Control of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase by Endogenously Synthesized Sterols in Vitro and in Vivo*

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Isolated rat hepatocytes converted mevalonolactone into sterol intermediates and fatty acids 6- to 8-fold faster than mevalonate salt at concentrations less than $6 \times 10^{-8}$ M. Incubation of hepatocytes for 3 h normally results in induction of 3-hydroxy-3-methylglutaryl-CoA reductase. This increase in enzyme activity was inhibited by mevalonolactone and by mevalonate salt; at each concentration between $6 \times 10^{-4}$ M and $6 \times 10^{-8}$ M the lactone was a more effective inhibitor than the salt. The increase in enzyme activity was completely prevented by $6 \times 10^{-4}$ M lactone, and at this concentration the cells synthesized from the lactone an amount of sterol per hour which approximated that leaving the cells in the same period.

Administration of mevalonolactone to intact rats resulted in a dose-dependent inhibition of hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity. At the highest dose (400 mg of (RS)-mevalonolactone/200 g of rat) enzyme activities declined 85% within 45 min and were still suppressed below normals after 28 h. Mevalonolactone treatment resulted in increases in liver cholesterol content and in the cholesterol ester concentration of liver microsomes. The results demonstrate that the activity of hepatic 3-hydroxy-3-methylglutaryl-CoA reductase can be controlled by the rate of endogenous sterol synthesis both in vitro and in vivo.

The rate-limiting enzyme for cholesterol biosynthesis from acetate is generally considered to be 3-hydroxy-3-methylglutaryl-CoA reductase (1, 2). This enzyme exhibits a circadian rhythm of activity with a 5- to 10-fold amplitude (3-6) which results from changes in the rate of synthesis of the enzyme in the presence of a constant rate of enzyme degradation (4, 5). The mechanisms involved in both the rapid increase in enzyme synthesis during the circadian rise and the complete cessation of enzyme synthesis during the circadian decline from the peak at midnight (4, 5) are unknown.

Administration of exogenous cholesterol to intact animals

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1 The abbreviation used is: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.
Regulation of 3-Hydroxy-3-methylglutaryl-CoA Reductase

3-Hydroxy-3-methylglutaryl-CoA Reductase - This enzyme was assayed in microsomes isolated from liver or hepatocytes in a 1 ml incubation as described previously (27). The [3-14C]HMG-CoA used in the assay was prepared by the method of Goldfarb and Pito (23) and had a specific activity of 0.34 Ci/mol. The [14C]mevalonate formed during the assay was isolated by chromatography on Bio-Rad AG1-X8 formate column (24) after hydrolysis with base of residual [3-14C]HMG-CoA and addition of [5-3H]mevalonate as the internal standard (23). Specific activities of the reductase are given as nanomoles of mevalonate formed/min/mg of microsomal protein; 1 enzyme unit catalyzed the reduction of 1 nmol of HMG-CoA/min.

Mevalonolactone and Sodium Mevalonate - Solutions of RS-unlabeled or [2-14C]mevalonolactone (pH < 6.0) were divided into two halves; 1 N NaOH was added to one half to pH > 9.0, and a volume of water equal to that of the NaOH solution used was added to the second half. The solutions were incubated at 37° for at least 30 min. Addition of 100 μl of the lactone or mevalonate solution to incubations of hepatocytes had no effect on the pH of the medium. The second half. The solutions were incubated at 37° for at least 30 min. Addition of 100 μl of the lactone or mevalonate solution to incubations of hepatocytes had no effect on the pH of the medium. The specific activity of the [2-14C]mevalonate (or lactone) was 99 mCi/mol.

Mevalonolactone concentrations were determined spectrophotometrically with mevalonate kinase coupled with pyruvate kinase and lactate dehydrogenase as described (27).

Mevalonate Kinase - This enzyme was purified from pig liver by a modification of the method of Levy and Popják (25). The enzyme catalyzed the phosphorylation of mevalonate at the initial rate of 5 pmol/minute (25).

