Regulation of Hepatic 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase and Cholesterol Synthesis*

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SUMMARY

Rat liver microsomal 3-hydroxy-3-methylglutaric acid (HMG)-coenzyme A reductase (mevalonate:NADP oxidoreductase (acylating CoA), EC 1.1.1.34) exhibits a cyclic rhythm with peak activity at midnight. HMG-CoA reductase from microsomes of rats killed at noon and midnight has similar properties. Cycloheximide completely prevents the 5- to 10-fold rise in activity which occurs from 6 p.m. to midnight, suggesting that the rise in activity is due to synthesis of new enzyme. The effect of cycloheximide is paralleled by short duration cholesterol feeding, which blocks the cyclic rise in activity.

Cholesterol does not appear to act as a feedback inhibitor of HMG-CoA reductase. Mixing experiments indicate that the livers of cholesterol-fed rats do not contain a soluble inhibitor of HMG-CoA reductase. Cholesterol-rich lipoproteins isolated from livers of cholesterol-fed rats and added to incubations at concentrations from 0.01 to 10 mM cholesterol did not inhibit HMG-CoA reductase from control animals.

After 4 hours of cholesterol feeding, liver cholesterol concentration begins to increase and acetate incorporation into cholesterol begins to decrease sharply. The decline in cholesterol synthesis measured in a liver mince closely parallels the decline in HMG-CoA reductase activity. The observed changes in HMG-CoA reductase activity thus appear to be an accurate reflection of changes in the intracellular rate of cholesterol synthesis.

After 10 hours of cholesterol feeding, acetate incorporation into cholesterol and HMG-CoA reductase activity are 22% and 19% of control values, respectively. The incorporation of acetyl-CoA into HMG-CoA and of mevalonic acid into cholesterol is unchanged. Under these conditions HMG-CoA reductase appears to be the sole regulatory site between acetyl-CoA and cholesterol.

Several lines of evidence implicate the reaction catalyzed by 3-hydroxy-3-methylglutaric acid (HMG)-CoA reductase (mevalonate:NADP oxidoreductase (acylating CoA), EC 1.1.1.34) as a control point in cholesterol synthesis. Dietary cholesterol depresses hepatic cholesterol synthesis from acetate (2-4) under conditions in which synthesis from mevalonate and ketone body production (5-7), or HMG-CoA synthesis (8) from acetate remains relatively unaffected. Various factors can dramatically alter HMG-CoA reductase activity. Fasting (9) or dietary cholesterol (1, 10, 11) reduces reductase activity while injection of Triton (12, 13) or thyroxine (14) increases it. Striking cyclic variations in HMG-CoA reductase activity which may be related to its regulatory role have been reported (1, 13, 15). The cyclic rise in reductase activity is prevented by injection either of cycloheximide (1) or of puromycin (13).

The methods reported in this paper yield a stable, active source of rat liver HMG-CoA reductase. Our data provide direct evidence that control of cholesterol synthesis is exerted at the HMG-CoA reductase reaction. The physiological significance of most alterations in enzyme activity remains to be established (16). Using a mince of whole liver which incorporates acetate into cholesterol, we have shown that the alterations in HMG-CoA reductase activity measured in isolated microsomes closely parallel alterations in the intracellular rate of cholesterol synthesis from acetate. Our investigations suggest that cholesterol affects the rate of sterol synthesis by changing the amount, not the activity, of HMG-CoA reductase.

MATERIALS AND METHODS

Materials—Chemicals were obtained from the sources indicated: sodium acetate-1-14C, Amersham; mevalonic acid-2-14C lactone, Calotonic; HMG-3-14C, Tracerlab; glucose 6-phosphate (potassium salt), Sigma, mevalonic acid lactone, and acetoxyacetic acid, Nutritional Biochemicals; NADP+ and glucose 6-phosphate dehydrogenase, Sigma; and HMG, K and K Laboratories. The tissue press was from the Harvard Apparatus Company. Chromatography sandwich chamber and thin layer chromatographic sheets were from Eastman. The lyophilizer was from Frigidaire for Science, Oceanside, New York.

