JCI The Journal of Clinical Investigation

3-Hydroxy-3-methylglutaryl coenzyme A reductase in anencephalic and normal human fetal liver.

B R Carr, … , W E Rainey, J I Mason

J Clin Invest. 1985;76(5):1946-1949. https://doi.org/10.1172/JCI112192.

Research Article

In previous investigations, we have found that the liver appears to be the major source of cholesterol in the human fetus, and, in particular, a principal source of circulating low density lipo-protein-cholesterol (LDL-C). LDL-C plasma levels are low in the normal fetus, most likely due to the rapid uptake and metabolism by the fetal adrenal as precursor for steroid hormone biosynthesis. In contrast, in the anencephalic fetus the adrenals are atrophic, the rate of estrogen and glucocorticoid production is low, and the levels of LDL-C in fetal plasma are high. The purpose of the present investigation was to determine the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the primary rate-limiting enzyme of cholesterol biosynthesis, in anencephalic liver and normal fetal liver. We found that the specific activity of HMG-CoA reductase in normal fetal liver microsomes was 0.428 +/- 0.054 nmol mevalonate formed times mg-1 protein X min-1 (mean +/- SE, n = 9). The rate of HMG-CoA reductase in anencephalic liver microsome preparations was 10-fold less (0.040 +/- 0.003) (mean +/- SE, n = 7) P less than 0.001. Furthermore, we detected HMG-CoA reductase (97,000-mol wt protein) in normal human fetal liver after SDS PAGE and immunoblotting by using a monoclonal antibody directed against HMG-CoA reductase. We were unable to detect any significant quantity of HMG-CoA reductase protein in anencephalic […]

3-Hydroxy-3-methylglutaryl Coenzyme A Reductase in Anencephalic and Normal Human Fetal Liver

Bruce R. Carr, William E. Rainey, and J. Ian Mason
The Cecil H. and Ida Green Center for Reproductive Biology Sciences, and the Departments of Obstetrics and Gynecology, Cell Biology, and Biochemistry, The University of Texas Health Science Center, Dallas, Texas 75235

Abstract

In previous investigations, we have found that the liver appears to be the major source of cholesterol in the human fetus, and, in particular, a principal source of circulating low density lipoprotein-cholesterol (LDL-C). LDL-C plasma levels are low in the normal fetus, most likely due to the rapid uptake and metabolism by the fetal adrenal as precursor for steroid hormone biosynthesis. In contrast, in the anencephalic fetus the adrenals are atrophic, the rate of estrogen and glucocorticoid production is low, and the levels of LDL-C in fetal plasma are high. The purpose of the present investigation was to determine the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the primary rate-limiting enzyme of cholesterol biosynthesis, in anencephalic liver and normal fetal liver. We found that the specific activity of HMG-CoA reductase in normal fetal liver microsomes was 0.428±0.054 nmol mevalonate formed times mg^{-1} protein × min^{-1} (mean±SE, n = 9). The rate of HMG-CoA reductase in anencephalic liver microsome preparations was 10-fold less (0.040±0.003) (mean±SE, n = 7) $P < 0.001$. Furthermore, we detected HMG-CoA reductase (97,000-mol wt protein) in normal human fetal liver after SDS PAGE and immunoblotting by using a monoclonal antibody directed against HMG-CoA reductase. We were unable to detect any significant quantity of HMG-CoA reductase protein in anencephalic fetal liver, which indicates that low reductase activity was due to low amounts of enzyme protein rather than inactive enzyme. In summary, we conclude that the low levels of cholesterol synthesis observed in anencephalic fetal liver are probably due to both the high levels of LDL-C in fetal plasma as well as the presence of low circulating levels of estrogens and glucocorticoids and that these factors regulate cholesterol synthesis both in vivo and in vitro in fetal liver. This occurs most probably by the modulation of the amount of HMG-CoA reductase, a primary rate-limiting and regulatory enzyme of the cholesterol biosynthetic sequence.