Hydrolysis of Mevalonolactone at Near Physiological pH

Utilization of [2-14C]Mevalonolactone and Sodium [2-14C]-Mevalonate by Hepatocytes - Addition of (RS)-[2-14C]mevalonolactone or sodium [2-14C]mevalonate to isolated rat hepatocytes resulted in incorporation of 14C into nonsaponifiable lipids, digiotor-prefectible sterols, and fatty acids. Six to nine times more 14C was incorporated into all three lipid fractions from [14C]mevalonolactone than from [14C]mevalonate at each of the concentrations tested between 6 x 10^-8 M and 6 x 10^-4 M (Table I). Incorporation of 14C into nonsaponifiable lipids was maximal at 6 x 10^-5 M mevalonolactone and 8 x 10^-3 M mevalonate (data not shown).

Hydrolysis of Mevalonolactone at Near Physiological pH Values - It is known that the substrate for mevalonate kinase (EC 2.7.1.36) is mevalonate, not the lactone (26). Further, the lactone is stable at low pH but is hydrolyzed rapidly at a pH greater than 9.0. In order to determine both the ratio of mevalonolactone to mevalonic acid in aqueous solution and the rapid hydrolysis of mevalonolactone in water by stomach tube. Controls were not treated after it was found that administration of isotonic NaCl solution by stomach tube had no effect on HMG-CoA reductase.

RESULTS

In the first experiments on the possible regulatory function of endogenously synthesized cholesterol on levels of HMG-CoA reductase, a solution of mevalonolactone in water was added to hepatocytes incubated under conditions (13, 17) that led to a loss of intracellular cholesterol and caused invariably in 3 h a 3- to 4-fold rise in the activity of the reductase. A concentration of 6 x 10^-4 M mevalonolactone prevented completely the increase in enzyme activity. When this experiment was repeated, however, with a solution of the salt (sodium mevalonate), the increase in enzyme activity was only partially prevented. Such results were consistent with a greater incorporation of mevalonolactone into sterols compared to mevalonate. Indeed, in 1962 Fumagalli et al. (31) had reported that in liver slices the lactone was a slightly better substrate than the salt for sterol synthesis. These differences were at first puzzling since it was known that the first enzyme acting on mevalonate, mevalonate kinase, was inactive with the lactone (26).

In order to explain the difference between the effect of the lactone and salt on the activity of HMG-CoA reductase, it had to be assumed that the neutral lactone was taken up by the cells faster than the charged mevalonate and that it was subsequently hydrolyzed rapidly in the cells. Before proceeding to the further study of the role of endogenously synthesized cholesterol in the regulation of levels of HMG-CoA reductase, we examined the utilization of mevalonolactone and mevalonate by the hepatocytes for sterol synthesis and the rate of nonenzymic hydrolysis of mevalonolactone at various pH values.

Hydrolysis of Mevalonolactone at Near Physiological pH Values - It is known that the substrate for mevalonate kinase (EC 2.7.1.36) is mevalonate, not the lactone (26). Further, the lactone is stable at low pH but is hydrolyzed rapidly at a pH greater than 9.0. In order to determine both the ratio of mevalonolactone to mevalonic acid in aqueous solution and...
the rate of hydrolysis of the lactone as a function of pH, the lactone was incubated with mevalonate kinase, buffered between pH 6.7 and 7.5, and the components of the coupled assay system (phosphoenolpyruvate, pyruvate kinase, lactate dehydrogenase, and NADH) which measures the rate of formation of ADP from ATP in the mevalonate kinase reaction (25). Oxidation of 1 mol of NADH corresponds to the conversion of 1 mol of mevalonate to 5-phosphomevalonate.

With the amount of mevalonate kinase used, 0.46 µmol of sodium (R)-mevalonate (added as the RS mixture) was completely phosphorylated at pH 7.4 within 5 to 7 min (Fig. 1 and Table II). However, addition of a solution of lactone at the same concentration and pH, resulted in rapid phosphorylation of only 25% of the added substrate. The remainder was phosphorylated slowly, at a rate of approximately 0.84%/min (Fig. 1 and Table II). Completion of the phosphorylation of (RS)-mevalonolactone required approximately 90 min.