Preparation of Substrate—DL-HMG-3-14C anhydride, m.p. 101.5-102°, was synthesized in 65 to 70% yield by the dicyclohexylcarbodiimide method of Goldfarb.† HMG-CoA was prepared from the anhydride as described by Hilz et al. (17). HMG-CoA reductase was obtained from rat liver microsomes and assayed as described previously (18). The methods reported in this paper yield a stable, active source of rat liver HMG-CoA reductase. Our data provide direct evidence that control of cholesterol synthesis is exerted at the HMG-CoA reductase reaction. The physiological significance of most alterations in enzyme activity remains to be established (16). Using a mince of whole liver which incorporates acetate into cholesterol, we have shown that the alterations in HMG-CoA reductase activity measured in isolated microsomes closely parallel alterations in the intracellular rate of cholesterol synthesis from acetate. Our investigations suggest that cholesterol affects the rate of sterol synthesis by changing the amount, not the activity, of HMG-CoA reductase.

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†‡ Details available on request from Dr. Stanley Goldfarb, Mc-Argle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706.
CoA solutions stored at pH 5.5 to 6.0 at -20°C are stable for several months.

Animals—Female Wistar rats weighing 75 to 125 g were used for all experiments. For a given experiment the weight range generally did not exceed 15 g. Rats were kept in a windowless room with a controlled light-dark cycle (light 6 a.m. to 6 p.m.; dark 6 p.m. to 6 a.m. EST) and maintained on a stock diet and water ad libitum. Rats were kept two to a cage for at least 3 days prior to each experiment. Stock diet containing 5% cholesterol was used where indicated.

Preparation of Microsomes—Rats were killed by a sharp blow at the base of the skull. Their livers were excised into ice-cold, pH 6.0, ESM solution (30 mM EDTA, 70 mM NaCl, 10 mM β-mercaptoethanol) (18), weighed, and minced in a Harvard tissue press into 3.5 ml of ESM solution, pH 8.0, per g of liver. Minced liver tissue was homogenized with four strokes of a tight fitting Teflon pestle in a Potter-Elvehjem homogenizer. Mitochondria and their associated HMG-CoA lyase activity (3-hydroxy-3-methylglutaryl CoA deacetoacetate lyase, EC 1.1.3.4) were then removed by two successive 15-min centrifugations at 12,000 × g. The supernatant liquid was centrifuged at 48,000 × g for 1 hour in a Sorvall RC-23 centrifuge with an SS-34 rotor. The heavy microsomal pellet obtained is either resuspended in one half to the original homogenate volume of pH 6.0 ESM solution or transferred without addition of ESM solution to a 15-ml centrifuge tube and frozen in a Dry Ice-acetone bath. Quick-frozen pellets stored at -20°C retain full HMG-CoA reductase activity for at least 3 weeks. Microsomes resuspended in ESM solution lose considerable activity on freezing. The specific activity (nanomoles per min per mg of microsomes) of HMG-CoA reductase is generally slightly higher in the 48,000 × g pellet than in microsomes isolated by centrifugation at 105,000 × g for 90 min. To localize HMG-CoA reductase further, smooth and rough microsomes were separated on a discontinuous sucrose gradient (19, 20). We confirm the observation of Cudler, Nolte, and Wieland (14) that the rough microsomes contain over 95% of the total HMG-CoA reductase activity. Reductase in the 48,000 × g pellet had a specific activity of 0.548 ± 0.021 nmole per min per mg while reductase in rough microsomes from the same liver had a specific activity of 0.690 ± 0.042 nmole per min per mg. We therefore used the 48,000 × g pellet, which is relatively simple to prepare, throughout this work.

Assay of HMG-CoA Reductase Activity—Fresh or quick frozen pellets resuspended in pH 6.0 ESM solution were used as the source of enzyme. Each experimental point represents data obtained by combining livers from two rats prior to homogenization. Analyses were conducted in triplicate unless otherwise stated and the mean values and standard error of the mean are reported. Each incubation mixture contained, in 1.0 ml of ESM solution, pH 6.8: 30 μmoles of glucose 6-phosphate, 2.0 enzyme units of glucose 6-phosphate dehydrogenase, 3.0 μmoles of NADP+, 300 μmoles of DL-HMG-3,4-CoA (specific activity of the three preparations used was 390, 410, and 450 cpm per nmole), and 0.8 to 10 mg of microsomal protein. Samples were incubated for 15 min at 37°C, deproteinized with 0.2 ml concentrated HCl, frozen, and lyophilized overnight (14 hours). The mevalonolactone was dissolved in 0.5 ml of acetone–H2O, 9:1 (v/v), isolated by thin layer chromatography, and counted as described by Shapiro, Imbmul, and Rodwell (21). The RF for mevalonolactone must be determined for each commercial supplier or lot of thin layer chromatographic plates. The thin layer chromatographic assay gives identical results with either lyophilization or ether extraction. The recovery of added mevalonic acid-2-14C was 66.9 ± 1.9% (standard deviation) for 16 experiments performed over several months.

