In Vitro and in Vivo Phosphorylation of Rat Liver 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase and Its Modulation by Glucagon*

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Rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34) has been purified to homogeneity by a rapid procedure which employs HMG-CoA affinity chromatography. The purification of HMG-CoA reductase from solubilized enzyme can be completed in less than 24 h with a yield of 35%. Isolated HMG-CoA reductase migrated as a single band on sodium dodecyl sulfate gel electrophoresis with an apparent molecular weight of 51,000 ± 1,800. Antibodies prepared against purified HMG-CoA reductase gave a single immunoprecipitin line with microsomal, partially purified, and purified HMG-CoA reductase when analyzed by Ouchterlony immunodiffusion.

The in vitro phosphorylation of HMG-CoA reductase was studied utilizing a purified enzyme system containing electrophoretically homogeneous HMG-CoA reductase and reductase kinase. With this system, phosphorylated HMG-CoA reductase contained approximately 4 mol of phosphate/tetramer of 200,000 molecular weight. Purified 32P-labeled HMG-CoA reductase could be dephosphorylated with a phosphoprotein phosphatase.

To demonstrate that HMG-CoA reductase undergoes phosphorylation in vivo, rats were injected with 32P and hepatic HMG-CoA reductase isolated by immunoprecipitation with a monospecific antibody to HMG-CoA reductase and by purification of the enzyme to homogeneity. Analysis of 32P-labeled immunoprecipitates and purified HMG-CoA reductase by sodium dodecyl sulfate electrophoresis revealed a single peak of radioactivity co-migrating with purified HMG-CoA reductase establishing that HMG-CoA reductase can undergo phosphorylation in vivo.

Glucagon administration in vivo resulted in a 10- to 12-fold increase in hepatic cyclic AMP content with no change in [32P]ATP specific activity, a 2-fold increase in 32P incorporation into HMG-CoA reductase, and a 35 to 40% decrease in enzymic activity.

The enzymic activity of microsomal reductase kinase increased 2-fold following glucagon administration. Dephosphorylation of reductase kinase was associated with loss of enzymic activity.

These combined results represent the initial demonstration of the modulation of the in vivo phosphorylation of hepatic HMG-CoA reductase and suggest that phosphorylation-dephosphorylation of HMG-CoA reductase is an important physiological mechanism for the short term regulation of cellular cholesterol biosynthesis.

We have reported previously that the catalytic activity of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34), the rate-limiting enzyme of cholesterol biosynthesis, can be modulated in vitro by a phosphorylation-dephosphorylation reaction mechanism (1). In those studies, we utilized partially purified HMG-CoA reductase and reductase kinase for analysis of the phosphorylation reaction. To demonstrate the phosphorylation of HMG-CoA reductase, partially purified enzyme was radiolabeled with 32P, and the 32P-labeled HMG-CoA reductase isolated by immunoprecipitation with a monospecific antibody prepared against chicken liver HMG-CoA reductase. The radiolabeled immunoprecipitated enzyme had an identical electrophoretic migration as purified HMG-CoA reductase when analyzed by sodium dodecyl sulfate electrophoresis (1).

In the present report, we describe an extremely simple and rapid method for the purification of rat liver HMG-CoA reductase and demonstrate the phosphorylation of purified HMG-CoA reductase by reductase kinase. In addition, we also present evidence that hepatic HMG-CoA reductase undergoes phosphorylation in vivo. The in vivo phosphorylation of HMG-CoA reductase was shown to be increased by glucagon administration indicating that polypeptide hormones may modulate the enzymic activity and degree of phosphorylation of hepatic HMG-CoA reductase.

EXPERIMENTAL PROCEDURES

HMG-CoA reductase kinase was purified to homogeneity from rat liver microsomes (2); phosphoprotein phosphatase was partially purified according to the method of Brandt et al. (3); HMG-CoA, agarose-hexane-HMG-CoA, and ATP were purchased from P-L Biochemicals; [γ-32P]ATP, [32P]phosphoric acid, and [14C]HMG-CoA from New England Nuclear; NADPH, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and NADP from Sigma. Glucagon was a gift from Eli Lilly and Co.