Similar assays done between pH 6.7 and 7.5 indicated that less than 25% of the lactone was rapidly converted to phosphomevalonate and that the major portion of the lactone was phosphorylated slowly (Table II). The rate of this slow phosphorylation increased as the pH was raised; the rate was zero at pH 6.7 and 0.8%/min at pH 7.5 (Table II).

Since the mevalonate was completely phosphorylated by 7 min, we interpret the data to indicate that (a) in solutions buffered between pH 6.7 and 7.5 the ratio of mevalonolactone to acid is about 3:1, and that (b) the phosphorylation which occurred after 7 min was a direct measure of the rate of hydrolysis of the lactone.

Differential Uptake of Mevalonolactone and Sodium Mevalonate by Hepatocytes—In order to explain the greater synthesis of lipids in rat hepatocytes from the lactone as compared to mevalonate (Table I), we considered that the less polar lactone may enter the cell more easily than the acid. This possibility was examined by an experiment in which the pure (R)-[5-14C]mevalonolactone or sodium (R)-mevalonate was added to a concentrated suspension of hepatocytes and the rate of disappearance of each substrate from the medium was measured. The data in Table III leave no doubt that the lactone was taken up by the hepatocytes much faster than the mevalonate. The finding that in the presence of the salt the 14C content of 0.1 ml of medium at zero time was greater than in incubations performed in the absence of cells (133,900 dpm), Table III indicates that mevalonate was at first partially excluded from the cells. In contrast, in the time taken to separate the cells from the medium, the lactone content of the medium fell to 48,800 dpm and may indicate that the cells not only take up, but also concentrate the lactone.

**Inhibition of Activity of HMG-CoA Reductase in Hepatocytes by Mevalonolactone and Sodium Mevalonate—** There is no published information on whether the activity of HMG-CoA reductase is affected by the rates of endogenous cholesterol synthesis. We have reported previously that incubation of rat hepatocytes in a medium containing amino acids and 1.5% sodium (R)-[5-14C]mevalonate (added as the RS mixture) was completely phosphorylated at pH 7.4 within 5 to 7 min (Fig. 1 and Table II). Completion of the phosphorylation of (RS)-mevalonolactone required approximately 90 min.

**Effect of pH on synthesis of phosphomevalonate from mevalonolactone and sodium mevalonate with mevalonate kinase**

**(RS)-Mevalonolactone or mevalonate (0.994 pmol) was added to the 3-ml assay as described in the legend to Fig. 1, buffered between pH 6.7 and 7.5, and kept at 37° (25). The pH of the 0.1 ml Tris buffer was determined at the beginning and end of the incubation. The conversion of (R)-mevalonate to phosphomevalonate was determined as described by Pogáč (25). The initial rate of phosphorylation of (R)-mevalonolactone or (R)-mevalonate was calculated from the slope of the tracing during the first 2 min; the slow rate was calculated from the slope after a 15-min incubation period. The percentage of (R)-mevalonate phosphorylated at the stated times was determined by comparison of the A400 to that given by mevalonate at 15 min.**

**Table II**

<table>
<thead>
<tr>
<th>Substrate added as</th>
<th>pH of assay</th>
<th>Total (R)-mevalonate phosphorylated at</th>
<th>Rate of conversion of (R)-mevalonate to phosphomevalonate</th>
<th>%</th>
<th>%/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactone</td>
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<td></td>
<td>5</td>
<td>0.57</td>
</tr>
<tr>
<td>Lactone</td>
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<td>10 min</td>
<td></td>
<td>15</td>
<td>0.84</td>
</tr>
<tr>
<td>Salt</td>
<td>7.2</td>
<td>10 min</td>
<td></td>
<td>10</td>
<td>0.57</td>
</tr>
<tr>
<td>Lactone</td>
<td>7.44</td>
<td>15 min</td>
<td></td>
<td>15</td>
<td>0.84</td>
</tr>
<tr>
<td>Salt</td>
<td>7.50</td>
<td>15 min</td>
<td></td>
<td>10</td>
<td>0.57</td>
</tr>
</tbody>
</table>