Sterol Synthesis in Liver Preparation—Livers were excised into 0.1 M potassium phosphate buffer, pH 7.0, containing 0.15 M NaCl, 7.5 mM MgCl2, 30 mM nicotinamide, 1.0 mM EDTA, and 10 mM β-mercaptoethanol, minced through a Harvard tissue press (small openings), and weighed. A portion of liver was used for preparation of microsomes as described above. The remaining tissue was added to 4.0 ml of Krebs-Ringer phosphate buffer, pH 7.4 (29), per g of tissue and shaken for 20 sec on a Vortex mixer to dissociate clumps of cells. Two milliliters of liver mince were added to 2.0 ml of Krebs-Ringer phosphate buffer, pH 7.4, in 25-ml Erlenmeyer flasks. Flasks were flushed with 95% O2-5% CO2 and cappd and 10 μmoles (4.0 μCi) of sodium acetate-1-14C or 2.5 μmoles (17 μCi) of mevalonic acid-2-14C were injected through the cap. Incubations were for 40 min at 37°C, with shaking. The reaction was stopped by adding 1.0 ml of saturated KOH and the incubation mixture was saponified by autoclaving for 1 hour at 120°C. The reaction mixture was transferred to a separatory funnel, the flask was washed with 5.0 ml of methanol and 10 ml of hexane-ether, 1:1 (v/v), and the mixture was extracted with 110 ml of hexane-ether. The extract was washed twice with 25 ml of water and evaporated to dryness; the residue was taken up in 2 × 5.0 ml of chloroform-methanol, 2:1 (v/v) and transferred to a graduated centrifuge tube. An aliquot was removed for determination of cholesterol concentration (23) and the remainder was evaporated to dryness in a heating block. The residue was dissolved in acetone-ethanol, 1:1 (v/v) and 1 drop of 10% acetic acid and 2.0 ml of 0.5% digitonin were added. After about 15 hours, the precipitate was sedimented by centrifugation and successively washed with 3.0 ml of acetone, 3.0 ml of acetone-ether, 1:1 (v/v), and 3.0 ml of ether, and dried. The dry sterol digitonide was dissolved in 2.0 ml of hot methanol and counted in dioxane scintillation fluid. Over-all recovery of cholesterol-14C was 85 to 90%.

HMG-CoA Synthesizing System—Microsomes were prepared as for HMG-CoA reductase incubations but with 0.1 M sodium phosphate, pH 7.0, in place of ESM solution and centrifugation at 70,000 × g for 90 min. HMG-CoA synthase was solubilized as described by Rudney (24) by resuspending the 70,000 × g pellet in 0.1 M sodium phosphate, pH 7.0, containing 1.0 mM EDTA. The microsomes were removed by centrifugation at 70,000 × g for 90 min and the supernatant liquid containing the HMG-CoA synthesizing system was carefully decanted. Each 1.0-ml incubation mixture contained 100 μmoles of sodium phosphate (pH 7.0), 1.0 μmole of β-mercaptoethanol, 440 μmoles of acetyl-l-14C-CoA (106 cpm per nmole), and 0.2 ml of supernatant fraction. Triplicate incubations were for 15 min at 37°C. Reactions were stopped by addition of concentrated KOH, the incubation mixtures were acidified, and carboxylic acids were extracted with ether (4 × 10 ml) from a sodium sulfate slurry as described by Lyen and Grasell (25). Ether extracts were evaporated to dryness, and the residue was taken up in 0.5 ml of methanol and chromatographed on activated silica gel in methyl ethyl ketone. The area containing HMG (RF 0.55 to 0.90) was scraped off and counted in dioxane scintillation fluid.
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FIG. 1. Effect of concentration of microsomal protein on the rate of mevalonate synthesis. The indicated quantities of microsomal protein from livers of rats killed at noon (O—O) or at midnight (■—■) were incubated in duplicate and assayed as described under "Materials and Methods."

