Abstracts
HMG-CoA Reductase III
June 4, 1983, San Francisco, California

HMG-CoA Reductase III.

The 33 abstracts below summarize research presented as talks and posters at "Reductase III". This one-day minisymposium, which focused on the regulation of HMG-CoA reductase, was held at the Hilton Hotel, San Francisco on June 4, 1983 in conjunction with the annual meeting of the American Society of Biological Chemists. Sponsorship was provided by the participants and by a contribution from the Merck Institute. Two prior minisymposia, "Reductase I and II", were held in Atlantic City, New Jersey in 1972 and in Toronto, Canada in 1979. Abstracts of research presented at "Reductase II" appeared in *J. Lipid Res.* **20:** 1044–1049.

"Reductase I" emphasized methods of assay for microsomal HMG-CoA reductase and the circadian rhythm in reductase activity. "Reductase II" emphasized in vitro phosphorylation-dephosphorylation of the enzyme and its possible physiological significance. "Reductase III" marked the emergence of the molecular biology of HMG-CoA reductase, the widespread recognition that the solubilized protein that we all have been studying is a proteolytic fragment of the native protein, and the discovery that reductase is a glycoprotein. We have witnessed the transition from descriptive biochemistry through delineation of the convertor enzymes to a point where we have some understanding of reductase as an interconvertible, covalently modulated regulatory enzyme. Our colleagues now are poised to determine the ultimate structure of reductase and the genetic basis of its regulation. We express our gratitude to Michael Beach, Karen Evenson, John Gill, Peter Kennelly, Rex Parker, and Phadungchom Wathanaronchai for invaluable assistance, and our sincere thanks to all participants at "Reductase III" for making this a memorable occasion.

Victor W. Rodwell and David M. Gibson, Organizers

(Reprints of these abstracts are NOT available)

1. SOLUBILIZATION AND PARTIAL PURIFICATION OF MEMBRANE-BOUND HMG-CoA REDUCTASE FROM A PLANT SOURCE. Thomas J. Bach and Harry Rudney. *Dept. of Biological Chemistry, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267.*

HMG-CoA reductase (HMGR) regulates the synthesis of mevalonic acid (MVA), a key precursor of the myriad polyisoprenoid substances functional in plant cell biochemical processes. In earlier work by Bach, T. J., 1981. Ph.D. Thesis, Karlsruhe Contrib. *Plant Physiol.* **10:** 1–219. ISSN 0173-3133, it was demonstrated that HMGR activity in radish seedlings (*Raphanus sativus*) is present in membrane fractions obtained by centrifugation at 16,000 *g* (P 16) and 105,000 *g* (P 105). The activity in each fraction appeared to be highly and independently regulated by light, phytohormones and herbicides. Further investigation of these effects requires purification of the enzyme. Published procedures for solubilization or purification of the rat liver and yeast enzyme were ineffective in releasing the enzyme from plant membranes. We have developed a new protocol for enzyme purification based on detergent extraction of membranes isolated from 4-day-old, dark-grown radish seedlings. Deep frozen P 16 (s.a. 173.6 pmol/mg/min) was thawed and incubated in the presence of 2% polyoxyethylene ether (Brij W-1) and 20% glycerol for 60 min at 37°C which resulted in a more than 80% activation of HMGR activity. After centrifugation at 190,000 *g* for 1 hr, the activity (413.1 pmol/min/mg) was found in the supernatant. The enzyme activity was precipitated between 0 and 37% $(NH_4)_2SO_4$ saturation, and was found in the floating lipid/carotenoid layer. This lipid/protein fraction was dissolved in a small quantity of K-phosphate/KCl/EDTA/DTE buffer containing 2% Brij W-1, and stored at −20°C (551.7 pmol/min/mg). The thawed fraction was diluted to a KCl concentration of 50 mM and loaded on DEAE-Sephadex A-50. Carotenoids and the bulk quantity of protein were found in the break-through fraction. HMGR activity was eluted at 400 mM KCl (2,225 pmol/min/mg). After dilution of KCl in the presence of 1% Brij, the solution was loaded on Blue Dextran-agarose and eluted at 500 mM KCl (8,306 pmol/min/mg). Chromatography on HMG-CoA-hexane agarose resulted in a s.a. of 56,360 pmol/min/mg, which represents a 325-fold purification. To the best of our knowledge, this is the first report on a successful purification of HMGR from a plant. The principal solubilization and purification protocol employed here could be used to isolate the enzyme from rat liver

microsomes in its native form. Supported by a NATO Fellowship (DAAD) to T.J.B. and grant no. AM-12402 from NIADDK.

2. STUDIES OF GENES CONTROLLING THE ACTIVITY OF HMG-CoA REDUCTASE IN *SACCHAROMYCES CEREVISIAE*. Michael Basson and Jasper Rine. *Dept. of Biochemistry, Univ. of California, Berkeley, CA 94720.*

We have developed a simple method for the isolation of recombinant clones controlling the activity of specific enzymes by exploiting gene dosage effects created by multi-copy yeast-*E. coli* plasmid shuttle vectors containing yeast genomic libraries. Yeast cells, transformed with such a library, were treated with compactin in order to select for compactin-resistant transformants. Several were isolated and shown to be resistant due to the presence of the plasmid and not due to chromosomal mutations. By restriction mapping the inserts in five independent clones, we determined that each contained a different overlapping piece of the yeast genome. Several independent lines of evidence indicate that this clone overproduces HMG-CoA reductase. 1) Compactin inhibits the activity of the yeast enzyme. 2) By radiochemical assays, cells containing this clone contain 8 to 14 times more HMG-CoA reductase activity than do isogenic cells without the plasmid. 3) This enhanced activity is inhibited by antibody raised against yeast HMG-CoA reductase. By blot hybridizations to genomic DNA we know that our clone is present in the yeast genome as a single copy. Analysis of RNA hybridization blots indicates the presence of three genes on our clone. By deletion mapping we have established that a 3.3 kb RNA is responsible for overproduction of enzyme activity. Some deletions appear to affect regulatory regions of this gene. Furthermore message level of this gene shows modest regulation by exogenous sterol regulation. Our working hypothesis is that this clone is the structural gene for yeast HMG-CoA reductase.

3. MECHANISMS INVOLVED IN THE IN VIVO MODULATION OF HEPATIC 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE (R), REDUCTASE KINASE (RK), REDUCTASE KINASE KINASE (RKK) AND PHOSPHOPROTEIN PHOSPHATASE (PP) BY MEVALONOLACTONE (MVL). Zafarul H. Beg, John A. Stonik and H. Bryan Brewer, Jr. *Molecular Disease Branch, NHLBI, NIH, 9000 Rockville Pike, Building 10, Room 7N117, Bethesda, MD 20205.*

It has been previously demonstrated that the enzymic activity of rat liver R is modulated in vitro and in vivo by a bicyclic cascade system involving reversible phosphorylation of R and RK (*Federation Proc.* 1982. **41:** 2634–2638.). Administration of MVL (~600 mg/kg) to rats is known to be associated with a rapid biphasic inhibition of R. The first phase (20 min) of reversible inhibition (~30%) was due to enhanced R phosphorylation. The second phase (60 min) of MVL inhibition of R activity (~80%) was an irreversible process, not involving a decrease in quantity of purified R protein in comparison to controls. These data suggest the presence of an additional new mechanism for the regulation of R activity. The increase in R phosphorylation (20 min) was associated with an increased phosphorylation and activation (2–3-fold) of both cytosolic and microsomal RK. Increased activation (phosphorylation) of RK was catalyzed by RKK which was significantly activated (2–3-fold) following the administration of MVL. The mechanism of the in vivo activation of RKK is not yet known. Both RK and RKK activities remained elevated for 60 min after MVL administration. In addition, a relative increase in the degree of phosphorylation of R and RK occurred due to a significant decrease in PP activity. A 35% and 54% reduction in PP activity (expressed in terms of R dephosphorylation) occurred 20 and 60 min after MVL administration, respectively. These combined data indicate that MVL-mediated short-term in vivo inhibition of R activity occurred by two mechanisms: (1) an increase in the degree of phosphorylation of both R and RK due to increased activities of RK and RKK; (2) a decrease in the dephosphorylation of both R and RK due to a reduction in PP activity. These combined effects would favor an increase in the steady state level of the phosphorylated forms of both R and RK resulting in a reduction in the enzymic activity of R. These results also represent the initial demonstration that the activity of *RKK* is modulated in vivo providing a mechanism for the regulation of the activities of both RK and R.

4. HMG-CoA REDUCTASE: A GLYCOSYLATED, INTEGRAL ER PROTEIN. Deborah A. Brown and Robert D. Simoni. *Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305.*

A variant cell line exhibiting 100× the parental level of reductase activity was selected by its resistance to 225 μM compactin from the parental BHK cell line. Reductase was immunoprecipitated from extracts of these cells using an antiserum raised in rabbits against rat liver reductase. In some experiments, mRNA was isolated from the variant cells and translated in the reticulocyte lysate or wheat germ system. Reductase was then immunoprecipitated from the translation mixture. Reductase is shown to be an integral membrane protein by the following criteria: 1) solubilization of the intact 92K protomer only in the presence of detergent, 2) synthesis on membrane-bound polysomes, and 3) co-translational but not post-translational association with dog pancreatic microsomes added to the in vitro translation. The in vitro and in vivo forms of the 92K protomer are indistinguishable on SDS-PAGE. However, V8 protease digestion reveals a few differences. This apparent paradox may be resolved by the finding that ^{3}H-mannose specifically labels reductase. Also, reductase isolated from cells grown in the presence of tunicamycin migrates faster on SDS-PAGE than reductase from untreated cells. Studies are underway to determine if cleavage of an N-terminal signal sequence occurs. Cleavage of a signal sequence and addition of carbohydrate may compensate to give a mature subunit with the same molecular weight as the in vitro form. (Supported by NIH grant HL 26502.)

