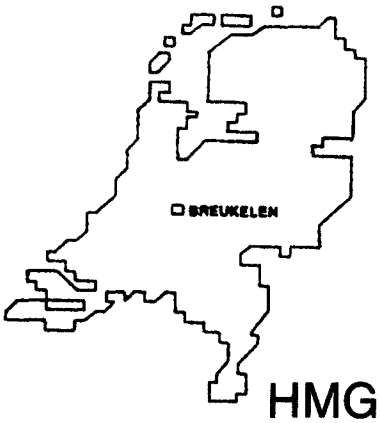

Abstracts
HMG-CoA Reductase IV
August 24, 1985, Breukelen, The Netherlands

HMG-CoA Reductase IV

Reductase IV, the fourth in a series of satellite meetings devoted to the enzymology and regulation of HMG-CoA reductase (EC 1.1.1.34), was held at Nijenrode Castle, Breukelen, The Netherlands, on August 24, 1985, immediately prior to the 13th International Congress of Biochemistry at Amsterdam. Responsibility for preparations for the meeting were shared by Victor Rodwell (West Lafayette, IN), David Gibson (Indianapolis, IN), and Math Geelen (Utrecht, The Netherlands). Generous financial support was provided by the following Industrial Sponsors: Abbott Laboratories, Boehringer-Mannheim Biochemicals, Bristol-Myers Pharmaceutical Research and Development Division, Paul Masson Vineyards, Merrell-Dow Pharmaceuticals, Merck, Sharp & Dohme Research Laboratories, Nattermann, Sankyo Corporation, Ltd., G. D. Searle & Co., and Wyeth Laboratories, Inc.

Located in quiet country near Amsterdam, Nijenrode Castle provided a coordinated and picturesque setting for Reductase IV. The meeting, opened by Prof. Dr. Simon Van den Burgh, representing the 13th I.U.B. Congress, included oral presentations, posters and a discussion session, and concluded with a reception and dinner, accompanied by pre-baroque music performed on period instruments.

A summary of the proceedings of the meeting is in press in the European Journal of Biochemistry. Presented below are 35 abstracts that summarize work by attendees at the meeting.

(Reprints of these abstracts are NOT available)

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1. EFFECT OF A CHOLESTEROL DIET AND FURTHER SUPPRESSION ON THE LIPID COMPOSITION AND HMG-CoA REDUCTASE ACTIVITY OF CHICK LIVER MICROSOMAL MEMBRANES. M. J. Alejandre, M. Garcia-Gonzalez, D. Gonzalez-Pacanowska, E. Garcia-Peregrin, and J. L. Segovia. *Dept. of Biochemistry, Univ. of Granada, Spain.*

It has been reported that the physicochemical state and the lipid composition of the endoplasmic reticulum are possible factors involved in the physiological control of reductase activity similar to what occurs with other integral membrane proteins. In the present study, chicks have been used as experimental animals due to their ability to absorb efficiently relatively high amounts of dietary cholesterol. Feeding a short-term cholesterol diet for 3 or 6 hours to 21-day-old animals induced a partial inhibition of reductase activity, an increase in the C/P molar ratio and a mobilization of microsomal fatty acids. When cholesterol was included in the diet from hatching, similar effects but of a greater magnitude were observed. Refeeding a cholesterol-free diet during different time periods after the 3 hour cholesterol treatment gave rise to a partial recovery of both HMG-CoA reductase activity and C/P molar ratio and produced profound modifications in the fatty acid composition. Contrarily, inhibition in reductase activity and modifications in the lipid composition of microsomes were maintained when the cholesterol-free diet was given to animals submitted to longer cholesterol treatments (6 hours or 21 days). The present observations indicate that a close relationship appears to exist between changes in the microsomal membrane C/P molar ratio and fatty acid composition and modifications in HMG-CoA reductase activity.