FIG. 2. Mevalonate synthesis as a function of incubation time. Duplicate incubations were conducted for the indicated times under otherwise standard conditions but using either 6.5 mg of microsomal protein from livers of rats killed at noon (O—O) or 2.1 mg of microsomal protein from livers of rats killed at midnight (■—■).

Lipoprotein Isolation—Rat liver low and very low density lipoproteins from control and cholesterol-fed rats were isolated by flotation from a ρ = 1.07 density solution as described by Ewing, Freeman, and Lindgren (26).

Fig. 3. Lineweaver-Burk plots for HMG-CoA reductase. Triplicate incubations carried out at the indicated concentrations of DL-HMG-CoA under otherwise standard conditions contained either 1.96 mg of microsomal protein from livers of rats killed at noon (O—O) or 0.89 mg of microsomal protein from livers of rats killed at midnight (■—■). Kinetic constants, obtained from computer least square fits by using our "Dataplot" program (28) on a Control Data Corporation 3500 computer, were: $K_m = 5.4 \times 10^{-5} \text{M}$ for DL-HMG-CoA and $V_{max} = 0.215 \text{n mole per min per mg for reductase from microsomes of rats killed at noon (O—O)}$ and $K_m = 3.63 \times 10^{-5} \text{M}$ for DL-HMG-CoA and $V_{max} = 0.357 \text{n mole per min per mg for reductase from microsomes of rats killed at midnight. Standard errors, which were less than 10%, in all cases, are omitted for simplicity.}$

Units of Activity—HMG-CoA reductase activity is expressed either as nanomoles of mevalonic acid synthesized per min per mg of microsomal protein or in two cases also as nanomoles per g of liver (15). Protein was determined by the method of Lowry et al. (27).

RESULTS

Properties of HMG-CoA Reductase—HMG-CoA reductase of microsomes isolated from rats killed at noon or midnight has similar properties. In both cases the rate of mevalonate synthesis is proportional to enzyme concentration up to 12 mg of protein per ml (Fig. 1). The decrease in activity at high protein concentration resembles that reported by Kandutsch and Saucier (13) for mouse liver HMG-CoA reductase. The reaction is linear for 20 min by using microsomes from rats killed either at noon or at midnight (Fig. 2). Lineweaver-Burk double reciprocal plot of reductase activity in microsomes isolated from rats killed at noon or at midnight gave highly similar $K_m$ values for DL-HMG-CoA (Fig. 3).

Effects of Dietary Cholesterol on Cholesterol Synthesis and on HMG-CoA Reductase Activity—Gould and Swerr (29) have shown that prolonged feeding of cholesterol depresses cholesterol synthesis at sites beyond mevalonic acid. Although Siperstein and Fagan (8) have shown that conversion of acetate to HMG-CoA is unaffected by dietary cholesterol, White and Rudney (30, 31) have provided evidence that under certain conditions HMG-CoA synthase (3-hydroxy-3-methylglutaryl-CoA acetoacetyl-CoA-lyase (CoA-acetylating), EC 4.1.3.5) may be a regulatory enzyme in cholesterol synthesis. We attempted to determine
respectively. Standard errors for HMG-CoA reductase assays were performed as described under "Materials and Methods." The experiment was conducted from 8 a.m. to 6 p.m. EST during which time reductase activity in control rats was constant. Control values for HMG-CoA reductase activity and for acetate incorporation into cholesterol were the average of triplicate assays using normal fed rats killed at 0, 4 and 10 hours. Heavy and light vertical lines represent the standard error of the mean for triplicate assays of liver cholesterol content (O—O) and of cholesterol synthesis from acetate (●—●), respectively. Standard errors for HMG-CoA reductase assays (O—O) were less than 5% in all cases and are omitted.

the number and location of control points in cholesterol synthesis by simultaneous study of the decrease in microsomal HMG-CoA reductase activity and the over-all rate of sterol synthesis following cholesterol feeding. As shown in Fig. 4, reductase activity and cholesterol synthesis decline in parallel. After 10 hours of cholesterol feeding the incorporation of acetate-1-14C into cholesterol had declined to 22% of control values while HMG-CoA reductase activity was 19% of control activity. The decrease in HMG-CoA reductase activity associated with the rise in liver cholesterol (Fig. 4) reflects the operation of intracellular controls on cholesterol synthesis. The data also suggest that the decrease in the rate of HMG-CoA reduction accounts for the decrease in the rate of cholesterol synthesis from acetate. Incorporation of acetate-1-14C into cholesterol (Fig. 5) is unchanged under the above conditions. This indicates that, for short duration cholesterol feeding, HMG-CoA reductase, the first enzyme unique to polyprenoid synthesis, is the sole regulatory site in sterol synthesis. Continuing cholesterol feeding for several days may result in regulation at additional sites including HMG-CoA synthase (30, 31) and sites beyond mevalonic acid (8, 29). We therefore investigated the molecular mechanisms by which cholesterol decreases the rate of HMG-CoA reductase.

