

*Abstracts**Regulation of HMG-CoA Reductase
July 7, 1979, Toronto, Canada*

HMG-CoA reductase II. Regulation of HMG-CoA reductase

HMG-CoA Reductase II, a scientific meeting devoted to current concepts and research on the regulation of HMG-CoA reductase, was held on July 7 in the Hotel Toronto, Toronto, Ontario, Canada. The designation "HMG-CoA Reductase II" recognizes our first meeting held in conjunction with the 1972 FASEB meeting in Atlantic City, NJ. A satellite meeting of the 11th International Congress of Biochemistry, HMG-CoA Reductase II was organized by V. W. Rodwell (Purdue University) and D. M. Gibson (Indiana University) and supported financially by grants from the International Congress of Biochemistry and from G. D. Searle and Co. of Chicago, IL. The organizers also acknowledge the cooperation of the editors of the *Journal of Lipid Research* for premeeting publicity and for printing these abstracts.

The abstracts summarize research presented in poster or oral form. Oral presentations, organized into four sessions, were chaired by H. Rudney (Cincinnati), J. R. Sabine (Adelaide), J. L. Nordstrom (Houston), and D. M. Gibson (Indianapolis). Attendance at the meeting numbered approximately ninety, and was international in character. So also was the science. Of the two dozen papers presented, slightly less than half were from the United States, three from Spain, two each from Canada, England, West Germany, and Japan, and one each from Australia and India. Discussion was spirited, and the meeting concluded with an open discussion of current problems in this area of research. The organizers thank all those who by their participation made this a meeting to remember.

(Reprints of these abstracts are NOT available.)

1. DEVELOPMENTAL PATTERN AND DIURNAL RHYTHM OF NEONATAL CHICK HMG-CoA REDUCTASE. M. J. Alejandre, H. Ramirez, J. L. Segovia, M. D. Suarez, and E. Garcia-Peregrin. *Dept. of Biochemistry, Univ. of Granada, Spain.*

HMGR was studied for the first time in developing avian tissues. Microsomes from brain, liver, and intestine from newly hatched chicks were used for enzyme preparations.

The developmental pattern of HMGR showed no significant differences in chick brain and intestine, remaining more or less constant up to 10 days and declining thereafter. However, very little MVA was formed in liver until about 4 days of life; then there was a sharp increase up to 10 days, followed by a less pronounced increase until 20 days. In chicks exposed to a normal lighting schedule (12 hr light, 12 hr dark) and fed ad libitum, HMGR activity of liver and of intestine was minimal during the dark phase and attained peak values near the midpoint of the light phase. No diurnal rhythm was observed in chick brain HMGR. The phase rhythms observed in chick liver and intestine were contrary to those reported for the same tissues of rat. However, as in the rat, the amplitude of oscillations was more pronounced in liver than in intestine.—Supported by a grant from the CAICT.

2. IN VITRO AND IN VIVO REGULATION OF HMGR AND REDUCTASE KINASE (RK) ACTIVITIES BY REVERSIBLE PHOSPHORYLATION (PHOS)–DEPHOSPHORYLATION (DEPHOS). Zafarul H. Beg, John A. Stonik, and H. Bryan Brewer, Jr. *Molecular Disease Branch, NIH, Bethesda, MD.*

Rat liver HMGR can be modulated in vitro by a Phos–Dephos reaction mechanism (Beg et al. 1978. *Proc. Natl. Acad. Sci.* **75**: 3678). The microsomal enzyme RK, which catalyzes the Phos of HMGR, was purified to homogeneity utilizing absorption chromatography, gel filtration, and thin-layer isoelectric focusing ($P_1 = 5.6 \pm 0.2$). Purified RK migrated as a single band on aqueous and SDS gel electrophoresis, and had a monomeric and oligomeric molecular weight of 65,000 and 380,000, respectively. Incubation of phosphatase-treated RK with [32 P]ATP and Mg^{2+} and a cytosolic protein kinase, reductase kinase kinase (RKK), resulted in a time-dependent increase in radioactivity and ability to Phos (inactivate) HMGR. Incubation of 32 P-labeled RK with phosphatase was associated with a loss of 32 P and a decrease in ability to Phos HMGR. Analysis of 32 P-labeled RK by gel electrophoresis revealed all radioactivity to be in the position of the protein band and enzymatic activity of purified RK.

Thus, two protein kinases, RK and RKK, are involved in a bicyclic cascade system modulating the catalytic activity of HMGR. SDS gel electrophoresis of purified ^{32}P -labeled HMGR isolated from rats injected with ^{32}P confirmed in vivo Phos of HMGR. In vivo Phos of HMGR was significantly stimulated by glucagon. These results strongly support the concept that HMGR activity is regulated in vivo by a Phos-Dephos mechanism.