2. PROPERTIES OF DETERGENT-SOLUBILIZED AND PURIFIED HMGR FROM RADISH SEEDLINGS. T. J. Bach. *Botanical Inst. (Plant Physiology) Univ. of Karlsruhe, D-7500 Karlsruhe, FRG.*

HMGR (EC 1.1.1.34) was solubilized with Brij W-1 from a heavy membrane fraction, sedimented at 16,000 × *g* from a cell-free homogenate of 4-day-old, dark-grown radish seedlings. The solubilized enzyme was further purified through ammonium sulphate precipitation followed by column chromatography on DEAE-Sephadex A-50, Blue Dextran–agarose and HMG-CoA–hexane–agarose, all in the presence of detergent, which did not interfere with the chromatographic procedures used (Bach and Rudney JLR 24, 1404 (1983), and Bach et al. EJB, submitted). Sucrose density centrifugation suggested an apparent molecular weight of 180 kD with subunits of 45 kD (SDS-PAGE). The enzyme was stable at 67.5°C for 30 minutes in the presence of glycerol, DTE and detergent. Studies on enzyme stability and activation indicate that the radish enzyme is a hydrophobic protein with free thiol groups that are essential for full activity. The activation energy was estimated to be 22 kcal (Arrhenius plot). When both HMG-CoA and NADPH concentrations were varied, intersecting patterns were obtained with double reciprocal plots. The apparent K_m-values were 1.5 μM ([S]-HMG-CoA), and 27 μM (NADPH). Concentrations of NADPH greater than 150 μM caused substrate inhibition at low HMG-CoA concentrations. Analysis of these data and the product inhibition pattern

obtained with NADP, HS-CoA and MVA suggest a sequential mechanism with HMG-CoA being the first substrate binding to the enzyme, followed by NADPH. After release of NADP a second NADPH binds to the enzyme–mevaldate–thiohemiacetal–CoA-complex. The preliminary data suggest MVA being the first product released from the enzyme, followed by HS-CoA and the second NADP. The relatively specific inhibition by NADP of radish HMGR might be a possibility to directly regulate HMGR activity in vivo through the intracellular NADP/NADPH ratio. Another possibility could be the regulation through the activity of ubiquitous thioredoxins which use NADPH as a hydride donator for the reduction of disulfide bonds, thereby leading to an activation of HMGR. Studies to accelerate the purification procedure of HMGR not only from plants but also from other sources, such as yeast, by using a novel affinity material are under current progress.

3. PARTIAL PROTEIN SEQUENCE OF *PSEUDOMONAS* M 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE.

M. J. Beach, M. A. Hermodson, and V. 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 sole source of carbon (Gill et al. *J. Bacteriol.* (1984) 160, 294–298). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is induced in this organism when it is grown on mevalonate as the sole carbon source. This catabolic enzyme, which exists as a tetramer of identical MW 43,000 subunits, has been purified in quantities suitable for protein sequencing. Reduced, iodoacetamide-alkylated HMG-CoA reductase fragments were generated by CNBr digestion. Peptides were initially separated on Sephadex G-50, followed by purification utilizing reverse phase HPLC. Purified peptides were then sequenced by automated Edman degradation. Approximately 50% of the primary sequence has been obtained. Thus far, the primary sequence of *Pseudomonas* M HMG-CoA reductase does not appear to exhibit any obvious homology with that of Chinese hamster ovary cell UT-1 HMG-CoA reductase (Chin et al. (1984) Nature 308, 613–617). In addition, our sequence data has allowed us to design and synthesize two mixed oligonucleotide probes. These probes are currently being used in the selection of recombinant HMG-CoA reductase clones from a *Pseudomonas* M lambda library. (Supported by NIH predoctoral training grant GM07211 (MJB) and Public Health Service grant GM19223).

