The purpose of this study was to evaluate low density lipoprotein (LDL) receptor activity on skin fibroblasts from rhesus monkeys with either spontaneous or diet-induced hypercholesterolemia in order to determine whether a defect in LDL receptor function was associated with the hyperbetalipoproteinemia in either of these conditions. This was of particular interest in the spontaneously hypercholesterolemic animals since animals with this genetically mediated disorder have many of the phenotypic characteristics of familial hypercholesterolemia in man. Skin fibroblasts were grown from two spontaneously hypercholesterolemic rhesus monkeys that maintained plasma cholesterol concentrations of >700 mg/dl while consuming a cholesterol-free diet. Control monkeys were normocholesterolemic (plasma cholesterol concentrations <150 mg/dl) while consuming a cholesterol-free diet, but were either hypo- or hyperresponsive to dietary cholesterol with plasma cholesterol concentrations ranging from 250 to 1000 mg/dl. Skin fibroblasts from all spontaneously hypercholesterolemic and control animals bound, internalized, and degraded 125I-LDL normally. Cells from spontaneously hypercholesterolemic animals were larger and expressed more LDL receptors per cell when maximally stimulated than did normal cells. Likewise, differences in LDL receptor function were not correlated with hypo- or hyperresponsiveness to dietary cholesterol in control animals. The LDL receptors also functioned normally in delivering cholesterol to cells from all but one of the spontaneously cholesterolemic animals as seen by the suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Cells from this animal (No. 1108) showed a relative defect in regulation of HMG-CoA reductase activity, but no obvious relationship of this defect to an explanation of the spontaneous hypercholesterolemia was found. Consequently, spontaneous hypercholesterolemia, in the two rhesus monkeys studied here, even though having many of the phenotypic characteristics of familial hypercholesterolemia in human beings, was not associated with a similar defect in LDL receptor function.

In 1968, Morris and Fitch (1) described the occurrence of spontaneous hypercholesterolemia in two male rhesus monkeys consuming diets devoid of cholesterol. Subsequently, the hypercholesterolemia of these animals was shown to result from increased concentrations of LDL1 of normal composition and size (2). Furthermore, spontaneous hypercholesterolemia appears to be a form of primary hyperbetalipoproteinemia since the hypercholesterolemia was not secondary to other diseases such as diabetes mellitus, nephrosis, or hypothyroidism (1). The hyperbetalipoproteinemia in these animals appears also to be genetically mediated since one of these monkeys has sired an offspring with spontaneous hyperlipoproteinemia (3) and the offspring from both animals have been shown to be significantly more susceptible to diet-induced hypercholesterolemia than are offspring from normal rhesus monkeys (4). Since these animals have phenotypic characteristics remarkably similar to those of human beings with familial hypercholesterolemia, it is important to know whether they also have a similar defect in LDL receptor function.

Familial hypercholesterolemia in man is an autosomal dominant disorder characterized by hyperbetalipoproteinemia, xanthomatosis, and premature coronary heart disease (5). The primary metabolic defect is a deficiency in LDL receptor function. The mechanism whereby the lack of functioning LDL receptors results in hyperbetalipoproteinemia is unknown. Thus, the existence of an animal model with an LDL receptor defect similar to familial hypercholesterolemia in man would provide a powerful tool for investigation of the link between the receptor defect and the hyperbetalipoproteinemia. Conversely, the identification of animals with genetically mediated spontaneous hyperbetalipoproteinemia associated with normal LDL receptor function would provide a unique animal model in which to study abnormalities in LDL and cholesterol metabolism leading to hyperbetalipoproteinemia, but not associated with abnormal LDL receptor function.

Hyperbetalipoproteinemia can also be produced experimentally in a number of animal species by feeding cholesterol. This does not provide a model for familial hypercholesterolemia, however, since even in animals that are genetically hyperresponsive to dietary cholesterol, the LDL receptor function appears to be normal (6).

The purpose of this study was to determine whether the two rhesus monkeys with spontaneous hypercholesterolemia possessed an LDL receptor defect similar to that which occurs in human familial hypercholesterolemia.

1 The abbreviations used are: LDL, low density lipoproteins; HDL, high density lipoproteins; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HDL4, an abnormal HDL species produced as a result of cholesterol feeding.
In familial hypercholesterolemia in man or if they represent a form of familial hyperbetalipoproteinemia associated with normal LDL receptor function. Control animals used for this study were rhesus monkeys having normal plasma cholesterol concentrations while consuming cholesterol-free diets, but possessing a variable degree of individuality of hyperbetalipoproteinemia in response to dietary cholesterol.