Introduction

We have previously suggested that the principal source of cholesterol in the fetus is by way of fetal synthesis of cholesterol de novo (1, 2). Evidence to support this conclusion is derived from studies that demonstrate that no more than 20% of fetal cholesterol is derived from the maternal compartment (3–5) and that the fetal dietary source of cholesterol by way of swallowing amniotic fluid is negligible (6). We reported in a survey of various human fetal tissues that the adrenal, liver, and testicular tissue exhibited high rates of cholesterol synthesis. On the basis of the size of the liver and its rapid rate of growth during fetal life as well as its high rate of cholesterol synthesis, we concluded that the liver is the major site of cholesterol formation in the fetus (2). Furthermore, since the adult liver is a major source of lipoprotein biosynthesis and secretion, it seemed likely that in the fetus, the liver might be an important source of lipoprotein-cholesterol for extrahepatic tissues (7). In fact, fetal liver tissue in vitro secretes lipoproteins (8).

The fetal adrenal gland secretes large quantities of steroid hormones, and a major source of cholesterol for adrenal steroid hormone biosynthesis appears to be low density lipoprotein-cholesterol (LDL-C)[1] both in vitro and in vivo. The fetal adrenal gland is a major site of plasma LDL use and the activity of fetal adrenal is an important determinant of the levels of LDL in plasma (1, 9, 10). In the normal human fetus, the adrenal glands secrete large quantities of steroid hormones, and, presumably, LDL-C uptake by the adrenals is high; as a consequence, LDL-C levels in fetal plasma are low. It follows that the rate of formation of cholesterol in fetal liver, if rate limiting, then may regulate in part the rate of steroid secretion by the fetal adrenal glands and the formation of estrogens by the fetal-placental unit.

Estrogens and glucocorticoids, both found in large quantities in fetal plasma near term, stimulate cholesterol synthesis by fetal hepatocytes in vitro (11). In contrast, LDL-C inhibits cholesterol synthesis by hepatocytes maintained in vitro (12). However, since fetal plasma LDL-C levels fall during gestation, the lack of negative feedback or cholesterol synthesis would support an overall increase in the rate of cholesterol biosynthesis by the fetal liver in vivo (13).

In contrast to the normal fetus, in the anencephalic fetus the adrenals are atrophic and the rate of adrenal steroidogenesis and, consequently, the rate of estrogen production by the fetal placental unit are low (14, 15). Furthermore, since the rate of steroidogenesis is low, the adrenals are not using LDL-C and the levels of LDL-C in fetal blood are up to three times that of the normal fetus throughout gestation (9, 10, 16, 17). Since the hormonal milieu of the anencephalic fetus is the opposite of that of the normal fetus (i.e., low estrogen and adrenal hormones and elevated LDL-C levels), we have estimated the level of cholesterol synthesis in the livers of anencephalic fetuses by determining the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in liver microsome preparations. We compared the results obtained in the anencephalic preparations to those obtained in normal fetal liver. Finally, we compared the amounts of two other liver microsomal enzymes, namely NADPH cytochrome c (P450) reductase and glucose-6-phosphatase in normal and anencephalic fetal liver.

Received for publication 8 April 1985 and in revised form 8 July 1985.

J. Clin. Invest.

0021-9738/85/11/1946/04 $1.00
Volume 76, November 1985, 1946–1949

1. *Abbreviations used in this paper:* HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL-C, low density lipoprotein-cholesterol.

Methods

Liver tissue. Liver tissue was obtained from abortuses of 12–18 wk gestation delivered by induced abortion (dilatation and extraction). Tissues were obtained in accordance with the Donors Anatomical Gift Act of the State of Texas after obtaining consent in writing from the woman who was to undergo the abortion. Human adrenals were also obtained from seven anencephalic fetuses (17–46 wk gestation) within 1 h of death. The anencephalic fetuses were delivered vaginally after spontaneous or oxytocin-induced labor or by cesarean section. The tissues were placed immediately in ice-cold Waymouth-Gey's culture medium as previously described (18).