Failure of Cholesterol to Inhibit HMG-CoA Reductase Activity—The insolubility of cholesterol in water has led to the use of propylene glycol suspensions (10, 11, 32) of cholesterol to test its ability to inhibit HMG-CoA reductase. These suspensions are nonphysiological and their failure to inhibit (10, 11, 32) is therefore not conclusive. Linn (10) observed that mixing microsomes isolated from normal and cholesterol-fed rats did not result in inhibition of the reductase in the normal microsomes. Since an excess of a soluble inhibitor of HMG-CoA reductase might not

![Graph](https://via.placeholder.com/150)

**Fig. 4.** Decrease in HMG-CoA reductase activity and cholesterol synthesis from acetate following cholesterol feeding. HMG-CoA reductase assays, cholesterol synthesis incubations, and cholesterol assays were performed as described under "Materials and Methods." The experiment was conducted from 8 a.m. to 6 p.m. EST during which time reductase activity in control rats was constant. Control values for HMG-CoA reductase activity and for acetate incorporation into cholesterol were the average of triplicate assays using normal fed rats killed at 0, 4 and 10 hours. Heavy and light vertical lines represent the standard error of the mean for triplicate assays of liver cholesterol content (O—O) and of cholesterol synthesis from acetate (●—●), respectively. Standard errors for HMG-CoA reductase assays (O—O) were less than 5% in all cases and are omitted.

![Graph](https://via.placeholder.com/150)

**Fig. 5.** Effect of dietary cholesterol on incorporation of mevalonate-2,4-14C into cholesterol and of acetetyl-1,4C-CoA into HMG-CoA. Conditions for incubations and analyses are described under "Materials and Methods." Shown are liver cholesterol content (●—●) and incorporation of isotope from mevalonate into cholesterol (O—O) or from acetyl-CoA into HMG-CoA (O—O). For mevalonate incorporation data, control values are the mean of nine incubations performed in triplicate at 0, 4, and 10 hours (8 a.m. to 6 p.m. EST). For acetyl-CoA incorporation, control values are the mean of nine incubations performed in triplicate at 2, 6, and 10 hours. Vertical lines represent the standard error of the mean for triplicate incubations.

**Table I**

<table>
<thead>
<tr>
<th>Source of rat liver homogenate for microsome isolation</th>
<th>Specific activity of HMG-CoA reductase</th>
<th>Fraction of normal-fed control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ................................................................</td>
<td>0.130 ± 0.008</td>
<td>(100)</td>
</tr>
<tr>
<td>Cholesterol-fed (24 hrs) ..................................</td>
<td>0.016 ± 0.0002</td>
<td>12</td>
</tr>
<tr>
<td>Three-quarters normal + one-quarter cholesterol-fed ..</td>
<td>0.128 ± 0.005</td>
<td>98</td>
</tr>
<tr>
<td>One-half normal + one-half cholesterol-fed ..........</td>
<td>0.133 ± 0.005</td>
<td>103</td>
</tr>
</tbody>
</table>
TABLE II
Effect of lipoprotein from cholesterol-fed rats on HMG-CoA reductase activity

Rats were fed a 5% cholesterol diet for 3 days prior to isolation of low and very low density lipoproteins. Lipoproteins from livers of normal-fed and cholesterol-fed rats were isolated by flotation as described under "Materials and Methods." Lipoproteins isolated from normal rats contained 16 µg of cholesterol per mg of protein while those from cholesterol-fed rats contained 1120 µg of cholesterol per mg of protein. Microsomes were isolated from control rats killed at midnight. Lipoprotein from cholesterol-fed rats was added to incubations to give the indicated cholesterol concentrations. After preliminary incubation for 5 min at 37°C, with shaking, substrate was added to initiate the reaction. Incubations were performed as described under "Materials and Methods" but with shaking. The control value is the mean of six incubations. Specific activity is expressed ± the standard error of the mean for triplicate incubations. Lipoprotein cholesterol (1 mM) from normal rats added to incubations was also noninhibitory (HMG-CoA reductase activity of 1.27 ± 0.07).

