Overexpression of Low Density Lipoprotein (LDL) Receptor Eliminates LDL from Plasma in Transgenic Mice

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A complementary DNA encoding the human low density lipoprotein (LDL) receptor under control of the mouse metallothionein-I promoter was injected into fertilized mouse eggs, and a strain of mice expressing high levels of LDL receptors was established. After administration of cadmium, these mice cleared intravenously injected ^{125}I-labeled LDL from blood eight to ten times more rapidly than did normal mice. The plasma concentrations of apoproteins B-100 and E, the two ligands for the LDL receptor, declined by more than 90 percent after cadmium treatment, but the concentration of another apoprotein, A-I, was unaffected. Therefore, overexpression of an endocytotic receptor can dramatically lower the ambient concentration of its ligand in vivo.

HORMONES AND OTHER LIGANDS ARE KNOWN TO REGULATE their own receptors, but we have only recently recognized that the converse is also true, that is, that receptors can determine the concentrations of their own ligands. The latter control comes about because the receptors transport their ligands into cells by receptor-mediated endocytosis, thereby removing them from the blood (*1*). The most clear-cut example occurs in the low density lipoprotein (LDL) receptor system. LDL receptors, located predominantly in the liver, mediate the rapid removal of LDL from plasma. When LDL receptors are deficient as a result of genetic or acquired defects, the concentration of LDL in plasma rises. When the number of LDL receptors is increased by drug or hormone therapy, plasma LDL declines (*1*). Other receptors that mediate endocytosis include those for hormones such as insulin and follicle-stimulating hormone and transport proteins such as transferrin (iron) and transcobalamin (vitamin B12) (*1*). Whether these receptors affect the plasma concentrations of their ligands is unknown, largely because it is difficult to change the number of receptors experimentally in whole animals and because animals with genetic defects in these receptors have not been identified.

This issue can be explored through the study of transgenic animals. In the first use of this methodology for physiologic studies, Palmiter *et al.* (*2*) injected the gene for rat growth hormone under control of the mouse metallothionein I promoter into the pronuclei of fertilized mouse eggs. The resultant transgenic mice produced high levels of growth hormone, grew abnormally large, and transmitted this trait in a dominant fashion to their offspring. Subsequently, when this fusion transgene was injected into eggs from dwarf mice with a genetic deficiency of growth hormone, the phenotypic manifestations of this defect were corrected (*3*).

Transgene technology has also been used to explore enhancer elements required for tissue-specific gene expression, the immunologic effects of altered expression of cell surface antigens and immunoglobulins, and the proliferative actions of oncogenes (*4*). In our studies we have used the transgene technology to determine whether overexpression of an endocytosis-mediating receptor, the LDL receptor, would increase the clearance of the ligand, LDL, from the blood, thereby lowering the plasma LDL level.

Injection of LDL receptor transgene. The construct for the LDL receptor transgene that was injected into the male pronuclei of fertilized mouse eggs contained the mouse metallothionein-I gene promoter fused to the complete coding region of the complementary DNA (cDNA) for the human LDL receptor (Fig. 1). The sequence for transcription termination was derived from the human growth hormone gene, which was attached to the LDL receptor cDNA through a short linker. No intron was present in this construct.

A total of 674 eggs were injected with the transgene and transplanted into pseudopregnant females. Among the 80 offspring, 20 (25 percent) showed integration of the transgene as determined