3. INTERCONVERTIBLE FORMS OF HMGR IN VITRO AND THE IN VIVO ACTIVITY OF THE ENZYME. J. Berndt, R. Henneberg, and M. Löwel. *Gesellsch. f. Strahlen- und Umweltforschung, Abtlg. für Zellchemie, München, West Germany.*

Microsomal (or solubilized) HMGR in vitro could be inactivated by ATPMg and reactivated by a cytosolic protein (0–40% AS). F^- (50 mM) completely inhibited the reactivation. To study the in vivo activity of HMGR, livers from rats (rhythm peak or nadir, fasted (40 hr) or cholesterol-fed (16 hr)) were homogenized *a*) at 0° , *b*) at $0^\circ + \text{F}^-$, *c*) at 25° , or *d*) at $25^\circ + \text{F}^-$. HMGR in the microsomes was inactivated and then reactivated. There was no difference in the activities between *a*, *c*, and *d*. Activities from *b* always were very low (10–20% compared to those of *a*, *c*, and *d*). HMGR from *b* could be inactivated only very little; HMGR from *a*, *c*, and *d* could be inactivated to the same level as could *b*. Inactivated *a*, *b*, *c*, and *d* could be reactivated to the original level of *a*, *c*, and *d*, but not substantially higher; *a*, *c*, and *d* could not be activated directly (no prior inactivation). From these experiments one has to conclude 1) HMGR is not activated during liver homogenization; 2) HMGR in vivo is to a high extent in its active (i.e., dephosphorylated) form; 3) HMGR activity found at $0^\circ + \text{F}^-$ most likely does not reflect the enzyme's activity in vivo.

4. REGULATION OF HMG-CoA REDUCTASE AS STUDIED USING SPECIFIC INHIBITORS. Akiro Endo. *Fermentation Laboratory, Tokyo Noko Univ., Fuchi-shi, Tokyo, Japan.*

ML-236B (compactin) is a specific competitive inhibitor of reductase (K_i 1.0×10^{-8} M). This drug produces a marked reduction of plasma cholesterol levels in hens, rabbits, dogs, monkeys, and humans, including patients with familial and nonfamilial hypercholesterolemia, at doses of 0.5–5 mg/kg, while no effects are detected in mice, rats, or hamsters at doses as high as 500–2000 mg/kg. Adenosine-2'-monophospho-5'-diphosphoribose, which was found to be a reversible inhibitor of reductase in this laboratory, inhibits the enzyme competitively with respect to NADPH and uncompetitively with respect to HMG-CoA. By using these two specific inhibitors, the mechanism of action of reductase of rat liver was studied. The results indicate that the two substrates bind to the enzyme effectively in an ordered manner; HMG-CoA and reductase first interact to make a binary complex, which, in turn, forms a ternary complex with one molecule of NADPH. Considered together with the results of the product inhibition study, and assuming that a hemithioacetal of mevaldate and CoA is an intermediate of the reaction, a bi uni uni ter ping pong mechanism is proposed as a model of the overall reaction.

5. SYNCHRONOUS REGULATION OF MICROSOMAL ENZYMES OF CHOLESTEROL BIOSYNTHESIS. J. L. Gaylor, J. T. Billheimer, M. A. Longino, and J. M. Trzaskos. *Univ. of Missouri, Columbia, MO 65211.*

Activities of HMGR and the microsomal enzymes that catalyze the biosynthesis of cholesterol from lanosterol vary in concert. The effects are not mediated by phosphorylation-dephosphorylation. The changes depend upon two cytosolic proteins: a neutral protein of MW 12,400, which is identical to Z-protein of liver, and a basic protein of MW 11,300, which is identical to SCP₂ described by Scallen and co-workers. Neutral protein synchronously stimulates specific activities of several microsomal enzymes of cholesterol biosynthesis, binds a wide variety of substances such as fatty acids, acyl-CoA, HMG-CoA, mevalonate, and heme, and associates with membranes. In the presence of the neutral protein, fatty acids are inhibitory and the oxidases are maximally stimulated by heme. Unlike neutral protein, the basic protein does not affect activities in the absence of ligands; however, when cholesterol is added, synchronous inhibition is observed. These effects are not observed with solubilized enzymes. Thus, metabolite-dependent modulation of activities must be associated with interactions of the enzymes, metabolites, and cytosolic proteins in the membrane. A general mechanism for end-product inhibition by cholesterol esters in the membrane is being developed. The neutral protein is not present in the cytosol of rapidly growing Morris hepatomas. Lack of regulation of cholesterol biosynthesis in hepatomas and other tumors may be due to abnormal cytosolic rather than microsomal proteins.— Supported by grant AM-21336.

