Steroid-secreting cultured mouse adrenal cells of the Y-1 clone were shown to possess a high affinity cell surface receptor for plasma low density lipoprotein (LDL). Binding of either human or mouse LDL to the receptor was followed by the uptake of the lipoprotein and the hydrolysis of its protein and cholesteryl ester components within lysosomes. In the absence of adrenocorticotropin (ACTH), the LDL-derived cholesterol suppressed the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, enhanced the rate of incorporation of $^{14}$C-oleate into cholesteryl esters, and suppressed the activity of the LDL receptor. In the presence of ACTH, the LDL-derived cholesterol was converted to 21-carbon steroids (chiefly, 11-$\beta$-hydroxy-20-$\alpha$-dihydroprogesterone).

When the mouse adrenal cells were grown in the presence of ACTH but in the absence of lipoproteins, the availability of cholesterol became rate-limiting for steroid synthesis. The subsequent addition of LDL to the culture medium caused a large intracellular accumulation of cholesteryl esters and enhanced the rate of steroid secretion by 4-fold. Under these conditions, more than 75% of the secreted steroid was derived from LDL that entered cells through the receptor-mediated pathway. When the rate of steroid secretion was high, the amount of cholesterol that could be derived from LDL was not sufficient to suppress completely the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase or to stimulate fully the incorporation of $^{14}$C-oleate into cholesteryl esters.

High density lipoprotein (HDL) derived from either human or mouse plasma did not bind to the LDL receptor on mouse adrenal cells as indicated by its inability to compete effectively with $^{125}$I-LDL for binding and degradation. As a result, HDL did not increase the cholesterol content of the adrenal cells nor was it able to suppress the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase or provide cholesterol substrate for steroid synthesis.

Cultured cells that secrete steroids require cholesterol not only for plasma membrane synthesis but also as a substrate for steroid production. The most thoroughly studied example of such a steroid-secreting cell is the mouse adrenal cell line that was initially derived from a functional adrenal cortical mouse tumor (1) and adapted to tissue culture by Buonassissi et al. (2). Clones of this cell line were prepared by Yasumura et al. (3). One of these, the Y-1 clone, secretes large amounts of 21-carbon steroids in response to adrenocorticotropin (3, 4).

In an extensive series of studies, Kowal demonstrated that the Y-1 adrenal cells have apparently lost the 21-hydroxylase enzyme and therefore do not produce corticosterone, the normal major secretory product of mouse adrenal glands (4-7). Rather, when stimulated with ACTH, cells of the Y-1 clone produce large amounts of 11-$\beta$-hydroxy-20-$\alpha$-dihydroprogesterone (11-$\beta$-hydroxy-DHP) as their main secretory steroid (4, 5).

The rate-limiting step in the conversion of cholesterol to adrenal steroids is generally believed to be the process mediating the entry of cholesterol into the mitochondria where the side chain of the steroid is cleaved to form pregnenolone (8, 9). Kowal showed that in the Y-1 adrenal cells the cholesterol substrate for mitochondrial side chain cleavage could be provided either by endogenously synthesized cholesterol or by cholesterol that entered the cells from the culture medium (4). Kowal also demonstrated that when steroid synthesis was stimulated by ACTH there was a secondary enhancement in the incorporation of radioactivity from $[^{14}]$Cacetate or $[^{14}]$Cglucose into cellular $[^{14}]$C-cholesterol which in turn was converted into $[^{14}]$C-labeled steroids (4). Since ACTH did not stimulate the incorporation of radiolabeled mevalonate into cholesterol, Kowal concluded that the ACTH-mediated stimulation of cholesterol biosynthesis involved an early step in the cholesterol synthetic pathway (4).

The studies of Kowal relating cholesterol metabolism to steroid synthesis in the Y-1 adrenal cells were conducted in culture medium that contained serum lipoproteins (4-7). These studies were performed prior to the recognition that mammalian cells possess a specific mechanism for the uptake of cholesterol from certain of these cholesterol-carrying lipoproteins. As delineated in cultured human fibroblasts (re-
viewed in Refs. 10 and 11). This uptake process involves a high affinity plasma membrane receptor that specifically binds low-density lipoprotein (LDL) and facilitates its uptake into the cell by adsorptive endocytosis. Once inside the cell, the protein and the lipoprotein components of LDL are hydrolyzed in cellular lysosomes, and the resultant free cholesterol is used by the fibroblasts for plasma membrane synthesis. The free cholesterol derived from the hydrolysis of LDL also becomes available to regulate three events in cellular cholesterol metabolism: (a) it suppresses endogenous cholesterol synthesis by reducing the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (12); (b) it enhances the formation of cellular cholesterol esters by activating a microsomal fatty acyl-CoA:cholesterol acyltransferase (13, 14); and (c) it exerts a feedback suppression on the synthesis of the LDL receptor, thus preventing excessive uptake of LDL by the cells (15, 16).

The current studies were conducted to determine whether a receptor-mediated process for the uptake of lipoproteins exists in the cultured Y-1 mouse adrenal cells and to define the relationship between such a lipoprotein uptake process and the regulation of steroid synthesis. The results indicate that the cultured mouse adrenal cells possess a cell surface receptor that allows them to derive cholesterol from LDL and that this receptor-mediated process has the capability of supplying more than 75% of the cholesterol used for steroid synthesis in these cells.