**Table III**

Comparison of rate of uptake of mevalonolactone and mevalonate into rat hepatocytes

L-6 rat hepatocytes (at a concentration of approximately 1 g of cells, wet weight, diluted to 2 ml with modified Swinn S-7 (Tris medium) were incubated with (R)-[5-14C]mevalonolactone or mevalonate (11.0 Ci/mol). The substrate concentration in the 2.5 ml of assay was 4.39 × 10⁻⁴ M. At the indicated times samples of the cell suspension were removed and centrifuged at 600 rpm × 3 min in a hemacytometer tube. The packed cell volume was 51% of the total volume under these conditions. The supernatant (medium) was removed and recentrifuged at approximately 1200 rpm × 10 min. A sample (0.1 ml) of this supernatant was mixed with 0.9 ml of ethanol, the precipitate removed by centrifugation, and an aliquot (0.5 ml) removed to determine its 14C Content. Incubations performed in the absence of hepatocytes and made up to 2.6 ml with buffer gave 105,200 dpm/0.1 ml of medium.

**Regulation of 3-Hydroxy-3-methylglutaryl-CoA Reductase**

![Figure 1](https://example.com/figure1.png)

**FIG. 1.** Rate of phosphorylation of mevalonate or mevalonolactone by mevalonate kinase. (RS)-Mevalonolactone or mevalonate (0.92 µmol) was added to a 3-ml reaction mixture containing mevalonate kinase (0.1 unit) and components of a coupled assay system at 37°, buffered at pH 7.44 (25). The change in absorption at 340 nm was determined spectrophotometrically. A change of absorption of 0.1 A unit is equivalent to the phosphorylation of 0.0485 µmol of mevalonate in 3 ml. Left, changes observed after addition of sodium mevalonate; right, after addition of the same amount of mevalonolactone to the reaction mixture.
bovine serum albumin results in loss of cellular cholesterol into the medium and induction of HMG-CoA reductase activity (13, 17). Further, the degree of induction of the enzyme after 3 h was directly proportional to the initial rate, and hence amount, of cholesterol leaving the cell (17).

The normal induction of the enzyme during a 3-h incubation was inhibited when mevalonolactone or sodium mevalonate were included at concentrations between \(8 \times 10^{-3}\) M and \(6 \times 10^{-6}\) M (Fig. 2). The increase in enzyme activity was completely inhibited by the lactone at \(6 \times 10^{-4}\) M or by sodium mevalonate at \(8 \times 10^{-4}\) M (Fig. 2). At each substrate concentration the lactone was a more effective inhibitor of enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2). However, the most marked difference between the effect of lactone and salt on enzyme activity than the salt (Fig. 2).

Efflux of cellular cholesterol into the medium during a 3-h incubation was not significantly affected by either form of the substrate although cells incubated with the lactone at \(6 \times 10^{-4}\) M did show repeatedly an increased sterol efflux of approximately 6%.

In ten different cell preparations incubation of basal cells under standard conditions resulted in a mean efflux of cellular cholesterol into the medium of 18.96 ± 3.7 µg of cholesterol in 3 h/1.1 x 10^7 cells, or approximately 6.3 µg of cholesterol/h/1.1 x 10^7 cells. Analyses of the digitonin-precipitable [14C]sterols from different cell preparations incubated with [14C]lactone at \(6 \times 10^{-4}\) M indicated that the cells synthesized from the added [14C]lactone approximately 10.7 µg of 3β hydroxysterols/h/1.1 x 10^7 cells (range 9 to 13 µg).

Hence, under conditions in which the increase in enzyme activity was completely prevented by mevalonolactone, the cells synthesized an amount of sterol approximating that leaving the cells.