4. REVERSIBLE PHOSPHORYLATION OF HMG-CoA REDUCTASE: MODULATION OF ENZYMIC ACTIVITY BY MULTIPLE KINASES.

Z. H. Beg, J. A. Stonik, and H. B. Brewer, Jr. *Molecular Disease Branch, NHLBI, NIH, Bethesda, MD 20205.*

The synthesis of cholesterol and other polyisoprenoid compounds is regulated by the rate-limiting enzyme, HMG-CoA reductase (HMGR). Proteolysis of the native insoluble enzyme (MW ~ 100,000) results in a soluble active fragment (MW ~ 53,000). We have previously established that the enzymic activity of rat and human HMGR is modulated by a bicyclic cascade system involving phosphorylation and dephosphorylation of both HMGR and reductase kinase. We have also demonstrated that in intact rats the activity of reductase kinase kinase is modulated in vivo by short-term (20 min) administration of mevalonolactone providing a mechanism for the regulation of the enzymic activities of both reductase kinase and HMGR (PNAS 81: 7293–7297, 1984). Recently, we have identified two new systems for the short-term regulation of HMGR. A Ca^{2+} activated and phospholipid-dependent protein kinase (C kinase) is able to phosphorylate and inactivate both microsomal native (MW ~ 100,000) and purified soluble HMGR (MW ~ 53,000). Maximal phosphorylation of purified HMGR was associated with the incorporation of 1.05 ± 0.02 mol of phosphate per mol of native enzyme. Incubation of ^{32}P-HMGR with purified phosphatase caused loss of ^{32}P and reactivation of enzymic activity. Tumor-promoting phorbol ester, phorbol 12-myristate 13-acetate, stimulated the protein kinase C catalyzed phosphorylation of HMGR, suggesting a possible in vivo mechanism for HMGR regulation (JBC 260: 1682–1687, 1985). A third new mechanism for the modulation of HMGR involves a Ca^{2+}, calmodulin-dependent kinase (CMK). We have purified and characterized a new low molecular form of CMK from rat brain cytosol. The purified CMK phosphorylated and concomitantly inactivated (75%) both purified and native HMGR. Phosphorylation was associated with the incorporation of one mol of phosphate per mol of native enzyme. Autoradiograms of the ^{32}P-phosphorylated native and purified HMGR following immunoprecipitation and $NaDodSO_4$-gel electrophoresis revealed a single band which corresponded to the ~100,000 and ~53,000 molecular forms, respectively. The significance of these findings will be discussed. The elucidation of multiple kinase systems for the modulation of HMGR activity by covalent phosphorylation provides new insights into the molecular mechanisms involved in the regulation of cholesterol biosynthesis.

5. OXYSTEROL BINDING PROTEIN (OSBP): A NOVEL STEROL BINDING PROTEIN IN NORMAL RAT LIVER CYTOSOL WITH CHARACTERS SIMILAR TO HTC CELL OSBP. FIRST ASSAY OF PURIFICATION IN HTC CELLS.

F. Beseme, M. E. Astruc, R. Defay, and A. Crastes de Paulet. *INSERM U.58, 60 rue de Navacelles, 34100 Montpellier, France.*