6. PURIFICATION AND PROPERTIES OF DIFFERENT HMG-CoA REDUCTASE PHOSPHATASES FROM RAT LIVER. Gregorio Gil, Mercedes Sitges, and Fausto G. Hegardt. *Dept. of Biochemistry, Univ. of Barcelona School of Pharmacy, Barcelona-28, Spain.*

From 105,000 *g* supernatant of rat liver homogenate, different phosphatases (Phases) with activating action on HMG-CoA reductase have been obtained. The purification process involved the following steps. 1) Chromatography on DEAE-cellulose. At this point, two different phosphatase activities eluted, the first (I) with 150 mM KCl and the second (II) with 200 mM KCl. 2) Both phosphatase pools were independently precipitated with ammonium sulfate, and 3) were chromatographed on gel filtration (BioGel A 0.5 m). The molecular weights were similar (about 200,000). With this procedure, the two Phases called Ia and IIa had been purified 420- and 770-fold, respectively. Other aliquots of the I and II preparations were treated independently with ethanol, then the precipitate was extracted with buffer and applied to a Sephadex column. The eluted fractions called Ib and II b have molecular weights of nearly 35,000. The fractions at this stage had been purified 940- and 1260-fold, respectively. The four preparations activated the inactive reductase. The kinetics of this activation depended on the concentration of Phases and on time.

7. REGULATION OF VERTEBRATE HMG-CoA REDUCTASE IN VITRO VIA REVERSIBLE MODULATION OF ITS CATALYTIC ACTIVITY. Carolyn F. Hunter and Victor W. Rodwell. *Dept. of Biochemistry, Purdue Univ., West Lafayette, IN 47907.*

We have investigated the comparative biochemistry of *in vitro* regulation of HMG-CoA reductase (EC 1.1.1.34) in microsomal preparations from livers of nine vertebrates (rat, mouse, gerbil, hamster, rabbit, pig, frog, chicken, and fish). In all instances, HMGR activity was rapidly and profoundly decreased by addition of MgATP. The protein(s) that mediates MgATP-dependent inactivation of HMGR thus appears to be present in liver tissue of all vertebrates. HMGR activities were restored to near or above initial levels following removal of MgATP and incubation with rat liver cytosol HMGR activator. Restoration of HMGR activity was blocked by NaF and P_i, known inhibitors of phosphoprotein phosphatase activity. Liver cytosol of species other than the rat exhibits HMGR activator activity. The enzymes that catalyze modulation of HMGR activity thus are ubiquitous in vertebrate liver. Most HMGR in vertebrate livers appears to be present in a catalytically inactive or latent form *in vivo*. For a given species, the fraction present in the active form appears to be quite constant from preparation to preparation. Species to species, from 20 to 45% of HMGR appears to be in the active form *in vivo*.—Supported by NIH grant HL 19223 and Indiana Heart Association grant 0851. C. F. Hunter was supported by a Minority Fellowship from Purdue University.

8. SHORT-TERM MODULATION OF HMG-CoA REDUCTASE BY REVERSIBLE PHOSPHORYLATION. Thomas S. Ingebritsen, Math J. H. Geelen, Rex A. Parker, and David M. Gibson. *Dept. of Biochemistry, Indiana Univ. School of Medicine, Indianapolis, IN 46223.*

We have demonstrated the presence in rat liver of a bicyclic system in which the activities of both HMG-CoA reductase (HMGR) and HMGR kinase (RK) are regulated by reversible phosphorylation. In the phosphorylated form HMGR is inactive while RK is active. RK is phosphorylated by a second kinase referred to as RK kinase (RKK). Dephosphorylation of both HMGR and RK is catalyzed by liver phosphorylase phosphatase (P). In the present studies, the properties of the HMGR modulating enzymes have been further characterized. Both RK and RKK were found to be predominantly cytosolic enzymes. The two kinases were separated by chromatography on DEAE-cellulose. RK and RKK activities were unaffected by either cAMP or the heat-stable protein kinase inhibitor protein. Dephosphorylation of both HMGR and RK by P was severely inhibited by the spontaneously active P inhibitor protein from rat liver and rabbit skeletal muscle. Dephosphorylation of HMGR and RK was also inhibited by a second heat-stable inhibitor protein from skeletal muscle; it is active only after phosphorylation by cAMP-dependent protein kinase. These studies further support the bicyclic model for regulation of HMGR and suggest an *in vivo* mechanism for regulation of the system by insulin and glucagon through the phosphoprotein phosphatase.—Supported by NIH grants AM 21278 and AM 19299 and the Showalter Foundation.