Determination of the specific activity of HMG-CoA reductase in fetal liver microsomes. Liver tissues were homogenized with a Teflon-glass homogenizer in 3 ml of a solution that contained sucrose (0.25 M), potassium phosphate buffer (10 mM, pH 7.4), EDTA (1 mM), and mercaptoethanol (10 mM). The homogenate was centrifuged at 15,000 *g* for 10 min at 4°C. The supernatant fraction was centrifuged at 100,000 *g* for 60 min. The microsome-enriched pellet was rinsed in cold buffer (the buffer used for homogenization, omitting mercaptoethanol) and stored in liquid nitrogen until assayed.

HMG-CoA reductase activity was assayed by a modification of the method of Brown et al. (19; see also reference 20). Optimal conditions for the assay of HMG-CoA reductase in liver tissue were determined previously (20). The data presented are the average of duplicate assays. A statistical analysis of the data was made using the Student's *t* test.

SDS PAGE and immunoblotting of HMG-CoA reductase and NADPH cytochrome c reductase. One-dimensional electrophoresis was conducted on a 7.5% polyacrylamide gel containing 0.1% (wt/vol) SDS with a variation to the method of Laemmli (22). Samples were applied to the gel in sample buffer (15% SDS, 8 M urea, 10% [wt/vol] sucrose, 62.5 mM Tris-HCl, pH 7.4, 100 mM dithiothreitol, 2 mM β-mercaptoethanol, 0.05% bromophenol blue) after incubation for 5 min at 37°C. Electrophoresis was carried out at 12–15 mA at 4°C for 12–15 h. Proteins were transferred from SDS slab gels on to nitrocellulose paper according to the method of Burnette (23) in a Trans-Blot cell apparatus (Bio-Rad Laboratories, Richmond, CA). Electrophoresis was carried out at 200 mA for 16 h at 4°C with an electrode buffer of Tris base (20 mM), glycine (150 mM), and methanol (20%). Antibody incubation with nitrocellulose was carried out using a variation of the method of Johnson and Elder (24). The nitrocellulose sheet was preincubated for 30 min at room temperature in BLOTTO buffer (50 mM Tris-HCl, pH 7.4, 80 mM NaCl, 5% [wt/vol] Carnation evaporated milk and 0.2% [vol/vol] Nonidet P-40). BLOTTO buffer containing the primary antibody (10 μg/ml) was added for a 1-h incubation at room temperature. The sheet was then washed three times with BLOTTO buffer for a total of 15 min. Fresh BLOTTO solution containing ^{125}I-labeled rabbit anti-mouse IgG (10^6 cpm/ml) was added for 1 h. The sheet was washed three times for a total of 15 min, blotted dry, wrapped in plastic wrap, and placed under Kodak Omat-X film for 3 d at −70°C. Mouse monoclonal anti-HMG-CoA reductase (primary antibody) and ^{125}I-labeled rabbit anti-mouse IgG were generously provided by Drs. R. G. W. Anderson and L. Liscum (University of Texas Health Science Center) (25). Rabbit polyclonal NADPH cytochrome *c* reductase antibody was provided by Drs. B. S. Masters (Medical College of Wisconsin, Milwaukee, WI) and M. R. Waterman (University of Texas Health Sciences Center). Mouse leydig (I-10) tumor cells when grown in lipoprotein-deficient medium contain a high amount of HMG-CoA reductase (Rainey, W. E., and J. I. Mason, unpublished observations). Thus, these cells were used as an additional marker for HMG-CoA reductase blotting and localization. Mouse leydig (I-10) tumor cells were obtained from the American Type Tissue Culture Collection (Rockville, MD) and were grown in Ham's F-12 and Dulbecco's modified Eagles media (1:1; vol/vol) containing lipoprotein-depleted fetal calf serum (1%) and Hepes (10 mM) buffer, pH 7.2. These cells were solubilized in the sample buffer and applied to the gel.

