse apoB transgenic mice that were heterozygous for the knockout mutation. Those mice were intercrossed to generate mouse apoB transgenic mice that were homozygous for the knockout mutation (genotypes MBTg2/2, apoB2/2 or MBTg2/2, apoB2/2). Mice that were homozygous for the knockout mutation were detected by demonstrating a "double dose" of the neo gene (inserted into the apoB locus in the knockout mice), as compared with the "single dose" of neo in mice heterozygous for the knockout mutation. For these studies, tail DNA was digested with HindIII, and Southern blots were probed simultaneously with a neo probe and a cDNA probe for the α-tocopherol transport protein (8). The intensity of the neo signal relative to the α-tocopherol transport protein signal was assessed using a Phos-
Determination of Transgene Copy Number—To determine transgene copy number, tail DNA from F1 transgenic mice (line 6690) and non-transgenic littermates was digested with EcoRI, and Southern blots were performed using probe D (see Fig. 1). Transgene copy number was determined using a Phosphorimager, based on the amount of radioactivity in the apoB band on the Southern blot.

Assessing the “Intactness” of the Transgene within the Mouse Genome—To determine whether intact copies of the 87-kb transgene had been incorporated into the mouse genome, Southern blot analysis was performed using NruI-cleaved, high molecular weight DNA from F1 mice of transgenic lines 6690 and 6691. The strategy was to identify an 88-kb NruI fragment within the genomic DNA. Both of the NruI sites

**Fig. 4. Lipids and lipoproteins in transgenic and nontransgenic mice.** A, comparison of the lipids and lipoproteins in six 10-week-old female transgenic mice and six female nontransgenic littermate control mice. B, comparison of the lipids and lipoproteins in MBTg+/-,apoB-/- mice and age- and sex-matched nontransgenic control mice. Bars are means, and lines represent standard deviations.

**Fig. 5. Analysis of the distributions of cholesterol (A), triglycerides (B), and apoB48 and apoB100 (C) in the plasma of 8-week-old mouse apoB transgenic mice.** Pooled plasma samples from six female transgenic mice and six female nontransgenic littermate controls were size-fractionated on an FPLC column, as described under “Materials and Methods.” Cholesterol and triglycerides were determined by an enzymatic assay (panels A and B), and the distributions of apoB48 and apoB100 were determined by Western blots of SDS-polyacrylamide gels using a rabbit antibody to mouse apoB and the Enhanced Chemiluminescence Western blotting detection reagents (Amersham Corp.) (panel C). The angled appearance of the apoB48 and apoB100 bands in the lower blot in panel C was due to the angled placement of the gel on the blotting paper before electrophoretic transfer to nitrocellulose.
are located within the 95-kb MluI fragment (see Fig. 1). Liver cell suspensions were prepared from transgenic and nontransgenic mice, and the cells were embedded and DNA prepared in agarose plugs (9). The plugs were digested with NruI, and the DNA was size-fractionated on a pulsed-field 1% agarose gel in 0.5 × TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA) at 6 V/cm for 15.16 h at 14°C, with initial and final switching times of 0.22 and 21.79 s, respectively. The gel was blotted onto a nylon membrane, and the membrane was hybridized to probe D (see Fig. 1).

RNA Analysis—Six-week-old F1 transgenic offspring from founder 6691 (and nontransgenic littermate controls) were sacrificed, and total cellular RNA was prepared from liver and intestine (duodenum) using the Totally RNA kit (Ambion, Austin, TX). Three amounts of RNA (0.25, 1.0, and 4.0 μg) were loaded on a nondenaturing 1% agarose membrane using a slot-blot apparatus (Schleicher and Schuell, Keene, NH), and the membrane was hybridized to a probe containing sequences from exon 25 of the mouse apoB gene (probe D in Fig. 1) as well as a probe for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (to control for the amount of RNA bound to the membrane). The intensity of the apoB signal, relative to the GAPDH signal, was assessed using a phosphorimager.