**Regulatory Sterol Pool Controlling Reductase Activity**—Previous studies from this laboratory have shown that the increase in activity of the reductase after 3 h in the presence of lecithin dispersions at less than 300 µg of phospholipid/ml was determined by the amount of cholesterol leaving the cell (17). At lecithin concentrations above 300 µg/ml this relationship was no longer true, and we have suggested that at the very high rate of cholesterol loss from the cells other factors (e.g. rate of mRNA transcription) may become rate-limiting in determining the extent of induction of HMG-CoA reductase.

When hepatocytes were incubated for 3 h with up to 1500 µg of lecithin/ml, the amount of cholesterol lost from the cells to the medium increased with increasing concentrations of lecithin (Fig. 3). However, the increased activity of HMG-CoA reductase after 3 h was linearly proportional to the sterol leaving the cells only at lecithin concentrations of less than 300 µg/ml (Fig. 4). At 1500 µg of lecithin/ml, the reductase activity increased by 2.6 units during the 3-h incubation (Fig. 4). The cells, which initially contained approximately 260 µg of cholesterol, lost 89 µg of cholesterol into the medium during this 3-h period (Fig. 4).

Addition of (RS)-mevalonolactone (2.4 x 10^{-3} M) to cells incubated with 1500 µg of lecithin/ml had no significant effect on efflux of cholesterol into the medium (Fig. 4). However, the increase in enzyme activity was inhibited significantly; enzyme activity increased by 1.3 units during the 3-h incubation in experimental compared to the 2.6 units in controls (Fig. 4).

Analysis of the [14C]content of the nonsaponifiable lipids from cells incubated for 2 h with [14C]mevalonolactone at 2.4 x 10^{-3} M indicated that lecithin at 1500 µg/ml did not affect the rate of sterol synthesis from the lactone; experimental values were 106% of controls incubated without lecithin.

Hence, since cells synthesize from the mevalonolactone approximately 32 µg of sterol/3 h (see above), then the net loss of cholesterol from cells incubated with the lactone will be 57 µg in 3 h (i.e. 89 - 32 µg).

If reductase levels were determined by the total cell cholesterol pool, we would predict from Fig. 4 that a net loss of 57 µg of cholesterol would result in an increase in enzyme activity of approximately 2.0 units. However, the data shown in Fig. 4, which is representative of the results obtained in three experiments, indicate that the reductase was induced by only 1.3 units.

**Inhibition of Circadian Rhythm of HMG-CoA Reductase in Vivo by Mevalonate**—It seemed pertinent to determine whether reductase activity in intact animals could also be controlled by endogenously synthesized sterol.

Mevalonolactone was given to rats via stomach tube and the animals were killed 5.5 h later at the time of the expected peak reductase activity. The normal circadian rise of hepatic HMG-CoA reductase activity was partially prevented at doses of
Regulation of 3-Hydroxy-3-methylglutaryl-CoA Reductase

(RS)-mevalonolactone above 25 mg/200 g of body weight (Table IV, Experiment B) and completely prevented by doses greater than 100 mg/200 g of body weight (Table IV). At a dose of 400 mg/200 g of rat, reductase activities were less than 2% of untreated control animals killed at a time of peak activity (Table IV, Experiment A), and were significantly lower than the normal basal levels (Table IV, Experiment B).

In these same animals, increasing doses of mevalonate caused increased concentration of cholesterol in the liver and an increase in the microsomal cholesteryl ester content (Table IV). A significant increase in liver cholesterol concentration (about +0.5 mg of cholesterol/g of tissue) was observed 5.5 h after administration of the highest dose of lactone (Table IV).

The time course of the inhibition of the reductase after intubation into the stomach of 400 mg of (RS)-mevalonolactone indicates that enzyme activity declined very rapidly after treatment and that this inhibition of the enzyme persisted for at least 28 h (Fig. 5). At all times that enzyme levels were inhibited the cholesteryl ester content of the microsomes was enhanced (Table V).

Animals killed only 45 min after dosing with mevalonate had reductase levels less than 15% of controls (Table V, Experiment B) indicating that the treatment must have caused enzyme inactivation, since the half-life of degradation of HMG-CoA reductase is known to be 2 to 4 h (5, 9, 32). Microsomal cholesterol ester concentration also was significantly increased within 45 min of mevalonolactone administration (Table V).