### EXPERIMENTAL PROCEDURES

**Materials** — [1-14C]Oleic acid (51.8 mCi/mmmol), m-3-hydroxy-3-methyl[14C]glutaryl coenzyme A (18.8 mCi/mmol), cholesterol [1-14C]Olate (44 mCi/mmol), and Aquason solubility fluid were purchased from New England Nuclear Corp. Sodium [3H]laurate-free in 0.05 N NaOH was obtained from Schwarz/Mann. [12, 3H]Cholesterol (65 mCi/mmol), [14-14C]progesterone (44 mCi/mmol), and Aquason solubility fluid were purchased from Amersham/Searle Corp. 7-Ketosteroids and stigmasteryl were obtained from Steraloids. Pepsone from Sterponios griucus, B grade (45,000 units/g) was obtained from Calbiochem. Cholesterol and cholesterol olate were purchased from Applied Science. Authentic 11-β-hydroxy-20α-dihydroprogesterone (14-2H) was the gift of Proleutroph E. W. Klyne and Dr. D. N. Kirk, MRC Steroid Reference Collection, Department of Chemistry, Westfield College, Hampstead, London. Chloroquine diphosphate, sodium heparin (Grade II), progesterone, pregnenolone, and 20α-dihydroprogesterone were purchased from Sigma Chemical Co. Aminogluthethimide was provided by Ciba. Adrenocorticotropic hormone (ACTH; 1-39) was obtained from the National Institute for Medical Research, Pharmaceutical Co. Methylene chloride (glass-distilled) was purchased from Burdick and Jackson Laboratories, Inc. Whole mouse plasma was purchased from Pet-Freeze Biologicals, Inc. (Rogers, Ark.). Ham's F-12 medium with l-glutamine (Catalogue No. 12-815) was obtained from Microbiological Associates. Horse serum (Catalogue No. 80b) was purchased from Grand Island Biological Co. Other tissue culture supplies, thin layer and gas-liquid chromatographic materials, and reagents for assays were obtained from sources as previously reported (12, 17). Gas-liquid chromatography of steroids and fatty acids was performed using a Hewlett-Packard model 5750 research chromatograph with flame ionization detector and Hewlett-Packard model 3370 B integrator (17).

**Cells** — The ACTH-responsive mouse adrenal tumor line (Y-1 clone) was obtained from the American Type Culture Collection. Cells were grown in monolayer and maintained in a humidified incubator (5% CO2 at 37°C in 75 cm2 flask containing 10 ml of growth medium consisting of Ham's F-12 medium supplemented with 15% (v/v) horse serum, 2.5% (v/v) fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). All experiments were carried out using the similar formation of cell stocks from flask were dissocated with 0.05% trypsin-0.02% EDTA solution and were seeded (Day 0) at a concentration of 2.5 × 106 cells/dish into Petri dishes (60 × 15 mm) containing 5 ml of the above growth medium with 15% horse serum and 2.5% fetal calf serum. On Day 3, the medium was replaced with 3 ml of fresh growth medium containing 15% horse serum and 2.5% fetal calf serum. On Day 6, the medium was switched to 2 ml of Medium A (Ham's F-12 medium containing 10% (v/v) human lipoprotein-deficient serum (final protein concentration, 5 mg/ml). All experiments were initiated on Day 7. The cells had been cultured for 24 h in the presence of lipoprotein-deficient serum.

**Lipoproteins** — Human and mouse LDL (density 1.019 to 1.063 g/ml), human and mouse HDL (density 1.085 to 1.215 g/ml), and human lipoprotein-deficient serum (density > 1.215 g/ml) were prepared by differential ultracentrifugation (18) as previously described (12). Both the human and mouse plasma were processed within 36 h of bleeding. For human LDL and HDL, the mass ratio of total cholesterol to protein content was 1.6:1 and 1.3:1, respectively. For mouse LDL and HDL, the mass ratio of total cholesterol to protein content was 1.6:1 and 1.7:1, respectively. Human [3H]LDL specific activity: 200 to 450 cpm/μg protein was used as previously described (19). For experiments, the [3H]LDL was diluted with unlabeled human LDL to give the final concentration and specific activity indicated in the figure legends.

**Preparation of [3H]Cholesteryl Linolate-LDL**—[3H]Cholesterol was reacted with an excess of linoleic acid in dry pyridine as described by Goodman (20). In order to enhance the efficiency of incorporation of the [3H]cholesterol linolate into LDL, an improved method was developed for purifying the [3H]cholesterol linolate so as to eliminate traces of contaminating material that interfered with the testing procedure. Following the purification procedure, contents of the reaction vials were transferred to a 250-ml separatory funnel with five 4-ml aliquots of heptane. This organic solution was then extracted in the following sequence, the lower aqueous phase being discarded after each separation: two extractions with 10 ml of 0.5 N HCl; one extraction with 10 ml of H2O; two extractions with 10 ml of 0.05 N NaOH in 50% ethanol; and one extraction with 10 ml of H2O. Next, the 20-ml heptane solution was extracted with 8 ml of methanol, dissolving the lower phase. This extraction was repeated twice with 8 ml of a lower phase solvent generated from a mixture of heptane/methanol (3:2). The resultant upper organic phase was then evaporated to dryness in a disposable glass test tube (13 × 100 mm). The evaporated residue was dissolved in 0.15 ml of benzene and applied to a silicic acid/Cellite (2:1) column (0.6 × 12 cm) pre-equilibrated with benzene. Purified [3H]cholesterol linolate was eluted with an additional 2.65 ml of benzene. The final [3H]cholesterol linolate was kept 99% pure as determined by thin layer chromatography on silica gel using benzene/ethyl acetate (9:1) as the developing solvent system and was recovered with a 90% yield.

The purified [3H]cholesterol linolate was incorporated into LDL using the procedure previously described (12, 21). Forty microcuries (0.93 nmol) of the purified [3H]cholesterol linolate was evaporated to dryness in a disposable glass test tube (13 × 100 mm). The residue was dissolved in 0.1 ml of dimethylsulfoxide, and the clear solution was agitated on a Vortex mixer for 1 min. Next, 0.9 ml of a buffer consisting of 20 mM Tris/chloride, pH 7.4, 0.15 M NaCl, and 0.3 mM sodium EDTA was added, followed by 0.9 ml of 25 μl of protein (25 μl of a solution containing 0.15 M NaCl and 0.3 mM sodium EDTA). This solution was then incubated for 2 h at 40°C, after which it was dialyzed at 4°C for 48 h against four changes of 4 liters of buffer consisting of 20 mM Tris/chloride, pH 7.4, 0.15 M NaCl, and 0.3 mM sodium EDTA. The solution was then removed from the dialysis tubing and centrifuged in a Beckman Microfuge (5 min, 4°C, 12,000 rpm). The resultant supernatant contained about 0.9 mg of protein and an average of 60% of the originally added [3H]cholesterol linolate. The final specific activity averaged 50,000 cpm/nmol of total cholesteryl linolate in LDL.