HMG-CoA reductase activity was determined as described earlier (1, 2) except that the incubation mixture contained bovine serum albumin (5 mg/ml), 0.2 M KCl, and 0.15 M potassium phosphate (pH 6.9). Antibodies to purified rat HMG-CoA reductase (500 μg, two injections) were raised in a goat by intradermal injection. The γ-globulin fraction of the antiserum was isolated by the method of Steinbaugh and ABrand (4). Immunodiffusion was performed in 1% agar gel (Hyland immunodiffusion plates). Polyacrylamide gel elec-

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The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SDS, sodium dodecyl sulfate.

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In vivo 32P-labeled HMG-CoA reductase was isolated from solubilized hepatic microsomal membranes obtained from rats injected with 32P. The solubilized radiolabeled protein samples were incubated with antisera prepared against purified HMG-CoA reductase (antibody concentration 2-fold greater equivalence) for 30 min and 18 h at 37°C and 4°C, respectively, and the samples centrifuged (1500 rpm, 45 min). The immunoprecipitates were washed three times with 0.9% NaCl, dissolved in 10 mM Tris-HCl (pH 8.0) containing 1% SDS, 40 mM dithiothreitol, 1 mM EDTA, 10 μg/ml of pyronin Y, incubated for 45 min at 70°C, and analyzed by SDS-gel electrophoresis (5.6% acrylamide, pH 7.4) (7). Following electrophoresis gels were either sliced (1- to 2-mm sections) and 32P radioactivity determined or stained for protein with Coomassie blue. The migration of the radiolabeled protein was compared to the electrophoretic position of purified HMG-CoA reductase and other proteins of known molecular weight.

Male Sprague-Dawley rats, used for isolation of HMG-CoA reductase, were housed for 3 weeks in a light-controlled room (dark cycle, 6:30 a.m. to 6:30 p.m.). The animals had free access to food (Purina Chow) and water. Rats were decapitated at 12:30 p.m. and hepatic microsomes isolated as described earlier (1).

RESULTS

Purification of HMG-CoA Reductase—HMG-CoA reductase was solubilized from rat liver microsomes as described earlier (8) except Buffer A contained glycerol as reported by Edwards et al. (9). The solubilized enzyme (198 mg) was fractionated with ammonium sulfate (35 to 50%). The precipitate (64 mg) was dissolved in 5 ml of 50 mM KCl, 40 mM potassium phosphate, 30 mM EDTA, 0.1 mM sucrose, and 2 mM dithiothreitol, pH 7.2 (Buffer A), containing 1 mM KCl and 30% glycerol. The solution was heated at 65°C for 30 min and centrifuged (15,000 × g). The supernatant was diluted (2-fold) with Buffer A and precipitated with ammonium sulfate (0 to 50%). This precipitate (5 mg) was dissolved in 50 ml of diluted 1:1 Buffer A containing 20% glycerol (Buffer B) and applied to an affinity column of HMG-CoA (1.2 × 2.5 cm), equilibrated with Buffer B at room temperature. The column was washed with 50 ml of Buffer A containing 20% glycerol followed by 25 ml of Buffer A containing 200 μM HMG-CoA. All HMG-CoA reductase activity was confined to the fractions eluted with HMG-CoA. The enzyme was concentrated (0.2 mg/ml) and stored in Buffer A containing 50% glycerol at −70°C.

A summary of the purification scheme for rat liver HMG-CoA reductase is shown in Table I. HMG-CoA reductase was purified approximately 6,000-fold with an overall yield of 35%.

This procedure can be completed within 24 h. Aqueous polyacrylamide gel electrophoresis of purified HMG-CoA reductase demonstrated a single electrophoretic band (Fig. 1A), and analysis of enzymic activity in gel slices (2 mm) revealed that greater than 98% of the reductase activity was associated with the single protein band. On SDS-gel electrophoresis purified HMG-CoA reductase migrated as a single electrophoretic band with a monomer molecular weight of 51,000 ± 1,800 (Fig. 1B). An antibody prepared in a goat against purified rat liver HMG-CoA reductase formed a single immunoprecipitin line of identity with microsomal, solubilized, and purified HMG-CoA reductase (Fig. 1C).

The purified enzyme had a specific activity of 5,205 nmol/min/mg of protein, and an apparent Kₐ for D-HMG-CoA and NADPH of 0.86 μM and 38 μM, respectively. The specific activity of HMG-CoA reductase purified by this procedure from cholestyramine-fed rats (5 weight % diet, 3 days) was higher than cycled normal rats and ranged from 10,000 to 15,000 nmol/min/mg of protein.