9. EFFECTS OF MEVALONOLACTONE AS A MODULATOR OF HMG-CoA REDUCTASE. Charlene Jett and James E. Miller. *Dept. of Biological Research, Searle Laboratories, Chicago, IL 60680.*

When mevalonolactone (M) is administered to male rats, liver HMG-CoA reductase (HMGR) activity can be reduced quickly in a dose-dependent fashion. To evaluate the *in vivo* effect of M on rat liver HMGR, two test systems were used to alter HMGR levels. In one model, rats were given a low-cholesterol diet enriched with sucrose (68%). In the second model, rats were given 20,25-diazacholesterol·HCl (DNC) at a dose of 5 mg/kg per day for 7 days to increase HMGR with concurrent desmosterol accumulation due to a block in cholesterol biosynthesis. If M were to simply act as a precursor of new cholesterol, HMGR activity would be expected to be sensitive to M in the sucrose-enriched diet while, in the DNC-treated rats, HMGR should show little response to M due to the predominate formation of desmosterol in this system. However, in both tests, M (100 mg/kg) decreased HMGR by 60% or more over concurrent control treatments. These results suggest that M exerts an effect on HMGR independent of the action of newly synthesized cholesterol. Additional studies with DNC and M were undertaken to determine effects on the activation state of HMGR. After treatment, livers were removed and sectioned for preparation of HMGR in the presence (Ra) and absence (Rt) of 50 mM NaF. Control rats had an activity ratio for HMGR of Ra/Rt = 0.25 while M-treated rats had a Ra/Rt = 0.46. An interpretation of the data is that M may directly influence the activation state of HMGR *in vivo*. The DNC system with M may allow further understanding of HMGR.

10. REGULATION OF CHOLESTEROL BIOSYNTHESIS IN ISOLATED HEPATOCYTES BY CHYLOMICRON REMNANTS. M. R. Lakshmanan, Richard A. Muesing, John C. LaRosa, and Richard L. Veech. *NIAAA, Rockville, MD 20852 and The George Washington Univ. School of Medicine, Washington, DC 20037.*

Dietary cholesterol (CH) is known to markedly suppress hepatic CH synthetic rate and has already been shown to inhibit HMGR activity (Higgins and Rudney, 1973. *Nature New Biol.* **246**: 60). Dietary CH enters the circulation primarily as chylomicrons. Nervi et al. (1975. *J. Biol. Chem.* **250**: 4145) showed that an intravenous infusion of chylomicrons to rats markedly inhibited hepatic CH synthesis after 12 hr *in vivo*. Since chylomicrons are partially hydrolyzed by extrahepatic lipoprotein lipase before being taken up by the liver, we have tested the undegraded chylomicrons from rat and human and the remnants of these chylomicrons (prepared *in vitro* by treatment with either postheparin plasma or with purified bovine milk lipoprotein lipase) for their ability to suppress CH synthetic rate in isolated hepatocytes from meal-fed rats. These hepatocytes had a CH synthetic rate of $0.052 \pm 0.004 \mu\text{mol acetyl units min}^{-1} \text{g}^{-1}$ on the basis of the incorporation of ³H from ³H₂O. Whereas the undegraded chylomicrons from either rat or human failed to inhibit CH synthesis, the remnants of these chylomicrons markedly inhibited CH synthesis during 1 hr of incubation with the hepatocytes. Thus, the chylomicron remnants from humans and rats caused 50 and 75% inhibition of CH synthesis at protein concentrations of 100 and 325 $\mu\text{g/ml}$ of incubation mixture, respectively. The effect of chylomicron remnants upon hepatocyte HMGR activity is currently under investigation.

11. REGULATION OF HAMSTER ADRENAL HMG-CoA REDUCTASE ACTIVITY. J-G. Lehoux and Benjamin Preiss. *Faculty of Medicine, University of Sherbrooke, Sherbrooke, Québec, Canada.*

Under physiological conditions, hamster adrenals possess a high HMG-CoA reductase activity (at 1900 hr, peak activity levels are about 50 nmol of mevalonate formed per 30 min) with a circadian rhythm coinciding with that of plasma corticosteroids. In contrast to the rat, adrenals of the hamster are relatively lacking in esterified cholesterol; this finding is particularly important since it is this fraction that fluctuates in response to stress in the rat. Administration of 4-aminopyrazolopyrimidine to hamsters resulted in decreased plasma cholesterol but did not provoke a reduction in adrenal cholesterol content or in any increase in HMG-CoA reductase activity. Combined treatment with ACTH and aminoglutethimide resulted in a significant increase in the adrenal free cholesterol content and in a decrease in HMG-CoA reductase activity. In conclusion, these observations suggest that 1) hamster adrenals are independent of exogenous cholesterol and 2) in this species, the endogenous free cholesterol of the gland may well be involved in regulating its own synthesis.—Supported by the Medical Research Council of Canada.