Oxysterols are known to inhibit sterol synthesis, DNA synthesis and growth in numerous cell lines. They could act by binding to a specific protein (OSBP) followed by nucleus transfer of the complex. OSBPs have been demonstrated by us in the cytosol of normal human lymphocytes, rat embryo fibroblasts and HTC cells, and by Kandutsch et al. in L cells and other mouse cells. Several sterol carrier proteins such SCP_1 or SCP_2 are known in rat liver and, since OSBP is thought to be involved in cholesterogenesis regulation, we tried to evidence such a protein in rat liver cytosol. A macromolecular component of proteic nature able to specifically bind 25-hydroxycholesterol (25-OH-chol) was evidenced by using a cell-free system assay: precipitation of cytosol proteins by ammonium sulfate (25–40% saturation), incubation with 25 nM [^{3}H]25-OH-chol with or without an excess of unlabeled sterol (300 nM), separation of unbound sterol by Sephadex LH20 gel filtration and/or ultracentrifugation on linear sucrose gradient. The characters of liver OSBP were closely similar to those of HTC cell OSBP (indicated in parentheses): sedimentation coefficient about 8S (8S), molecular weight (MW) of the 25-OH-chol–protein complex: 152,000 daltons (160,000), K_D about 10×10^{-9} M (6.9×10^{-9} M), maximal binding site number: 150 fmoles/mg P (320); as in HTC cells, liver OSBP bound specifically the cholesterol derivatives oxidized on the side-chain; the C_7-oxidized compounds were weak competitors, whereas cholesterol, squalene and oxido-squalene were not recognized. In HTC cells, OSBP was partially purified through a first step of ion-exchange chromatography: cytosol proteins were layered on a DE-52 column and eluted by a linear salt gradient (0 to 0.4 M KCl). Binding assay with [^{3}H]25-OH-chol was performed on each fraction. OSBP eluted in a single peak at 0.08 M KCl and the 25-OH-chol–protein complex had the same S and MW as the complex formed with nonpurified OSBP. This step gave a 40-fold purification with a recovery of 65% of the binding capacity. Experimental conditions for more purification are now in progress. This work was supported by a contract INSERM (no. 824020).

6. FATE OF MEVALONATE AND REGULATION OF HMG-CoA REDUCTASE IN 16 HOURS REGENERATING RAT LIVER. G. Bruscalupi, S. Leoni, M. T. Mangiantini, M. Marino, M. Minieri, S. Spagnuolo, M. Valbonesi, F. Hemming, and A. Trentalance. *Dept. of Cellular and Developmental Biology, Univ. of Rome I "La Sapienza", Rome, Italy and Dept. of Biochemistry, Nottingham Univ. and Med. Sch., Nottingham, U.K.*

In the regenerating rat liver, which is a well synchronized system of in vivo proliferation, the correlation between cholesterol biosynthesis and HMG CoA reductase (HMG CoA R) activity is lost. In fact, at S phase of the first cell cycle, which occurs 16 hrs after partial hepatectomy, cholesterogenesis is depressed while HMG CoA R activity remains high (Trentalance and colleagues, 1984), as well as the production of labeled mevalonate from acetate as detected by a cold mevalonate "trap" (Bruscalupi and colleagues, 1985). The fate of mevalonate and the regulation of HMG-CoA R activity have been studied in this system. The fate of mevalonate has been studied following the conversion of labeled mevalonate in different end products of its metabolism. A lower catabolism to CO_2, an unmodified conversion to isopentenyl tRNA and ubiquinone, and a higher incorporation into dolichol have been observed. The in vitro regulation by phosphorylation-dephosphorylation mechanisms and the membrane-mediated control of the enzyme activity do not appear to be significantly changed. The in vivo treatment, with some compounds active on the enzyme of quiescent liver, shows an unmodified sensitivity to cholesterol and mevalonate and a different sensitivity to Triton WR-1339 and mevinolin. The diurnal rhythm of enzyme activity is maintained. The results, showing the preferential diversion of mevalonate into dolichol and a different sensitivity of HMG-CoA R to some effectors, support a further characterization of the enzyme in this system. Trentalance, A. & colleagues, Biochim. Biophys. Acta 794, 142, 1984. Bruscalupi, G. & colleagues, Cell. Mol. Biol., 1985, in press.

7. PURIFICATION TO HOMOGENEITY OF CYTOSOLIC HMG-CoA REDUCTASE KINASE FROM RAT LIVER. C. Caelles, A. Ferrer, N. Massot, V. E. Calvet, and F. G. Hegardt. *Dept. of Biochemistry, Univ. of Barcelona Sch. of Pharm., 08028 Barcelona, Spain.*