Determination of the activity of glucose-6-phosphatase in fetal liver microsomes. Liver microsome preparations were prepared as discussed previously for HMG-CoA reductase assay. The activity of glucose-6-phosphatase was determined in two normal and two anencephalic liver microsome preparations by the method of Beaufay et al. (26). The assays were run in triplicate at two dilutions and reported as mean±SE in units of μmoles of phosphate formed per milligram protein per minute.

Materials. The following compounds were purchased from New England Nuclear (Boston, MA): D,L-3-[glutaryl-3-^{14}C]hydroxy-3-methylglutaryl coenzyme A, D,L-[mevalonic-5-(*N*)-^{3}H]mevalonic acid DBED salt, and Omnifluor. NADP$^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, dithiothreitol, and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). The following materials were obtained from Grand Island Biological Co. (Grand Island, NY): Waymouth's MB 752/1 medium, Gey's Balanced Salt Solution. Analytical precoated thin-layer silica gel chromatography plates were obtained from Whatman, Inc. (Clifton, NJ). Other chemicals used were of analytical grade.

Results

The specific activities of HMG-CoA reductase in seven individual anencephalic liver microsome preparations of fetuses between 17 and 46 wk gestational were determined. The values obtained ranged from 0.025 to 0.060 nmol mevalonate formed times mg^{-1} microsomal protein × min^{-1}. The reductase activities obtained did not vary significantly with gestational age. The specific activities of HMG-CoA reductase in nine individual normal fetal liver membrane preparations were determined. The range of gestational ages (12–18 wk) was less than that observed in the anencephalic fetuses, and the results obtained did not vary significantly within this gestational period (0.162–0.704 nmol mevalonate formed times mg^{-1} microsomal protein × min^{-1}).

The average specific activity of HMG-CoA reductase in anencephalic fetal liver was 0.040±0.003 nmol × mg^{-1} protein × min^{-1} (mean±SE, n = 7). In contrast, the average specific activity of HMG-CoA reductase in normal fetal liver microsome preparations was 10-fold greater (0.428±0.054 nmol × mg^{-1} protein × min^{-1} [mean±SE, n = 9]) than the results obtained for anencephalic fetal liver (Fig. 1).

To determine whether the reduction in reductase activity was due to an inactive enzyme or reduced amounts of enzyme protein, we used the immunoblotting technique to compare the amount of HMG-CoA reductase protein in fetal and anencephalic liver homogenates. In fetal liver homogenates, a 97,000-mol-wt protein was readily detected, but no similar protein was detectable in the anencephalic liver sample (Fig. 2). Furthermore, there was no evidence of the lower molecular weight forms (53,000 or 66,000) of the enzyme (25). The immunoblotted hu-

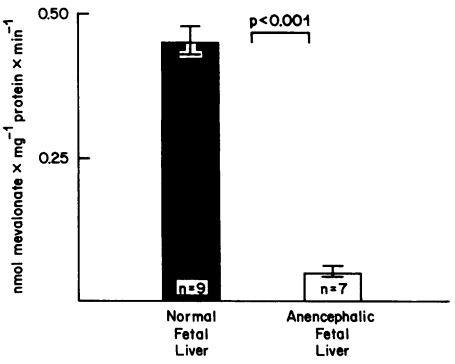

Figure 1. The specific activity of HMG-CoA reductase in anencephalic and normal human fetal liver microsome preparations. The results obtained are the mean±SE of duplicate assays obtained from nine normal (solid bar) and seven anencephalic (open bar) fetal liver preparations.

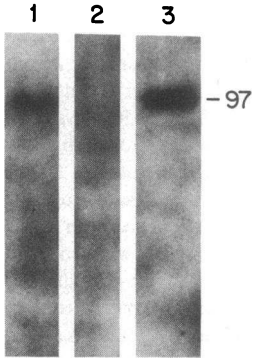

Figure 2. SDS PAGE and immunoblotting of HMG-CoA reductase from normal (lane *1*) and anencephalic (lane *2*) fetal liver homogenates and a homogenate of mouse leydig (I-10) tumor cells (lane *3*). Samples containing 200 μg of protein were subjected to electrophoresis in 7.5% polyacrylamide gels containing 0.1% SDS. The proteins were transferred electrophoretically to nitrocellulose paper and incubated with monoclonal anti-HMG-CoA reductase antibody (10 μg/ml) and then incubated with ^{125}I-labeled rabbit anti-mouse IgG (10^6 cpm/ml). The dried filters were processed for autoradiography as indicated in the Methods. The location of the molecular weight standard phosphorylase b (Bio-rad Laboratories, 97 [97,000]) is indicated.