For the RNase protection assays, total RNA was isolated from liver and intestine (duodenum) of F1 mice from lines 6690 and 6691 by homogenization in guanidine isothiocyanate (10) using the RNAzol method (Tel-test, Inc., Friendswood, TX). A 245-bp antisense RNA transcript was prepared with XbaI-linearized plasmid pBSmB245 and T7 RNA polymerase, using the MAXIscript kit (Ambion). Plasmid pBSmB245 contains a XbaI/Msal fragment from exon 25 of the mouse apoB gene. The RNase protection assay was performed using the RPA II Ribonuclease protection assay kit (Ambion). The samples were electrophoresed on denaturing 6% polyacrylamide gels. Dried gels were analyzed by autoradiography and with the phosphorimager.

Analysis of an Exon 26 DNA Polymorphism in the Transgene—To determine the molecular basis of the exon 26 DNA polymorphism in the transgene, a 399-bp segment of the 5′ portion of exon 26 of the mouse apoB gene. The RNase protection assay was performed using the RPA II Ribonuclease protection assay kit (Ambion). The samples were electrophoresed on denaturing 6% polyacrylamide gels. Dried gels were analyzed by autoradiography and with the phosphorimager.

Analysis of the Plasma Lipoproteins—To determine the molecular basis of the exon 26 DNA polymorphism in the transgene, a 399-bp segment of the 5′ portion of exon 26 of the mouse apoB gene was enzymatically amplified, both from p649 and from genomic DNA from nontransgenic mice. The 399-bp DNA fragments were cloned into the TA3PCR vector using the TA cloning kit (Invitrogen, San Diego, CA) and sequenced using an Applied Biosystems 373A DNA sequencer (Foster City, CA).

Generation of a Rabbit Antiserum to Mouse apoB—Mouse LDL (d = 1.006–1.063 g/ml) were prepared by ultracentrifugation (11). The LDL (3 ml) were mixed with 6 ml of sonication buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 2% sodium deoxycholate), and protease inhibitors (aprotinin [final concentration, 100 KI units/ml], leupeptin (0.1 mM), and phenylmethylsulfonyl fluoride (1 mM)) were added to the solution. After sonication the solution with six 10-s pulses and one 15-s pulse using a Branson Sonifier 450 sonicator (power output = 2, with the micro tip), 1.5 ml of the solution was overlaid onto each of six SW41 ultracentrifuge tubes containing a 10–40% sucrose gradient. The sucrose gradient was prepared in buffers containing 100 mM ammonium bicarbonate, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, and the aforementioned protease inhibitors. Following ultracentrifugation in an SW41 rotor at 35,000 rpm for 60 h at 10°C, fractions (0.5 ml each) were unloaded from the gradient and analyzed by electrophoresis on a 4–15% polyacrylamide-SDS gel. Low density lipoproteins were visualized by staining the gels with the Bio-Rad Silver Stain Plus kit. Those fractions containing exclusively apoB48 and apoB100 were pooled and used to immunize a human apoB transgenic New Zealand White rabbit (12). The rabbit was immunized with 10 μg of mouse apoB in complete Freund's adjuvant followed by booster immunizations of 5 μg of protein in incomplete Freund's adjuvant at 4, 6, and 14 weeks. Serum samples were taken weekly after the final immunization. The rabbit was sacrificed, and serum was collected at 19 weeks. Rabbit serum was screened for binding to mouse LDL by an enzyme-linked immunosassay. The specificity of the antibody for mouse apoB48 and apoB100 was confirmed by Western blots of plasma proteins that had been size-fractionated on an SDS-polyacrylamide gel.