**Measurement of Steroids Secreted into Culture Medium** — The medium (2 ml) from one Petri dish of cultured adrenal cells was agitated on a Vortex mixer for 10 s in a test tube containing 3 ml of methylene chloride and 2 μg of stigmasterol as an internal standard. The phases were separated by centrifugation (15 min, 24 000 rpm). The top aqueous phase was drawn off with a Pasteur pipette, and the organic phase was re-extracted with 2 ml of 0.1 N NaOH followed by 2 ml of H2O. The final bottom phase was evaporated to dryness and dissolved in 5 μl of ice-cold chloroform, and 1.5 μl was injected into a 1.8 by 10 cm glass gas chromatography column packed with 3% OV-17 on 100 to 120 mesh Gas-Chrom Q. The column was run at 260°C as previously described (17). Quantitation was achieved by using the internal standard area ratio technique employing stigmasterol as the internal standard (17). Average elution...
times for the steroids were: pregnenolone, 360 s; unresolved peak containing both progesterone and 20α-dihydroprogesterone, 427 s; cholesterol, 563 s; stigmastanol, 815 s; and 11-β-hydroxy-DHP, 981 s. The steroid content of the culture medium is expressed as micrograms of steroid per mg of total cell protein.

Measurement of Cellular Content of Free and Esterified Cholesterol. The content of free cholesterol was determined by a previously described method in which the steroids were extracted from washed cell pellets with chloroform/methanol (21). The free and esterified cholesterol fractions were separated on silicic acid/Celite columns, and the cholesterol content of each fraction was measured by gas-liquid chromatography (following alkaline hydrolysis of the cholesteryl ester fraction) (17). Correction for procedural losses, which averaged 25%, was made by utilizing [1-14C]cholesterol, [4-14C]cholesteryl stearate, and stigmasterol as internal standards (17). The relative composition of the major fatty acyl components of the cholesteryl esters in plasma LDL and in adrenal cells was determined by gas-liquid chromatography of the fatty acid methyl esters as previously described (Method 2, Ref. 22).

Surface Binding, Intracellular Accumulation, and Proteolytic Degradation of [3H]Cholesteryl Linoleate-LDL by Intact Adrenal Cells—Monolayers of adrenal cells were incubated at either 4°C or 37°C with [3H]LDL in 2 ml of Medium A. After the indicated interval, the medium was removed, the intact [3H]LDL contained in the medium was washed with chloroform/acetone, the resulting supernatant was extracted with chloroform and hydroquinone to remove free iodine (23), and an aliquot of the supernatant fraction was counted to determine the amount of [3H]-labeled, trichloroacetic acid-soluble material formed by the cells released into the medium (24). Degradative activity represents the cell-dependent rate of proteolysis and is expressed as the nanograms of [3H]-LDL degraded to acid-soluble material per mg of total cell protein.

The amount of [3H]LDL bound to the surface of the cells and the amount of [3H]LDL contained within the cells were determined as previously described for fibroblasts (25). Following removal of the incubation medium, the monolayers were washed six times at 4°C with an albumin-containing buffer after which a solution containing sodium bicarbonate (10 mM) was added to each dish (25). The dishes were incubated at 4°C for 1 h; the heparin-containing medium was removed, and an aliquot was placed in a well-type β counter to determine the amount of [3H]-LDL that had been bound to the cell surface and had been released by heparin into the supernatant solution. After the heparin wash, the cells were dissolved in 0.1 N NaOH, and an aliquot was counted to determine the total amount of [3H]LDL that had entered the cell and had therefore not been released by heparin (25). Another aliquot was used to measure the content of total cell protein. Surface binding and intracellular accumulation were expressed as the autoradiograms of [3H]LDL per mg of total cell protein. In some experiments the data are expressed as specific high affinity [3H]LDL receptor binding and receptor-mediated degradation of [3H]-LDL. In these cases, values for nonspecific binding and nonspecific degradation were subtracted from the observed, total values. The specific binding values were determined by incubating parallel monolayers with [3H]-LDL in the presence of a 10- to 15-fold excess of unlabeled human LDL (24, 25). In each case where this correction was made, the values for the nonspecific processes were less than 10% of the observed total values.

Hydrolysis of [3H]Cholesteryl Linoleate-LDL and Incorporation of [3H]Cholesterol into [3H]Steroids by Intact Adrenal Cells—Monolayers were incubated at 37°C with [3H]cholesteryl linoleate-LDL in 2 ml of Medium A, after which the medium was removed and the secreted steroids were extracted as described above for measurement of the total steroid content except that [3H]progesterone (50 μg, 500 cpm), 10 μg of cholesteryl oleate, and 50 μg each of cholesterol, pregneno lone, and 20α-dihydroprogesterone were added to each tube. The evaporated organic extract was dissolved in 30 μl of benzene and spotted directly on plastic-backed silica gel (without guespin) thin layer sheets. The sheets were developed with chloroform/diethyl ether (9:1) in a solvent-saturated tank. The steroids were visualized by iodine vapor with the following RF values: 11-β-hydroxy-DHP, 0.05; 20α-dihydroprogesterone, 0.20; pregnenolone, 0.25; cholesterol, 0.33; progesterone, 0.45; and cholesteryl oleate, 0.75. Each steroid spot was cut out and counted in 10 ml of Aquasol. Calculations were performed on a computer by subtracting the radioactivity of the cholesteryl oleate standard, which averaged 65%. Results are expressed as picomoles of [3H]-steroid formed per mg of total cell protein.

After removal of the medium, each monolayer was washed as previously described (21), and the cells were extracted with chloroform/methanol (2:1) after the addition of an internal standard containing [14C]cholesterol (30 μg, 500 cpm) and unlabeled cholesteryl oleate (30 μg). The free and esterified [3H]cholesterol were separated by thin layer chromatography on silica gel sheets using benzene/ethyl acetate (9:1). Hydrolytic activity is expressed as picomoles of [3H]cholesterol formed per mg of total cell protein. Calculations were performed for the recovery of [14C]cholesterol from each sample, which averaged 85%.

Assay of 3-Hydroxy-3-methylglutaryl-CoA Reductase Activity in Cell-free Extracts—The rate of conversion of 3-hydroxy-3-methyl-3-[14C]glutaryl-CoA to [14C]mevalonate was measured in extracts of detergent-solubilized adrenal cells as previously described for fibroblasts (19) except for the following modifications: 30 to 75 μg of solubilized protein was assayed; incubation was 1 h at 37°C; and a final [14C]HMG-CoA concentration of 67 μM (10,000 cpm/mmol) was used. The concentrations of n. HMG-CoA and TPNH giving half-maximal activities were about 10 μM and 50 μM, respectively. HMG-CoA reductase activity is expressed as picomoles of [14C]mevalonate formed per min per mg of detergent-solubilized protein.