5. LIVER REGENERATION AND MEMBRANE-MEDIATED CONTROL OF HMG-CoA REDUCTASE. Giovanna Bruscalupi, Giovanna Curatola, Silvia Leoni, Maria Teresa Mangiantini, Silvana Spagnuolo, Anna Trentalance and Giovanna Zolese. *Dept. of Cellular and Developmental Biology, Univ. of Rome and Dept. of Biochemistry, Univ. of Ancona, Italy.*

It has been reported that the physicochemical state and the lipid composition of the endoplasmic reticulum are modulating factors in the physiological control of reductase activity. The modification of membrane lipid composition is reflected by the Arrhenius activation energy of the membrane-bound reductase: this relationship allows us to use the temperature-dependence of the enzyme activity as a probe of reductase-lipid interaction. Since during liver regeneration the specific activity of reductase enhanced twice at 16 hr after partial hep-

atectomy (onset of the first cell cycle S phase; sham operated animals as controls), the relationship between the kinetic characteristics of the reductase and the physical state and biochemical composition of the microsomal membrane at that replicative step were studied. No change was observed in substrate dependence (K_m) while the disappearance of the 28°C break in temperature dependence and an increased activation energy were detected. Electron Paramagnetic Resonance (EPR) of the mobility freedom of the lipid probes (5NS, 16NS) and protein probe (MSL) showed a modified fluidity of the hydrophobic core; the superficial region of the membrane kept unaltered. No modification was detected in the bulk biochemical composition. These results suggest an altered structural organisation of the microsomal membrane proteins that could explain the increased Ea of the reductase, but other mechanisms must be responsible for the modified specific activity. A relationship between the physical state and the Ea of the enzyme has been detected also in the regenerating liver, but the membrane-mediated control is not the mechanism used by the regenerating liver to regulate the HMG-CoA reductase activity in that critical phase of the cell cycle.

6. PURIFICATION AND PROPERTIES OF CYTOSOLIC AND MICROSOMAL HMG-CoA REDUCTASE PHOSPHATASES FROM RAT LIVER. Victoria E. Calvet, Mercedes Sitges, Teresita Royo, Guillermina Asins, Gregorio Gil, and Fausto G. Hegardt. *Dept. of Biochemistry. Univ. of Barcelona, School of Pharmacy, Barcelona-28, Spain.*

Reductase phosphatases, enzymes that dephosphorylate and activate HMG-CoA reductase have been purified from rat liver cytosol and microsomes. The purification steps of the cytosolic enzymes are: 1) DEAE-cellulose chromatography, 2) Aminohexyl Sepharose 4B chromatography, 3) Bio-Gel A 1.5 m filtration. Along these stages, four protein phosphatases are separated. Three of them I_V, I_R, and II_R show reductase phosphatase, glycogen synthase phosphatase and phosphorylase phosphatase activities; the fourth enzyme I_X shows only phosphorylase phosphatase activity. The purification steps for the microsomal protein phosphatases are: 1) solubilization from microsomes; 2) DEAE-cellulose chromatography which separates two different activities; one phosphatase is excluded from the column and the other one is retained; 3) the excluded activity (Ex) is purified by the next stages; 3A) phosphocellulose chromatography; 4A)Aminohexyl Sepharose 4B chromatography; 5A) Sephadex G-100 filtration. The phosphatase activity retained by DEAE-cellulose is purified on 3B) Aminohexyl Sepharose, which separates two distinct peaks I_M and II_M; 4B) gel filtration on Sephadex G-100 and Bio-Gel A 1.5 m, respectively. Protein phosphatases Ex and I_M exhibit phosphatase activity using reductase, glycogen synthase and phosphorylase as substrates; while the phosphatase II_M presents only reductase phosphatase activity. The optimum pH of the reductase phosphatases are comprised between 6 and 6.5, except the phosphatase I_V whose optimum pH is 8.5. All these protein phosphatases are activated in the presence of 5 mM Mg^{2+} or Mn^{2+}. Phosphorylase phosphatase I_X becomes reductase phosphatase in the presence of these cations. Reductase phosphatases are inhibited by chelators of divalent ions, such as F^-, PPi^- adenine nucleotides, dicarboxylic acids and by the reductase substrates (NADPH, HMG-CoA and CoA). The ethanol treatment of the cytosolic phosphatases produces low molecular weight forms (35000 and 72000). The specificities towards the three different substrates of these postethanolic phosphatases remain the same, respectively, as that before the ethanolic treatment.

7. REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL CoA (HMG-CoA) REDUCTASE mRNA LEVELS IN RAT LIVER. Catherine F. Clarke, Peter A. Edwards, Show-Fung Lan and Alan M. Fogelman. *UCLA School of Medicine, Los Angeles, CA.*

A key regulatory step in cholesterol biosynthesis is the conversion of 3-hydroxy-3-methylglutaryl CoA to mevalonate, a reaction catalyzed by the microsomal enzyme HMG-CoA reductase. The mass and activity of reductase in rat liver can be induced over 250-fold following addition of cholestyramine and mevinolin to the diet (Tanaka et al., 1982. *J. Lipid Res.* **23:** 1026–1031). Under these dietary conditions reductase maintains a diurnal cycle of activity. We have isolated liver RNA from rats fed cholestyramine ± mevinolin and quantitated reductase mRNA by immunoprecipitation of total in vitro translation products and gel electrophoresis. RNA isolated from rats fed rat chow with 5% cholestyramine and 0.1% mevinolin had functional reductase mRNA levels which were 5.7-fold higher than in rats fed 5% cholestyramine and were 16-fold higher than in rats fed 5% cholestyramine and given mevalonalactone by stomach intubation 3 hr before death. RNA isolated from animals killed at different times during the light and dark cycle showed changes in functional reductase mRNA levels which mirrored the changes in reductase activity. Rats fed rat chow with 5% cholestyramine and 0.05% mevinolin and killed in the middle of the 12-hr light period had a 4- to 5-fold decrease in levels of reductase mRNA when compared with similar rats killed at the 8th hr of the dark period. A rhythm of reductase mRNA levels (varying by 2- to 3-fold over the course of the light–dark cycle) was also observed in animals fed rat chow with 5% cholestyramine. These observed differences in functional reductase mRNA levels were found to be similar in degree to differences in reductase activity. When the RNA was treated with CH_3HgOH (a reversible denaturant) prior to in vitro translation, we found that incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable proteins increased approximately 2-fold, while the [^{35}S]methionine incorporated into immunoprecipitable reductase increased by 2- to 6-fold. RNA isolated from untreated rats killed at the nadir of the diurnal rhythm of enzyme activity produced no detectable amounts of reductase, even after CH_3HgOH pretreatment.

8. STIMULATION OF THE PROLIFERATION OF MADIN-DARBY CANINE KIDNEY (MDCK) CELLS BY HIGH DENSITY LIPOPROTEINS AND THEIR INDUCTION OF HMG-CoA REDUCTASE ACTIVITY. David C. Cohen, Sharon Massoglia, and Denis Gospodarowicz. *Cancer Research Institute, Univ. of California Medical Center, San Francisco, CA 94143.*

MDCK cells seeded on extracellular matrix- (ECM)- coated dishes in a medium supplemented with high density lipoproteins (HDLs, 750 μg protein/ml) and transferrin (10 μg/ml) have a proliferative rate, final cell density, and morphology similar to those of cells grown in serum-supplemented medium. The mitogenic stimulus provided by HDLs is not limited by the initial cell seeding density, nor is it limited in time, since cells grown in medium supplemented with transferrin and HDLs grew for at least 50 generations. HDLs are required in the medium in order for cells to survive, since cells actively

proliferating in the presence of medium supplemented with HDLs and transferrin begin to die soon after being transferred to medium supplemented only with transferrin. Low density lipoprotein (LDL) at low concentrations (from 2.5 to 100 μg protein/ml) is mitogenic for MDCK cells. Above 100 μg protein/ml, LDL is cytotoxic and therefore cannot support cell proliferation optimally. A close association between the ability of HDLs to support cell proliferation and their ability to induce the activity of HMG-CoA reductase is observed. Reductase activity is 18 times higher (70 pmol/min/10^6 cells) in proliferating cells than in confluent, nondividing cells (4 pmol/min/10^6 cells). The reductase activity of sparse cells is more sensitive to induction by HDLs (8-fold higher than control cells) than that of confluent cells (2-fold higher than control levels). The dose–response relationships between the abilities of HDLs to support proliferation and to induce HMG-CoA reductase activity are similar. The time courses of the stimulation of proliferation and the increase in enzyme activity of sparse, quiescent cells after exposure to HDLs are parallel. The reductase activity of sparse MDCK cells is induced 6-fold by exposure to compactin, a competitive inhibitor of reductase. This induction of reductase is prevented by mevalonic acid, not affected by LDL, and synergistically enhanced by simultaneous exposure to HDLs. HDLs effect a rescue from the cytotoxic effect of compactin, whereas LDL does not. More specifically, cells proliferating in the presence of HDLs are 100 times more resistant to the toxic effects of compactin than are cells exposed to LDL. These results suggest that the induction of reductase activity by HDLs may play a role in mediating the proliferative effect of HDLs. The significance of the increased mevalonate made available by higher levels of reductase appears not to lie in the bulk provision of cellular cholesterol, but rather in the provision of a specific pool of endogenously synthesized sterol, or in one or more of the nonsterol products of mevalonate. In cells which proliferate in response to HDLs, such as the MDCK and vascular endothelial cells (*J. Biol. Chem.* 1982. **257:** 9429) the induction of reductase appears to be a consistent and essential feature of a possibly pleiotypic metabolic response to HDLs.