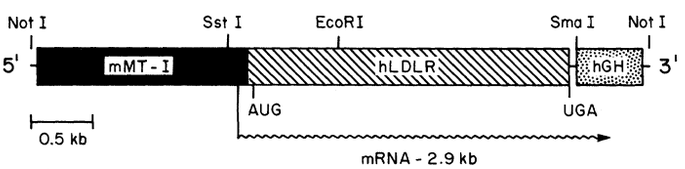

Fig. 1. LDL receptor transgene. The promoter and transcription initiation sites derived from the mouse metallothionein I gene were contained in a 1.7-kilobase (kb) fragment (black box) derived from plasmid pMThGH 111 (*2*). The protein coding region was contained in a 2.6-kb fragment derived from plasmid pLDLR2 (*7*) and included the complete coding region of the LDL receptor and 27 base pairs (bp) of 5′ untranslated sequence (striped box). The transcription termination signal from the human growth hormone gene (stippled box) was contained in a 625-bp Sma I–Eco RI fragment derived from pMThGH 111 (*2, 15*). The thin lines represent polylinker sequences. Several landmark restriction enzyme sites are indicated above the schematic. The transgene, contained in a 5-kb linear Not I DNA fragment, was purified free of vector sequences (*16*) and microinjected into fertilized C57/SJL F2 hybrid mouse eggs (*17*). Mice that contained the transgene were identified by dot hybridization of DNA from tail tissue (*18*). The calculated size of the mRNA transcribed from the hybrid gene is approximately 2.9 kb.

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by dot hybridization of DNA obtained from tail homogenates. These transgenic mice were subjected to partial hepatectomy at approximately 5 to 6 weeks of age. Eight of these 20 mice produced messenger RNA (mRNA) derived from the transgene in the liver as determined by blot hybridization. One of these mice (designated 93-4) showed extremely high levels of mRNA. This mouse was the only one that produced amounts of human LDL receptor protein that were detectable by immunoblotting of liver membranes (see below). To establish a line of mice expressing the human LDL receptor, we bred the founder mouse; analysis of tail tissue indicated that seven of the eight offspring were positive for LDL receptor DNA. Liver biopsies were performed on four of these offspring, and two of them produced mRNA derived from the transgene. One of the mRNA-positive mice was used for further breeding. Twenty of 40 offspring from this mouse contained DNA from the transgene, and all of the DNA-positive offspring that were tested expressed human LDL receptor mRNA in the liver. Quantitative analysis of the tail blots indicated that the genome of the founder contained approximately 11 copies of the transgenic DNA. The nonexpressor offspring of this mouse contained eight copies, and the expressor offspring contained three copies. We interpreted these data to indicate that the transgene in the founder mouse had integrated into two separate chromosomal sites, one containing three copies and the other containing eight copies and that only the three-copy integration site gave rise to mRNA. The eventual strain that was established contained these three copies integrated at a single site. These genes were expressed in subsequent generations as a single autosomal dominant trait.