Incorporation of [1-14C]Oleate into Cholesteryl Esters by Intact Adrenal Cells—Monolayers were incubated at 37°C with 0.1 mM [1-14C]oleate bound to albumin in 2 ml of Medium A (21). After the indicated time, the cells were washed, harvested, and extracted with chloroform/methanol (2:1), and the cholesteryl [14C]oleate was isolated by thin layer chromatography as previously described (13). Esterification activity is expressed as the picomoles of cholesteryl [14C]oleate formed per mg of total cell protein. Correction for procedural losses, which averaged 15%, was made by utilizing [1]cholesteryl oleate as an internal standard added prior to the chloroform/methanol extraction.

The results of experiments on the incorporation of the labeled fatty acid into cholesteryl esters are expressed as picomoles per mg of total cell protein. Surface binding was determined as described above for intact adrenal cells. The protein concentrations of extracts and whole cells were determined by a modification of the method of Lowry et al. (26) using bovine serum albumin as a standard. The concentration of ACTH in horse serum, fetal calf serum, and human lipoprotein-deficient serum was measured by direct radioimmunoassay (27).

RESULTS

To study the relation between plasma lipoproteins and adrenal steroid secretion, a protocol was established in which the mouse Y-1 adrenal cells were grown for 6 days in medium containing 15% horse serum and 2.5% fetal calf serum. The cells were then incubated for 24 h in medium containing 10% human lipoprotein-deficient serum so as to deprive the cells of an exogenous source of cholesterol. Fig. 1 shows the gas-liquid chromatographic profile of the steroids that accumulated over 24 h in the culture medium of adrenal cells that were incubated in lipoprotein-deficient serum supplemented with ACTH, or LDL, or both, as indicated. Peak 1 corresponded to the elution time of an authentic sample of 11-β-hydroxy-20α-dihydroprogesterone (11-β-hydroxy-DHP). The Peak 1 material also showed a migration pattern identical with that of authentic 11-β-hydroxy-DHP when subjected to thin layer chromatography as described under "Experimental Procedures." Peak 2 corresponded to cholesterol. Peak 3 contained both 20α-dihydroprogesterone and progesterone. Peak 4 contained pregnenolone. Also shown in Fig. 1 is the elution time of stigmasterol, which was added to the culture medium as an internal standard.

The tracing in Fig. 1 illustrates the striking increase in Peak 1 that occurred when ACTH plus LDL were added to adrenal cells previously cultured in lipoprotein-deficient serum. The concentration of ACTH giving a maximal stimula-
The increase in the cholesterol content of the culture medium, as determined by gas-liquid chromatography, was used as an index of adrenal steroid secretion. The rate of secretion of 11-β-hydroxy-DHP was more than 3-fold higher than the secretion rate during the first 24-h interval. In contrast, the rate of secretion of pregnenolone and progesterone plus 20α-dihydroprogesterone, all of which are believed to be precursors of 11-β-hydroxy-DHP, was constant during the 72-h interval. In the experiments reported below, the secretion of 11-β-hydroxy-DHP was used as an index of adrenal steroid secretion.

When the mouse adrenal cells were grown in the absence of ACTH and lipoproteins, the addition of increasing amounts of human LDL caused a slight, but dose-related increase in the secretion of 11-β-hydroxy-DHP (Fig. 3A). When the cells were grown in the presence of ACTH but in the absence of lipoproteins, a moderate amount of 11-β-hydroxy-DHP was secreted (18 μg/mg of cell protein over 48 h) (Fig. 3B). Under these conditions, the addition of increasing amounts of human LDL caused a marked stimulation in the rate of steroid secretion. These data suggest that in the absence of plasma lipoproteins the availability of cholesterol constitutes a limiting factor in the secretion of adrenal steroids and that this deficiency can be overcome by LDL.

To study further the relation between cholesterol metabolism and steroid hormone secretion in the cultured adrenal cells, the effects of ACTH and lipoproteins on the activity of HMG-CoA reductase were determined. Adrenal cells grown in the absence of lipoproteins and ACTH exhibited a moderately high level of HMG-CoA reductase activity (250 pmol·min⁻¹·mg⁻¹) (Fig. 4). Under these conditions, the addition of human LDL suppressed the activity of HMG-CoA reductase by more than 90% over a 24-h interval. When ACTH was present, HMG-CoA reductase activity in the absence of LDL was extremely high (600 pmol·min⁻¹·mg⁻¹). Under these conditions, the addition of LDL produced only a partial suppression of enzyme activity even at high levels of the lipoprotein. In the presence of 240 μg of protein/ml of LDL, the activity of HMG-CoA reductase was more than 10-fold higher in the presence of ACTH than it was in the absence of the hormone (Fig. 4).

These data suggest that the activity of HMG-CoA reductase in the adrenal cells is governed by a balance between the demand for cholesterol for steroid synthesis, as dictated by ACTH, and the supply of exogenous cholesterol available in the form of LDL. Thus, under conditions in which steroid secretion is high, the amount of cholesterol derived from LDL was not sufficient to completely suppress HMG-CoA reductase activity.

In addition to human LDL, mouse LDL also had the ability to suppress HMG-CoA reductase in the mouse adrenal cells when added in the absence of ACTH (Fig. 5A). In contrast, neither human nor mouse HDL caused a significant suppression of HMG-CoA reductase activity at concentrations up to 150 to 300

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**Fig. 1.** Gas-liquid chromatograms of methylene chloride extracts of culture medium from mouse adrenal cells. On Day 7, each monolayer of adrenal cells received 2 ml of Medium A containing the following additions: A, none; B, ACTH, 100 milliunits/ml; C, LDL, 50 μg of protein/ml; or D, ACTH, 100 milliunits/ml plus LDL, 50 μg of protein/ml. After incubation at 37°C for 24 h, the medium was removed, extracted with methylene chloride, and subjected to gas-liquid chromatography as described under "Experimental Procedures." Peak 1, 11-β-hydroxy-DHP; Peak 2, cholesterol; Peak 3, progesterone plus 20α-dihydroprogesterone; Peak 4, pregnenolone. The increase in the cholesterol content of the culture medium (Peak 2) in Panels C and D is attributable to the LDL that was added to the medium.