9. PURIFICATION AND PROPERTIES OF REDUCTASE KINASE FROM RAT LIVER. Albert Ferrer, Francesc Pla, Diego Haro, Pedro Marrero and Fausto G. Hegardt. *Dept. of Biochemistry. Univ. of Barcelona, School of Pharmacy, Barcelona-28. Spain.*

Reductase kinase, the enzyme which phosphorylates and inactivates HMG-CoA reductase, has been purified by solubilization from rat liver microsomes and chromatography on Affi-Gel Blue, phosphocellulose, Bio-Gel A 1.5 m and agarose-hexane-ATP. The enzyme appears to be homogeneous by criteria of PAGE both with and without SDS. The holoenzyme has an apparent M_r of 480,000 and its unique subunit an M_r of 105,000. Incubation of homogeneous reductase with homogeneous reductase kinase and [γ-^{32}P]ATP 0.2 mM (2220 cpm/pmol) produces a time-dependent inactivation of the enzymatic activity. The immunoprecipitate of ^{32}P-labeled reductase electrophoresed by SDS-PAGE showed a unique ^{32}P-band with identical R_f as the subunit of reductase. Under the conditions used the stoichiometry of phosphate incorporation is 0.2 moles per mol, assuming that reductase is a tetramer of M_r 200,000. Incubation of ^{32}P-labeled reductase with reductase phosphatase IIa resulted in a time-dependent loss of protein-bound ^{32}P radioactivity and an increase in enzymatic activity. Reductase kinase does not exhibit mevalonate kinase activity. Reductase kinase when incubated with 0.5 mM [γ-^{32}P]ATP was autophosphorylated, increasing its enzymatic activity by a factor of 2. Under the conditions used 0.78 moles of phosphate were covalently bound per mol of reductase kinase. Tryptic treatment of immunoprecipitated ^{32}P-labeled reductase phosphorylated by reductase kinase, produced only one ^{32}P-phosphopeptide which had the same R_f (0.72) as one of the two tryptic phosphopeptides previously reported by us. Recent studies have led to the purification from rat liver cytosol of a new protein with reductase kinase activity. This enzyme phosphorylates and inactivates reductase when incubated with [γ-^{32}P]ATP. This preparation does not show, in addition, mevalonate kinase activity.

10. PURIFICATION AND PROPERTIES OF *PSEUDOMONAS* HMG-CoA REDUCTASE. John F. Gill, Jr. and Victor W. Rodwell. *Dept. of Biochemistry, Purdue Univ., West Lafayette, IN 47907.*

Pseudomonas M was isolated from soil by elective culture on (R,S)mevalonate as the sole source of carbon. Under these conditions, HMG-CoA reductase was induced in *Pseudomonas* M. The enzyme was purified by ammonium sulfate fractionation, ion-exchange chromatography on DEAE-Sephacel and affinity chromatography (Blue Dextran Sepharose and HMG-CoA-Hexane-Agarose) to a state judged to be homogeneous on silver-stained SDS-polyacrylamide gels. Purification was facilitated using a rapid spectrophotometric assay monitoring NAD^+ reduction by (R,S)mevalonate and CoA to form NADH and HMG-CoA (Bensch and Rodwell. 1970. *J. Biol. Chem.* **245:** 3755). The final specific activity was estimated to be as high as 60,500 nmol NAD^+ reduced/min/mg of protein, which represents a 25-fold overall purification and 54% recovery of initial activity. The high yield of homogeneous protein (approximately 15 mg from 4000 mg of dry cells) should facilitate future structural studies on *Pseudomonas* reductase. The molecular weight of the holoenzyme was 178,000 as judged by gel filtration chromatography and the molecular weight of the subunit was estimated to be 43,000 on SDS-polyacrylamide gels. The holoenzyme thus appears to exist as a tetramer. A partial amino terminal sequence has been obtained. This sequence data represents the first such report for HMG-CoA reductase from any organism. Antibody prepared in rabbits against homogeneous *Pseudomonas* reductase inactivated reductase activity (both in crude extracts and in purified form). However, antibody to the *Pseudomonas* enzyme failed to inactivate rat liver reductase. [Supported by grants from the N.H.L.B.I. (HL 19223) and the American Heart Association, Indiana Affiliate].

11. ROLE OF PROTEIN PHOSPHATASES IN THE REGULATION OF HMG-CoA REDUCTASE. T. S. Ingebritsen. *Dept. Pharmacology, Univ. of Colorado Health Sci. Center, Denver, CO 80262.*

Previous experiments have demonstrated that hepatic HMG-CoA reductase is regulated by a bicyclic phoshorylation system in which the reductase is phosphorylated (inactivated) by a cytosolic protein kinase, termed reductase kinase. This protein kinase is itself a phosphoprotein which is only active after phosphorylation by a second cytosolic protein kinase, termed reductase kinase kinase. In the present studies, the nature of the protein phosphatases in liver extracts acting on reductase and reductase kinase has been investigated. These studies demonstrate that all of the protein phosphatase activity in liver

extracts acting on the two phosphoproteins is explained by three enzymes, termed protein phosphatases 1, 2A and 2C. An important conclusion from these studies is that the protein phosphatases acting on reductase and reductase kinase are not unique to this phosphorylation system but are also active on phosphorylated proteins involved in the regulation of glycogen metabolism, glycolysis, gluconeogenesis, fatty acid synthesis and protein synthesis. The activity of protein phosphatase 1 is controlled by two heat-stable regulatory proteins termed inhibitors-1 and -2. Phosphorylation of inhibitor-1 on a specific threonine residue by cAMP-dependent protein kinase results in its activation and the inhibition of protein phosphatase 1. Inhibitor-2 and the catalytic subunit of protein phosphatase 1 have been shown to form a 1:1 complex which is inactive. Incubation of this complex with MgATP and a protein kinase termed glycogen synthase kinase 3 results in the phosphorylation of inhibitor-2 on a threonine residue. Phosphorylated inhibitor-2 dissociates from the complex leading to the reactivation of protein phosphatase 1. Protein phosphatase 2A is resolved into three species, termed protein phosphatases $2A_0$ (M_r = 210,000), $2A_1$ (M_r = 210,000) and $2A_2$ (M_r = 150,000), following chromatography on DEAE-cellulose. Each species contains the same M_r = 38,000 catalytic subunit. Protein phosphatases $2A_1$ and $2A_2$ have been purified to homogeneity from several tissues including rat liver. Protein phosphatase $2A_1$ contained two additional subunits M_r = 60,000 and 55,000 while protein phosphatase $2A_2$ contains only the M_r = 60,000 subunit complexed with the catalytic subunit. Protein phosphatase $2A_0$ has not yet been purified to homogeneity and its subunit composition is unknown. However, this species is inactive and its activity is only expressed after dissociation of the M_r = 38,000 catalytic subunit from the high molecular weight complex. This result suggests that a mechanism(s) for regulating protein phosphatase 2A may exist in vivo. Protein phosphatase 2C is a Mg^{2+}-dependent enzyme which consists of a single subunit (M_r = 43,000). The role of these three protein phosphatases in the regulation of HMG-CoA reductase will be discussed. (Supported by the Colorado Heart Association and grant BRSG-05357 awarded by the Biomedical Research Grant Program, N.I.H.)

12. REGULATION OF HMG CoA-REDUCTASE (HMGR) ACTIVITY BY A POSSIBLE ENZYME-LIPID INTERACTION. H. St. Jenke, M. Löwel and J. Berndt, *GSF, Inst. of Toxicology and Biochemistry, 8042 Neuherberg, West Germany.*

Feeding 0.05% polychlorinated biphenyls (PCBs) results in an increase of rat liver HMGR activity within 9 days, followed by a decrease towards normal levels. The PCBs are incorporated into the microsomal membrane; after 9 days of PCB feeding the concentration is about 2 mol% related to phospholipids. The effect on lipid levels in serum of PCB-fed rats is as follows: triglycerides are initially increased but come back to normal values; phospholipids and nonesterified cholesterol reach a maximum after about 7 days and maintain this level for more than 3 weeks. A completely different situation is found with the lipids in the microsomes from PCB-fed rats: triglycerides are elevated very rapidly and stay at this level; phospholipids do not differ from the controls; while the cholesterol content is markedly decreased throughout the entire feeding period. This decrease might be even greater considering a compartmentalization of cholesterol in the microsomal membrane. Thus, the cholesterol/phospholipid ratio, which may influence membrane-bound enzymes, is changed. Measurements of fluorescence intensity, using parinaric acid as a probe, in microsomes from PCB-fed rats suggest small changes in the microsomal membrane structure. Moreover, immunotitrations of microsomal-bound HMGR from PCB-fed rats show higher equivalence points than HMGR from untreated controls. With the solubilized enzyme, however, no increase in the equivalence points was observed. This indicates that in PCB-treated rats pre-existing HMGR becomes activated, possibly by a modification of its microenvironment rather than by changes in enzyme quantity. In vitro transfer of cholesterol to microsomes reveals a loss in HMGR activity concomitant with a lowering of the equivalence point, an effect which is also not observed with the solubilized enzyme. These results demonstrate lipid compositional changes in liver microsomes of PCB-fed rats. Simultaneously there is a transient stimulation of microsomal HMGR, which we suggest to be probably the consequence of an altered enzyme-lipid interaction. The decrease after prolonged PCB feeding could be due to a counter-regulation at the level of transcription.