MATeRIALS AND METHODS

Sodium $^{[125]}$iodide (approximately 17 Ci/mg, carrier free, low $p$H), DL-3-hydroxy-3-methyl-8-[3-$^3$H]glutaryl-CoA ($25$ mCi/mmol), and DL-[5-$^5$H]mevalonic acid (N,N' -dibenzylethylene diamine salt) were purchased from New England Nuclear, Boston, MA. Tissue culture supplies were obtained from Flow Laboratories, Rockville, MD. Kyro EOB, a synthetic nonionic detergent that solubilizes plasma membranes, but not endoplasmic reticulum of cultured cells (7), was a gift from Dr. D. H. Hughes, Miami Valley Research Laboratories, Procter and Gamble Co., Cincinnati, OH.

Isolation and Characterization of Lipoproteins—Lipoproteins were isolated from the pooled plasma of six adult male rhesus monkeys consuming a diet containing 0.05% cholesterol and having plasma cholesterol concentrations of 180 to 220 mg/dl. The animals were fasted for 16 h and blood was collected in tubes containing EDTA (final concentration 1 mg/dl). LDL and HDL were isolated from the plasma by the combined ultracentrifugal-agarose column chromatography of Jowsey et al. (8). The isolated lipoproteins were concentrated by dialysis against a solution of 30% sucrose and 0.1% EDTA. The sucrose was removed by exhaustive dialysis against phosphate buffer consisting of 8 g of NaCl, 0.2 g of KCl, 0.2 g of KH$_2$PO$_4$, and 1.15 g of Na$_2$HPO$_4$ per liter, pH 7.4.

Iodination of LDL—LDL was iodinated with $^{125}$I by the iodine monochloride method of McFarlane as modified by Bilheimer (9). Following iodination, the LDL was dialyzed exhaustively against phosphate buffer containing 0.1% EDTA. The initial dialysis solution contained 0.1 M NaI in order to reduce the specific activity of remaining Na$^{125}$I, while all subsequent dialysis solutions contained only the above-described phosphate buffer. The iodinated LDL was sterilized through a 0.45-pm Millipore filter and stored refrigerated.

After incubation, 1 ml of the culture medium was removed and added to a scintillation vial containing scintillation fluid consisting of 2,5-diphenyloxazole and 10% of 2,5-diphenyloxazole in toluene. All samples were counted in a Beckman LS-230 liquid scintillation counter to a 2 sigma error of <2%. An external standard channel method was used to correct for quenching and all results were corrected for recovery of the $^3$H-labeled mevalonic acid internal standard.

RESULTS

Table I shows plasma cholesterol concentrations of the spontaneously hypercholesterolemic and control rhesus monkeys from which skin fibroblasts were obtained. The spontaneously hypercholesterolemic animals had been hypercholesterolemic for more than 10 years with plasma cholesterol concentrations ranging from 449 mg/dl (1) to >700 mg/dl (2). The control animals were normocholesterolemic for this species while consuming monkey chow, but were selected because they exhibited a marked individuality in plasma cholesterol response to dietary cholesterol. Hyperresponsive animals had an average plasma cholesterol concentration of >900 mg/dl, while hyporesponsive animals maintained plasma cholesterol concentration of vitamins (Eagle's vitamins), 10% fetal bovine serum, 25 mm sodium bicarbonate, 100 nm l-glutamine, 1 mg of a-04+)-glucose/ml. 100 KIU of penicillin/ml, and 100 $\mu$g of streptomycin/ml.

The outgrowth of skin fibroblasts from the explants was dissociated with 0.05% trypsin and 0.02% EDTA and was maintained in 75-cm$^2$ flasks at 37°C in a humid atmosphere of 5% CO$_2$ and 95% air. Cells were transferred at each passage with a 1:3 split and used for experiments between the 4th and 12th passage. For experiments, 2 to 8 $\times$ $10^6$ cells were transferred to 60-mm tissue culture dishes. The culture medium was changed every 3 days and experiments were commonly initiated after 7 days. At this time, the cells that were plated at lower density were in the late log phase of growth (100 to 200 $\mu$g of cell protein/dish), while those plated at higher density were confluent (300 to 500 $\mu$g of cell protein/dish). The growth state of the cells is indicated for each experiment.