**Fig. 2.** Quantitation of steroids secreted by mouse adrenal cells during successive 24-h intervals after addition of ACTH to the culture medium. On Day 7 (zero time), each monolayer received 2 ml of Medium A containing 200 milliunits/ml of ACTH and 20 μg of protein/ml of LDL. Every 24 h the medium was replaced with fresh medium containing ACTH and LDL. At the indicated interval, the medium from duplicate dishes was removed for measurement of its steroid content, and the cells were harvested for measurement of their total protein content. Each value represents the average of these duplicate dishes.
Steroid Metabolism in Cultured Mouse Adrenal Cells

FIG. 3 (left). Enhancement of steroid secretion by human LDL in mouse adrenal cells previously incubated in lipoprotein-deficient serum. On Day 7, each monolayer received 2 ml of Medium A in the absence (A) or presence (B) of 200 milliunits/ml of ACTH. On Day 8 (zero time), the medium in each monolayer was replaced with 2 ml of fresh medium of the same composition but containing the indicated concentration of human LDL. After incubation at 37° for an additional time as indicated, the medium was removed for measurement of its content of 11-β-hydroxy-DHP and the cells were harvested for measurement of their total protein content. Each value represents the average of duplicate incubations.

FIG. 4 (right). Suppression of HMG-CoA reductase activity in mouse adrenal cells by human LDL. On Day 7, each monolayer received 2 ml of Medium A in the absence (A) or presence (B) of 200 milliunits/ml of ACTH. On Day 8, the medium was replaced with 2 ml of fresh medium of the same composition but containing the indicated concentration of human LDL. After incubation at 37° for 24 h, the cells were harvested for measurement of HMG-CoA reductase activity. Each value represents the average of duplicate incubations.

FIG. 5. Comparison of the ability of human and mouse LDL and HDL to suppress HMG-CoA reductase activity in mouse adrenal cells. On Day 7, each monolayer received 2 ml of Medium A and the indicated concentration of one of the following lipoproteins: Experiment A, ☐, none; ●, human LDL; ▲, mouse LDL; ○, mouse HDL. Experiment B, ☐, none; ●, human LDL; ○, human HDL. After incubation for 24 h at 37°, the cells were harvested for measurement of HMG-CoA reductase activity. Each value represents the average of triplicate incubations (☐) or single incubations (●, ○, ▲).

FIG. 6 (left). Stimulation of [14C]oleate incorporation into cholesteryl [14C]oleate by human LDL in mouse adrenal cells incubated in the absence and presence of ACTH. On Day 7, each monolayer received 2 ml of Medium A in the absence (A) or presence (B) of 200 milliunits/ml of ACTH. On Day 8, the medium was replaced with 2 ml of fresh medium of the same composition but containing the indicated concentration of LDL. After incubation at 37° for 6 h, each monolayer was pulse-labeled for 2 h with 0.3 mCi [14C]oleate/albumin (12,000 cpm/nmol), after which the cells were harvested for determination of their content of cholesteryl [14C]oleate. Each value represents a single incubation.

FIG. 7 (right). Comparison of the ability of mouse and human LDL and HDL to stimulate [14C]oleate incorporation into cholesteryl [14C]oleate in mouse adrenal cells. On Day 7, each monolayer received 2 ml of Medium A and the indicated concentration of one of the following lipoproteins; ☐, none; ●, human LDL; ▲, mouse LDL; ○, mouse HDL. After incubation for 8 h at 37°, each monolayer was pulse-labeled for 2 h with [14C]oleate/albumin (12,000 cpm/nmol), after which the cells were harvested for determination of their content of cholesteryl [14C]oleate. Each value represents a single incubation.

The data in Figs. 4 and 6 indicate that in the presence of ACTH the ability of LDL either to suppress HMG-CoA reductase or to activate cholesterol esterification by lipoproteins was specific for human and mouse LDL as opposed to HDL (Fig. 7). The data in Figs. 4 and 6 indicate that in the presence of ACTH the ability of LDL either to suppress HMG-CoA reductase or to activate cholesterol esterification by lipoproteins was specific for human and mouse LDL as opposed to HDL (Fig. 7).

In human fibroblasts (13) and lymphoid cells (28), the addition of LDL to the culture medium causes an enhancement in the rate at which the cells incorporate [14C]oleate into cholesteryl [14C]oleate. This is due to an activation of a microsomal acyl-CoA:cholesterol acyltransferase by excess cholesterol derived from LDL (14). The data in Fig. 6 show that a similar stimulation of cholesterol esterification occurred in adrenal cells incubated with human LDL. In the presence of ACTH, the stimulation of cholesterol esterification by LDL was blunted, again suggesting that the lipoprotein was not able to supply as much excess cholesterol to the cells when the rate of steroid synthesis was high. As with the suppression of HMG-CoA reductase, the stimulation of cholesterol esterification by lipoproteins was specific for human and mouse LDL as opposed to HDL (Fig. 7).
inhibiting the side chain oxidation of cholesterol (7, 29, 30). Fig. 8A shows that increasing concentrations of aminoglutethimide progressively inhibited the secretion of $^{11}$β-hydroxy-DHP in the presence of ACTH and LDL. In the absence of aminoglutethimide, the concentration of 11α-hydroxy-DHP was much higher in the presence of ACTH than in its absence (Fig. 8D). As the concentration of aminoglutethimide was increased, the activity of HMG-CoA reductase was increased, however, the activity of HMG-CoA reductase in the ACTH-treated cells declined so that eventually it came to equal the activity in the absence of ACTH. Similar effects of aminoglutethimide on cholesterol synthesis from $^{14}$C-oleate in the presence of serum lipoproteins have previously been reported by Kwawal (4). The converse phenomenon was seen with respect to cholesterol esterification (Fig. 8C). In the absence of aminoglutethimide, the activation of this process by LDL was blunted by ACTH, and the effectiveness of ACTH also diminished as the concentration of aminoglutethimide was increased. Neither aminoglutethimide at concentrations up to 400 μg/ml nor ACTH had any effect on HMG-CoA reductase activity in human fibroblasts either in the absence or presence of LDL (data not shown).

To determine whether the selective ability of LDL to suppress HMG-CoA reductase activity and to activate cholesterol esterification in the adrenal cells was due to a high affinity cell membrane receptor as in human fibroblasts, a series of experiments was conducted using human $^{125}$I-LDL. Monolayers of adrenal cells were incubated with $^{125}$I-LDL at either 4° or 37° and washed extensively to remove nonspecifically bound material. The cells were then incubated at 4° with a solution containing heparin, an agent that has been shown to release $^{125}$I-LDL from its cell-surface receptor in fibroblasts (25). The amount of heparin-releasable, $^{125}$I-LDL and the amount of $^{125}$I-LDL that remained associated with the cell after heparin treatment (heparin-resistant $^{125}$I-LDL) were determined separately (25).