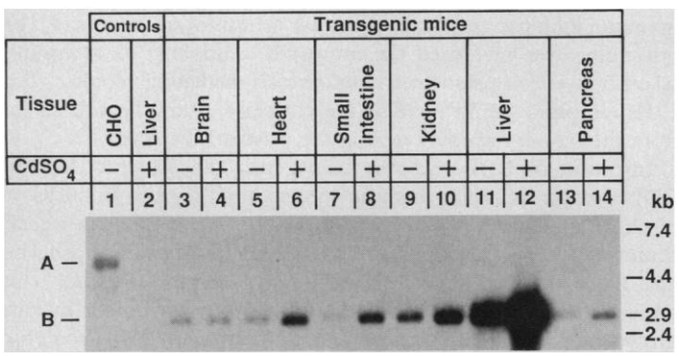

Fig. 2. Blot hybridization of human LDL receptor mRNA in tissues of transgenic mice. One transgenic mouse was killed without heavy metal treatment. A second transgenic mouse and one normal mouse were treated with $CdSO_4$ (1 mg/kg) intraperitoneally 12 hours and 6 hours before these animals were killed. Total RNA was isolated by the guanidinium isothiocyanate–CsCl procedure (*19*), size-fractionated on 1.5 percent agarose gels after denaturation with glyoxal, and transferred to nylon membranes (*20*). Uniformly ^{32}P-labeled single-stranded cDNA probes (>10^9 cpm/μg) were prepared by primer extension from phage M13 templates (*21*). A mixture of two cDNA probes corresponding to nucleotides 1358 to 2154 and 2155 to 2544 of pLDLR2 (*7*) were used. Hybridizations were carried out at 42°C for 12 to 16 hours in 50 percent (v/v) formamide containing (per milliliter) 5× Denhardt's solution (1× consists of 0.02 percent polyvinylpyrrolidone, 0.02 percent Ficoll, and 0.02 percent bovine serum albumin); 5× SSPE (1× SSPE consists of 150 m*M* NaCl, 10 m*M* NaH_2PO_4, and 1 m*M* EDTA, *p*H 7.4), 1 percent SDS, and 100 μg of denatured salmon sperm DNA, 1 μg of polyadenylic acid, and 10^6 cpm of [^{32}P]DNA probe. The filters were washed briefly in 2× SSC (1× SSC consists of 150 m*M* NaCl, 15 m*M* sodium citrate) and 1 percent SDS at room temperature; then in 0.1× SSC and 1 percent SDS at 68°C for 90 minutes; and exposed to XAR-5 film with an intensifying screen at −70°C for 8 hours. Total RNA (10 μg) was placed in each well, except in the lanes containing RNA from heart, where 5 μg of total RNA was used. (Lane 1) RNA extracted from a Chinese hamster ovary (CHO) cell line (TR-715 cells) (*22*) expressing a plasmid containing a full-length human LDL receptor cDNA, including the entire 3′ untranslated region; (lane 2) RNA from liver of a normal mouse treated with $CdSO_4$; (lanes 3 to 14) various tissues from the transgenic mice treated with or without $CdSO_4$ as indicated. Band A denotes the complete 5.3-kb human LDL receptor mRNA transcript; band B denotes the 2.9-kb human LDL receptor mRNA transcribed from the transgene shown in Fig. 1. The data represent one of two experiments that gave similar results.

LDL receptor mRNA. A hybridization blot of total RNA from tissues of two transgenic mice was probed with a mixture of ^{32}P-labeled fragments of the human LDL receptor cDNA (Fig. 2). The mRNA extracted from Chinese hamster ovary (CHO) cells that were transfected with an expressible full-length cDNA for the human LDL receptor is 5.3 kilobases (kb) in length, including an unusually long 3′ untranslated region of 2.5 kb (lane 1). The liver of a control mouse did not contain any mRNA that hybridized with the human probe under the conditions used (lane 2). All of the examined tissues from the transgenic mice expressed human LDL receptor transgenic mRNA (lanes 3 to 14). The size of this mRNA was 2.9 kb, which is consistent with the size predicted from the transgene, which has a truncated 3′ untranslated region (Fig. 1). By far the largest amounts of mRNA were found in the liver. Moderate amounts of mRNA were found in the kidney, small intestine, and heart; smaller amounts were found in the brain and pancreas. When the relative sizes of these organs are taken into account, it is clear that most of the transgene mRNA was present in the liver of these animals. In all tissues except the brain, the amount of mRNA was increased when the animals were treated for 12 hours with cadmium sulfate ($CdSO_4$), a treatment known to stimulate transcription from the metallothionein promoter (*2*). In the liver of the transgenic mice, the calculated amount of LDL receptor mRNA, as determined by solution hybridization, rose from 630 molecules per cell to 3300 molecules after $CdSO_4$ treatment (*5–8*).

LDL receptor protein. To demonstrate the LDL receptor protein in liver, we solubilized liver membranes with detergents, performed sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis, transferred the proteins to nitrocellulose, and incubated