Inactivation and Reactivation of Homogeneous Rat HMG-CoA Reductase—When electrophoretically homogeneous HMG-CoA reductase was incubated with purified reductase kinase, 4 mM ATP plus 10 mM MgCl₂, a time-dependent inactivation of reductase activity was observed (Fig. 2). Incubation of inactivated HMG-CoA reductase with phosphoprotein phosphatase was associated with a time-dependent increase in enzymic activity of HMG-CoA reductase (Fig. 2). These results are consistent with inactivation-reactivation of microsomal HMG-CoA reductase reported earlier (1).

In Vitro Phosphorylation of Homogeneous Rat HMG-CoA Reductase—Purified HMG-CoA reductase did not undergo phosphorylation in the presence of ATP plus MgCl₂ indicating that the isolated enzyme did not undergo autophosphorylation and that HMG-CoA reductase had been purified free of contamination with reductase kinase. This is in contrast to purified rafte kinase which readily undergoes autophosphorylation (data not shown). Incubation of purified HMG-CoA reductase with reductase kinase and [γ-32P]ATP plus MgCl₂ was associated with a time-dependent increase in protein-bound radioactivity and decrease in enzymic activity. The inhibition of enzymic activity was dependent on ATP concentration with a decrease in HMG-CoA reductase activity (30

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TABLE I

| Data presented are representative of five separate experiments. |

| Fraction                        | Protein | Activity | Specific Activity | Purification | Yield |
|---------------------------------|---------|----------|-------------------|--------------|-------|
| Microsomal suspension           | mg      | units    | units/mg          | fold%        | %     |
| Soluble fraction                | 3028    | 2516     | 0.83              | 1            | 100   |
| Ammonium sulfate precipitation  (35-50%) | 198     | 2164     | 10.9              | 13           | 86    |
| Heat treatment (65°C, 30 min)  + ammonium sulfate precipitation (0-50%) | 64      | 1761     | 27.5              | 33           | 70    |
| HMG-CoA affinity chromatography | 4.97    | 1432     | 288               | 347          | 57    |
|                                 | 0.171   | 890      | 5205              | 6271         | 35    |

*One unit is defined as the amount of enzyme that catalyzes 1 mmol of HMG-CoA to mevalonate in 1 min.

*Isolated from 100 g of liver.
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min) of 28% and 78% with 0.2 mM and 1 mM [γ-32P]ATP, respectively. To confirm that HMG-CoA reductase had been radiolabeled with 32P, 32P-labeled samples were analyzed by SDS-gel electrophoresis, and radioactivity (>98%) was associated with the single electrophoretic band of purified HMG-CoA reductase (Fig. 3). Assuming that HMG-CoA reductase is a tetramer of 200,000 molecular weight (lo), an average of 3.68 ± 0.56 (N = 8) mol of phosphate were incorporated/mol of tetramer. Treatment of 32P-labeled HMG-CoA reductase

Fig. 2. Inactivation and reactivation of homogeneous HMG-CoA reductase. Inactivation, incubations were performed at 37°C in duplicate in 100 μl containing 50 mM β-glycerophosphate (pH 7.0), 10 mM dithiothreitol, 4 mM ATP, 10 mM MgCl2, and purified HMG-CoA reductase (0.01 mg/ml). The reaction was started by the addition of highly purified reductase kinase (0.6 mg/ml). Control incubations contained no ATP. At the indicated times EDTA and components of reductase assay (70 μl) were added and HMG-CoA reductase activity determined (●). Reactivation, at the end of 30 min of inactivation 30 mM EDTA was added, the incubation mixture (100 μl) diluted to 200 μl with 50 mM imidazole, 5 mM EDTA, 1 mM dithiothreitol buffer (pH 7.4), and dephosphorylation initiated by the addition of 0.15 mg of phosphoprotein phosphatase (1). At the indicated time intervals 50 mM NaF was added and HMG-CoA reductase activity determined. Control incubations contained phosphoprotein phosphatase plus 50 mM NaF (○).