**DISCUSSION**

The data indicate clearly that the activity of HMG-CoA reductase both in isolated rat hepatocytes and in the intact animal is dependent on the rate of endogenous sterol synthesis. The increase in cholesterogenesis in the presence of mevalonate, and in the absence of changes in the rate of sterol efflux across the plasma membrane, leads to a decline in the activity of HMG-CoA reductase (Fig. 2 and Table V). This, we believe, is the first evidence of regulation of the reductase activity by endogenously synthesized sterol.

Further, we have presented data suggesting that de novo synthesized sterol, or possibly an intermediary product of sterol biosynthesis, enters a specific pool within the hepatocyte and regulates the activity of the reductase.

There is now much evidence accumulated from the study of human leukocytes (19, 33), rat liver hepatocytes (13, 17), and mouse L-cell fibroblasts (14, 22) showing that the increase in activity of HMG-CoA reductase was determined by the loss of intracellular sterol. The experiments of Jakoi and Quartordt (16) on whole animals argue for the same thesis in respect to liver HMG-CoA reductase even though the sterol loss from the

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**TABLE IV**

<table>
<thead>
<tr>
<th>Time of treatment</th>
<th>Mevalonolactone dose</th>
<th>Reductase activity at L-10</th>
<th>D-3.5</th>
<th>D-6</th>
<th>Liver cholesterol concentration</th>
<th>Microsomal CE as percentage of lip total cholesterol</th>
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<tr>
<td>D-0.5</td>
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<td>0.93 ± 0.06</td>
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<td>2.05 ± 0.07</td>
<td>3.24 ± 0.19</td>
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<td>T1-0.5</td>
<td>400 (3)</td>
<td>0.015 ± 0.01</td>
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<td>2.73 ± 0.15</td>
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<td>L-10</td>
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<td>L-10</td>
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<td>0.04 ± 0.01</td>
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</tr>
</tbody>
</table>

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**Fig. 4.** Hepatocytes were incubated for 3 h with various concentrations of lecithin (PC) dispersions as described in the legend to Fig. 3. Incubations containing the highest concentration of lecithin examined (1500 µg of lecithin/ml) contained either 0 or 2.4 mM mevalonolactone (MVA) as indicated. HMG-CoA reductase activities in the cell microsomes were determined as described under "Experimental Procedures."

**Fig. 5.** Inhibition of the circadian rhythm of HMG-CoA reductase activity by mevalonolactone. Rats were either untreated or given 400 mg of mevalonolactone (MVA) at the 10th h of the light period. Hepatic reductase activities were determined during the following 28 h. Enzyme activities are given as nanomoles of mevalonate synthesized/min/mg of microsomal protein.
Liver of the animals was accomplished by unusual means (intravenous infusion of lecithin). We have demonstrated here that, by providing a readily utilisable substrate for cholesterol synthesis, and thus replenishing the sterol content of the cells, the increased activity of HMG-CoA reductase can be prevented even in the face of continued sterol loss. Abolishing the circadian rise of HMG-CoA reductase in the liver of the rat by the administration of mevalonolactone which resulted in increased cholesterol content in the liver, demonstrates for the first time, we believe, the important role of endogenous synthesis of cholesterol in the regulation of HMG-CoA reductase and gives strong support to the hypothesis first proposed by Rothblat (14, 18) and extended by Fogelman et al. (19) and Edwards et al. (17) that the rise and fall of HMG-CoA reductase in a variety of cells is part of a homeostatic mechanism guarding the cell against excessive loss or accumulation of cholesterol. Thus, a common mechanism may control the activity of the reductase and the overall rate of cholesterol synthesis in many cell types. We propose that the reductase activity is determined both by the relative rates of sterol flux into and out of the cell and also by the rate of endogenous sterol synthesis.