Determination of $^{131}$I-LDL Binding, Internalization, and Degradation—Cells were washed twice with phosphate-buffered saline and incubated for up to 48 h with culture medium in which the fetal bovine serum had been replaced with 2.5 $\mu$g of protein/ml of lipoprotein-deficient bovine serum plus the desired concentrations of the $^{131}$I-LDL or other lipoproteins, was added to the dishes and incubated for up to 24 h at 37°C or 4°C. Incubations were performed with gentle mixing on an oscillating mixer at 60 oscillations/min.

After incubation, 1 ml of the culture medium was removed for determination of proteolytic degradation by treatment with trichloroacetic acid and hydrogen peroxide followed by removal of free iodine by extraction with chloroform as described by Goldstein and Brown (11).

The cells were then washed extensively with albumin-containing Tris buffer as described by Goldstein and Brown (11) and finally twice with Tris buffer without albumin. The cells were removed from the dishes by digestion with 1 ml of 1 M NaOH. Aliquots of the NaOH solution were taken for counting, using a Searle anaLytic model 1185 gamma counter, and for protein determination by the method of Lowry (12) using bovine albumin as the standard.

Determination of HMG-CoA Reductase Activity—The cells were washed extensively as described above. Cells were removed from the dishes by scraping with a rubber policeman, and HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, was assayed by the method of Brown et al. (7). HMG-CoA reductase was routinely assayed for 90 min with approximately 75 $\mu$g of cell protein/tube and a DL-3-hydroxy-3-methyl-8-[3-$^3$H]glutaryl-CoA substrate concentration of 30 $\mu$M. For all cell lines studied, the reaction was linear for incubations up to 120 min and for cell protein concentrations up to 300 $\mu$g/tube. At 30 $\mu$M substrate concentration, the reaction exhibited zero order kinetics. Following incubation, approximately 60,000 dpm of $^3$H-labeled mevalonic acid were added as an internal standard and 2.5 $\mu$g of mevalonic acid lactone were added for thin layer chromatography. The mevalonic acid lactone was separated by thin layer chromatography using a solvent system of benzene and acetone (1:1). After visualization with iodine, the mevalonic acid lactone was scraped directly into counting vials containing scintillation fluid consisting of 6 g of 2,5-diphenyloxazole, 100 ml of Bio-Solv (Beckman), and 30 ml of water/liter of toluene.

All samples were counted in a Beckman LS-230 liquid scintillation counter to a 2 sigma error of <2%. An external standard channels ratio method was used to correct for quenching and all results were corrected for recovery of the $^3$H-labeled mevalonic acid internal standard.

2 Animals 1108 and 1648 are the same animals that have been identified in earlier publications (1, 3, 4) as numbers 263 and 279, respectively.
concentrations of approximately 250 mg/dl while consuming the identical diet containing 1.056 mg of cholesterol/kcal.

The growth characteristics of skin fibroblasts from the spontaneously hypercholesterolemic animals compared with those of four of the control cell lines are shown in Fig. 1. Even though differences were seen in the rate of growth of individual lines of fibroblasts from the control animals, there were no systematic differences in growth characteristics that correlated with the phenotype of these animals (hyporesponder versus hyperresponder). Fibroblasts from animals with spontaneous hypercholesterolemia grew more slowly than did control cells and attained a lower cell density when confluent. Thus, at confluency, the cells from spontaneously hypercholesterolemic animals would be expected to occupy a greater surface area than would the control cells. This impression was supported by the results of a preliminary study in which cell size was calculated from scanning electron micrographs of control and spontaneously hypercholesterolemic cells. The average area of the spontaneously hypercholesterolemic cells was approximately 25% greater than that for control cells (5556 μ2 compared with 4290 μ2 for control cells, as calculated from the mean of 100 of each cell type). Even though individual cells from the spontaneously hypercholesterolemic animals occupied a larger area of the culture dish than did individual control cells, both cell types contained equivalent amounts of protein/cell and, in all cases, the increase in cell protein paralleled the increase in cell numbers. Consequently, we have utilized the amount of cell protein as a measure of the number of cells for the expression of the biochemical data. It is well established that differences in cell number can influence LDL receptor number (13); thus, we were careful to conduct these studies on cells that had attained an equal degree of confluency. For some experiments, cells were confluent while for other experiments cells were in the late log growth phase. This is indicated for each experiment.