12. INHIBITION OF MICROSOMAL AND SOLUBLE HMG-CoA REDUCTASE BY OLEIC ACID AND OLEOYL CoA. G. M. Lehrer, S. R. Panini, D. H. Rogers, and Harry Rudney. *Dept. of Biological Chemistry, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267.*

Rat liver HMG-CoA reductase was inhibited by oleic acid and oleoyl CoA. Fifty percent inhibition occurred with 1.3 μmol of oleic acid or 0.059 μmol of oleoyl CoA per unit of enzyme activity. NADP, NADPH, or an NADPH-generating system could partially prevent the oleic acid inhibition when added prior to the inhibitor. Similarly, 10 μM HMG-CoA could prevent the oleoyl CoA inhibition completely but could only partially prevent the oleic acid inhibition. Treatment of the microsomes by the freeze-thaw technique, which solubilized the enzyme, had the effect of decreasing the inhibition at lower concentrations of oleic acid. On the other hand, the freeze-thaw supernatant fraction containing most of the enzyme activity was more sensitive to inhibition than the microsomes. The sensitivity to inhibitors increased with the degree of purification of the enzyme and the same protection phenomena, with prior substrate addition, were observed. These data show that membrane structure plays a minimal role in the inhibition and point to a regulatory mechanism involving a complex interplay of substrate levels and natural inhibitors which could be released by the action of enzymes on membrane and cytosolic lipids.—Supported by grants from USPHS, NIAMDD-12402 and NHLBI-20428.

13. THE EFFECT OF CYCLIC MONO- AND DI-TERPENES ON HMG-CoA REDUCTASE. Bruce Middleton, Richard Clegg, and David White. *Dept. of Biochemistry, Queen's Medical Centre, Nottingham NG7 2UH, England.*

Rowachol (Rowa Ltd., Bantry, Eire), a proprietary choleric comprising menthol, pinene, menthone, borneol, camphene, and cineole in olive oil dissolves cholesterol gallstones in man. Administration of Rowachol at a dose of 2

g/kg body weight to rats 17 hr prior to peak hepatic HMGR activity caused an immediate choleresis, decreased the biliary lithogenic index, and reduced peak hepatic HMGR activity by $60 \pm 5\%$. This inhibition of peak reductase activity persisted over at least two diurnal cycles. The individual terpenes were administered to rats at a dose of 3 mmol/kg body weight under the same time schedule. Menthol and cineole inhibited peak hepatic HMGR activity by $74 \pm 5\%$ while the related cyclic terpenes menthone and borneol inhibited reductase activity by $59 \pm 6\%$. Camphene and pinene had little or no effect on the peak activity of the enzyme. Menthol and cineole administered at the time of peak hepatic HMGR activity had no inhibitory effect on the enzyme when measured 2 hr after dosing.

14. THE RELATION BETWEEN CHANGES IN THE CONCENTRATION OF FREE CHOLESTEROL IN THE ENVIRONMENT OF HMGR AND ITS ACTIVITY IN THE LIVER MICROSOMAL FRACTION FROM RATS IN VARIOUS EXPERIMENTAL CONDITIONS. K. A. Mitropoulos. *Medical Research Council Lipid Metabolism Unit, Hammersmith Hospital, London W12 0HS, England.*

The activity of HMGR in the microsomal fraction is influenced by the fluidity of the endoplasmic reticulum membrane and effects of changes of the concentration of free cholesterol on membrane fluidity may be interpreted. Thus HMGR in the microsomal fraction could be used as a probe to detect changes in the concentration of free cholesterol in the enzyme's environment. The increased supply of cholesterol to the hepatocyte in rats that follows the administration of dietary cholesterol, the sharp and considerable increase in hepatic cholesterologenesis after the injection of a load of mevalonic acid, or the administration of unsaturated fat results in a considerable decrease in the activity of HMGR in liver microsomes. In all three conditions, changes in the characteristics of the Arrhenius plots of the enzyme are consistent with an increase in the concentration of free cholesterol in the environment of the enzyme. In contrast, conditions associated with increased efflux of cholesterol from the hepatocyte like the administration of a cholestyramine-supplemented diet, the ligation of the bile duct, or the intravenous injection of Triton WR-1339 are associated with a considerable increase in the activity of HMGR in the microsomal fraction. In all three conditions, changes in the characteristics of Arrhenius plots of the enzyme are consistent with a decrease in the concentration of free cholesterol in the environment of HMGR.