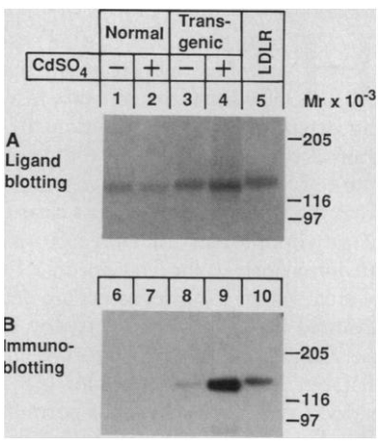

Fig. 3. Detection of human LDL receptor protein in transgenic mice by ligand blotting (**A**) and immunoblotting (**B**). Detergent-solubilized mouse liver membranes were prepared from the same control and $CdSO_4$-treated mice that were studied in the experiment of Fig. 2. The membranes were prepared as described (*23*), except that the following protease inhibitors were present during tissue homogenization and all subsequent procedures: 1 m*M* phenylmethanesulfonyl fluoride, 1 m*M* 1,10-phenanthroline, 0.1 m*M* leupeptin, pepstatin (at 1 μg/ml), and aprotinin (at 0.5 μg/ml). The extracts (100 μg of protein per lane) were subjected to electrophoresis in SDS–7 percent polyacrylamide gels under nonreducing conditions and transferred to nitrocellulose filters (*24*). For ligand blotting (*25*), the filters were incubated for 1 hour at 37°C with ^{125}I-labeled rabbit β-VLDL (5 μg of protein per milliliter; 3× 10^5 cpm/μg), washed, and exposed to XAR-5 film at −70°C with an intensifying screen for 10 hours. For immunoblotting (*24*), the filters were incubated for 1 hour at room temperature with a rabbit polyclonal antibody (immunoglobulin G fraction, 10 μg/ml) specific for the human LDL receptor (*26, 27*), washed, probed with ^{125}I-labeled goat antibody to rabbit immunoglobulin G (5 × 10^6 cpm/ml; ~10^7 cpm/μg), and exposed to film for 5 hours. (Lanes 1 and 6) Membranes from normal mouse; (lanes 2 and 7) normal mouse treated with $CdSO_4$; (lanes 3 and 8) transgenic mouse; (lanes 4 and 9) transgenic mouse treated with $CdSO_4$; (lanes 5 and 10) CHO cells transfected with a plasmid bearing the human LDL receptor coding region as described in the legend to Fig. 2.

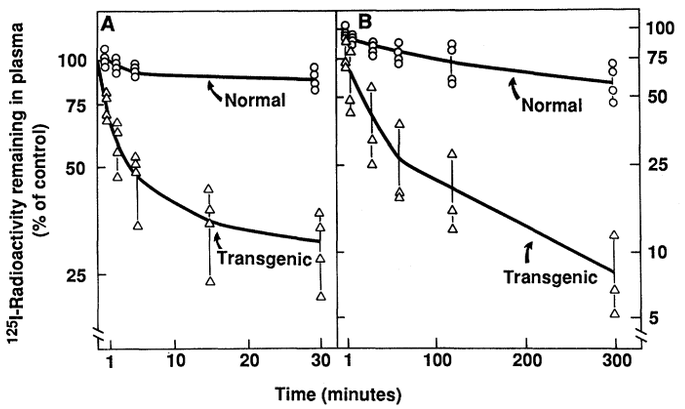

Fig. 4. Disappearance of ^{125}I-labeled LDL from the circulation in normal (○) and transgenic (△) mice. ^{125}I-Labeled human LDL (570 to 720 cpm per nanogram of protein) was prepared as described (*28*). Normal and transgenic mice were treated with $CdSO_4$ as described in the legend to Fig. 2 and anesthetized with sodium pentobarbital (90 mg/kg). Each mouse received an intravenous bolus via the external jugular vein of 0.1 ml of 0.15*M* NaCl containing bovine serum albumin (2 mg/ml) and 15 μg of ^{125}I-labeled LDL. Blood was collected by retro-orbital puncture and placed in EDTA-coated tubes at the times indicated, and a portion (5 μl) of the plasma was counted for its ^{125}I content. The "100 percent of control" represents the mean value for plasma ^{125}I in the normal mice at 1 minute after injection. Values for individual mice are indicated by open circles (normal mice) or triangles (transgenic mice), and a line is drawn through the mean of the values for each group at each time. (**A**) Data are from four transgenic mice (two male, two female) and from four normal littermates of the transgenic mice (two male, two female) of 7 weeks of age. Animals were not fasted. (**B**) The transgenic mice (but not the normal mice) received 300 μg of unlabeled human LDL as carrier mixed with the 15 μg of ^{125}I-labeled LDL before injection. The apo B in plasma was measured in each group 1 minute after each mouse was injected. Apo B concentrations (determined by rocket immunoelectrophoresis as in the legend to Fig. 7) were 25 ± 1.6 mg/dl (mean ± SE) in the transgenic group and 35 ± 2.8 in the normal group. During the 5-hour experiment, the animals could move freely in their cages, without access to food or water. The transgenic group consisted of two females and one male; the normal group consisted of two mice of each sex who were littermates of the transgenic mice. The mice were 7 weeks old.