man enzyme had a similar molecular weight to the reductase of mouse leydig (I-10) tumor cells. As mentioned previously, mouse leydig (I-10) tumor cells exhibit a large increase in synthesis of HMG-CoA reductase when grown in lipoprotein-deficient medium (Rainey, W. E., J. I. Mason, unpublished observations).

Furthermore, we wished to determine whether the low level of enzyme (HMG-CoA reductase) was unique for anencephalic liver and the possible mechanisms whereby its activity was lowered by determining the activity or amount of other liver microsomal enzymes. As presented in Fig. 3, the amounts of microsomal NADPH cytochrome *c* reductase were similar in normal and anencephalic liver microsomes. Similar results were also observed when the activities of glucose-6-phosphatase were compared in anencephalic and normal fetal liver microsomes (Table I).

Discussion

In the present investigation, we observed a high rate of cholesterol synthesis on the basis of estimates of the specific activity of HMG-CoA reductase in microsomal preparations of normal fetal liver (0.428±0.054 nmol × mg^{-1} protein × min^{-1}). These results were similar to those reported in a preliminary study (0.58±0.18 nmol × mg^{-1} protein × min^{-1}) (20). In our investigation, advancing gestational age did not influence levels of reductase activity.

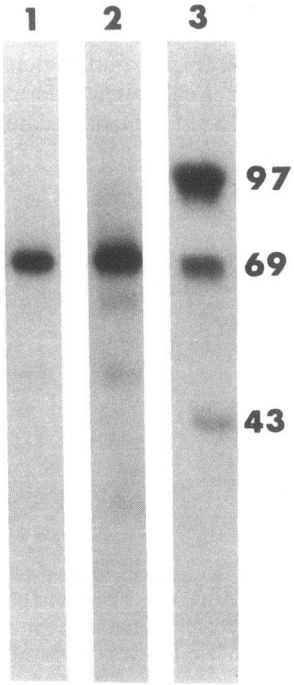

Figure 3. An immunoblot after SDS PAGE of microsomal NADPH cytochrome *c* (P450) reductase from normal (lane *1*) and anencephalic (lane *2*) fetal liver microsomes. Samples (50 μg protein) were subjected to electrophoresis in 7.5% polyacrylamide gels containing 0.1% SDS. The proteins were transferred electrophoretically to nitrocellulose paper and incubated with polyclonal NADPH cytochrome *c* reductase antibody (10 μg/ml) and then incubated with ^{125}I-labeled goat anti-rabbit IgG (10^6 cpm/ml). The dried filters were then processed for autoradiography. Radioactive protein standards (molecular weight × 10^3) were chromatographed in lane *3*.

Table I. Activities of Glucose-6-Phosphatase (μmol × mg^{-1} protein × min^{-1}) of Anencephalic and Normal Fetal Liver Microsome Preparations

	Glucose-6-phosphatase activity (mean±SE)
Normal 1	0.157±0.001
Normal 2	0.092±0.001
Anencephalic 1	0.176±0.001
Anencephalic 2	0.109±0.002

The results of our investigation demonstrate that the specific activity of HMG-CoA reductase was 10-fold less in anencephalic fetal liver microsomal preparations than observed in normal fetal liver. These differences were not due to inactive enzyme (27), but due rather to decreased amounts of enzyme protein. A plausible mechanism to explain the low level in HMG-CoA reductase of anencephalic liver is the presence of a high concentration of LDL-C circulating in fetal plasma. LDL-C serves to down-regulate cholesterol synthesis in fetal liver hepatocytes in vitro (12). In the normal fetus, LDL-C concentrations are one-third that observed in anencephalic fetal plasma (9). Another explanation is that in the normal fetus, as gestation advances, the estrogen and glucocorticoids formed by the fetal-placental unit stimulate cholesterol synthesis in the fetal liver, as it has been previously demonstrated to occur in human fetal hepatocytes maintained in culture (11). Thus, in the absence of significant estrogen milieu and in the presence of a high level of LDL-C, cholesterol synthesis in the anencephalic liver is low.