15. THE EFFECT OF INSULIN ON CULTURED HUMAN LYMPHOCYTES: EXAMINATION OF THE ACTIVITY OF 3-HYDROXY-3-METHYLGLUTARYL CoA REDUCTASE (HMGR) AND LOW DENSITY LIPOPROTEIN (LDL) RECEPTOR ACTIVITY. H. Nakayama, M. Keimatu, Y. Kurihara, N. Manda, T. Sasaki, and S. Nakagawa. *Second Dept. of Medicine and Second Dept. of Biochemistry, Hokkaido Univ., Sapporo, Japan.*

Human lymphocytes were grown in culture media that contained 10% fetal calf serum (FCS) or 10% lipid-deficient serum (LDS) for 24 and 48 hr with or without insulin (100–10,000 $\mu\text{U/ml}$). The HMGR activity in cells grown in FCS was extremely low, but in cells grown in LDS for 24 or 48 hr it was 17 and 49 pmol/min per mg protein, respectively.

Culturing cells in LDS with increasing amounts of insulin resulted in a dose-dependent increase in HMGR activity. LDL binding in the cells grown in LDS was increased to $44 \pm 13.8\%$ in the presence of insulin ($1000 \mu\text{U/ml}$) compared with controls ($n = 6, P < 0.01$). Insulin also increased LDL degradation (3 hr) ($33 \pm 6.8\%$, $n = 6, P < 0.001$). Large insulin doses ($10,000 \mu\text{U/ml}$) did not increase LDL binding and degradation. These results show that relatively small doses of insulin result in an increase in both HMGR activity and LDL receptor activity when lymphoid cells are grown in LDS.

16. REGULATION OF HOMOGENEOUS HMG-CoA REDUCTASE BY KINASE AND PHOSPHATASE. Gene C. Ness. *Dept. of Biochemistry, College of Medicine, Univ. of South Florida, Tampa, FL 33612.*

Studies from several laboratories have confirmed the original observations of Beg et al. (1973. *Biochem. Biophys. Res. Commun.* **54**: 1362) that rat liver microsomal reductase activity is decreased by incubation with MgATP. However, in studies with partially purified reductase, the degree of inactivation was always less than that seen with the microsomal enzyme. We have recently developed a procedure that consistently yields homogeneous reductase preparations (*Arch. Biochem. Biophys.* In press). Incubation of homogeneous reductase with MgATP and a partially purified preparation of kinase from rat liver cytosol resulted in up to 98% inactivation of reductase activity. Upon the addition of EDTA and a phosphatase preparation from liver cytosol, reductase activity was restored to twice the original activity. Inactivation of reductase required MgATP, depended on kinase concentration, was blocked by EDTA, did not occur with β, γ -methylene ATP but did occur with ADP. The ADP effect was likely due to the presence of adenylate kinase as addition of AMP markedly decreased the inactivation observed with ADP. The activity of purified homogeneous reductase was further increased by phosphatase. The activation by phosphatase was time- and concentration-dependent and could be blocked by KF. These findings give further support to the proposal that reductase activity is regulated by phosphorylation-dephosphorylation.—Supported in part by USPHS grant HL 18094.

17. HMG-CoA REDUCTASE ACTIVITY IN ADRENAL MITOCHONDRIA. Benjamin Preiss and Jean-Guy Lehoux. *Départements de Biochimie et de Gynécologie-Obstétrique, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.*

We have recently reported (1978. *Can. J. Biochem.* **56**: 958) that beef adrenal mitochondrial fractions contained a low level of reductase activity, 18 pmol/mg protein per min, which rose to 31 pmol/mg per min after purification on a sucrose gradient. Mitochondrial pellets from hamster adrenal homogenates were found to contain much higher reductase activities. The crude mitochondrial fraction from hamster adrenals was further purified on a linear sucrose gradient (24–56% w/v). Three fractions were obtained, ordered by increasing density, containing the following specific activities of HMGR in nmol/mg protein per min: 0.15, 0.08, and 0.27. The heaviest band also contained all the measurable cytochrome *c* oxidase activity in the gradient and was enriched 1.9-fold in HMGR compared to the crude mitochondrial pellet. No HMGR activity could be detected

in rat adrenal mitochondria, while hamster adrenal microsomes had about 55 times the specific HMGR activity of rat microsomes at 11:00 AM.—Supported by the Banting Research Foundation.

18. PREVENTION OF INACTIVATION BY ATP-Mg OF HMG REDUCTASE. T. Ramasarma, Rajan George, and A. S. Menon. *Dept. of Biochemistry, Indian Institute of Science, Bangalore 560012, India.*

The inactivation of HMG-CoA reductase on preincubating rat liver microsomes with ATP-Mg (1 mM) could be prevented competitively when adenosine, 5'AMP, cyclic AMP, or Ca^{2+} (10 mM) was added in the preincubation mixture. The inactivation effect of ATP-Mg was lost on heating microsomes at 50°C for 10 min.