them with ^{125}I-labeled β-migrating very low density lipoprotein (β-VLDL), which is a high affinity ligand for the LDL receptor (9) (Fig. 3A), or with a polyclonal antibody specific for the human LDL receptor (Fig. 3B). Under these nonreducing conditions, the apparent molecular weight (M_r) of the human LDL receptor, as observed in CHO cells transfected with human LDL receptor cDNA, was 130,000 (lane 5).

The liver of the normal mouse contained a detectable amount of ^{125}I-labeled β-VLDL binding activity that migrated with an apparent M_r of 125,000 (lane 1). The transgenic mouse produced a receptor that was intermediate in M_r between that of the mouse receptor and the normal human LDL receptor (lane 3). In a normal mouse that was treated with $CdSO_4$, the amount of LDL receptor protein was not increased (lane 2). However, the transgenic mouse injected with $CdSO_4$ expressed much higher levels of LDL receptor protein than did the noninjected littermate (lane 4). As would be expected, the polyclonal antibody that is specific for the human LDL receptor did not detect the mouse LDL receptor (lanes 6 and 7). The receptor in the transgenic mouse reacted with the polyclonal antibody (lane 8), and the amount was much higher in the $CdSO_4$-treated animal (lane 9).

Function of receptors encoded by transgene. Transgenic mice were treated with $CdSO_4$ and injected intravenously with ^{125}I-labeled LDL. We then measured the rate of disappearance of the ^{125}I-labeled LDL from plasma. In normal mice that were treated with $CdSO_4$, there was little removal of ^{125}I-labeled LDL throughout the 30-minute duration of the experiment (Fig. 4A). In contrast, in the transgenic mice treated with $CdSO_4$, more than 50 percent of the labeled LDL was removed within 10 minutes.

In the $CdSO_4$-treated transgenic mice, the circulating LDL was less than 1 mg/dl (see below), and hence the tracer amounts of ^{125}I-labeled LDL were not diluted by an endogenous LDL pool as they were in normal mice. To control for this variable, we repeated the experiment on the removal of ^{125}I-LDL under identical conditions except that the transgenic mice were injected with an amount of unlabeled human LDL that was calculated to bring the plasma LDL concentration to the range seen in normal mice. The experiment was also extended for a longer time (Fig. 4B). In the transgenic mice, 1 minute after the injection of unlabeled LDL, the mean plasma concentration of apo B-100, the major protein component of LDL, rose from <1 to 25 mg/dl as compared to a mean value of 35 mg/dl in the normal mice (legend to Fig. 4). Despite this nearly normal concentration of apo B-100, the transgenic mice continued to remove the tracer ^{125}I-labeled LDL rapidly from the blood. After 300 minutes a mean of 8 percent of the injected radioactivity remained in the blood of the transgenic mice as compared with 60 percent in the normal mice (Fig. 4B). The total amount of apo B in the plasma of the injected mice also declined by more than 90 percent by 300 minutes as determined by immunoelectrophoresis.