The circadian rise of HMG-CoA reductase in the liver of the rat is clearly related to the eating habits of this animal and may be explained by the assumption that it results from the partial depletion of the cholesterol content of the organ through the conversion of cholesterol into bile acids.

Choleresis, i.e. the flow of new liver bile, is a well known physiological response to eating. Thus, choleresis may deplete the cholesterol stores of the liver through synthesis of bile acids and secretion of cholesterol itself into the bile. The enzyme regulating bile acid synthesis, cholesterol 7α-hydroxylase, shows also a daily rise and fall of activity (34, 35), as does the HMG-CoA reductase. The hydroxylase rise precedes that of the reductase and it reaches a maximum 2 to 3 h before the middle of the dark period by which time its levels are declining (34, 35). Hence, this decline in the rate of cholesterol catabolism plus the enhanced rate of cholesterol synthesis occurring at this time (3, 36) would be expected to inhibit the synthesis of HMG-CoA reductase and result in a decline of enzyme activity. Such a decline is observed experimentally (Fig. 5).

This hypothesis is also in accord with the fact that enforced bile acid synthesis either by a bile fistula or by administration of cholestyramine results in elevation of liver HMG-CoA reductase activity or cholesterol synthesis from acetate (37, 38).

It is pertinent to point out that at midnight when reductase specific activity is maximal and approximates 1.0 unit/mg of microsomal protein it can be calculated, given that the microsomal content of liver approximates 20 mg of protein/g of tissue (39), that the liver synthesizes 7.7 μg of cholesterol/h/0.1 g of tissue assuming that all the mevalonate formed is converted into cholesterol. Synthesis of HMG-CoA reductase is undetectable during the subsequent 8 h after peak enzyme activity (5).

The increase in enzyme activity is also completely suppressed when isolated rat hepatocytes are incubated with 6 x 10⁻⁴ M mevalonolactone (Fig. 2). It can be calculated from studies with the same concentration of [1°C]mevalonolactone that the cells synthesize approximately 10 μg of cholesterol/h/0.1 g of tissue. This close correlation between the rate of cholestero genesis and subsequent decline in activity of HMG-CoA reductase in both isolated hepatocytes and in the intact rat is in support of the above proposal for control of HMG-CoA reductase.

The finding that mevalonolactone is converted by intact hepatocytes into sterols at a rate 6- to 8-fold that of the salt (Table I) is essentially in agreement with the report of Fumagalli et al. since these authors found that incubations of liver slices with [1°C]mevalonolactone or mevalonate resulted in slightly more labeled sterol with the lactone as substrate (31). These results could be partially explained by a more rapid entry into the cells of the less polar lactone (Table III).

It is possible that the rate of noncatalyzed hydrolysis of the lactone within the cell is sufficient to saturate the enzymes of the sterol biosynthetic pathway even though this rate is slow in vitro at pH 7.5 (Table II). An alternative idea, currently under investigation is that the cell contains a mevalonolactone hydrolase.

An inverse correlation between the rate of cholesterolgenesis and the cholesterol concentration in the liver was first reported by Gould and Swyrly (10). More recently Harry et al. (8) and Edwars and Gould (9) demonstrated that cholesterol feeding resulted in decreased reductase levels and increased concentrations of cholesteryl esters in the liver microsomes.

Mevalonolactone feeding resulted in both a rapid decline in reductase activity and a rapid increase in total liver cholesterol and in the microsomal cholesteryl esters (Tables IV and V). The consistent finding that microsomal sterol esters are enhanced after lactone feeding (Tables IV and V) may indicate that these esters have a specific role in regulation of reductase activity. The increase in liver cholesterol content 5.5 h after incubation of 400 mg of lactone (+0.5 mg/g of tissue) (Table IV) is consistent with approximately 5% of the (R)-mevalonolactone administered being converted in cholesterol and retained in the liver.

Reductase levels declined by more than 85% 45 min after intact rats were given 400 mg of mevalonolactone (Table V). The lactone must result in increases rates of enzyme inactiva-
tion, since the half-life of degradation of the enzyme is reported to be between 2 and 4 h (5, 9, 32).

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