Analysis of the Plasma Lipoproteins—Total cholesterol, HDL cholesterol, and triglyceride levels were measured in fresh plasma samples after a 4-h fast. Plasma lipoprotein electrophoresis was performed on 1% agarose gels (13). The agarose gels were either stained for neutral lipids with Fat Red 7B or used for Western blots with a 125I-labeled

![Fig. 6. RNA slot blot demonstrating the relative amounts of apoB mRNA in the livers (A) and intestines (B) of transgenic and nontransgenic mice. With one of the nontransgenic mice, the 0.25-μg liver RNA sample was not loaded onto the membrane.](image-url)
To analyze the distribution of cholesterol, triglycerides, and apoB within the plasma lipoproteins, plasma (250 μl pooled from six animals of the same genotype) was fractionated on a Superose 6 10/30 column (Pharmacia Biotech Inc.) using the automated Bio-Rad Biologic System (Bio-Rad). The column was eluted at a flow rate of 0.5 ml/min, and 96 fractions (0.25 ml each) were collected in a 96-well plate. Consecutive fractions were pooled to obtain 48 fractions (0.5 ml each), and cholesterol and triglyceride concentrations in each fraction were measured using colorimetric methods (4). The distribution of mouse apoB in each fraction was analyzed on Western blots of 4% polyacrylamide-SDS gels, using the rabbit antibody to mouse apoB and the Enhanced Chemiluminescence Western blotting detection reagents (Amersham Corp.).

Pathological Studies—Mice lacking apoB synthesis in the intestine (genotype MBTg<sup>1</sup>/0, apoB<sup>2/2</sup>), as well as wild-type littermates, were sacrificed at 10 days of age. Sections of 10 μm thickness were prepared from paraffin-embedded liver and intestine and stained with hematoxylin and eosin.

RESULTS

To generate transgenic mice expressing mouse apoB, the 95-kb Milu fragment of p649 was microinjected into murine zygotes (Fig. 1). Six founder mice were identified by Southern blot analysis and the two with the highest levels of plasma apoB, 6690 and 6691, were selected for breeding and further analysis (Fig. 2, A and B). Using Southern blots of pulsed-field agarose gels, we demonstrated that both of the transgenic lines contained intact copies of the transgene (Fig. 2C). In line 6690, the transgene copy number was assessed by phosphorimager analysis of a Southern blot as ~20. High copy numbers were also found in the human apoB transgenic mice generated with a P1 bacteriophage clone (1).

Transgenic mice from line 6691 had increased amounts of β-migrating lipoproteins on an agarose gel (Fig. 3A), and Western blots demonstrated increased levels of mouse apoB (Fig. 3B). Using chemical techniques, the concentration of mouse apoB in the plasma of the nontransgenic mice was 10 mg/dl. Quantitative analysis of the Western blots of agarose gels with a phosphorimager revealed a 3-fold increase in the amount of plasma apoB in the transgenic animals (line 6691) compared to nontransgenic mice.
with nontransgenic controls. Therefore, based on the analysis of apoB in the plasma of the transgenic mice (line 6691) was ~30 mg/dl. The plasma apoB levels in transgenic line 6690 were approximately 2-fold higher than those in line 6691, ~60 mg/dl. The increased amounts of apoB in the plasma of the transgenic mice were associated with significantly increased amounts of plasma triglycerides and significantly decreased amounts of HDL cholesterol, compared with nontransgenic littermates (Fig. 4). Fractionation of the plasma by fast phase liquid chromatography (FPLC) revealed that the transgenic mice had increased amounts of LDL cholesterol (Fig. 5A). The FPLC studies revealed that the LDL of the transgenic animals was strikingly enriched in triglycerides (Fig. 5B), accounting for the increased plasma triglyceride levels in these animals. Western blot of the FPLC fractions revealed differences in the quantity of apoB48 and apoB100 in transgenic and nontransgenic animals, but there were no significant differences in the particle size distribution of apoB100 and apoB48 (Fig. 5C). Most of the apoB48 and apoB100 were located in LDL-sized lipoproteins.

To assess levels of apoB mRNA in the liver and intestines of transgenic animals (line 6691), we used RNA slot blots (Fig. 6).

Quantitative analysis of the RNA slot blots using a phosphorimager indicated that the levels of the transgenic mice had a 3-fold increase in the amount of apoB mRNA (Fig. 6A). However, no increase in the amount of intestinal apoB mRNA was observed in the transgenic mice (Fig. 6B).