The data in Fig. 9 show that when $^{125}$I-LDL was allowed to bind to adrenal cells at 4°, a saturable binding process was observed. Most of the $^{125}$I-LDL bound to the cells was released by heparin. Apparent saturation of the heparin-releasable surface binding occurred at an $^{125}$I-LDL concentration between 100 and 200 μg of protein/ml and apparent half-maximal binding occurred at about 25 μg of protein/ml.

When $^{125}$I-LDL was incubated with adrenal cells at 37°, a steady state was reached during which the amount of heparin-releasable, surface-bound $^{125}$I-LDL and the cellular content of heparin-resistant $^{125}$I-LDL remained constant (Fig. 10, A and B). During this steady state period, $^{125}$I-LDL continued to bind to the receptor and enter the cell, but the rate of entry was balanced by an equal rate of proteolysis and with excretion from the cell of $^{125}$I-labeled trichloroacetic acid-soluble material (Fig. 10C). This degradation of $^{125}$I-LDL appeared to occur within cellular lysosomes since it could be inhibited by the lysosomal inhibitor chloroquine (Fig. 10C) (21, 31). When degradation was inhibited in this manner, intact $^{125}$I-LDL continued to accumulate within the cell throughout the experiment (Fig. 10B). The kinetic data for these processes in mouse adrenal cells are very similar to the processes for $^{125}$I-LDL binding, internalization, and lysosomal degradation previously observed in human fibroblasts (11, 15, 25, 31).

The specificity of the binding and internalization processes for LDL at 37° was indicated by the experiment shown in Fig. 11. Unlabeled human LDL, but not HDL, competed effectively...
Steroid Metabolism in Cultured Mouse Adrenal Cells

FIG. 10. Time course of binding (A), internalization (B), and degradation (C) of $^{131}$I-LDL in mouse adrenal cells in the absence and presence of chloroquine. On Day 7, each monolayer received 2 ml of Medium A containing 100 milliunits/ml ACTH. On Day 8 (zero time), the medium was replaced with 2 ml of fresh medium of the same composition but containing 24 $\mu$g of protein/ml of human $^{131}$I-LDL (24 cpm/ng) in the absence (0) or presence (0) of 20 $\mu$m chloroquine. After incubation at 37°C for the indicated time, the medium was removed and its content of $^{131}$I-labeled trichloroacetic acid-soluble material (C) was measured. The cell monolayers were then washed by the standard procedure, and the amounts of heparin-releasable (A) and heparin-resistant (B) $^{131}$I-LDL were determined. Each value represents the average of duplicate incubations.

For the $^{131}$I-LDL binding sites as indicated either by measurement of the amount of heparin-releasable $^{131}$I-LDL (Fig. 11A) or by measurement of the amount of $^{131}$I-LDL internalized by the cell (Fig. 11B).

In human fibroblasts the cell surface LDL receptor has been shown to be exquisitely sensitive to destruction by pronase and other proteolytic enzymes (15, 24). Fig. 12 shows that a similar destruction of high affinity surface binding sites also occurred in adrenal cells after incubation for a brief period with pronase at concentrations less than 5 $\mu$g/ml. To determine whether destruction of the binding sites would also prevent the biologic actions of LDL, the ability of the lipoprotein to stimulate the incorporation of $^{14}$C-choleate into cholesteryl $^{14}$C-choleate was measured. Fig. 13 shows that in pronase-treated cells the LDL-mediated stimulation of cholesterol esterification was inhibited. On the other hand, the ability of the adrenal cells to respond to 7-ketocholesterol plus cholesterol, a mixture that stimulates cholesterol esterification in the absence of the LDL receptor in fibroblasts (13, 14), was not affected by pronase treatment.

In human fibroblasts the LDL receptor is subject to regulation so that its activity becomes suppressed when excess intracellular cholesterol has accumulated (15, 16). Table I shows that a similar regulation is demonstrable in the cultured adrenal cells using measurements of either heparin-releasable $^{131}$I-LDL binding or the rate of degradation of $^{131}$I-LDL as indices of receptor activity. In the absence of ACTH, the addition of unlabeled LDL caused a 69% suppression of $^{131}$I-LDL binding and a 76% suppression of the rate of $^{131}$I-LDL degradation. The addition of ACTH induced an enhanced receptor activity in the absence of LDL. Moreover, in the presence of ACTH the suppression of receptor activity by unlabeled LDL was blunted. These data are similar to the previous data regarding the regulation of HMG-CoA reductase activity by LDL in the presence and absence of ACTH (see Figs. 4 and 8).
To follow directly the fate of the cholesteryl esters of LDL, the lipoprotein was labeled with \(^{3}H\)cholesterol linoleate by a technique that does not impair the ability of the lipoprotein to bind to the LDL receptor (21, 22). Fig. 14 shows that when this \(^{3}H\)cholesterol linoleate-LDL was added to adrenal cells the cellular content of esterified cholesterol reached a steady state level within 2 h (Fig. 14A). The liberated \(^{3}H\)cholesterol progressively accumulated in the cell over a 7-h period (Fig. 14B). In the presence of chloroquine, the hydrolysis of the \(^{3}H\)cholesterol esters was inhibited (Fig. 14B), suggesting that these esters, like the protein component of LDL, were hydrolyzed within cellular lysosomes (21, 22).

The data in Fig. 15 show that after a more prolonged incubation with a low level of \(^{3}H\)cholesterol linoleate-LDL, the liberated \(^{3}H\)cholesterol was converted to 11-\(\beta\)-hydroxy-\(^{3}H\)DHP and secreted into the culture medium. Whereas ACTH did not greatly alter the steady state cellular content of free or esterified \(^{3}H\)cholesterol, it did cause a marked stimulation of the rate of conversion of the \(^{3}H\)cholesterol to 11-\(\beta\)-hydroxy-\(^{3}H\)DHP.

In addition to providing substrate for steroid synthesis, LDL also caused an accumulation of cholesteryl esters in the cultured mouse adrenal cells. Fig. 16 shows that when adrenal cells were grown in the presence of ACTH but in the absence of lipoproteins the cellular content of cholesteryl esters was very low. The addition of human LDL produced a large increase in cellular cholesteryl esters, whereas human HDL had little effect. Table II shows the relative distribution of the major fatty acids of the cholesteryl esters that accumulated in adrenal cells incubated with human LDL. This distribution resembles that of the LDL with which the cells were incubated, suggesting that a large fraction of the accumulated cholesteryl esters represent unhydrolyzed cholesteryl esters derived from LDL, rather than LDL-derived cholesterol that has been hydrolyzed and re-esterified.