The time course of binding of 125I-LDL to cells from spontaneously hypercholesterolemic and control animals was determined by incubation with 10 μg of 125I-LDL protein/ml at 4°C or 37°C for up to 480 min. At 4°C, the 125I-LDL binds to LDL receptors, but is not internalized (11, 14). Identical experiments were carried out at 37°C, a temperature at which binding, internalization, and proteolytic degradation of the 125I-LDL occur (11). At 4°C, as shown in Fig. 2, all cell lines showed a rapid time-dependent binding of 125I-LDL for up to approximately 1 h, after which binding approached a plateau. The cells from the spontaneously hypercholesterolemic animals bound about twice as much 125I-LDL/cell as did the control cells. These differences in binding cannot be explained by differences in the degree of confluency since the experiment was done while all of the cells were confluent. The greater amount of LDL bound in the spontaneously hypercholesterolemic cells can be partially explained by the fact that these cells were larger than were control cells, thus providing a greater surface for binding. However, since the difference in surface area between spontaneously hypercholesterolemic and control cells was only about 25%, they would also appear to have more LDL receptors per cell than the control cells.

Results of the time course of binding plus internalization of 125I-LDL at 37°C are also shown in Fig. 2. Under these conditions, total cell-associated 125I-LDL increased relatively linearly for 120 to 480 min, depending on the specific cell line. The rate of uptake of 125I-LDL was greater in the cells from spontaneously hypercholesterolemic animals than in the control cells during the initial 120 min of incubation, which is consistent with the greater binding of 125I-LDL observed at 4°C. This difference became less apparent, however, as a steady state was approached with longer periods of incubation at 37°C.

The effect of incubation time on 125I-LDL proteolytic degradation at 37°C is shown in Fig. 3. After an initial 2-h lag period during which little 125I-LDL degradation occurred, all cell lines showed a progressive, nonsaturable increase with time in the amount of 125I-LDL that was degraded. There was no detectable degradation when cells were incubated at 4°C (data not shown). Generally, the cell lines with the greatest initial rate of increase of bound plus internalized 125I-LDL at 37°C (cell lines 1108, 1648, and 458) also degraded the largest amount of LDL. At the steady state in cells incubated at 37°C, there was little correlation between the amount of cell-associated and degraded 125I-LDL with the amount of 125I-LDL bound at 4°C. This may reflect differences in the steady state flux of LDL into cells in culture that is independent of the total number of LDL receptors that can be expressed upon incubation with lipoprotein-deficient serum.
LDL Receptor Function in Hypercholesterolemic Monkeys

FIG. 2. The effect of time of incubation at 4°C and 37°C on \(^{125}\)I-LDL binding and internalization by skin fibroblasts from spontaneously hypercholesterolemic and control rhesus monkeys. All cells were grown in 60-mm tissue culture dishes to confluence before being washed twice with phosphate buffer and incubated for 48 h with lipoprotein-deficient serum containing culture medium. The \(^{125}\)I-LDL was added to all dishes at a final concentration of 10 \(\mu\)g/ml. The cells were then incubated at 4°C or 37°C for the times shown and analyzed for bound (4°C) or bound and internalized (37°C) \(^{125}\)I-LDL. All points represent the mean of duplicate determinations. Four dishes containing no cells were also incubated for four different periods of time in each group to serve as controls. The radioactivity from these control dishes was subtracted from that of the dishes containing cells. The numbers adjacent to the lines on the graphs indicate the animals from which cells were obtained.

FIG. 3. The effect of time of incubation at 37°C on \(^{125}\)I-LDL degradation by skin fibroblasts from spontaneously hypercholesterolemic and control rhesus monkeys. The cells were incubated at 37°C as described in the legend to Fig. 2. Following incubation, 1 ml of the culture medium was analyzed for trichloroacetic acid-soluble \(^{125}\)I radioactivity for determination of the proteolytic degradation of \(^{125}\)T-LDL. All points are the means of duplicate determinations.

The influence of LDL concentration on the binding and internalization of \(^{125}\)I-LDL is shown in Fig. 4. The binding and internalization showed typical saturation kinetics for all cell lines regardless of the phenotype of the animals from which they were obtained. All cell lines were half-saturated at \(^{125}\)I-LDL concentrations of 5.8 to 12.5 \(\mu\)g of LDL protein/ml of culture medium. There was considerable variability, however, among cell lines in the maximum amount of \(^{125}\)I-LDL bound and internalized at saturation, with no obvious relationship among the cells from animals of different phenotypes.