To further evaluate the issue of apoB mRNA levels, we performed RNase protection studies of liver and intestine RNA samples, using a riboprobe from exon 26 of the mouse apoB gene (both the 6690 and 6691 lines). In the nontransgenic animals, as well as the transgenic animals, we observed the expected 245-bp protected fragment. This fragment was of equal intensity in transgenic and nontransgenic animals (Fig. 7). In addition, the liver RNA from transgenic animals yielded protected fragments of approximately 151 and 91 bp. DNA sequencing revealed that the transgene contained two single-nucleotide substitutions, separated by one nucleotide, in exon 26 of the apoB gene (the exact DNA sequence changes were determined and are given in the legend to Fig. 7). Thus, the smaller protected fragments in the RNase protection experiment represented apoB mRNA derived from the transgene, which had been cleaved at the mismatched bases. In a separate RNase protection assay, the apoB mRNA levels were normalized to the amount of mRNA for GAPDH (data not shown). Quantitative analysis of apoB mRNA levels (relative to GAPDH mRNA levels) using a phosphorimager indicated a 3-fold increase in hepatic apoB mRNA in the transgenic mice (total radioactivity in the 245-, 151-, and 91-bp bands) compared with the nontransgenic mice (the 245-bp band). With the intestinal RNA, the endogenous 245-bp protected fragment was observed in both transgenic and nontransgenic mice; however, the 151- and 91-bp bands (indicative of transgene expression) were not observed.

To evaluate intestinal transgene expression further, the transgenic mice (line 6691) were mated with apoB knockout mice to obtain mice that carried the transgene and were homozygous for the knockout of the endogenous apoB gene (genotype MBTg<sup>10</sup>,apoB<sup>−/−</sup>). In the presence of the mouse apoB transgene, we demonstrated homozygosity for the apoB knockout mutation by showing that these animals had a double dose of the neo gene, relative to a signal from an unrelated single-copy gene (Fig. 8, A and B). The MBTg<sup>10</sup>,apoB<sup>−/−</sup> mice had a striking phenotype. Within 24 h of birth, these animals developed a completely white abdominal cavity and manifested growth retardation (Fig. 9). The white abdominal cavity was due to the failure to synthesize apoB in the intestines, which caused a massive accumulation of lipids in the villus enterocytes (Fig. 10). Of note, the MBTg<sup>10</sup>,apoB<sup>−/−</sup> mice had increased levels of triglycerides and decreased levels of HDL cholesterol, compared with nontransgenic mice (Fig. 4B). FPLC fractionation of the plasma of the MBTg<sup>10</sup>,apoB<sup>−/−</sup> mice revealed that these animals had an increased amount of LDL cholesterol, compared with nontransgenic animals, and a marked reduction in HDL cholesterol (Fig. 11A). The LDL of the MBTg<sup>10</sup>,apoB<sup>−/−</sup> mice was enriched in triglycerides (Fig. 11B). The size distribution of apoB48- and apoB100-containing lipoproteins was similar in the nontransgenic mice and the MBTg<sup>10</sup>,apoB<sup>−/−</sup> mice (Fig. 11C).

**DISCUSSION**

In this study, we used a 95-kb MluI fragment from a mouse apoB P1 clone, p649, to produce transgenic mice that overexpress mouse apoB. As judged by Southern blots of pulsed-field gels of high molecular weight liver DNA, intact copies of the transgene were incorporated into the genomes of the transgenic lines that we investigated. Documentation of transgene expression was straightforward, even though the transgenic animals also expressed apoB from their endogenous apoB al-
leles. The apoB transgene used in this study was isolated from a strain RIII genomic library, and it contained two single nucleotide substitutions within the 5' portion of exon 26. The existence of this pair of DNA sequence polymorphisms made it possible to document transgene expression unequivocally using RNase protection assays. The RNase protection studies, as well as the RNAslot blot studies, indicated that the hepatic levels of apoB mRNA in transgenic line 6691 were increased 3-fold, compared with nontransgenic mice.