13. CHARACTERIZATION OF RAT LIVER 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE SOLUBILIZED IN THE PRESENCE OF INHIBITORS OF PROTEOLYSIS. Peter J. Kennelly, Daniel G. Sherban, Karl G. Brandt and Victor W. Rodwell. *Dept. of Biochemistry, Purdue Univ., West Lafayette, IN 47907.*

Solubilization of 3-hydroxy-3-methylglutaryl coenzyme A reductase (reductase, E.C. 1.1.1.34) by traditional freeze-thaw methods is dependent on the action of proteases (Ness et al. 1981. *Biochem. Biophys. Res. Commun.* **102:** 81–85) and apparently yields a degraded form of the enzyme. We have used nonionic detergents (Lubrol WX or Polyoxyethylene ether type W1) to solubilize rat liver reductase in the presence of the proteolytic inhibitors leupeptin (which blocks the freeze-thaw solubilization), EGTA and PMSF (Kennelly et al. 1983. *Biochemistry.* **22:** 2784–2788.). Detergent-solubilized reductase has been partially purified (approximately 40-fold) using sucrose density gradient ultacentrifugation and dye-ligand chromatography. In contrast to the proteolytically degraded form(s) of reductase, detergent-solubilized reductase is heat-labile and is readily inactivated by cytosolic reductase kinase. This inactivation requires both ATP and ADP and is reversed by treatment with a low molecular weight phosphoprotein phosphatase. Detergent-solubilized reductase catalyzes the following reactions: 1) HMG-CoA + 2 NADPH + 2 H^+ = Mevalonate + CoA + 2 $NADP^+$. 2) Mevaldate + NADPH + H^+ = Mevalonate + $NADP^+$. 3) Mevaldate + $NADP^+$ + CoA = HMG-CoA + NADPH + H^+. Reaction 2 is stimulated up to 4-fold by CoA. Preliminary experiments suggest a differential effect of reductase kinase treatment on the ability of reductase to catalyze the reactions above. We used cytosolic reductase kinase plus Mg-nucleotides (Harwood and Rodwell. 1982. *J. Lipid Res.* **23:** 754–761) to completely inactivate Reaction 1. Under these conditions, Reaction 3 was also completely blocked, but the rate of Reaction 2 did not significantly decrease. Thus, phosphorylation-dephosphorylation appears to control reductase activity by modulating the first partial reaction. (Supported by a grant from the N.H.L.B.I. (HL 19223). P.J.K. is supported by an N.S.F. graduate fellowship (8166394)).

14. EFFECT OF ACTH ON THE SYNTHESIS OF HMG-CoA REDUCTASE IN HAMSTER ADRENOCORTICAL TISSUE. Jean-Guy Lehoux and Andrée Lefebvre. *Dept. of Biochemistry, Faculty of Medicine, Sherbrooke Univ., Sherbrooke, Quebec, Canada, J1H 5N4.*

Under a controlled light-dark cycle, the hamster plasma corticosteroids level followed a diurnal rhythm with minimum values in the morning and a maximum at 1900 hr. Plasma ACTH levels were 7.3 ng/dl at 1100 hr and 14.2 ng/dl at 1900 hr. HMG-CoA reductase activity was also twice more elevated at 1900 hr than at 1100 hr. 75% of the reductase appears to be in an inactive form. The inactive form can be activated by a rat liver phosphorylase phosphatase preparation and also by an adrenal fraction obtained (in the same way). Another microsomal fraction inhibited the reductase activity in the presence of Mg^{2+} and ATP. (*Life Sci.* 1982. **31:** 867–873). Time-course experiments revealed that in vivo co-administration of synthetic ACTH (Duracton, 0.5 IU/100 g body weight) produced a significant increase in adrenal HMG-CoA reductase activity (after a 60-min lag period); the expressed form of the reductase was increased twofold by 120 min post-treatment; the total concentration of the enzyme measured after its preincubation in the presence of rat liver phosphorylase phosphatase was increased 2.3 and 4.1 times at 120 and 180 min post-treatment, respectively. Ratios of expressed reductase/total reductase did not increase during the experiment, suggesting that the increased activity was mainly due to de novo synthesis of the reductase. The effect of co-administration of Actinomycin D or cycloheximide with ACTH was also studied. Actinomycin D did block the reductase activity. However, cycloheximide prevented the effect of ACTH on plasma corticosteroids production as well as on HMG-CoA reductase activity. Cycloheximide alone produced a significant decrease (50%) in the total reductase activity 60 min after its administration, suggesting a rapid turnover of the enzyme. Cycloheximide, or cycloheximide and ACTH induced an accumulation of free cholesterol (1.9–2.1-fold) in adrenals, whereas ACTH alone decreased their content by 35%. In conclusion, we have demonstrated increased net synthesis of HMG-CoA reductase in hamster adrenals by ACTH. Because of the rapid turnover of the enzyme, however, it is not possible to completely rule out that ACTH acts at the level of the activation of the inactive form of the reductase. Supported by a grant from the Medical Research Council of Canada.

15. MECHANISM OF RAPID MODULATION OF HMG-CoA REDUCTASE IN ISOLATED HEPATOCYTES DURING FETAL AND PERINATAL LIFE. Silvia Leoni, Laura Conti De Virgiliis, Luciana Dini, Maria Teresa Mangiantini, Silvana Spagnuolo and Anna Trentalance. *Dept. of Cellular and Developmental Biology, Univ. of Rome, Rome, Italy.*

Short term and long term regulatory mechanisms have been reported in the control of cholesterogenesis and of the activity of HMG-CoA reductase. Which mechanism drives the changes observed in rat liver HMG-CoA reductase activity at different stages of intrauterine and perinatal life is still unknown: in fact the growth process suggests an increased amount of the enzyme, consistent with an increased activity, but a low activity is detectable at some developmental stages. In hepatocytes isolated at 16th day of gestation, or just before and after birth, or during lactation, we followed the reductase activity in microsomes prepared without NaF (−NaF-R), or in the presence of 50 mM NaF (+NaF-R), and the cholesterol synthesis. −NaF-R and + NaF-R showed similar patterns: the activity was low at 16th day of gestation, increased before the birth, returned to lower values at birth, and decreased during lactation. The activation state (as detectable from +NaF/−NaF ratio) was quite different; there was an increasing rate of NaF inhibition, that peaked at birth. The pattern of cholesterol synthesis was more fitting in that of +NaF-R activity than in that of −NaF-R activity. This relationship is particularly evident at birth, where +NaF-R activity and therefore cholesterol synthesis dropped, and −NaF-R was still higher. The activity of hepatic reductase is regulated by a short-term mechanism during development, involving phosphorylation-dephosphorylation processes. This mechanism, markedly active at birth, could mediate the hormonal changes occurring during perinatal life.

16. IMMUNOTITRATION AND IMMUNOBLOTTING OF RAT LIVER MICROSOMAL HMG-CoA REDUCTASE. Gene C. Ness, Clare E. Phillips and Duane C. Eichler. *Dept. of Biochemistry, College of Medicine, Univ. of South Florida, Tampa, FL 33612.*

Monospecific antisera to homogeneous, albeit proteolytically modified, HMG-CoA reductase was produced in male New Zealand White rabbits. About 500 μg of purified reductase in 50 mM potassium phosphate buffer, pH 7.1, was concentrated to a volume of 400 μl, mixed with 1 ml of Freund's Complete Adjuvant, and injected into the toe pads. Two weeks later, 100 μg of purified enzyme in a volume of 400 μl was mixed with 1 ml of Freund's Incomplete Adjuvant and injected subcutaneously. Two weeks later and at weekly intervals thereafter, the rabbits were bled and IgG fractions were prepared. The specificity of the antisera was examined by Ouchterlony double diffusion analysis. Single lines of precipitation were observed when the antisera was diffused against homogeneous reductase and against impure enzyme from various stages in the purification procedure. Further evidence that the antisera was monospecific was obtained from immunoblotting studies. A single band was observed in SDS extracts of microsomes. This monospecific anti-reductase IgG was used for immunotitration studies of lysosome-free microsomes isolated in 0.25 M sucrose. Equivalent amounts of enzyme activity were used rather than constant amounts of microsomal protein. Diluted microsomes were incubated with IgG for 10 min at 25°C. Residual reductase activity was then determined at 37°C. The amount of anti-reductase IgG required to neutralize a unit (1 nmol of mevalonate formed per min) of reductase activity was 7 and 8 μg, respectively, for microsomes from fasted and cholestyramine-fed rats. Similar results were observed in other dietary and hormonal states. Using immunoblotting techniques, the question of the native subunit size of the rat liver enzyme was investigated. Briefly, SDS extracts were run on gradient SDS slab gels, electrophoretically transferred to a nitrocellulose sheet, incubated with a 1 to 100 dilution of anti-reductase IgG (3 mg/ml), incubated with goat anti-rabbit IgG conjugated with horseradish peroxidase and finally incubated with peroxidase substrate solution. Purified proteolytically modified reductase gave a broad band at 50,000. Lysosome-free microsomes isolated in 0.25 M sucrose gave a subunit molecular weight of 100,000. Addition of leupeptin or EGTA did not affect this value. (Supported in part by NIH grant HL 18094.)