<table>
<thead>
<tr>
<th>Cholesterol added as Lipoprotein</th>
<th>Specific activity of HMG-CoA reductase</th>
<th>Fraction of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>(nmol/min/mg)</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>1.10 ± 0.07</td>
<td>(100)</td>
</tr>
<tr>
<td>0.01</td>
<td>1.11 ± 0.01</td>
<td>96</td>
</tr>
<tr>
<td>0.1</td>
<td>1.11 ± 0.01</td>
<td>96</td>
</tr>
<tr>
<td>1.0</td>
<td>1.25 ± 0.05</td>
<td>108</td>
</tr>
<tr>
<td>10</td>
<td>1.30 ± 0.04</td>
<td>112</td>
</tr>
</tbody>
</table>

Fig. 6. Cyclic variation in HMG-CoA reductase activity. Rats were kept in a windowless room with lights on from 6:00 a.m. to 6:00 p.m. and off from 6:00 p.m. to 6:00 a.m. (EST). Microsomes isolated from rats killed at the indicated times were quick-frozen prior to assay the following day. Vertical lines represent the standard error of the mean for triplicate incubations of reductase from normal fed (○—○), cholesterol-fed (□—□), and cycloheximide-injected (n—n) rats.

Fig. 7. Effect of dietary cholesterol and of cycloheximide on the cyclic variation in HMG-CoA reductase activity. Cholesterol-fed rats were fed a 5% cholesterol diet beginning at 9 a.m. and continuing throughout the experiment. At 6 p.m. cycloheximide (0.5 mg/100 g) was injected intraperitoneally into paired rats fed control diet. Microsomes isolated from rats killed at the indicated times were quick-frozen prior to assay the following day. Vertical lines represent the standard error of the mean for triplicate incubations of reductase from normal fed (○—○), cholesterol-fed (□—□), and cycloheximide-injected (n—n) rats.

...be present in microsomes, we have extended this observation by performing experiments with crude homogenates prepared from livers of normal and cholesterol-fed rats. As shown in Table I, microsomes isolated from mixed homogenates have unimpaired activity. It therefore appears that livers of cholesterol-fed rats do not contain a soluble inhibitor of HMG-CoA reductase.

To test further the possibility that cholesterol or a derived metabolite inhibits HMG-CoA reductase activity, we have obtained cholesterol in its physiological state by isolating the low and very low density lipoprotein fraction from the livers of normal and cholesterol-fed rats. As shown in Table II, neither inhibits HMG-CoA reductase activity of microsomes from livers of control rats. Cholesterol thus does not appear to be a feedback inhibitor of HMG-CoA reductase. We therefore investigated the alternative possibility that cholesterol affects the levels of HMG-CoA reductase.

Cyclic Variations in Reductase Activity—Both HMG-CoA reductase (1, 13, 15) and cholesterol synthesis (33) undergo cyclic variations. This is shown for HMG-CoA reductase with data obtained at shorter time intervals than previously reported (Fig. 6). In experiments performed over several months the activity at midnight ranged from 0.55 to 1.3 nmoles per min per mg of protein with the higher activities generally occurring in smaller (75 to 100 g) rats. Daytime activity (9 a.m. to 5 p.m.) is relatively constant and ranges from 0.125 to 0.150 nmoles per mg and, within limits, appears to be independent of the age and size of the rats. Cycloheximide completely prevents the cyclic rise in activity (Fig. 7). Actinomycin D (0.5 mg per
Cholesterol feeding suppressed the cyclic rise in HMG-CoA reductase activity (Fig. 7). However, in contrast to cycloheximide which completely prevented the rise in activity, cholesterol feeding produced a cyclic rhythm of markedly reduced amplitude. Both the absolute rise in activity and the rise relative to the initial activity are notably smaller in cholesterol-fed rats. The effect of cholesterol feeding on the cyclic rhythm differs from the effect of fasting (15). Although fasting markedly decreased reductase activity throughout the cycle, the rise relative to the initial activity was similar in normal and fasted rats. The suppression of the cyclic rise in the rate of HMG-CoA reduction is consistent with the possibility that cholesterol may act to repress the synthesis of HMG-CoA reductase.