The large differences in \(^{125}\)I-LDL binding capacity among individual cell lines appear to be a property of the cell lines themselves and not the result of differences in confluence since all cells were grown to confluence prior to addition of the \(^{125}\)I-LDL. Similar differences in \(^{125}\)I-LDL binding among cell lines have been demonstrated in other studies in which cells were also grown to confluence in order to minimize variation in cell density (15). These differences among cell lines are magnified by the processes of internalization and degradation since the variation among cell lines is much greater when measured at 37°C than at 4°C, even in identically prepared cells (Fig. 2).

The data presented on the binding, internalization, and degradation of \(^{125}\)I-LDL represent total \(^{125}\)I-LDL uptake and include both specific and nonspecific processes (14). Due to the limited amount of LDL available to us, we were unable to determine the contribution of specific and nonspecific processes at all of the LDL concentrations used in this study. As an alternative, we estimated the contribution of specific and
nonspecific binding in control and spontaneously hypercholesterolemic cells at a single concentration of \(^{125}\text{I}-\text{LDL}\). For this, cells were incubated for 3 h at 4°C or 37°C with 5 µg/ml of \(^{125}\text{I}-\text{LDL}\) in the presence or absence of a 10-fold excess of unlabeled LDL. Specific binding was calculated by subtracting the cell-associated \(^{125}\text{I}\) radioactivity obtained after incubation with the 10-fold excess of unlabeled LDL from that obtained without addition of the unlabeled LDL. We also corrected for the fact that a 10-fold excess of unlabeled LDL would be expected to remove only a maximum of 90% of the specifically bound LDL. For eight cell lines studied (two spontaneously hypercholesterolemic and six controls), nonspecific binding accounted for 7.8 ± 1.0% (± S.E.) of total cell-associated radioactivity at 37°C and 19.2 ± 4.1% at 4°C. There were no differences in the proportion of specific and nonspecific binding in cells from spontaneously hypercholesterolemic animals relative to control cells. Thus, even though the data on LDL metabolism presented in this study include both specific and nonspecific binding, the contribution of nonspecific binding to total binding appears small (particularly at 37°C) and would not be expected to introduce major errors in interpreting the binding data.

From the results presented thus far, it is clear that cells from both spontaneously hypercholesterolemic and control animals bind, internalize, and degrade LDL normally. We then questioned whether these processes effectively delivered cholesterol to the cells such that the LDL cholesterol could be used to regulate metabolic functions. This was done by determining the influence of LDL on HMG-CoA reductase activity.

Prior to addition of lipoproteins to cells in culture, we incubated the cells for 48 h with culture medium containing lipoprotein-deficient serum in order to deplete them of cholesterol and to stimulate HMG-CoA reductase activity maximally. Preliminary studies indicated that maximum stimulation of HMG-CoA reductase activity was achieved in all cell lines after incubation for 48 h with medium containing lipoprotein-deficient serum. Under conditions of maximum stimulation, HMG-CoA reductase activities averaged 56.9 (range 37.3 to 93.3) pmol of mevalonate produced/min/mg of cell protein.

Incubation with LDL for 6 h at concentrations up to 20 µg of protein/ml produced a LDL concentration-dependent suppression of HMG-CoA reductase activity in all cell lines except those of the spontaneously hypercholesterolemic cell line 1108 (Fig. 5). After 24 h of incubation, the suppression of HMG-CoA reductase activity was more pronounced, reaching levels of <10% of maximum activity at even the lowest concentration of LDL used (2 µg/ml). Under these conditions, and at the highest concentration of LDL used (22 µg/ml), the HMG-CoA reductase activity in cell line 1108 was suppressed to only about 50% of that seen in the other cells.

The influence of HDL, relative to LDL, on the activity of HMG-CoA reductase is shown in Fig. 6. For this experiment, we incubated the cells for 24 h with equivalent concentrations of cholesterol as LDL or HDL. Consistent with the results shown in Fig. 5, LDL suppressed HMG-CoA reductase activity to <10% of maximum in all cell lines except those from animal 1108, where suppression averaged about 50%. LDL was less effective than HDL in suppressing HMG-CoA reductase activity, but did suppress reductase activity significantly to levels of 30 to 60% of maximum. In cells from animal 1108, HDL actually stimulated HMG-CoA reductase activity at cholesterol concentrations <25 µg/ml. At higher concentrations, however, HDL reduced HMG-CoA reductase activity, even in cell line 1108, to a level equivalent to that produced by LDL. Thus, cells from animal 1108 appeared to possess an abnormality in the regulation of HMG-CoA reductase activity as evidenced by their reduced response to both LDL and HDL.