17. DNA-MEDIATED GENE TRANSFER OF RESISTANCE TO 25-OH CHOLESTEROL. Jeffrey L. Nordstrom, *Dept. of Biochemistry and Biophysics, Texas A & M Univ., College Station, TX 77843.*

To study genes that regulate cholesterol biosynthesis, we have begun to characterize the gene (or genes) that encodes resistance to 25-OH cholesterol. Total chromosomal DNA was isolated from variant hamster cells that exhibit resistance to 25-OH cholesterol (CHL-A2 cells, kindly provided by Harry Chen, Jackson Labs, ME). Mouse L-M cells were transformed with this DNA by the calcium-phosphate method (20 μg of Ca-PO_4-DNA precipitate was added to 5×10^5 cells). After 4 hr, selection pressure was exerted (0.3 μg/ml 25-OH cholesterol) and maintained for 3 weeks, after which time several resistant colonies were evident. No resistant colonies were observed when L-M cells were transformed with no DNA or with DNA from cells that were not resistant to 25-OH cholesterol. Resistant L-M cells exhibit typical fibroblast morphology, anchorage dependence and essentially unaltered growth in up to 0.5 g/ml 25-OH cholesterol. The phenotype is not lost when cells are grown for more than 10 generations in nonselective media. Analysis of the total nuclear DNA of resistant L-M cells by southern blot hybridization, using a probe specific for highly repetitious hamster DNA, indicates that resistant L-M cells have acquired hamster DNA sequences. The HMG-CoA reductase activity of L-M and resistant L-M cells, when grown in the absence of 25-OH cholesterol, is identical (210 pmol/min/mg). After 24 hr in the presence of 1.0 μg/ml 25-OH cholesterol, the reductase activity of L-M cells dropped to 18 pmol/min/mg but that of resistant L-M cells dropped only to 150 pmol/min/mg. Thus, in the presence of a high concentration of 25-OH cholesterol, L-M cells that were rendered resistant by transformation exhibit 8-fold higher reductase activity than sensitive L-M cells. Our results demonstrate that cultured cells may acquire resistance to 25-OH cholesterol by DNA-mediated gene transfer techniques. Thus, resistance to 25-OH cholesterol is a dominant selectable genetic trait. We confirm that resistance to 25-OH cholesterol is associated with altered regulation of HMG-CoA reductase. Current experimentation is underway to rescue and identify the gene (or genes) that encodes resistance to 25-OH cholesterol. This will enable us to determine whether resistance results from an alteration in the reductase gene or in a putative regulatory gene.

18. ROLE FOR ENDOGENOUS OXYSTEROLS IN THE REGULATION OF HYDROXYMETHYLGLUTARYL CoA REDUCTASE. S. R. Panini, R. C. Sexton and H. Rudney. *Dept. of Biological Chemistry, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267.*

Regulation of reductase was studied in cultured rat intestinal epithelial cells (IEC-6) using 3-β-(2-diethylaminoethoxy)-androst-5-en-17-one (U18666A), a steroidal inhibitor of cholesterol biosynthesis. We have demonstrated that U18666A is a potent inhibitor of 2,3-oxidosqualene cyclase leading to the accumulation of squalene-2,3:22,23-dioxide (SD) in IEC-6 cells and in human skin fibroblasts (Panini et al. 1983. *Federation Proc.* **42:** 1836). Treatment of cells for 24 hr with low levels of U18666A (5 to 100 ng/ml) led to progressive loss (up to 60%) of reductase activity. Further increases in the level of the drug, however, paradoxically lessened the inhibition such that at a level of 1 μg/ml, no inhibition of reductase activity was observed. Treatment with U18666A at 500 ng/ml for 24 hr, followed by fresh medium without the drug for 5 hr, caused a decrease in the cellular level of SD and a corresponding increase in the level of the polar sterol fraction as well as an increase in the degree of inhibition of reductase activity (from 40% to 75%). These results suggest that polar oxysterols derived from SD, rather than SD itself, may be the intracellular suppressor(s) of reductase in IEC-6 cells. Thus, the decrease in reductase activity seen at low levels of U18666A could be due to incomplete inhibition of cyclase, allowing cyclization of SD to oxysterols. To test this further, IEC-6 cells grown in lipoprotein-deficient medium were exposed to various concentrations of SD in the presence and the absence of 1 μg/ml U18666A. At a level of 1 μg/ml, SD caused considerable suppression (65–70%) of reductase activity. This effect was abolished by U18666A, indicating that cyclization of SD to oxysterols such as 24,25-oxidolanosterol and 24(S),25-epoxycholesterol (Nelson et al. 1981. *J. Biol. Chem.* **256:** 1067–1068) may be an essential prerequisite to its suppressive action. On the other hand, U18666A failed to prevent the decrease in reductase activity caused by 25-hydroxycholesterol (0.5 μg/ml), confirming that the drug interferes with the formation but not the subsequent action of the oxysterols. Two other known suppressors of reductase, namely low density lipoproteins (LDL) and mevalonolactone (MVL) were tested on IEC-6 cells in the presence and the absence of 1 μg/ml U18666A. The drug did not prevent the inhibition of reductase by MVL (1–5 mM). Surprisingly, the inhibitory effect of LDL (10–50 μg protein/ml) was totally abolished by U18666A. Degradation of ^{125}I-LDL was unaltered in the presence of the drug thus demonstrating the absence of any lysosomotropic effect in the action of U18666A. These data strongly suggest that the suppressive action of LDL on reductase is mediated by endogenous oxysterols generated from SD via partial inhibition of 2,3-oxidosqualene cyclase. (Supported by grant AM-12402 from NIAMDDK).

19. INHIBITORS OF CELL AUTOPHAGY AND PROTEOLYSIS AFFECT THE DECAY OF HMG-CoA REDUCTASE ACTIVITY IN ISOLATED HEPATOCYTES. Rex A. Parker, Karen J. Evenson and David M. Gibson. *Dept. of Biochemistry, Indiana Univ. School of Medicine, Indianapolis, IN 46223.*

Rat hepatocytes isolated at 5 hr into the dark phase of the diurnal cycle initially have a high total reductase activity which declines at least 50% for 1 or 2 hr during cell incubations. We have previously observed that glucagon enhances while insulin diminishes this basal decrease in total reductase activity (*J. Biol. Chem.* 1979. **254:** 9986). In the present study, we have demonstrated that incubation of hepatocytes with agents known to affect cellular protein degradation perturbed both the basal and glucagon-enhanced decay of reductase activity measurable in protein phosphatase-treated microsomes prepared from 17,000 *g* extracts. The inclusion of a mixture of amino acids (Eagle's MEM amino acid solution) blocked the decline of total reductase activity in a concentration-dependent manner (maximal at 1–2 × stock conc.). 3-Methyladenine, a specific inhibitor of autophagosome formation (Seglen and Gordon. 1982. *Proc. Natl. Acad. Sci. USA.* **79:** 1889) suppressed the loss of reductase up to 80% at 1 mM. The ionophore monensin (5 μM) blocked 60–80% of the decline, as did the microtubule inhibitors colchicine (10 μM) and vinblastine (1 μM). The thiol protease inhibitor leupeptin (200 μM) prevented up to 90% of the decline, and cycloheximide (10–100 μM) blocked up to 50% when added during cell isolation. In contrast, the lysosomotropic amines (ammonium

chloride, methylamine, neutral red) has little effect with the exception of chloroquine (5–100 μM) which paradoxically caused a rapid further diminution of reductase compared to controls. This effect of chloroquine was prevented by pretreatment with leupeptin, 3-methyladenine, or colchicine. The time-dependent decay of total reductase activity in the microsomal fraction was not accompanied by an increase in activity in other subcellular fractions. None of the compounds studied affected reductase activity when added directly to microsomes. The results indicated that reductase in the hepatocyte was degraded by way of the autophagic vacuolar-lysosomal system and suggested that a leupeptin- and cycloheximide-sensitive protease(s) was involved. (Supported by grants from NIH (AM 21278), the American Heart Association, Indiana Affiliate, and the Showalter Foundation.)

20. (R)3-HYDROXY-3-METHYLGLUTARYL COENZYME A: THE NOT-SO-INNOCENT BYSTANDER IN THE ENZYMATIC REDUCTION OF (S)HMG-CoA TO (R)MEVALONATE. Andrzej Pastuszyn, Christopher M. Havel, Terence J. Scallen and John A. Watson. *Univ. of New Mexico, Albuquerque, NM, and Univ. of California, San Francisco, San Francisco, CA.*

Chemical synthesis using radioactive 3-hydroxy-3-methylglutaryl anhydride and coenzyme A yields a racemic (RS) thioester. Although (S)HMG-CoA is the natural substrate for HMG-CoA reductase, it was assumed that (R)HMG-CoA was benign in the (RS) mixture. In the course of our efforts to develop an inexpensive, facile synthesis of (S)[^{14}C]HMG-CoA, we found that (R)HMG-CoA was a competitive inhibitor of reductase with K_i of 2.5 μM. The apparent K_m for (S)HMG-CoA was 1.5 μM. Rat liver reductase activity, with (S)HMG-CoA, was 1.8–2.0-fold higher than that found with the (RS) substrate. Our biological synthesis of (S)[^{14}C]HMG-CoA was based on the procedure described by Henning et al. (*Arch. Biochem. Biophys.* 1959. **83:** 259). The (S)[5-^{14}C]HMG-CoA (53 mCi/mmole; 300 μCi) synthesized was *1*) radiochemically pure by HPLC, *2*) completely reduced to (R)[^{14}C]mevalonate by *Halobacterium halobium* and rat liver reductase, and *3*) the latter was readily metabolized to [$^{14}CO_2$] by intact cells and cell-free extracts. Our results demonstrated that (R)HMG-CoA was *not* an innocent bystander in the reductase reaction. Thus, one should have serious concern about the use of (RS)HMG-CoA as a substrate in mechanistic studies. Furthermore, the 3–4-fold disparity between corrected C_2 flux into cholesterol and corrected C_2 flux into mevalonate (*J. Lipid Res.* 1974. **15:** 508) is increased further by a factor of 2.0. Therefore, if reductase is the rate-determining enzyme for sterologenesis, its in vivo activity must be even more inhibited than previously thought. (J.A.W. is an Established Investigator (80 231) of the American Heart Association. This research was supported by HL 16796.)