Reductase kinase, the enzyme that phosphorylates HMG-CoA reductase, is present in rat liver microsomes and cytosol. The cytosolic enzyme has been purified to homogeneity by chromatography on DEAE cellulose, Affi-Gel Blue, Ultrogel AcA 34, 2nd DEAE cellulose, ATP-hexane agarose and hydroxylapatite. The enzyme is very unstable through all chromatographic steps, and accordingly, it is advisable to carry as many steps as possible in the first day to avoid low yields. The presence of 5 mM dithiothreitol, 0.5 mM phenyl methyl sulphonyl fluoride and 1 mM benzamidine in buffers improves the stability of reductase kinase. The enzyme appears to be homogeneous by criteria of polyacrylamide gel electrophoresis with and without SDS. The holoenzyme has an apparent M_r of 205 Kd and its unique subunit an M_r of 107 Kd. Accordingly, a dimeric structure with identical subunits is proposed. The optimum pH is 7.5. The reductase kinase preparation is devoid of mevalonate kinase activity. Cytosolic reductase kinase is activated by ADP and in lesser amount by other nucleoside diphosphates. Taking the activity of ATP/ADP/Mg (2:2:15 mM) equal to 100%, ATP/CDP/Mg produces a 26%, ATP/UDP/Mg is 22%, ATP/Mg is 7.3% and ATP/GDP/Mg is 6.6%. The activation constant for ADP is 420 μM. Antibody prepared in rabbits against homogeneous reductase kinase immunoprecipitated the enzyme with loss of activity. Incubation of homogeneous reductase with cytosolic reductase kinase and 0.5 mM ^{32}P ATP and 2 mM ADP produces a time-dependent inactivation of the enzyme activity. The ^{32}P reductase electrophoresed by SDS PAGE showed an unique band with identical R_f as the subunit of reductase. ^{32}P appears to be bound to serine residues. The incubation of ^{32}P reductase with reductase phosphatases removes ^{32}P, this process being concomitant with the activation of reductase.

8. THE EFFECT OF INHIBITORS OF THE CHOLESTEROL SYNTHESIS ON HMG-CoA REDUCTASE ACTIVITY IN THE HUMAN HEPATOMA CELL LINE HEP G2. L. H. Cohen, A. Boogaard, and H. J. Kempen. *TNO Gaubius Inst. for Cardiovascular Res., Herenstraat 5d, 2313 AD Leiden, The Netherlands.*

Blocking the pathway to cholesterol at specific sites causes accumulation of intermediates before and depletion of metabolites behind the blockade. Both effects may influence the reductase activity and hence may give insight in regulatory mechanisms. We already showed that in Hep G2 cells compactin, an inhibitor of the reductase itself, gave rise to an induction of the reductase activity (measured after removal of the drug), which was prevented partially by LDL and totally by mevalonate (Biochem. J. 222 (1984) 35–39). In the experiments to be described here, we used U18666A as inhibitor of 2,3-oxidosqualene cyclase, buthiobate and ketoconazole as blockers of the C14-demethylation of lanosterol and triparanol, an inhibitor of desmosterol reductase. None of these substances inhibited the reductase activity in Hep G2 cell homogenates. After an 18–20 h incubation of the cells with different concentrations of the drug, the HMGCoA reductase activity was determined and the inhibition of the cholesterol synthesis and accumulation of intermediates were determined by incorporation of [^{14}C]acetate or [^{14}C]-mevalonate into nonsaponifiable lipids, identified by TLC (in the control without drugs the ^{14}C-label was incorporated only into cholesterol). In some cases accumulation of intermediates was confirmed by gas chromatographic analysis. Inhibition of the cholesterol synthesis by U18666A resulted in a decrease of the reductase activity with a minimum at 0.3–0.5 μM U18666A; however at concentrations higher than 3 μM a marked and concentration-dependent increase of the reductase activity was found. The accumulated ^{14}C-labeled intermediate formed at the lower concentration of U18666A was different from those formed at the high concentrations of the drug. The latter were probably 2,3-oxidosqualene and squalene-2,3:22,23-dioxide, whereas at the low U18666A concentration one component was found, which behaved as a polar sterol in TLC. This finding suggests that this compound may be involved in the lowering of the reductase activity. The U18666A-induced increase was additive to the activity enhancement of compactin and was not abolished by mevalonate. In the presence of 30 μM U18666A (^{14}C-acetate incorporation into cholesterol was $< 3\%$ of control) the increase due to compactin could be prevented by addition of mevalonate. This indicates the existence of a nonsterol effector in addition to a sterol dependent regulation. Surprisingly LDL, which lowered the reductase activity itself, enhanced the effect of U18666A at concentrations higher than 3 μM. Incubation of the cells with buthiobate or ketoconazole resulted in a concentration-dependent decrease of the reductase activity accompanied with a lanosterol accumulation. No other nonsaponifiable lipid was detected. With triparanol we also found a concentration-dependent lowering of the reductase activity, now accompanied with an increase of the desmosterol content of the cells. In this case as well as in the previous described one it is possible that from the accumulated sterols metabolites are formed which suppress the reductase activity. Another explanation may be that the sterol accumulating in the membranes directly influences the reductase activity.