Mouse LDL, like human LDL, produced an accumulation of cholesteryl esters in the mouse adrenal cells and also facilitated the secretion of 11-\(\beta\)-hydroxy-DHP in the presence of ACTH (Table III). On the other hand, mouse HDL, like human HDL, was apparently unable to deliver its cholesterol to the adrenal cells and hence this lipoprotein did not produce an accumulation of esterified cholesterol nor did it stimulate the secretion of 11-\(\beta\)-hydroxy-DHP.

It has been reported that one of the actions of ACTH in rat

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**Fig. 13. Reduction in the LDL-mediated stimulation of cholesteryl \(^{14}C\)esterate formation by prior treatment of mouse adrenal cells with pronase.** On Day 7, each monolayer received 2 ml of Medium A. On Day 8, each monolayer was washed once with 3 ml of warm phosphate-buffered saline and then incubated at 37°C for 40 min in 2 ml of growth medium containing no serum and either no pronase (open symbols) or 2.5 \(\mu\)g/ml of pronase (closed symbols). Each monolayer was then washed once with 3 ml of ice-cold Medium A. Each monolayer was then incubated at 37°C with 2 ml of Medium A containing 0.1 mm \(^{14}C\)oleate-albumin (10,500 cpm/nmol) and one of the following additions: C, 10 \(\mu\)l of ethanol; C, A, 400 \(\mu\)g of protein/ml of human LDL and 10 \(\mu\)l of ethanol; or C, A, a mixture of 2 \(\mu\)g/ml of 7-ketocholesterol and 20 \(\mu\)g/ml of cholesterol added in 10 \(\mu\)l of ethanol. At the indicated time, the cells were harvested for determination of their content of cholesteryl \(^{14}C\)oleate. Each value represents the average of duplicate incubations.

**Table I**

<table>
<thead>
<tr>
<th>Treatment of Cells</th>
<th>Treatment of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>pronase</td>
</tr>
</tbody>
</table>

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**Fig. 14. Uptake (A) and hydrolysis (B) of \(^{3}H\)cholesterol linoleate-LDL by mouse adrenal cells in the presence and absence of chloroquine.** On Day 7, each monolayer received 2 ml of Medium A containing 200 milliliters/ml of ACTH. On Day 8 (zero time), the medium was replaced with 2 ml of fresh medium of the same composition but containing 1.2 \(\mu\)g of protein/ml of human \(^{3}H\)cholesterol linoleate-LDL in the absence (C) or presence (C) of 70 \(\mu\)M chloroquine. After incubation at 37°C for the indicated time, the cells were harvested for determination of their content of esterified (A) and free (B) \(^{3}H\)cholesterol. Each value represents the average of duplicate incubations.
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FIG. 15 (left). Uptake and hydrolysis of \(^{13}H\)-cholesteryl linoleate-LDL and secretion of 11-\(\beta\)-hydroxy-\(^{13}H\)DHP in mouse adrenal cells incubated in the absence (A) and presence (B) of ACTH. On Day 7, each monolayer received 2 ml of Medium A in the absence (A) or presence (B) of 200 milliunits/ml of ACTH. On Day 8 (zero time), the medium was replaced with 2 ml of fresh medium of the same composition but containing 1 ng of protein/ml of \(^{13}H\)-cholesteryl linoleate-LDL. After incubation at 37° for the indicated time, the medium was removed and its content 11-\(\beta\)-hydroxy-\(^{13}H\)DHP (A) was measured by thin layer chromatography. The monolayers were then washed and harvested for measurement of the cellular content of \(^{13}H\)-cholesteryl linoleate (B) and \(^{13}H\)-cholesterol (C) by thin layer chromatography. Each value represents the average of duplicate incubations.

FIG. 16 (center). Increase in the cellular content of esterified cholesterol in mouse adrenal cells after incubation with human LDL, but not with human HDL. On Day 7, each monolayer received 2 ml of Medium A containing 200 milliunits/ml of ACTH. On Day 8, the medium was replaced with 2 ml of fresh medium of the same composition but containing the indicated concentration of one of the following lipoproteins: \(\Box\), none; \(\bullet\), human LDL; or \(\mathbb{1}\), human HDL. After incubation for 24 h at 37°, the cells were harvested for measurement of their cholesterol ester content by gas-liquid chromatography. Each value represents the average of duplicate incubations.

TABLE II
Fatty acid composition of cholesteryl esters of human LDL and of mouse adrenal cells incubated with human LDL

| Fatty acid | Human LDL | Mouse adrenal cells
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>13</td>
<td>18.0</td>
</tr>
<tr>
<td>18:1</td>
<td>22</td>
<td>26.0</td>
</tr>
<tr>
<td>18:2</td>
<td>65</td>
<td>56.0</td>
</tr>
</tbody>
</table>

TABLE III
Stimulation of 11-\(\beta\)-hydroxy-DHP formation by mouse LDL in mouse adrenal cells

<table>
<thead>
<tr>
<th>Additon to medium</th>
<th>Lipoprotein concentration in medium</th>
<th>Free cholesterol in cells</th>
<th>Esterified cholesterol</th>
<th>11-(\beta)-Hydroxy-DHP in medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0 (\mu g) protein/ml</td>
<td>0 (\mu g) cholesterol/ml</td>
<td>8.7 (\mu g)</td>
<td>4.5 (\mu g) cell protein</td>
</tr>
<tr>
<td>Mouse LDL</td>
<td>55 (\mu g)</td>
<td>85 (\mu g)</td>
<td>14.5 (\mu g)</td>
<td>14.6 (\mu g) cell protein</td>
</tr>
<tr>
<td>Mouse LDL</td>
<td>220 (\mu g)</td>
<td>340 (\mu g)</td>
<td>21.9 (\mu g)</td>
<td>46.1 (\mu g) cell protein</td>
</tr>
<tr>
<td>Mouse LDL</td>
<td>660 (\mu g)</td>
<td>1020 (\mu g)</td>
<td>21.0 (\mu g)</td>
<td>63.8 (\mu g) cell protein</td>
</tr>
<tr>
<td>Mouse HDL</td>
<td>145 (\mu g)</td>
<td>65 (\mu g)</td>
<td>10.4 (\mu g)</td>
<td>1.3 (\mu g) cell protein</td>
</tr>
<tr>
<td>Mouse HDL</td>
<td>290 (\mu g)</td>
<td>170 (\mu g)</td>
<td>10.6 (\mu g)</td>
<td>3.2 (\mu g) cell protein</td>
</tr>
<tr>
<td>Mouse HDL</td>
<td>1160 (\mu g)</td>
<td>680 (\mu g)</td>
<td>9.9 (\mu g)</td>
<td>4.9 (\mu g) cell protein</td>
</tr>
</tbody>
</table>