To determine whether the inhibition of HMG-CoA reductase activity by LDL was due to contamination of the HDL with LDL, we tested the isolated HDL for the presence of LDL by immunodiffusion against antisera to rhesus monkey LDL. This procedure was sufficiently sensitive to detect 0.5% contamination of HDL protein with LDL protein, and none was detected.

Mahley and Innerarity (16) have described the presence of an abnormal HDL species, known as HDL\(_4\), that is produced in animals and man as a result of cholesterol feeding. HDL\(_4\) binds to the LDL receptor with as much as 100-fold greater affinity than does LDL and also results in the suppression of HMG-CoA reductase activity. Thus, even a small amount of contamination with HDL could provide an explanation for the small but consistent suppression of HMG-CoA reductase by HDL. We have been unable, however, to detect the presence of the arginine-rich apoprotein in rhesus monkey HDL using sodium dodecyl sulfate-polyacrylamide gel chromatography. This is consistent with the results reported by Rudel and Pitts (17). Thus, it seems unlikely that contamination by HDL\(_4\) can be the explanation for the LDL effect on HMG-CoA reductase activity.

![Figure 5](http://www.jbc.org/)

**FIG. 5.** The influence of time of incubation and LDL concentration on the activity of HMG-CoA reductase in skin fibroblasts from spontaneously hypercholesterolemic and control rhesus monkeys. Skin fibroblasts were grown and prepared for the addition of test media as described in the legend to Fig. 2 with the exception that the cells were approximately 80% confluent at the time of initiation of the experiment. After the 48-h incubation with lipoprotein-deficient serum, a subgroup of cells was analyzed for HMG-CoA reductase activity and expressed as 100%. Under these conditions of maximum stimulation, HMG-CoA reductase activity averaged 56.9 (range 37.3 to 93.3) pmol of mevalonate produced/min/mg of cell protein among the six cell lines studied. The indicated concentrations of LDL were added to all remaining dishes, incubated with the cells for 6 or 24 h, and analyzed for HMG-CoA reductase activity. All points in the 6-h study are the mean of duplicate determinations, while those in the 24-h study are the mean of five replicate determinations.
Fig. 6. The influence of LDL and HDL on the activity of HMG-CoA reductase in skin fibroblasts from spontaneously hypercholesterolemic and control rhesus monkeys. Skin fibroblasts were grown and prepared for incubation with test medium as described in the legend to Fig. 2. Cells were washed with phosphate buffer, incubated for 48 h with culture medium containing lipoprotein-deficient serum, and the HMG-CoA reductase activity of these cells was expressed in the figure as 100%. This medium was removed, 2 ml of culture medium containing lipoprotein-deficient serum and the indicated concentrations of cholesterol as LDL or HDL were added, and the cells were incubated at 37°C for 24 h. The cells were then harvested and analyzed for HMG-CoA reductase activity. Results are expressed as a percentage of the HMG-CoA reductase activity of the cells incubated with lipoprotein-deficient serum alone. All points represent the mean of five replicate determinations, except for those cells incubated with lipoprotein-deficient serum in which the results are the mean of 10 replicate determinations.

CoA reductase activity, although we cannot exclude the possibility that small amounts of HDL may have been present and may not have been detected by the methods used.

**DISCUSSION**

It seems apparent from the data presented that even though the two rhesus monkeys with spontaneous hypercholesterolemia have many of the phenotypic characteristics of familial hypercholesterolemia in man, they do not possess the LDL receptor defect characteristic of familial hypercholesterolemia. A number of pieces of evidence support this conclusion. Binding, internalization, and degradation of 125I-LDL by skin fibroblasts from spontaneously hypercholesterolemic animals occurred at rates similar to those seen for skin fibroblasts from six control rhesus monkeys. In cells from all of the animals studied, >92% of cell-associated 125I-LDL could be competed for by unlabeled LDL and, thus, could be accounted for by specific binding. Although we did not measure internalization of bound LDL directly, at least two pieces of evidence indicate that effective internalization occurred in cells from both control and spontaneously hypercholesterolemic animals. Cells incubated at 37°C accumulated over 10 times as much 125I-LDL as did cells incubated for the same period of time at 4°C (Fig. 2). Assuming that binding is approximately equal at 4°C and 37°C, as has been reported by others for human skin fibroblasts (18, 19) and by us for rhesus monkey smooth muscle cells (20), the approximately 10-fold greater amount of cell-associated 125I-LDL in cells incubated at 37°C must represent 125I-LDL that has been internalized. In addition, if internalization had not occurred in the spontaneously hypercholesterolemic cells, we would have expected a marked reduction in degradation as occurs in cells from a patient with a defect in internalization of receptor-bound LDL (21). This clearly was not the case, as effective degradation of 125I-LDL occurred in all cell lines (Fig. 3). Thus, it appears clear that LDL binding, internalization, and proteolytic degradation are not defective in cells from the spontaneously hypercholesterolemic animals nor are there systematic differences in LDL receptor function in animals with a wide range of individuality of response to dietary cholesterol (hyperclosponders versus hyporesponders).