Fig. 3. SDS-gel electrophoresis of purified HMG-CoA reductase phosphorylated in vitro. Purified HMG-CoA reductase was incubated for 1 h with 4 mM MgCl2 + 0.2 mM [γ-32P]ATP in a medium containing 2 mM dithiothreitol, 50 mM β-glycerophosphate (pH 7.0), and purified reductase kinase (0.8 mg/ml). Following incubation 1% SDS, 40 mM dithiothreitol, 1 mM EDTA, 10 μg of pyronin Y, and 10 mM Tris-HCl (pH 8.0) were added, and the reaction mixture heated at 70°C for 30 min. The samples were then analyzed by SDS-polyacrylamide gel electrophoresis (7). The gels were either sliced into 2-mm sections and 32P radioactivity determined or stained for protein with Coomassie blue. The electrophoretic band in the reference gel denotes the position of the 51,000-molecular weight subunit of HMG-CoA reductase.

Fig. 4. SDS-disc gel electrophoresis of [32P]HMG-CoA reductase labeled in vivo. Light-dark cycled rats (N = 4 per group) were injected intraperitoneally with 1 mCi of carrier-free 32P at 8:45 a.m. and 9:45 a.m. At 9:15 a.m., saline (control) and glucagon (400 μg) were injected subcutaneously. At 10:00 a.m. the rats were anaesthetized and received an additional intraperitoneal injection of saline (control) or glucagon (200 μg). The livers were removed from the animals at 10:15 a.m. Panel A, hepatic HMG-CoA reductase was purified from control and glucagon-treated rats through the heat treatment step (Table I) and dialyzed against 10 mM K2HPO4 buffer (pH 7.4) containing 0.2 mM dithiothreitol. [32P]HMG-CoA reductase was immunoprecipitated utilizing a monospecific antibody and the immunoprecipitates washed and dissolved in buffer containing SDS as described under "Experimental Procedures." The 32P-labeled immunoprecipitates of control (○) and glucagon-treated (●) rats were analyzed by SDS-gel electrophoresis (5.6% acrylamide) in a Tris-sodium acetate-EDTA-SDS (pH 7.4) system (7). No radioactivity was detected in the immunoprecipitates when nonimmune goat serum was employed. The electrophoretic mobility of purified HMG-CoA reductase is shown in the gel inset. Panel B, hepatic HMG-CoA reductase was purified to homogeneity by affinity chromatography as described under "Experimental Procedures." Radio-labeled HMG-CoA reductase protein, and enzymic activity eluted from the affinity column as a single symmetrical peak. The ratio of specific activity of HMG-CoA reductase from control and glucagon-treated rats was constant throughout the various steps of the purification procedures. Recovery of enzymic activity during purification from control and glucagon-treated rats averaged 32% (three experiments).

In Vivo Phosphorylation of HMG-CoA Reductase and Its Modulation by Glucagon—In order to demonstrate that HMG-CoA reductase undergoes phosphorylation in vivo rats were injected with 32P, liver microsomes isolated, and HMG-CoA reductase was either partially purified and immunoprecipitated or purified to homogeneity by affinity chromatography as described under "Experimental Procedures." Radiolabeled HMG-CoA reductase protein, and enzymic activity eluted from the affinity column as a single symmetrical peak. The ratio of specific activity of HMG-CoA reductase from control and glucagon-treated rats was constant throughout the various steps of the purification procedures. Recovery of enzymic activity during purification from control and glucagon-treated rats averaged 32% (three experiments).

Examination of the 32P-labeled immunoprecipitates and purified enzyme by SDS-gel electrophoresis revealed a single

(0.98 mg/ml) with phosphoprotein phosphatase (1.18 mg/ml) resulted in a release of the 32P radioactivity (>90%).

In Vivo Phosphorylation of HMG-CoA Reductase and Its Modulation by Glucagon—In order to demonstrate that HMG-CoA reductase undergoes phosphorylation in vivo rats were injected with 32P, liver microsomes isolated, and HMG-CoA reductase was either partially purified and immunoprecipitated or purified to homogeneity by affinity chromatography as described under "Experimental Procedures." Radio-labeled HMG-CoA reductase protein, and enzymic activity eluted from the affinity column as a single symmetrical peak. The ratio of specific activity of HMG-CoA reductase from control and glucagon-treated rats was constant throughout the various steps of the purification procedures. Recovery of enzymic activity during purification from control and glucagon-treated rats averaged 32% (three experiments).