One of the most intriguing aspects of these studies was that the mouse transgene, which contained 33 kb of 5'-flanking sequences and 11 kb of 3'-flanking sequences, was not expressed in the intestines, an organ where the endogenous gene is normally expressed at high levels. This finding, initially was shown by RNA slot blot and RNase protection studies, was further analyzed by breeding the transgenic mice with the apoB knockout mice. Transgenic mice that were homozygous for the knockout mutation (MBTg<sup>−/−</sup>,apoB<sup>−/−</sup>) could not synthesize apoB in the intestines and consequently developed a massive accumulation of fats within the villus enterocytes of the small and large intestines.

In a prior study (1), we found that a human apoB gene construct containing 19 kb of 5'-flanking sequences and 17.5 kb of 3'-flanking sequences did not confer intestinal expression of the apoB gene in transgenic mice. Although we suspected that the human transgene simply lacked the DNA sequences controlling intestinal expression of the gene, those studies did not allow us to exclude the possibility that the mouse was simply incapable of recognizing and utilizing the human sequences that control intestinal expression of the apoB gene. The current studies using a mouse transgene argue strongly against the latter possibility and, together with the human transgene studies, suggest that the intestinal element is probably located more than 33 kb 5' of the structural gene or more than 11 kb 3' from the gene. One hypothesis to explain the distant location of the cis-acting DNA sequence element controlling intestinal apoB expression is that the element might be shared with a neighboring gene. Sharing a tissue-specific element with an adjacent gene would not be unprecedented. For example, apoE and apoCI are known to share a DNA sequence element that governs liver expression of both genes (14).

The phenotype of the MBTg<sup>−/−</sup>,apoB<sup>−/−</sup> mice was similar to that of the HuBTg<sup>−/−</sup>,apoB<sup>−/−</sup> mice that we described earlier (15), both in terms of the intestinal pathology and the lipoprotein phenotype. However, two findings with the MBTg<sup>−/−</sup>,apoB<sup>−/−</sup> mice deserve to be underscored. First, those animals had higher levels of triglycerides in the plasma, compared with nontransgenic animals, despite the fact that they had severe intestinal pathology. Because fat absorption is markedly reduced in these mice (15), the high plasma triglyceride levels are probably maintained by prodigious rates of de novo lipogenesis in the liver. Second, the MBTg<sup>−/−</sup>,apoB<sup>−/−</sup> mice had markedly decreased levels of HDL cholesterol. This finding is consistent with the low levels of HDL cholesterol that are observed in human patients with abetalipoproteinemia (16) and suggests a critical role for normal chylomicron formation in the maintenance of normal plasma concentrations of HDL.

The studies of the mouse apoB transgenic mice helped to...
clarify the lipoprotein phenotype associated with increased apoB production by the liver. High levels of human apoB expression in transgenic mice resulted in a large increase in the amount of LDL cholesterol in the plasma, as well as decreased levels of HDL cholesterol (1, 4). However, since human apoB binds very poorly to the mouse LDL receptor (5), one could make a strong argument that the LDL accumulation in these animals might be caused, in large part, by defective clearance of the human LDL from the plasma. The current study, involving overexpression of mouse apoB in mice, provides a portrayal of hepatic apoB overexpression that is free of caveats regarding the intrinsic metabolic properties of a heterologous apoB. The transgenic mice that overproduce mouse apoB in the liver had increased plasma levels of LDL cholesterol and decreased levels of HDL cholesterol, strongly suggesting that these findings represent hallmark metabolic features of apoB overexpression by the liver.

Finally, our prior studies of the human apoB transgenic mice revealed that those animals had a marked triglyceride enrichment of the LDL (1). This unexpected finding suggested the possibility that lipolysis of triglycerides within the human apoB-containing lipoproteins might be defective in the mouse. The fact that we observed triglyceride enrichment of the LDL in the mouse apoB transgenic mice casts serious doubt on that potential explanation. It seems more likely that the triglyceride enrichment of the LDL might be due to relatively low levels of hepatic triglyceride lipase in the mouse to or to the production of small, triglyceride-rich nascent lipoproteins by the liver (1). This topic deserves further investigation.

Acknowledgments—We thank S. Taylor and K. Hoover for microinjection of mouse DNA, J. Taylor for pBSmB245G, G. Howard for editorial assistance, and J. Carroll and A. Corder for graphics.

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