19. IN VITRO PHOSPHORYLATION OF 3-HYDROXY-3-METHYLGLUTARYL-CoA (HMG-CoA) REDUCTASE: ANALYSIS OF ^{32}P -LABELED INACTIVATED ENZYME. David H. Rogers, Michael L. Keith, Victor W. Rodwell, and Harry Rudney. *Dept. of Biochemistry, Purdue Univ., West Lafayette, IN 47907 and Dept. of Biological Chemistry, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267.*

Rat liver microsomal HMG-CoA reductase (EC 1.1.1.34) has been shown to be inactivated in vitro by the addition of MgATP. Reactivation is effected by incubation with various cytosolic fractions or phosphatases and can be blocked by inhibitors of phosphoprotein phosphatase activity. Microsomes were incubated with sufficient $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ to insure that, if labeling of reductase protein occurred, it could be detected. The inactivated reductase was solubilized and purified to homogeneity. The purified, inactivated reductase contained between 1 and 4 mol of phosphate per mol of tetramer. The ^{32}P radioactivity was immunoprecipitable by antibody to pure rat liver reductase. The ^{32}P radioactivity comigrated with reductase activity and with protein on disc gel electrophoresis and with reductase monomer on SDS gels. This provides direct evidence that inactivation of reductase by MgATP results in the covalent modification of reductase by attachment of the terminal phosphate of ATP.—Sponsored by grants from the American Heart Association, Indiana Affiliate, and the USPHS (NIAMDD-12402, NHLBI-20428, and NHLBI-19223).

20. INTESTINAL HMGR REGULATION IN VITRO: ADVERSE EFFECTS OF CHOLESTEROL (C), BILE ACIDS (BA), AND LIPOPROTEINS (LP). A. Schneider, E. F. Stange, M. Alavi, H. Ditschuneit, and J. R. Poley. *Dept. of Internal Medicine and Dept. of Pediatrics, Univ. of Ulm, Federal Republic of Germany.*

Recently we suggested C as principal regulator of HMGR in rabbit intestine (GE 76, 5/2, 1254). Here we report on alternative regulators: C and/or BA and LP. *Methods.* Ileal mucosa of NZW rabbits was organ cultured for 24 hr (JCI 48, 1423). Viability was insured by linear incorporation of ^3H thymidine and ^{14}C leucine in DNA and protein, respectively. HMGR was measured before and after culture in media containing C, BA, mixed micelles of both, and lipoproteins. *Results.* HMGR increased during the culture period in lipid-free medium by 129%. 0.05 mM C had no effect, 0.25 and 0.5 mM suppressed HMGR by 22 and 47%, respectively ($P < 0.01$). However, glycodeoxycholic and glycocholic acids increased HMGR at 1 mM by 104%, and at 5 mM by 176% ($P < 0.01$). At higher concentrations both HMGR

and sucrase/alkaline phosphatase were suppressed, indicating toxicity. This BA-induced increase was completely reversible by adding 0.5 mM C in mixed micellar form. 0.2 mM LDL-C decreased HMGR by half, whereas VLDL and HDL stimulated up to 4-fold. *Conclusion.* Free, mixed micellar, and lipoprotein-bound C regulate intestinal HMGR effectively. The effect of bile acids alone is presumably due to their detergent action and is probably not physiological.

21. MEMBRANE-MEDIATED CONTROL OF HMG-CoA REDUCTASE (HMGR) AND THE REGULATION OF HEPATIC CHOLESTEROL SYNTHESIS. A. B. Sipat and John R. Sabine. *Univ. of Adelaide, Australia.*

Previous work from this and other laboratories suggests that the physico-chemical state (principally fluidity) of the membranes of the endoplasmic reticulum is a critical regulatory factor in the physiological control of hepatic HMGR. This has been designated 'membrane-mediated control'. We can now report several further aspects of this control mechanism. 1) It appears to be specific for HMGR, in that the activities of other microsomal enzymes show no response to changes in membrane fluidity. 2) It appears to be restricted to dietary feedback control, in that other physiological controls, e.g., fasting, diurnal rhythm, seem not to influence membrane fluidity. 3) In the feedback situation it begins to operate not until some time after gross enzyme activity has been depressed. 4) At lower assay temperatures, standard Michaelis-Menten kinetics do not apply to HMGR. Since membrane-mediated control has been so far the only molecular mechanism proposed to explain physiological regulation of HMGR in the liver, and since regulation of HMGR is critically defective in the liver tumor, further work in this area should provide additional insights into the control of and importance of cholesterol synthesis in health and disease.