We observed similar activities of other liver microsomal enzymes, namely NADPH cytochrome *c* reductase and glucose-6-phosphatase in anencephalic and normal fetuses. These results suggest that the low activity of HMG-CoA reductase is not reflective of a general decrease of liver function. Moreover, we previously have measured similar rates of activity of estrone sulfate 16α-hydroxylase in microsomal preparations of anencephalic and normal fetal livers (28). Furthermore, the masses of livers of anencephalic fetuses are not significantly different to those of the normal fetus, except for a slight decrease near term (29).

In conclusion, we were unable to determine if the low level of HMG-CoA reductase of anencephalic fetal liver was due singularly to the elevated LDL-C of fetal plasma or the deficiency of estrogens and glucocorticoids. These factors may both serve to regulate cholesterol synthesis both in vivo and in vitro in human fetal liver. The low activity of HMG-CoA reductase was not due to inactive (e.g., phosphorylated) enzyme, but to decreased enzyme protein.

Acknowledgments

We would like to thank Nora Cline and Barbara Murry for their expert technical assistance and Lynne McDonnell for her editorial assistance in the preparation of this manuscript.

This work was supported in part by U. S. Public Health Service grant HD17814.

References

1. Carr, B. R., and E. R. Simpson. 1981. Lipoprotein utilization and cholesterol synthesis by the human fetal adrenal gland. *Endocrine Review.* 2:306–326.

2. Carr, B. R., and E. R. Simpson. 1982. Cholesterol synthesis in human fetal tissues. *J. Clin. Endocrinol. Metab.* 55:447–452.

3. Davis, M. E., E. J. Plotz, G. V. LeRoy, R. G. Gould, and H. Werbin. 1956. Hormones in human reproduction: metabolism of progesterone. *Am. J. Obstet. Gynecol.* 72:740–755.

4. Hellig, H., D. Gattereau, Y. Lefebvre, and E. Bolte. 1970. Steroid production from plasma cholesterol. I. Conversion of plasma cholesterol to placental progesterone in humans. *J. Clin. Endocrinol. Metab.* 30: 624–631.

5. Lin, D. S., R. M. Pitkin, and W. E. Connor. 1977. Placental transfer of cholesterol into the human fetus. *Am. J. Obstet Gynecol.* 128:725–739.

6. Das, S. K., H. W. Foster, P. K. Adhikary, B. B. Mody, and D. K. Bhattacharyya. 1975. Gestational variation of fatty acid composition of human amnionic fluid lipid. *Obstet. Gynecol.* 45:425–432.

7. Eisenberg, S., and R. I. Levy. 1975. Lipoprotein metabolism. *Adv. Lipid Res.* 1–89.

8. Zannis, V. I., D. M. Kuonit, and J. L. Breslow. 1982. Hepatic Apo-AII and Apo-E and intestinal Apo-AI are synthesized in precursor isoprotein forms by organ cultures of human fetal tissues. *J. Biol. Chem.* 257:536–544.

9. Parker, C. R., Jr., E. R. Simpson, D. W. Bilheimer, K. Leveno, B. R. Carr, and P. C. MacDonald. 1980. Inverse relationship between LDL-cholesterol and dehydroisoandrosterone sulfate in human fetal plasma. *Science (Wash. DC).* 208:512–514.

10. Parker, C. R., Jr., B. R. Carr, C. A. Winkel, M. L. Casey, E. R. Simpson, and P. C. MacDonald. 1983. Hypercholesterolemia due to elevated low-density lipoprotein-cholesterol in newborns with anencephaly and adrenal atrophy. *J. Clin. Endocrinol. Metab.* 57:37–43.