The tissues that took up the ^{125}I-labeled LDL were identified by gamma counting 30 minutes after injection of ^{125}I-labeled LDL into $CdSO_4$-treated normal and transgenic mice. In the liver, the uptake in the transgenic mice was more than ten times higher than that observed in the normal animals (Fig. 5). A significant increase in uptake was also observed in the spleen and adrenal gland. The kidney, testis, and ovary showed no significant difference in uptake between the normal and transgenic mice.

To determine whether the receptors in liver were expressed in parenchymal cells as opposed to sinusoidal cells, we injected LDL that was labeled with a fluorescent dye. After 30 minutes the animals were killed and sections of liver were examined by fluorescence microscopy. In the normal animal a small amount of fluorescent dye was found in scattered sinusoidal cells and in hepatocytes (Fig. 6A).

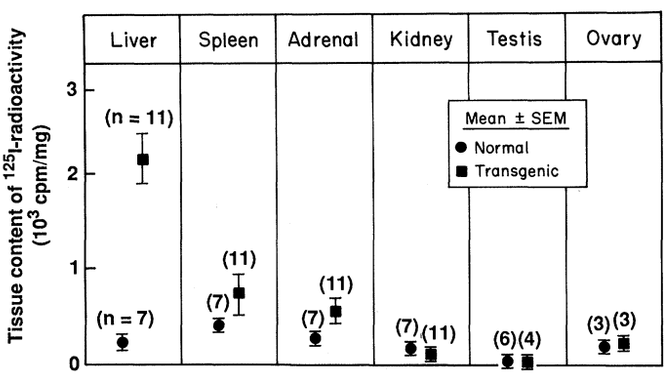

Fig. 5. Uptake of ^{125}I-labeled LDL in tissues from $CdSO_4$-treated normal (●) and transgenic (■) mice. Mice were injected intravenously with 15 μg of ^{125}I-labeled LDL after $CdSO_4$ treatment as described in the legend to Fig. 4. After 30 minutes, with the animals still under pentobarbital anesthesia, the ascending aorta was cannulated and the right atrium was slit. Mice were perfused over 3 to 4 minutes with about 25 ml of 0.15*M* NaCl containing bovine serum albumin (BSA) (2 mg/ml). The indicated tissues were then removed, placed into ice-cold 0.15*M* NaCl, and then dissected free of adherent fatty or fibrous tissue. The tissues were weighed, and the total content of ^{125}I was determined. The number of mice in each group is indicated in parentheses. Each group consisted of approximately equal numbers of males and females of 7 to 9 weeks of age.