21. REGULATION OF HMG-CoA REDUCTASE AND CHOLESTEROL SYNTHESIS IN H-4-II-E-C3 CULTURED HEPATOMA CELLS AND THEIR CYTOPLASTS. George Popják, Cheri Hadley and Aniko Meenan. *UCLA School of Medicine, Los Angeles, CA 90024.*

When the culture medium (Swim's S-77 + 5% fetal bovine + 10% horse serum = FG-medium) of confluent H4-II-E-C3 hepatoma cells (H4) is changed to a similar medium but supplemented with lipid-depleted sera (LD-medium), the H4 cells respond in 24 hr with a 3- to 5-fold and in 48 hr with a 5- to 6-fold induction of HMG-CoA reductase, or corresponding increases in incorporation of [^{14}C]acetate into cholesterol. Mevinolin, 1 μg/ml, added to LD-medium causes usually a further 3-fold induction of the reductase over and above that seen in LD-medium alone. This "superinduction" can be observed even in whole cells by an increased incorporation of [^{14}C]acetate into sterols, if the cells kept with mevinolin are first thoroughly washed and then incubated in mevinolin-free growth medium for at least 1 hr before another change of growth medium with [^{14}C]acetate. The superinduction of the reductase can be completely suppressed by 4 mM *R*-mevalonate (MVA), but only partially (60%) by as high a concentration of LDL as 110 μg/ml. Mevinolin, 1 μg/ml, causes in H4 cells kept even in FG-medium a 2- to 3-fold induction of the reductase in 24 hr. The half-life of the reductase after induction in LD-medium is about 1.5 hr, but it is about 4.0 hr after induction in LD-medium + mevinolin, as judged by decreased activity after addition of 4 mM *R*-mevalonate. H4 cells can be enucleated, at least partially, with cytochalasin B by the technique of Cavenee, Chen and Kandutsch (1981. *J. Biol. Chem.* **256:** 2675). The enucleated cells, the cytoplasts, retain all the sterol-synthesizing enzymes without impairment, as judged by incorporation of [^{14}C]acetate into sterols per mg protein. Cytoplasts were prepared from confluent H4 cells induced either by incubation for 24 hr in LD-medium or in FG-medium + mevinolin (1 μg/ml). Cytochalasin B-treated, but not enucleated cells and the cytoplasts, attached to the substratum, were made to recover in cytochalasin-free growth media for 1 hr at 37°C before testing the suppression of the reductase, or sterol synthesis, by the addition of 4 mM *R*-mevalonate. Whereas the cytochalasin-treated nucleated cells responded in 4 hr with 75–90% suppression, the cytoplasts responded with a 10–25% to a maximum of 40% suppression. The results are very similar to those reported by Cavenee et al. (1981) on the effects of 25-hydroxycholesterol in nucleated and enucleated CHO-1 cells. Since mevalonate suppresses the reductase only in vivo (Edwards et al. 1977. *J. Biol. Chem.* **252:** 1057), and since it appears that an interaction with the nucleus is required for the full effect of mevalonate to appear, it is postulated that a cytoplasmic substance derived from mevalonate acts as a natural regulator of the transcription of the reductase gene. (Supported by USPHS grant HL 12745).

22. APPARENT ENZYME-PHOSPHORYLATION-INDEPENDENT DOUBLING OF HAMSTER ADRENAL HMG-CoA REDUCTASE ACTIVITY DURING PREINCUBATION. Benjamin Preiss and Mario Delisle. *Univ. de Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4.*

The question what are optimal conditions for the assay of reductase activity in the adrenal gland has not received a satisfactory answer. Hamster adrenal microsomes were isolated at 10:00 AM routinely in 0.3 M sucrose, 5 mM DTT and the reductase assay was performed in a Tris-EDTA-KCl medium with 5 mM DDT. Protein was determined by the Bradford method, using a standard of bovine gamma globulins. Microsomes isolated in the absence of NaF were assayed immediately or preincubated for 30 min at 37°C. In a typical experiment, specific activities of reductase found, expressed in pmoles/mg protein/min, were 1410 and 2450 before and after preincubation, respectively, with 100 mM NaF present during preincubation and 67 mM NaF during the assay. Where NaF was omitted, activities were 1330 and 2700 before and after preincubation. When crude rat liver protein phosphatase was included during preincubation, the same preparation had an activity of 2710. The time course of the activation in the ab-

sence of NaF was 48% of full activity without preincubation, 60%, 85% and 100% after 3, 6, and 10 min preincubation, respectively. The effect of a lowered concentration of DTT on reductase activity was studied. Microsomes isolated and assayed with 0.5 mM DTT were compared to microsomes from matching adrenals prepared and assayed with 5 mM DTT. Microsomes in 0.5 mM DTT had 28% and 57% of the activity of preincubated 5 mM DTT microsomes before and after a 30-min preincubation, respectively. No HMG-CoA lyase activity was detected in microsomes either before or after preincubation.

23. FERMODULIN—A PROTEIN THAT INHIBITS HMG-CoA REDUCTASE IN THE PRESENCE OF Fe(II). T. Ramasarma, A. Satish Menon and S. Usha Devi. *Dept. of Biochemistry, Indian Institute of Science, Bangalore 560 012, India, and Institute for Toxicology, Univ. of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90033.*

Rapid inactivation of HMG-CoA reductase occurred when microsomes were incubated with $FeSO_4$ and the cytosolic fraction obtained from rat liver. The inhibitory effect was not due to any artificial loss of product or substrates of HMG-CoA reductase. Modulation of the activity of HMG-CoA reductase was obtained only in the combined presence of Fe(II) and the cytosolic protein (reminiscent of calmodulin) and this prompted naming it fermodulin. Using ammonium sulfate fractionation (30–60% saturation), heat treatment (50°C, 20 min), fractionation on CM-Sephadex (pH 6.0) and affinity chromatography on Fe-blue Sepharose matrix, fermodulin was purified to homogeneity which gave a single band on polyacrylamide gel electrophoresis. On filtration through a Sephacryl S-200 column, the protein gave a major peak with M_r 58,000 and a minor one at M_r 104,000, possibly a dimer. On SDS-polyacrylamide electrophoresis the protein resolved into two subunits of M_r 40,000 and 28,000. Fermodulin showed spectra of ultraviolet absorption and fluorescence typical of a tryptophan-containing protein and addition of $FeSO_4$ quenched the fluorescence. Using the Millipore filtration method, binding of 1.6 mole of ^{55}Fe-$FeCl_2$ per mole fermodulin (M_r 58,000) was demonstrated. Addition of purified fermodulin or Fe(II) by itself had no effect on the reductase activity in washed microsomes. Together they showed a concentration-dependent inhibition. This Fe(II)-dependent inhibition was not obtained with bovine serum albumin, transferrin, ferritin, superoxide dismutase, horseradish peroxidase or catalase. The inhibitory effect of fermodulin, at nonsaturating concentration, was potentiated by bicarbonate, ATP · Mg or ADP · Mg. Several lines of evidence suggested that the inhibition was not due to oxygen radicals or lipid peroxidation and that it might result out of a complex between Fe(II)-fermodulin-HMG-CoA reductase. Fermodulin appears to be a new intracellular iron-binding protein with a function of inhibiting HMG-CoA reductase when intracellular free iron concentration increases.

24. EVIDENCE THAT THE "ESSENTIAL" SULFHYDRYL GROUP OF HMG-CoA REDUCTASE IS INVOLVED IN THE BINDING OF HMG-CoA AND NOT NADPH. David Rogers and Harry Rudney. *Dept. of Biological Chemistry, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267.*

During immunochemical investigations of active-site-related conformational changes in rat liver HMG-CoA reductase (1982. *J. Biol. Chem.* **257:** 10650–10658), we found that the modification of enzyme antigenicity caused by the binding of HMG-CoA was prevented by omission of protective thiols from preincubation buffers. Using immunoinhibition of the reductase by a specific antibody as a probe for conformational changes in or near the catalytic site, we have studied the role of the "essential" sulfhydryl group(s) in the interaction of the enzyme with its substrates. In this technique, thiol-deficient microsomes (TDM, isolated without added thiols) were preincubated in the presence or absence of dithioerythritol (DTE) with various test compounds. Aliquots were then incubated with anti-reductase IgG followed by assay of the reductase activity remaining. The immunoinhibition titer is defined as the micrograms of IgG required to inhibit one unit of enzyme activity. An increased titer, then, indicates that the antibodies are less efficient in inhibiting the reductase, presumably as a result of a conformational change in the enzyme. Little difference was noted between the titer of TDM and that of control microsomes isolated with DTE from the same animal. The previously observed increases in the immunoinhibition titer upon exposure of the enzyme to low concentrations of HMG-CoA (5 μM) were completely eliminated in TDM. Addition of increasing amounts of DTE to the buffer during preincubation with substrate gradually restored the effect, reaching a maximum at 10 mM DTE. The activity of mevinolin, a potent inhibitor competitive with respect to HMG-CoA, was modulated in a similar fashion. In contrast, both NADPH and the competitive inhibitor, adenosine 2′-monophospho-5′-diphosphoribose (ATP ribose) increased the titer in TDM regardless of the presence of DTE. HMG and CoA had little effect on the titer of TDM with or without thiols; however, in equimolar combination, these substances caused a large increase in the titer dependent upon the presence of DTE. In sum, these observations suggest that the "essential" sulfhydryl group(s) of the reductase required for catalytic activity is located within that region of the enzyme broadly labeled as the HMG-CoA binding site. The conformational change and resulting effects of NADPH and ATP ribose on catalytic activity apparently do not involve a readily accessible sulfhydryl group analogous to that related to HMG-CoA binding. (Supported by grant AM-12402 from NIAMDDK).