11. Carr, B. R., and E. R. Simpson. 1984. Cholesterol synthesis by human fetal hepatocytes in monolayer culture: effect of hormones. *J. Clin. Endocrinol. Metab.* 58:1111–1116.

12. Carr, B. R., and E. R. Simpson. 1984. Cholesterol synthesis by human fetal hepatocytes in monolayer culture: effect of lipoproteins. *Am. J. Obstet. Gynecol.* 150:551–557.

13. Parker, C. R., Jr., B. R. Carr, E. R. Simpson, and P. C. MacDonald. 1983. Decline in the concentration of low-density lipoprotein-cholesterol in human fetal plasma near term. *Metabolism.* 32:919–923.

14. Benirschke, K. 1956. Adrenals in anencephaly and hydrocephaly. *Obstet. Gynecol.* 8:412–425.

15. Gray, E. S., and D. R. Abramovich. 1980. Morphologic features of the anencephalic adrenal gland in early pregnancy. *Am. J. Obstet Gynecol.* 137:491–495.

16. Carr, B. R., M. Ohashi, P. C. MacDonald, and E. R. Simpson. 1981. Human anencephalic adrenal tissue: low-density lipoprotein metabolism and cholesterol synthesis. *J. Clin. Endocrinol. Metab.* 53:406–411.

17. Ohashi, M., B. R. Carr, and E. R. Simpson. 1982. Low-density lipoprotein receptors in adrenal tissue of a human anencephalic fetus. *Early Hum. Dev.* 7:149–154.

18. Carr, B. R., P. C. MacDonald, and E. R. Simpson. 1980. The regulation of *de novo* synthesis of cholesterol in the human fetal adrenal gland by low density lipoprotein and ACTH. *Endocrinology.* 107:1000–1006.

19. Brown, M. S., S. E. Dana, and J. L. Goldstein. 1973. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins. *Proc. Natl. Acad. Sci. USA.* 70:2162–2166.

20. Carr, B. R., and E. R. Simpson. 1981. Synthesis of cholesterol in the human fetus: 3-hydroxy-3-methylglutaryl coenzyme A reductase activity of liver microsomes. *J. Clin. Endocrinol. Metab.* 53:810–812.

21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

22. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* 227:680–685.

23. Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:192–203.

24. Johnson, D. A., and J. H. Elder. 1983. Antibody directed to determinants of a moloney virus derived MCF GP70 recognizes a thymic differentiation antigen. *J. Exp. Med.* 159:1751–1756.

25. Liscum, L., R. D. Cumming, R. G. W. Anderson, G. N. DeMartino, J. L. Goldstein, and M. S. Brown. 1983. 3-Hydroxy-3-methylglutaryl-CoA reductase: transmembrane glycoprotein of the endoplasmic reticulum with N-linked "high-mannose" oligosaccharides. *Proc. Natl. Acad. Sci. USA.* 80:7165–7169.

26. Beaufay, H., A. Amar-Costesec, E. Feytmans, D. Thines-Sempoux, M. Wibo, M. Robbi, and J. Berthet. 1974. Analytical study of microsomes and isolated subcellular membranes from rat liver. I. Biochemical methods. *J. Cell Biol.* 61:188–200.

27. Beg, A. H., J. A. Stonik, and H. B. Brewer. 1984. Human hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: evidence for the regulation of enzymic activity by a byciclic phosphorylation cascade. *Biochem. Biophys. Res. Commun.* 119:488–498.

28. Milewich, L., P. C. MacDonald, A. Guerami, T. Midgett, W. Lassiter, N. V. Cline, and B. R. Carr. 1985. Characterization of human fetal liver 16α-hydroxylase activity. Proceedings of the 32nd Annual Meeting of the Society for Gynecologic Investigation, Phoenix, AZ. Abstract No. 101.

29. Lemire, R. J., J. B. Beckwith, and J. Warkany. 1978. Anencephaly. Raven Press, New York. 79–80.