DISCUSSION

The microsomal preparation described yields a stable, active source of HMG-CoA reductase. The specific activity of reductase in microsomes isolated from rats killed at noon is equal to that reported by Linn (18) and by Hamprecht, Nüssler, and Lynen (15). It exceeds by 15-fold that reported by Kawauchi and Rudney (32) and by about 7-fold that reported by Kandutsch and Sauier (13) for mouse liver HMG-CoA reductase. The specific activity of microsomal reductase from rats killed at midnight averaged about 1 n mole per min per mg, or one-third that reported by Kawauchi and Rudney (32) for 300-fold purified rat liver HMG-CoA reductase.

The decrease in the rate of HMG-CoA reduction seen following cholesterol feeding might be due to inhibition of HMG-CoA reductase by cholesterol or a cholesterol metabolite, or repression of HMG-CoA reductase synthesis, or to accelerated inactivation or degradation of existing reductase. The absence of a detectable soluble reductase inhibitor in livers of cholesterol-fed rats and the failure of cholesterol-rich lipoproteins to inhibit HMG-CoA reductase suggest that neither cholesterol nor a derived metabolite is a physiological inhibitor of the reductase.

The cyclic rhythm of HMG-CoA reductase activity closely resembles the idealized pattern for induction and degradation of a mammalian enzyme described by Schimke (34). Cyclic rhythms in activity have been reported for several hepatic enzymes including tyrosine transaminase and tryptophan pyrrolase (35, 36). The cyclic rise in HMG-CoA reductase activity might be due to release of inhibition of existing reductase, to activation of previously existing enzyme or proenzyme, or to de novo synthesis of new reductase protein. Both the similarity in basic kinetic properties of reductase from rats killed at noon and at midnight and the action of cycloheximide suggest that synthesis of new HMG-CoA reductase protein is responsible for the rise in activity.

The ability of cholesterol as well as cycloheximide to suppress the cyclic rise in HMG-CoA reductase activity is consistent with cholesterol acting to repress reductase synthesis. In the 9 a.m. to 5 p.m. period reductase activity remains relatively constant. In this time period the rate of HMG-CoA reductase synthesis presumably equals the rate at which reductase is degraded. From 6 p.m. to midnight when enzyme activity rises sharply the number of molecules of reductase synthesized should greatly exceed the number being degraded or inactivated. During this period the ratio of reductase activity in cholesterol-fed to that in control rats falls from 0.40 to 0.12. After midnight, reductase activity decreases in exponential fashion, implying that the number of enzyme molecules degraded exceeds the number synthesized. From midnight to 2 a.m., while reductase activity in both normal and cholesterol-fed rats falls rapidly, the ratio of cholesterol-fed to normal fed activity remains essentially constant (0.12 at midnight; 0.14 at 2 a.m.). The rate of reductase degradati 
on or inactivation may therefore be similar in normal and cholesterol-fed rats. Quantitative immunological studies of this parallel pattern will be necessary to definitively assign roles to alterations in the rates of enzyme synthesis and degradation. Our data, however, which strongly suggest that cholesterol affects the rate of HMG-CoA reduction by decreasing the level of HMG-CoA reductase, are consistent with cholesterol acting to repress reductase synthesis.

In intact cells the end products of amino acid degradation enter oxidative metabolism and are frequently difficult to measure. This contributes to the lack of information concerning the physiological significance of alterations in enzyme levels in mammalian systems (16). Cholesterol, the end product of this pathway, is readily assayed. This permits us to correlate changes in HMG-CoA reductase activity with changes in the rate of sterol synthesis from acetate in a liver mince. The excellent agreement between these parameters, which decline in parallel following cholesterol feeding, indicates that the observed alterations in reductase activity reflect changes in the intracellular rate of cholesterol synthesis and represent a true physiological control mechanism. By contrast, Kim and Miller (37) have reported that the induction of tyrosine transaminase and tryptophan pyrrolase was not associated with increased oxidation rates of 3H amino acids in vivo.

Periods of rapid rise and fall in HMG-CoA reductase activity may provide a useful system for the study of enzyme synthesis and degradation. These processes are largely dissociated in time without the use of inhibitors of protein or RNA synthesis which frequently have secondary effects.

REFERENCES

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