This does not mean, however, that the cells from the spontaneously hypercholesterolemic animals were normal. These cells occupied a significantly larger surface area of the culture dish while having the same protein content per dish as did the control cells. A similar difference in cell size has been described for skin fibroblasts from human beings with familial hypercholesterolemia (22). This may suggest potentially important differences in membrane function in both spontaneously and familial hypercholesterolemic cells that are independent of the presence or absence of LDL receptors.

Even though LDL binding, internalization, and degradation are normal in cells from spontaneously hypercholesterolemic animals, we wanted to know whether LDL could effectively deliver cholesterol to the cells and, if so, whether this cholesterol could act to regulate cellular functions such as HMG-CoA reductase activity. Other studies (23) have shown that it is the cholesterol pool within the cell that regulates HMG-CoA reductase activity. For all of the control cells and one of the spontaneously hypercholesterolemic cell lines (No. 1648), the receptor-mediated uptake of LDL was effective in delivering cholesterol to the cells in a manner that regulated HMG-CoA reductase activity normally. Cells from spontaneously hypercholesterolemic animal 1108, however, were considerably less efficient in the regulation of HMG-CoA reductase activity in response to LDL even though high affinity binding, internalization, and degradation proceeded normally.

In addition, cells from animal 1108 responded differently to HDL than did cells from the control animals or from those of the other spontaneously hypercholesterolemic animal (No. 1648). Incubation with HDL at equivalent cholesterol concentrations to LDL produced a level of suppression of HMG-CoA reductase activity of 20 to 50% of maximum activity, while LDL suppressed HMG-CoA reductase activity to greater than 90% of maximum. By knowing the composition of the LDL and HDL used for these studies and estimating the molecular weights to be 3.1 X 10^6 for LDL and 250,000 for HDL (24), we were able to calculate the molar concentration of LDL and HDL associated with the level of suppression of reductase activity from the data shown in Fig. 6. As little as 3.1 X 10^5 M LDL suppressed HMG-CoA reductase activity by 90%, while a concentration of 541 X 10^4 M HDL produced a mean suppression of reductase of approximately 40% in the control cells. Thus, even though HDL was able to suppress reductase activity partially, it was considerably less effective than was LDL. This is consistent with results by Miller et al. (25),
indicating that HDL reduces sterol synthesis in human skin fibroblasts only when it is added at high molar concentrations. The fact that HDL and LDL were both less effective in suppressing HMG-CoA reductase activity in cells from animal 1108 compared with the other cell lines is consistent with the conclusion that the defect in 1108 cells is not related to a defective LDL receptor mechanism. Instead, the reduced ability for suppression of HMG-CoA reductase in cells from animal 1108 appears to be related to one or more defective intracellular mechanisms such as an abnormality in the catalytic properties of the reductase, an abnormality in lysosomal cholesteryl ester hydrolase activity, abnormal compartmentalization of cholesterol within the cell such that it does not effectively regulate the activity of HMG-CoA reductase, or in an abnormal rate of loss of cholesterol from cells to culture medium secondary to an abnormality in plasma membrane function. Regardless of the explanation, a similar defect in regulation of HMG-CoA reductase activity was not found in cells from the other spontaneously hypercholesterolemic animal (No. 1648).

The fact that regulation of HMG-CoA reductase activity was normal in cells from animal 1648 and abnormal in those from 1108 suggests that these animals represent different genotypes for this disorder. These animals are phenotypically different from each other in at least two additional ways. Animal 1648 has extensive cutaneous and tendinous xanthomas while 1108 has none. Also, progeny from animal 1648 displayed a greater increase in total plasma cholesterol concentrations when fed dietary cholesterol than did progeny from animal 1108 (26). Thus, it seems unlikely that the abnormality in regulation of reductase activity can fully explain the marked hyperbetalipoproteinemia or the other phenotypic differences between the two animals with spontaneous hypercholesterolemia.