22. PURIFICATION OF A HEAT-STABLE INHIBITOR OF HMG-CoA REDUCTASE PHOSPHATASE FROM RAT LIVER. Mercedes Sitges, Gregorio Gil, and Fausto Hegardt. *Dept. of Biochemistry, Univ. of Barcelona School of Pharmacy, Barcelona-28, Spain.*

The occurrence of an inhibitor that acts on the HMG-CoA reductase phosphatase (Phase) could be demonstrated during the purification of this last enzyme from rat liver. During the DEAE chromatography of rat liver homogenate in order to purify the phosphatase (see accompanying abstract), a 5-fold activation (disinhibition) of the total Phase could be seen. The purification procedure of this heat-stable inhibitor involves several steps: 1) a heat treatment at 90°C for 10 min of the 50,000 g supernatant of rat liver homogenate; 2) precipitation with ammonium sulfate of the resulting supernatant; 3) chromatography on DEAE-cellulose; 4) precipitation with ammonium sulfate; and 5) gel filtration on Sephadex G-100. This process yielded a 920-fold purification. The purified inhibitor was active with four different preparations of the Phases. The kinetics of time-dependent inhibition depends on the concentration of both Phases and inhibitor. The role of the inhibitor on the cholesterol biosynthesis is under current investigation.

23. LIPID-PROTEIN INTERACTIONS IN THE REGULATION OF HMG-CoA REDUCTASE. Vana L. Smith, Lynn G. Brent, and Richard E. Thompson. *Dept. of Chemistry, North Texas State Univ., Denton, TX 76203.*

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is unique in the early part of the hepatic cholesterol biosynthetic pathway in that it is membrane-bound. Furthermore, both enzyme activity and the lipid composition of the endoplasmic reticulum to which the enzyme is bound have been shown to vary as a function of diet, drug therapy, and disease state. The experiments reported here utilize the temperature dependence of enzyme activity as a probe of HMGR-lipid interactions and all measurements were done using microsomal enzyme from Sprague-Dawley-derived rats which were fed Purina rat chow, rat chow supplemented 10% with coconut oil or 2% with cholestyramine, or a low-fat, high-carbohydrate diet, or from chow-fed, streptozotocin-diabetic rats. The results indicate that diabetes and dietary variations substantially influence the lipid composition of the endoplasmic reticulum, particularly the lecithin/cephalin and cholesterol/cholesterol ester ratios. This variation in membrane lipid composition is reflected in the Arrhenius activation energies of membrane-bound enzyme. These results indicate the relevance of membrane lipid-HMGR interactions in the regulation of HMGR activity as this activity responds within a physiologically relevant framework.—Supported by grants from the American Heart Association, Texas Affiliate, the R. A. Welch Foundation, an undergraduate fellowship from the R. A. Welch Foundation (V.L.S.) and a pre-doctoral fellowship from the North Texas Diabetes Research Institute (L.G.B.)

24. COPING WITH SUBSTRATE CLEAVAGE IN THE HMG-CoA REDUCTASE ASSAY. N. L. Young. *Dept. of Medicine, Cornell Univ. Medical College, New York, NY 10021.*

Substrate cleavage was monitored during the development of assays for reductase in freshly isolated human lymphocytes (HL) and rat small intestine (RSI). A simple method of cellulose TLC separation of [¹⁴C]HMG-CoA (*R_f* 0.2) from its metabolites (*R_f* > 0.5) with butanol-acetic acid-water 7:2:3 and scintillation counting with 0.6% PPO in toluene-NCS-water 100:10:1 was devised. In homogenates of HL and RSI, cleavage activity was very high (10 and 200 nmol HMG-CoA/(min·g tissue)) compared to reductase activity (0.002 and 1 nmol mevalonate/(min·g tissue)). Cleavage can interfere with the reductase assay by depleting substrate, by forming products that comigrate with mevalonolactone on TLC, and possibly by forming inhibitors of reductase. Cleavage activity was stable in ethanolamine buffer but declined during preincubation in phosphate buffer to 0.2 and 25 in HL and RSI, respectively. There was a concomitant increase in reductase activity to 0.02 and 4 in HL and RSI (that was only partially F⁻ suppressible) and a decrease in RSI inhibition of rat liver microsomal reductase from 90% to 13%. Here, substrate depletion could account for at most 4% inhibition. The major cleavage product by HL was acetoacetate, but a Lineweaver-Burke plot suggested more than one enzyme. Cleavage products migrating with mevalonolactone could be washed off, but subtracting radioactivity formed in the absence of NADP was easier. Thus interference with the reductase assay by cleavage enzymes can be made negligible by preincubating homogenate in phosphate buffer, by including an NADP minus control, and by keeping assay times short.