9. MEMBRANE FLUIDITY: A MECHANISM FOR THE MODULATION OF HMG-CoA REDUCTASE BY LOW DENSITY LIPOPROTEINS. P. J. Davis and M. J. Poznansky. *Dept. of Physiology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.*

Hydroxymethylglutaryl CoA reductase (HMGR) activity, a key regulatory step in cholesterol biosynthesis, is down-regulated in cells by exposure to low density lipoproteins. It was suggested that the fluidity of the endoplasmic reticulum membrane may modulate HMGR activity. To determine if changes in fluidity could account for the down-regulation of HMGR, we introduced changes in the lipid composition of microsomes from cultured human fibroblasts, both in vivo and in vitro, and monitored both HMGR activity and membrane fluidity (ESR order parameter of 12-nitroxystearate). Microsomes from cells incubated 24h in lipoprotein-deficient medium (LPDS microsomes) were enriched with a saturated phospholipid (dipalmitoyl phosphatidylcholine, DPPC) using a nonspecific lipid exchange protein. DPPC enrichment resulted in decreased HMGR activity and an increase in the ESR order parameter (decreased fluidity). Both the decrease in fluidity and the decreased activity were reserved when the DPPC-rich microsomes were subsequently enriched with more fluid egg phosphatidylcholine. The cholesterol level in LPDS microsomes was increased by incubating microsomal preparations with egg PC:cholesterol vesicles. Decreases in HMGR activity and fluidity were observed. Enrichment of these microsomes with epicholesterol, the 3-α-hydroxyl derivative of cholesterol, did not decrease fluidity nor did it reduce HMGR activity. Microsomes from cells incubated in the presence of low density lipoproteins did not show any HMGR activity. However, a depletion of cholesterol from these microsomes led to a dramatic enhancement of HMGR activity. These observations suggest that changes in the fluidity of the endoplasmic reticulum membrane can modulate cholesterol biosynthesis and that such changes in fluidity can result from physiologically relevant changes in membrane cholesterol levels. (Supported by the Canadian Heart Foundation and by the Alberta Heritage Foundation for Medical Research).

10. NEW SYNTHETIC INHIBITORS OF RAT LIVER HYDROXYMETHYLGLUTARYL CoA (HMG-CoA) REDUCTASE. R. Deana, G. Lippe, L. Cavallini, G. Quadro, and L. Galzigna. *Inst. of Biological Chemistry, Univ. of Padova and Medea Researches, Milano, Italy.*

A preliminary study (G. Lippe et al., in publication) of the assay procedure of HMG CoA reductase resulted in an original method based on an organic phase extraction of the reaction product. In the same study, the inhibition of HMG CoA reductase by diamide, an SH reagent, free HMG, and coenzyme A disulfide was studied in order to recognize the critical structural requirements of new synthetic inhibitors. The first synthetic compounds resulting from this approach are three molecules containing either thio-ether or thio-ester groups in chains of different length. The compounds appeared to act as inhibitors in a 0.5–5 mM concentration range and the highest percent inhibition obtained was 45%. A relatively long chain and thio-ether groups were identified as structural determinants for such inhibition.