Previous studies using skin fibroblasts from squirrel monkeys that were hypor hyperresponsive to dietary cholesterol suggested that there was no defect in regulation of cholesterol metabolism by LDL that could be correlated with the individuality of response to dietary cholesterol (6). Despite the fact that plasma cholesterol concentrations differed by nearly 700 mg/dl while the animals were consuming a cholesterol-containing diet, we could demonstrate no correlation of any parameter of LDL receptor function with the individuality of response to dietary cholesterol in the rhesus monkey of this study. Clearly, the factors responsible for individuality of control of plasma cholesterol concentration in response to dietary cholesterol do not appear to be mediated by either an absolute lack of LDL receptors or by an abnormality in their function. This is not surprising since these characteristics are evident only when the animals are challenged with dietary cholesterol (27, 28). This suggests that the hyperbetalipoproteinemia in hyperresponsive animals is mediated by other factors such as differences in cholesterol absorption (29), excretion, synthesis, distribution of absorbed cholesterol among body pools, or in the metabolism of the lipoproteins in response to the demand for transporting large amounts of absorbed dietary cholesterol. The potential importance of differences in lipoprotein metabolism is emphasized by the fact that the hypercholesterolemia resulting from cholesterol feeding in certain animals, including rhesus monkeys, is associated with an increased concentration of LDL of abnormal composition (28, 30). This is in contrast to the spontaneously hypercholesterolemic animals and human beings with familial hypercholesterolemia in which the hypercholesterolemia is largely the result of increased concentrations of LDL of normal composition (2).

Taken together, the data suggest that there must be a number of mechanisms whereby hyperbetalipoproteinemia can occur and that an abnormal LDL receptor function is not a requirement for all genetically mediated forms of hyperbetalipoproteinemia. Even in human beings with familial hypercholesterolemia, more than one mechanism is operating at any given time, as evidenced by the fact that in patients heterozygous or homozygous for this disorder there is a wide range of plasma cholesterol concentrations within a given genotype. Differences in LDL receptor function seem to dictate only a range of plasma LDL concentrations with the absolute concentration being determined by other factors such as diet, environment, etc. (31).

Rhesus monkeys that are hyperresponsive to dietary cholesterol, as well as the two rhesus monkeys with spontaneous hypercholesterolemia, all develop severe hyperbetalipoproteinemia associated with normal LDL receptor function. As with familial hypercholesterolemia in man, the spontaneously hypercholesterolemic monkeys develop their hyperbetalipoproteinemia while consuming an essentially cholesterol-free diet. Thus, the mechanism of hyperbetalipoproteinemia appears to be different from that of animals that develop hyperbetalipoproteinemia in response to dietary cholesterol and may suggest an abnormality in the rate of LDL synthesis or catabolism or in the metabolism of endogenously synthesized cholesterol by the animals with spontaneous hypercholesterolemia.

Although the animals with spontaneous hypercholesterolemia clearly do not provide the desired model of familial hypercholesterolemia in man, these studies have not eliminated the possibility that they may represent models for other dyslipoproteinemias existing in the human population. Since these animals are relatively hypertriglyceridemic for rhesus monkeys, 161 mg/dl versus 50 mg/dl for normal rhesus monkeys (1), it is possible that they may possess a similar genetic defect to that found in familial combined hyperlipoproteinemia in man (32). Regardless of whether the genetic mechanism of spontaneous hypercholesterolemia is found to be identical with that of a specific human familial hyperlipoproteinemia, these animals would appear to provide a powerful model for the study of alterations in the control of plasma LDL concentrations under conditions of normal LDL receptor function.

Acknowledgments—We acknowledge the excellent technical assistance of Ms. Molly Light, Ms. Grayce Greene, and Mrs. Patricia Hester. We also wish to thank Dr. Manford Morris from the University of Arkansas for allowing us to obtain skin biopsies from the two rhesus monkeys with spontaneous hypercholesterolemia.

REFERENCES

LDL Receptor Function in Hypercholesterolemic Monkeys

Low density lipoprotein receptor activity on skin fibroblasts from rhesus monkeys with diet-induced or spontaneous hypercholesterolemia.

L S Guertler and R W St Clair


Access the most updated version of this article at [http://www.jbc.org/content/255/1/92.citation](http://www.jbc.org/content/255/1/92.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/255/1/92.citation.full.html#ref-list-1](http://www.jbc.org/content/255/1/92.citation.full.html#ref-list-1)