Examination of the 32P-labeled immunoprecipitates and purified enzyme by SDS-gel electrophoresis revealed a single
peak of radioactivity migrating with an apparent molecular weight of 51,000 (±1,800) coincident with the migration of purified HMG-CoA reductase (Fig. 4 A and B). No immunoprecipitable radioactivity was detected on the gel when 32P-labeled HMG-CoA reductase was incubated with nonimmunized goat serum. These results establish that HMG-CoA reductase can undergo phosphorylation in vivo.

The effect of glucagon on the enzymic activity and extent of phosphorylation of HMG-CoA reductase was determined in rats injected with 32P and glucagon (1.5 mg/kg). The administration of glucagon was associated with a 35 to 40% decrease in the enzymic activity of HMG-CoA reductase, and a 10- to 12-fold increase in hepatic cyclic AMP content (Table II). Dephosphorylation of HMG-CoA reductase from control and glucagon-treated rats resulted in an increase in total enzymic activity to nearly identical levels. 32P-labeled HMG-CoA reductase was isolated by immunoprecipitation from partially purified enzyme from liver microsomes of rats injected with 32P and glucagon as outlined above. A comparison by SDS-gel electrophoresis of the 32P radioactivity of immunoprecipitated HMG-CoA reductase from control and glucagon-treated rats is illustrated in Fig. 4A. These findings were substantiated by the purification of hepatic HMG-CoA reductase to homogeneity from control and glucagon-injected rats (Fig. 4B). A single peak of 32P radioactivity coincident with purified HMG-CoA reductase was found when the samples were analyzed by SDS-gel electrophoresis (Fig. 4B). The incorporation of 32P radioactivity into hepatic HMG-CoA reductase was 2-fold greater in glucagon-treated than control rats (Table II, Fig. 4B). No change in hepatic ATP specific activity following glucagon administration was observed (Table II).

The ATP-dependent inactivation of the enzymic activity of HMG-CoA reductase by microsomal reductase kinase was increased approximately 2-fold in glucagon-treated rats as compared to control (Table III). Treatment of active (phosphorylated) reductase kinase with phosphoprotein phosphatase was associated with loss of enzymic activity and ability to inactivate HMG-CoA reductase (Table III).

**Table II**

| Group         | Cyclic AMP | ATP specific activity | Microsomal HMG-CoA reductase activity | 32P-Labeled HMG-CoA reductase |
|---------------|------------|-----------------------|--------------------------------------|-------------------------------|
|               | nmol/g     | dpm/pmol              | nmol/min/mg                           | dpm/mg                        |
| Saline        | 0.59 ± 0.18| 14.8 ± 1.3            | 0.132 ± 0.014                         | 15,250 ± 2,431                |
| Glucagon      | 4.98 ± 0.85| 13.9 ± 1.4            | 0.080 ± 0.009                         | 31,675 ± 1,890                |

* Representative data of eight separate experiments in which a total of 32 rats were used to determine the effect of glucagon on the enzymic activity of HMG-CoA reductase.

**Table III**

Effect of glucagon on enzymic activity of microsomal reductase kinase

Rats were injected with saline or glucagon as outlined in Fig. 4. Hepatic microsomes were isolated in a buffer containing 5 mM EDTA and 50 mM NaF, as described under Table II. Microsomal reductase kinase was prepared as described (2). For the preparation of dephosphorylated (inactive) reductase kinase, NaF was removed by dialysis against 50 mM imidazole, 5 mM EDTA, 1 mM dithiothreitol (pH 7.4) buffer and preincubated in the presence of phosphoprotein phosphatase for 37°C for 1 h (2). Control samples contained phosphoprotein phosphatase plus NaF. Active reductase kinase (0.3 mg) and phosphoprotein phosphatase-inactivated reductase kinase (0.6 mg) was preincubated at 37°C for 20 min in 200 µl containing microsomal HMG-CoA reductase (0.2 mg), 50 mM β-glycerophosphate (pH 7.0), 5 mM dithiothreitol, 50 mM NaF with or without ATP plus MgCl2 at the end of preincubation, HMG-CoA reductase activity was determined as described under "Experimental Procedures." Data represents the mean ± S.E. for results obtained from four animals in each experimental group.