25. GLUTATHIONE-DEPENDENT MODULATION OF RAT LIVER HMG-CoA REDUCTASE ACTIVITY BY REDUCED PYRIDINE NUCLEOTIDES. Joseph Roitelman and Ishaiahu Shechter. *Dept. of Biochemistry, Tel Aviv Univ., Tel Aviv, Israel.*

Rat liver microsomes devoid of free thiols were prepared in buffer HEPES in the presence of 30 μM leupeptin. The activation of HMG-CoA reductase by GSH or DTT in these microsomes was studied and compared to the activation by these thiols of reductase that was solubilized from the microsomes by the widely used freeze-thaw procedure. An increase of V_{max} in both solubilized and microsomal reductase was observed with increasing concentrations of the two activating thiols. Reactions of GSH-activated microsomal enzyme with increasing concentrations of NADPH showed sigmoidal kinetics with a Hill coefficient of 2.07 at 4 mM GSH. Increase of the activating GSH concentrations resulted in a gradual change towards normal Michaelis-Menten kinetics and a Hill coefficient of 1.05 was calculated at 25 mM GSH. With DTT, activation of the microsomal enzyme yielded similar results

except that a Hill coefficient of 1.1 was observed already at 2.5 mM DTT. When HMG-CoA reductase was solubilized, the cooperative pattern was abolished and normal Michaelis-Menten kinetics were observed with a Hill coefficient of 1.0, regardless of GSH concentration. Addition of 200 mM Pi to microsomal enzyme activated with 5 mM GSH resulted in a 1.65-fold increase in V_{max} and a decrease of the calculated Hill coefficient from 1.91 to 1.16. Normal enzyme kinetics were observed for HMG-CoA at all GSH concentrations. NADH serves as a positive modulator of microsomal, GSH-activated reductase activity. Addition of 200 μM NADH decreases the $S_{0.5}$ for NADPH from 60.0 μM to 30.4 μM. These results are compatible with a model in which HMG-CoA reductase activity is modulated by reduced pyridine nucleotides and GSH at their physiological hepatic concentrations. This work was supported by grant No. 1426 of The Council For Tobacco Research–U.S.A., Inc.

26. INHIBITION OF DEGRADATION OF HMG-CoA REDUCTASE BY MEVINOLIN. Michael Sinensky and Judith Logel. *Eleanor Roosevelt Institute, Box B129, 4200 E. 9th Avenue, Denver, CO 80262.*

Pulse and pulse-chase studies with ^{35}S-methionine on the effect of mevinolin on the turnover of HMG-CoA reductase in Chinese hamster ovary (CHO) fibroblasts were performed by radio-immune precipitation and SDS-PAGE methods. The results show no effect of short exposures (up to 24 hr) to mevinolin (0.5 μg/ml) on the synthesis of reductase but do show a dramatic lengthening of the half-life of the enzyme. In both the CHO-Kl wild-type cell and a CHO-Kl mutant (Mev-1) missing HMG-CoA synthase activity, the half-life of the reductase molecule is lengthened from approximately 2.5 hr to approximately 12 hr. The increase in reductase half-life in Mev-1 upon mevinolin treatment suggests that the block to mevalonate synthesis produced by this compound is not responsible for its effect on reductase half-life. Furthermore, studies of labeled amino acid release from total cellular protein to the culture medium indicate that mevinolin has no effect on turnover of bulk cellular protein and hence that the effect of mevinolin on reductase is specific. It was also found that short exposure (up to 5 hr) of cells to 25-hydroxycholesterol (0.5 μg/ml) inhibited reductase *activity* with a half-life of 2.5 hr in fibroblasts despite treatment with mevinolin so as to produce a half-life of the enzyme molecule of 12 hr. This result is consistent with an effect of 25-hydroxycholesterol on the catalytic efficiency of reductase in CHO cells in addition to its previously described capacity to rapidly block synthesis of reductase in these cells. It is of interest that the half-life of enzyme inactivation and degradation are identical upon treatment of CHO cells with 25-hydroxycholesterol, raising the possibility that the rate-limiting step in the two processes may be identical.

27. REGULATION OF HMG-CoA REDUCTASE BY A NEW CLASS OF NONCOMPETITIVE INHIBITORS. P. W. Stacpoole, H. J. Harwood, Jr., and C. E. Varnado. *Dept. of Medicine, Univ. of Florida, Gainesville, FL 32610.*

Dichloroacetate (DCA) reduces circulating cholesterol levels in animals and in patients with combined hypercholesterolemia or homozygous familial hypercholesterolemia. To investigate the mechanism of its cholesterol-lowering action, we studied the effects of DCA and its hepatic metabolites, glyoxylate and oxalate, on the activity of reductase obtained either from livers of healthy, reverse light-cycled rats or from peripheral blood leukocytes of healthy donors. Oral administration of DCA for 4 days to rats decreased reductase activity 46% (50 mg/kg/day) or 82% (100 mg/kg/day). A fall in reductase activity was observed as early as 1 hr after a single oral drug dose. The inhibitory effect of DCA was due to a reduction in both expressed enzyme activity and to the total number of reductase molecules present. With chronic administration, DCA inhibited ^3H_2O incorporation into hepatic cholesterol 38%. When liver microsomes were incubated with DCA, the pattern of inhibition of reductase activity was noncompetitive, but reversible, for both HMG-CoA (K_i 11.8 mM) and NADPH (K_i 11.6 mM). Glyoxylate was also a noncompetitive inhibitor for HMG-CoA (K_i 1.2 mM) and NADPH (K_i 2.7 mM). Oxalate had little effect on enzyme activity. The inhibition by glyoxylate was an effect exerted on the reductase itself, rather than on its regulatory enzymes, reductase kinase and reductase phosphatase. When microsomes from freshly isolated human leukocytes were incubated with 4 mM or 8 mM DCA or glyoxylate, reductase activity decreased 16%–34%. Conclusions: 1) the cholesterol-lowering effect of DCA is due, at least in part, to inhibition of endogenous cholesterol synthesis; 2) the probable mechanisms are by inhibition of expressed reductase activity by DCA per se and by conversion of DCA to an active metabolite, glyoxylate, which noncompetitively inhibits reductase; 3) DCA and related carboxylic acids may prove useful in regulating cholesterol synthesis and circulating cholesterol levels in man.

28. SHORT-TERM REGULATION OF HEPATIC CHOLESTEROL SYNTHESIS THROUGH PHOSPHORYLATION OF HMG-CoA REDUCTASE IN VIVO. Eduard F. Stange and John M. Dietschy. *Dept. of Internal Medicine, The Univ. of Texas Health Science Center at Dallas, Dallas, TX 75235.*

HMG-CoA reductase can be inactivated by a reductase kinase-dependent phosphorylation and activated by dephosphorylation through a phosphoprotein phosphatase in vitro. The physiological role of this process is still uncertain since the proportion of phosphorylated enzyme remains constant under conditions where total reductase activity and the rate of cholesterol synthesis varies as much as 50-fold (*J. Biol. Chem.* 1979. **254:** 5144–5149). Thus, it was suggested that long-term alterations in cholesterol synthesis are due to changes in the total amount of enzyme protein. The possibility remains, however, that reductase phosphorylation is important in short-term regulation of sterol synthesis. METHODS: Reductase was assayed in rat liver microsomes as described (*J. Biol. Chem.* 1979. **254:** 5144–5149) with modifications. Expressed activity was determined in the presence of 150 mM NaF during homogenization and assay; total activity was measured after preincubation of the microsomes with purified type 1 reductase phosphatase (kindly supplied by D. M. Gibson, Indianapolis). The specific activity of the phosphatase was 13 mU/μl (*Methods Enzymol.* 1981. **71:** 486). Digitonin precipitable sterol (DPS) synthesis was determined in liver slices in vitro with [^{14}C]octanoate as substrate (*J. Lipid Res.* 1979. **20:** 740–752) and in vivo with [^{3}H]water (*J. Lipid Res.* 1981. **22:** 551–569). RESULTS: Groups of rats received 1 ml of water (controls) or 1 ml of water containing 200 mg of mevalonolactone (MVL) intragastrically. In the control animals killed 20 or 60 min later, expressed reductase activity averaged 9.3% of total activity and reductase was fully activated with 1 μl of phospho-

protein phosphatase. There was no significant difference between the controls at the two time points. At 20 min, expressed activity in the animals receiving MVL was suppressed by 52% compared to controls (P = 0.004). In contrast, total activity, after full reactivation with 5 μl of phosphatase, was not significantly different between the two groups. At 60 min both expressed and total activity were decreased by 78 and 89%, respectively. When the animals were killed at 15 min and liver slices were incubated for another 15 min, [^{14}C]octanoate incorporation into DPS after MVL was only 11% of controls. Sixty min after the MVL administration DPS synthesis was 7% of controls, as judged in vitro. However, the overall incorporation of [^{3}H]water in vivo (including the incorporation into the administered MVL) was similar in both groups. Therefore, in the short-term (20 min) experiments, reductase phosphorylation led to rapid suppression of sterol synthesis from acetyl CoA while long-term regulation (60 min) of synthesis was related to total enzyme activity.