and bovine adrenal gland is to cause an enhancement in the activity of a neutral cholesteryl ester hydrolase (9, 32). To determine whether ACTH enhances the hydrolysis of cholesteryl esters in the cultured mouse adrenal cells, the cells were incubated in the presence of LDL plus \(^{14}C\)oleate so as to label the endogenously synthesized cholesteryl esters (Fig. 17). After removal of the LDL, the cells were incubated either in the presence or absence of ACTH and the monolayers were medium was replaced with 2 ml of fresh medium of the same composition but containing the indicated concentration of one of the following lipoproteins: \(\Box\), none; \(\bullet\), human LDL; or \(\mathbb{1}\), human HDL. After incubation for 24 h at 37°, the cells were harvested for measurement of their cholesterol ester content by gas-liquid chromatography. Each value represents the average of duplicate incubations.

Fig. 17 (right). Decline in cellular content of endogenously synthesized cholesteryl \(^{14}C\)oleate in mouse adrenal cells in the absence and presence of ACTH after removal of LDL from the culture medium. On Day 7, each monolayer received 2 ml of Medium A containing 0.1 mM \(^{14}C\)oleate/albumin (10,000 cpm/mol) and 60 \(\mu g\) of protein/ml of human LDL. On Day 8, the medium was removed and each monolayer received 2 ml of Medium A containing 0.1 mM \(^{14}C\)oleate/albumin in the absence (C) or presence (D) of 200 milliunits/ml of ACTH. After incubation at 37° for the indicated time, the cells were harvested for measurement of their content of cholesteryl \(^{14}C\)oleate. Each value represents the average of duplicate incubations.

TABLE II
Fatty acid composition of cholesteryl esters of human LDL and of mouse adrenal cells incubated with human LDL

| Measurement | Human LDL | Mouse adrenal cells
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesteryl ester content ((\mu g) cholesterol/mg protein)</td>
<td>39.4</td>
<td>43.1</td>
</tr>
<tr>
<td>Fatty acids (relative distribution (%))</td>
<td>16:0*</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>18:1</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>18:2</td>
<td>65</td>
</tr>
</tbody>
</table>

* Number of carbon atoms: number of double bonds.
that when LDL was removed from the medium cholesteryl 14C-oleate continued to accumulate for 4 h in the absence of ACTH, whereas such net accumulation ceased after 1 h in the cells receiving ACTH. Thereafter, the rate of decline in the cellular content of cholesteryl 14C-oleate was actually faster in the cells not incubated with ACTH. On the basis of this type of experiment, it was not possible to conclude that ACTH enhanced the hydrolysis of cellular cholesteryl esters in the cultured adrenal cells.

**DISCUSSION**

The data presented in the current paper show that cultured mouse adrenal cells of the Y-1 clone possess a specific mechanism for the uptake and utilization of cholesteryl esters from LDL. Like the previously described LDL pathway in human cells (10, 11), this adrenal uptake process involves an initial binding of LDL to a high affinity cell surface receptor from which the lipoprotein can be released by heparin treatment. The plasma membrane receptor in mouse adrenal cells binds human or mouse LDL, but not human or mouse HDL. The receptor-bound LDL is internalized by a temperature-dependent process. The protein and cholesteryl ester components of the LDL are then hydrolyzed in lysosomes as indicated by the observation that the hydrolytic process can be inhibited and intact LDL can be induced to accumulate within the cell by use of the lysosomal inhibitor chloroquine.

As in human fibroblasts (21, 22), the hydrolysis of the cho- lesteryl esters of LDL in the mouse adrenal cells generates free cholesteryl that acts to suppress HMG-CoA reductase activity, to activate the cellular cholesterol-estherifying process, and to cause a suppression in the number of LDL receptors. In the mouse adrenal cells, however, unlike in the fibroblasts, all of these processes are subject to an additional set of regulatory controls since some of the cholesteryl liberated from the hydrolysis of LDL is converted to steroids. Thus, when the adrenal cells are incubated with ACTH, the ability of LDL to suppress HMG-CoA reductase, to activate the cholesterol esterification system, and to suppress the activity of the LDL receptor are all blunted. ACTH appears to exert these modulating effects on LDL action by shunting cholesterol into the mitochondria where its side chain is cleaved. This formulation is supported by the experiments showing that aminoglutethimide, an inhibitor of mitochondrial cholesterol side chain cleavage (7, 29, 30), reduces the action of ACTH and thus allows LDL-derived cholesterol to exert its full effect on the cells.

The current data showing that ACTH reduces the LDL-mediated suppression of HMG-CoA reductase activity in the mouse adrenal cells offer a mechanism to explain Koval's previous observation that ACTH enhances cholesterol synthesis from radiolabeled acetate or glucose, but not from mevalonate (4). Moreover, by depriving the mouse adrenal cells of lipoproteins, it was possible in the current studies to create conditions in which the availability of cholesterol became rate-limiting for steroid synthesis. Under these conditions an amount of steroid equal to the total cellular content of cholesterol (about 10 to 15 μg/mg of protein) was secreted by the cells each 24 h. The measured activity of HMG-CoA reductase in the presence of ACTH but in the absence of lipoproteins (300 to 600 pmol·min⁻¹·mg⁻¹) would have been sufficient for the synthesis of about 15 to 30 μg of cholesterol/mg of protein/24 h, assuming that the enzyme was acting at its maximal velocity in the intact cell. When LDL was added to the lipoprotein-deprived adrenal cells, the rate of steroid secretion was stimulated by about 4-fold at a time when the activity of HMG-CoA reductase was actually somewhat reduced. These latter data suggest that in the presence of high levels of LDL at least 75% of the secreted steroid must have been synthesized from LDL-cholesterol.

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