**DISCUSSION**

The purification of solubilized HMG-CoA reductase from rat liver was achieved with a 12- to 16-h procedure employing affinity chromatography on HMG-CoA. The overall yield of approximately 35% is significantly higher than other published methods (10, 13-16). Ness et al. (13) independently reported the use of HMG-CoA affinity chromatography in the purification of HMG-CoA reductase. Their method, however, requires a time-consuming repetitive solubilization procedure and an additional step (Affi-Gel Blue chromatography) (13).

The present report describes the first demonstration of the in vitro phosphorylation of HMG-CoA reductase employing a system which utilizes electrophoretically homogeneous HMG-CoA reductase and reductase kinase. The demonstration and quantitation of the extent of phosphorylation of HMG-CoA reductase by a system which uses purified HMG-CoA reductase and reductase kinase eliminates several of the limitations encountered in the microsomal system (1, 17) (e.g. other specific (cAMP-dependent reductase (2) kinases, and nonspecific kinases, as well as sodium fluoride-resistant phosphatases, all of which may influence the degree of phosphorylation of HMG-CoA reductase).

Results obtained in the present study suggest that phosphorylation of HMG-CoA reductase occurs in vivo and may be important in the intracellular regulation of HMG-CoA reductase enzymic activity. In addition, the extent of phosphorylation and enzymic activity of hepatic HMG-CoA reductase were modulated in rats following glucagon administration. The increase in the degree of phosphorylation as well as
the decrease in enzymic activity of HMG-CoA reductase following glucagon administration was associated with an increase in intracellular concentrations of cyclic AMP, with no change in [32P]ATP specific activity. We have previously reported the presence of both a cyclic AMP-dependent and independent reductase kinase which catalyze the phosphorylation of HMG-CoA reductase and the reversible phosphorylation of reductase kinase (2). An elevation in cellular cyclic AMP concentration would be anticipated to enhance the phosphorylation of HMG-CoA reductase by the cyclic AMP-dependent reductase kinase. Furthermore, elevated cyclic AMP levels may also affect the activity of the phosphoprotein phosphatase inhibitory protein, thereby decreasing the phosphorylation of HMG-CoA reductase and reductase kinase as proposed earlier (2). Both these mechanisms would increase the phosphorylated or inactive form of HMG-CoA reductase, thereby decreasing intracellular cholesterol biosynthesis. However, other possible mechanisms by which glucagon may have been associated with an increased incorporation of [32P]P-labeled ATP may be anticipated to enhance the phosphorylation of HMG-CoA reductase and reductase kinase which catalyze the phosphorylation in a bicyclic cascade system (2). An elevation in cellular cyclic AMP concentration would be anticipated to enhance the phosphorylation of HMG-CoA reductase by the cyclic AMP-dependent reductase kinase. Furthermore, elevated cyclic AMP levels may also affect the activity of the phosphoprotein phosphatase inhibitory protein, thereby decreasing the phosphorylation of HMG-CoA reductase and reductase kinase as proposed earlier (2). Both these mechanisms would increase the phosphorylated or inactive form of HMG-CoA reductase, thereby decreasing intracellular cholesterol biosynthesis. However, other possible mechanisms by which glucagon may have been associated with an increased incorporation of [32P]P-labeled ATP cannot be ruled out definitely (for review see Ref. 18).

Recently, Ingebritsen et al. (19) reported changes in enzymic activity of HMG-CoA reductase and reductase kinase as well as cholesterol synthesis in in vitro suspension of rat liver cells following treatment with insulin and glucagon. Although the extent of phosphorylation of HMG-CoA reductase and reductase kinase was not demonstrated directly, the changes in enzymic activity correlate well with the in vivo data presented in the present report.

Recently, Brown et al. (20) proposed that changes observed in enzymic activity of HMG-CoA reductase isolated from rats subjected to long-term manipulation (e.g., prolonged feeding of cholesterol or cholesteryamine, fasting, and stress) were not attributable to changes in the degree of phosphorylation of the enzyme. These authors did not examine acute effects on enzyme activities and these findings are consistent with our earlier model (1) in which short-term regulation involves reversible phosphorylation, whereas long-term physiological modulation involves changes in the quantity of enzyme protein.

The combination of in vitro and in vivo results described in this report support our concept that the enzymic activity of HMG-CoA reductase and reductase kinase are modulated by reversible phosphorylation in a bicyclic cascade system (2). This mode of regulation may represent an important short-term mechanism for the regulation of cellular cholesterol biosynthesis (1, 2).

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