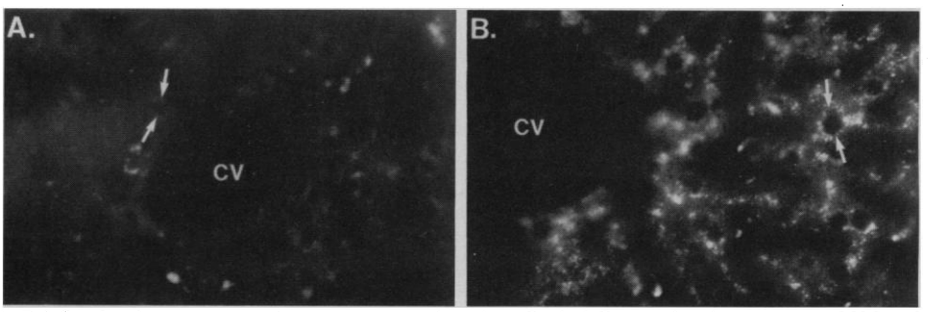

Fig. 6. Visualization of normal (**A**) and transgenic (**B**) mouse liver labeled by injection of fluorescent LDL in vivo. Mice were treated with $CdSO_4$ as described in the legend to Fig. 2 and injected intravenously with 15 μg of 3,3′-dioctadecylindocarbocyanine-labeled human LDL (*29*) in 0.1 ml of 0.15*M* NaCl and bovine serum albumin (2 mg/ml). After 30 minutes, the mice were perfused with 25 ml of 0.15*M* NaCl and BSA (2 mg/ml) and then with 2 percent (w/v) paraformaldehyde in phosphate-buffered saline (PBS) for 10 minutes. The organs were removed, fixed in 2 percent paraformaldehyde in PBS, and processed by a modification of a previously described method (*30*). Briefly, the tissue was blocked with ammonium chloride, washed with PBS, cryoprotected with 10 percent (v/v) dimethyl sulfoxide in PBS overnight at 4°C, frozen in isopentane cooled in liquid nitrogen, and stored at −120°C. Sections (~6 μm) were cut on an American Optical Cryogenic microtome at −20°C and photographed (Zeiss Photomicroscope III) with the filter package for rhodamine (magnification, ×615). Arrows denote nuclei of hepatic parenchymal cells. CV, central vein.

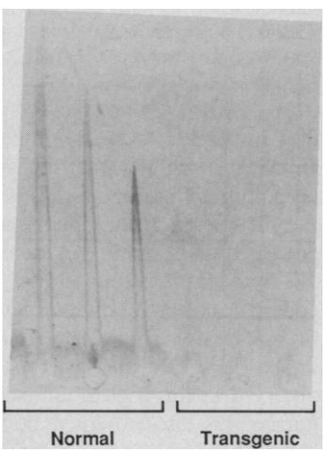

Fig. 7. Rocket immunoelectrophoresis (*31*) of mouse apo B in plasma of $CdSO_4$-treated normal and transgenic mice. Mouse plasma (5 μl) was placed in the wells of 0.5 percent agarose gels containing a polyclonal sheep antibody (0.3 mg/ml) immunoglobulin G fraction, directed against rabbit LDL. Electrophoresis was performed in 50 m*M* sodium barbital (*p*H 8.6) for 36 hours at 4 V/cm at 4°C. The gels were washed extensively with 0.3*M* and 0.15*M* sodium chloride, dried, and stained with Coomassie blue. The LDL was quantified by comparing the area of the rocket to several dilutions of an LDL standard that was prepared from normal mouse plasma by density gradient ultracentrifugation (d = 1.030 to 1.063 g/ml) (*10*). Each lane contains serum from a different mouse.

In the transgenic animal, abundant uptake of fluorescent dye was observed in hepatocytes throughout the organ (Fig. 6B). We conclude that the hepatic receptors in the transgenic animals are expressed predominantly in parenchymal cells.

Plasma apoprotein levels. The effect of the transgene on plasma LDL levels was determined by rocket immunoelectrophoresis, with a sheep antibody against rabbit LDL that cross-reacts with mouse apo B-100 (Fig. 7). The plasma of $CdSO_4$-treated normal mice showed relatively large amounts of apo B, as determined by the height of the "rockets." In $CdSO_4$-treated transgenic mice apo B was not detectable (Fig. 7). To quantify these data, we made a standard curve with known amounts of mouse LDL. We quantified apo B separately in male and female mice because the apo B in normal male mice was much higher than in females. Measurements were performed on at least five animals in each group balanced by age-matched controls. In normal male mice, the mean apo B concentration was 34 ± 2.6 mg/dl (mean ± SE). In male mice treated with $CdSO_4$, this value rose to 44 ± 2.2 mg/dl. In male transgenic animals without $CdSO_4$, the mean apo B was 24 ± 3.5 mg/dl (71 percent of normal), and it fell to less than 1 mg/dl after $CdSO_4$ treatment. In normal females the apo B was 14 ± 1.1 mg/dl, and it rose substantially to 53 ± 4.2 mg/dl after $CdSO_4$ treatment. In the transgenic females without $CdSO_4$ treatment, the level was 9.6 ± 0.7 mg/dl (72 percent of normal), and it fell to less than 1 mg/dl after $CdSO_4$ treatment.