29. CORRELATION OF IN VITRO INHIBITION OF LIVER HMG-CoA REDUCTASE WITH REDUCTION OF RAT SERUM TOTAL CHOLESTEROL BY 3-HYDROXY-3-METHYLGLUTARIC ACID DERIVATIVES. Luanne Stewart, James E. Miller, John Baran, Elaine Rohrbacher, Charlene Jett, and Beatrice Taite. *Searle Research and Development, G.D. Searle & Co. 4901 Searle Parkway, Skokie, IL 60077.*

The level of serum total cholesterol (TC) is controlled, in part, by the rate of new cholesterol biosynthesis. The major limiting step in cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR). A series of 3-substituted-3-hydroxyglutaric acids were prepared to determine structural requirements for inhibition of HMGR in vitro and in vivo reduction of TC. Inhibition of HMGR was determined using rat liver microsomes. Reduction of TC was measured by oral administration of test compounds to triton-treated male rats. Optimal inhibition ($IC_{50} \approx 50$ μM) of HMGR was observed with a free dicarboxylic acid with substituents (carbon 3) equivalent to 9–17 carbons in length. Inhibitory activity was lost with the anhydride(s), esters, with polar derivatives (carbon 3), and in the absence of the 3-OH group. Reduction of TC with doses up to 300 mg/kg was structure-dependent with maximum reduction (~30%) observed for compounds with a free dicarboxylic acid and a hydrophobic substitution (9–17 carbon equivalent) for the methyl group at carbon 3. Compounds that failed to inhibit HMGR did not reduce TC in the triton-treated rat. Thus, with this class of compounds, a correlation exists between chemical features required to inhibit HMGR and reduce TC.

30. REGULATION OF HMG-CoA REDUCTASE BY 25-HYDROXYCHOLESTEROL. Richard D. Tanaka, Peter A. Edwards and Alan M. Fogelman. *Dept. of Medicine and Biological Chemistry, UCLA, Los Angeles, CA.*

25-Hydroxycholesterol (25-OHC) inhibits cholesterol biosynthesis by inhibiting the activity of HMG-CoA reductase. We have used immunoprecipitation of radiolabeled enzyme from chicken myeloblasts to determine the mechanisms involved in this down regulation of enzyme activity. Addition of 25-OHC (5 μg/ml) to cell cultures caused an 80% decrease in reductase activity after 60 min. This rapid down regulation of reductase activity was due to rapid changes in the rates of reductase degradation and synthesis. 25-OHC induced a 350% increase in the specific rate of reductase degradation and caused a 70% decrease in the rate of reductase synthesis. These rapid changes in the rates of degradation and synthesis can account completely for the loss in enzyme activity due to 25-OHC treatment. The alteration in the rate of reductase synthesis was not correlated with concomitant changes in the levels of reductase mRNA, suggesting that 25-OHC inhibits the rate of enzyme synthesis by translational and not transcriptional regulation. The reductase synthesized by in vitro translation of mRNA consisted of two polypeptides (M_r = 102,000 and 94,000). Addition of the lysosomotropic agents, chloroquine and NH_4Cl, blocked the 25-OHC-induced increase in the rate of reductase degradation. These data suggest that the lysosomal system may be involved in the degradation of HMG-CoA reductase.

31. CORRELATION BETWEEN OXYSTEROL BINDING TO A CYTOSOLIC BINDING PROTEIN AND POTENCY IN THE SUPPRESSION OF HMG-CoA REDUCTASE. Fred R. Taylor and Andrew A. Kandutsch. *The Jackson Laboratory, Bar Harbor, ME 04609.*

Evidence for the hypothesis that a cytosolic oxysterol binding protein (*J. Biol. Chem.* 1981. **256:** 13068–13073) mediates the regulation of 3-hydroxy-3-methylglutaryl coenzyme A in cultured L cells (mouse fibroblasts) was obtained by correlating the apparent binding affinity of a wide range of oxysterols to their potency in suppressing reductase in cell cultures. Over 40 oxysterols encompassing a 300-fold range of activity have been tested. The two parameters were closely correlated for approximately 85% of the sterols. A few sterols, however, show poor binding when compared to their ability to repress the reductase in cell cultures. Among these are four with a keto function at C-3. For these sterols the discrepancy is explained by the fact that in cells the 3-ketone is rapidly converted (more than 75% in 5 hr) to the 3-beta hydroxy derivative which resulted in a much higher binding affinity. Sterols with a 3-keto-4-ene grouping are not reduced to a 3-beta hydroxy derivative in cells and therefore show no discrepancy in the two assays, while sterols of the 3,15-dione type do not require reduction to bind well to the binding protein and consequently display only slight activation in cell cultures despite substantial conversion. A satisfactory explanation for the disproportionately high activity of two other sterols in the repression of reductase in cells is presently under investigation.

32. RATES OF CHOLESTEROL SYNTHESIS IN VIVO REQUIRE FULLY ACTIVATED HMG-CoA REDUCTASE ACTIVITIES. Nancy L. Young. *Dept. of Medicine, Cornell Univ. Medical College, 1300 York Avenue, New York, NY 10021.*

Reductase in liver and small intestine is largely in the inactive, phosphorylated state by the time these organs are removed from the body of a rat and homogenized. However, the activation state in vivo may be different from that seen in vitro. The activity of reductase in vivo can be inferred from measurements of cholesterol synthesis in vivo. Male Wistar rats were studied at mid-dark, 3 weeks after injection of streptozotocin (Diabetic) or buffer (Control). Whole organ HMG-CoA reductase activity was measured (Young et al. *J. Lipid Res.* 1982. **23:** 266–275) in

liver microsomes and small intestine homogenates with and without NaF, i.e., without and with in vitro activation by endogenous phosphatase, in 11 diabetics and 10 controls. Whole organ cholesterol synthesis in vivo was measured (Young et al. *Diabetes.* 1983. **32:** 811–819) from [^{3}H]cholesterol formed by 80 min after injection of [^{3}H]water, in 3 diabetics and 4 controls. It was assumed that the specific activity of [^{3}H]NADPH equaled that of [^{3}H]water. Reductase activity in nmol MVA/min was converted to μg cholesterol/hr. Data are means ± SEM.

	Rate of Cholesterol Synthesis		
	From Reductase		
	+ F	− F	From [^{3}H]Cholesterol
	μg cholesterol/(hr × organ)		
Small intestine			
Diabetic	78 ± 8	275 ± 25	260 ± 7
Control	34 ± 4	129 ± 17	111 ± 6
Liver			
Diabetic	11 ± 3	70 ± 17	127 ± 57
Control	31 ± 4	305 ± 58	329 ± 45

Nonactivated reductase activity is too low in all cases to permit the rate of cholesterol synthesis measured in vivo. Correcting for incomplete recovery of endoplasmic reticulum in microsomes would raise hepatic reductase activity but not enough. Either the assay greatly underestimates reductase activity or reductase is rapidly inactivated just before homogenization of an organ. This may occur in response to catecholamine release or to low O_2/CO_2 in the tissue. This work was supported by NIH grant HL20488.

33. HMG-CoA REDUCTASE IN FRESHLY ISOLATED HUMAN MONONUCLEAR CELLS IS DECREASED BY POLYUNSATURATED FAT IN THE DIET. Nancy L. Young and Donald J. McNamara. *Cornell Univ. Medical College, 1300 York Ave., and The Rockefeller University, 1230 York Ave., New York, NY 10021.*

Polyunsaturated fat in the diet is known to decrease plasma cholesterol. To investigate the mechanism of this effect, we (Young, N. L., and B. Berger. 1981. *Methods Enzymol.* **71:** 498–509) measured HMG-CoA reductase activity in homogenates of mononuclear cells freshly isolated from men consuming a diet with either a high or low ratio of polyunsaturated to saturated fat (P:S) for 6 weeks. Mean P:S ratio, calculated from 9 day diet records at the end of the diet period, was 2.2 ± 0.3 in the high P:S group (n = 13), and 0.34 ± 0.03 in the low P:S group (n = 11). Blood was sampled before breakfast once a week during the last 3 weeks of the diet period. Mean plasma cholesterol was 244 ± 13 mg/dl in the high P:S group and 278 ± 9 in the low P:S group. Mean reductase activity was 521 ± 29 fmol/(min × mg protein) in the high P:S group and 577 ± 42 in the low P:S group. These differences were not significant due to wide variations between individuals within each group. However, upon linear regression analysis, significant relationships were detected. Thus, plasma cholesterol was negatively correlated with P:S in the combined groups: $r = -0.38$, $P = 0.03$. HMG-CoA reductase activity was also negatively correlated with P:S: $r = -0.48$, $P = 0.05$ in the high P:S group; $r = -0.85$, $P < 0.001$ in the low P:S group. This finding is consistent with the possibility that polyunsaturated fat increases the influx or decreases the efflux of cellular cholesterol thereby lowering plasma cholesterol and reducing HMG-CoA reductase activity by feedback inhibition. This work was supported by NIH grants HL20488 and HL25930.