Most of the cholesterol in mouse plasma is contained in high density lipoprotein (HDL), and only a small proportion is in LDL (*10*). The total plasma cholesterol concentrations in 22 $CdSO_4$-treated normal mice of both sexes averaged 119 ± 4.5 mg/dl (mean ± SE), whereas in 32 $CdSO_4$-treated transgenic mice the value was 68 ± 2.4 mg/dl (mean ± SE). These data are consistent with the possibility that $CdSO_4$ treatment of transgenic mice causes LDL, but not HDL, to disappear from plasma.

To test this hypothesis more directly, we isolated the total lipoprotein fraction from $CdSO_4$-treated control and transgenic mice by ultracentrifugation at a density of 1.21 g/ml (*10*). The proteins were then delipidated and subjected to electrophoresis on 3 to 14 percent gradient polyacrylamide gels in the presence of SDS. The proteins were visualized by staining with Coomassie blue. Treatment of the transgenic mice with $CdSO_4$ caused the complete disappearance of apo B-100 from plasma. The amount of apo E was reduced by more than 90 percent as judged by visual inspection. Both of these apoproteins are known to bind to LDL receptors (*1*). On the other hand, there was no apparent decline in the amount of apoprotein A-I, which is the major component of HDL. In normal mice, $CdSO_4$ did not affect the plasma level of any of these apoproteins. More detailed studies of lipoproteins await the availability of larger numbers of transgenic mice.

The in vivo expression of LDL receptors has been increased previously by administration of pharmacologic doses of 17α-ethinylestradiol to rats (*11*) and rabbits (*12*) and by administration of large doses of mevinolin to hamsters and rabbits (*13*). In the estradiol-treated animals and in the mevinolin-treated animals, plasma HDL, as well as LDL, declined. We speculated that this decline was attributable to the presence of apo E, a ligand for the LDL receptor, on the HDL of those animals. In the current study we found that direct overexpression of LDL receptors in mice did not lower HDL levels. This finding suggests that the lowering of HDL in the drug-treated animals was not a direct result of receptor overexpression. Alternatively, it is possible that the difference relates to species differences in lipoprotein metabolism between mice and the other species studied previously.

Extension to other receptors. Our current data show that overexpression of an endocytosis-mediating receptor in transgenic animals can lower the plasma concentration of the ligand for that receptor. In the case of the LDL receptor in our study, the most abundant expression was achieved in hepatocytes, and this led to the virtual disappearance of its two ligands, apo B-100 and apo E, from blood.

It is likely that overexpression of other receptors, such as the receptor for insulin or transferrin, would have similar effects. The question arises as to whether the overexpression of a receptor in a single tissue would cause a "ligand steal" syndrome that would deprive other organs of the ligand. Steiner *et al.* (*14*) showed that insulin is cleared from the blood by binding to the insulin receptor and that insulin molecules with low affinity for the receptor accumulate in blood. Would overexpression of the insulin receptor

in liver lead to the disappearance of insulin from blood, thereby depriving peripheral tissues of insulin and producing a diabetic syndrome? Would overexpression of the transferrin receptor in liver lead to the disappearance of iron-laden transferrin from blood, thereby depriving hematopoietic tissues of iron and producing anemia? These and other studies are now possible with the transgene technology.

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5. Solution hybridization was performed as described (*6*) except that the probe used was a 5′ ^{32}P-labeled oligonucleotide encompassing nucleotides 1659 to 1708 of the human LDL receptor cDNA (*7*). Hybridization was carried out for 16 hours at 60°C in a solution containing 22 m*M* tris-chloride (*p*H 7.5), 4 m*M* EDTA, 0.6*M* NaCl, 0.08 percent SDS, and salmon sperm DNA (100 μg/ml). Unhybridized single-stranded oligonucleotide was destroyed with S1 nuclease, and the protected fragments were precipitated with trichloroacetic acid. An in vitro generated SP-6 transcript (*8*) of the entire human LDL receptor cDNA was used as the standard. Data were calculated as mRNA molecules per cell as described (*6*).
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