11. CHARACTERIZATION OF MICROSOMAL PHOSPHATASES. W. F. Diven, J. Sweeney, and A. Sanghvi. *Dept. of Pathology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.*

The activity of HMG-CoA reductase has been shown to be regulated by phosphorylation/dephosphorylation and similar observations have been reported for cholesterol 7α-hydroxylase and ACAT. The microsomal enzymes which participate in this regulatory process have not been extensively studied. We have solubilized, separated and partially purified four phosphoprotein phosphatases from rat liver microsomes by cholate extraction, DEAE Sephadex chromatography and Sephadex G-200 chromatography. These enzymes have been characterized by their response to phosphatase inhibitors including fluoride, L-tartrate and a series of organomolybdate compounds. The results of these experiments indicate that these are kinetically distinct enzymes. These four enzymes have also been characterized by differences in their ability to activate HMG-CoA reductase and to inactivate cholesterol 7α-hydroxylase and ACAT. Our studies suggest that a number of microsomal phosphatases exist with differing but overlapping substrate specificities and that these enzymes may be important in the regulation of cholesterol and bile acid metabolism.

12. HMG-CoA REDUCTASE ACTIVITY OF CULTURED HUMAN SKIN FIBROBLASTS IN "SYNDROMATIC" PAUCITY OF INTRALOBULAR BILE DUCTS (ALAGILLE SYNDROME). J. Dupont, J. Raulin, D. Lapous, C. Loriette, and M. Gautier. *Food and Nutrition Dept., 107 MacKay Hall, Ames, IA, 50011, Nutrition Cellulaire, Université Paris 7, 2 place Jussieu, 75005 Paris, and INSERM U 56, Hôpital Bicêtre, 94270, Bicêtre, France.*

The syndrome was first described in 1949: children with this disease have normal extrahepatic bile ducts, but a decreased number of interlobular bile ducts. Since the 1975 publication by Alagille, Odièvre, Gautier and Dommergues, 80 children were seen (Hôpital de Bicêtre). Cholestasis may develop during the first 3 months of life, but will become apparent later. Hepatomegaly is always present, and xanthomas may be found at the surface of fingers, sign of severe intrahepatic cholestasis. The high, moderate or absent hyperbilirubinemia is associated with extremely high serum levels of cholesterol (1 to 2 g %). To establish correlation of this unusual level of cholesterol with the rate of mevalonate synthesis, HMG-CoA reductase activity was determined in some of the cultured skin fibroblasts from 2–3-year-old children. These values were compared with HMG-CoA reductase activity of control cultures from children of the same age. The present study was carried out in parallel at the Université Paris 7 and at the Iowa State University Ames using the same strains of skin fibroblasts cultured from children under observation at the INSERM U 56, Hôpital de Bicêtre, France.

13. CHOLESTEROL METABOLISM AND STEROL CARRIER PROTEIN. M. J. H. Geelen, A. C. Beynen, and W. J. Vaartjes. *Lab. of Veterinary Biochemistry and Dept. of Laboratory Animal Science, State Univ. of Utrecht, P.O. Box 80 177, 3508 TD Utrecht, The Netherlands.*

Rat liver cytosol contains a nonspecific lipid transfer protein or sterol carrier protein 2 [1]. The proposition that this protein is involved in cholesterol biosynthesis and esterification [1, 2] was not substantiated in a recent study [3]. Reuber H35 hepatoma cells were found to have higher rates of cholesterol biosynthesis and of cholesterol esterification, but had a 16-fold lower content of sterol carrier protein as compared to rat hepatocytes in culture. We also studied two inbred strains of rats with low or high response of serum cholesterol to a cholesterol-rich diet [4]. The high-cholesterol diet (2% cholesterol and 0.5% cholate, w/w) caused a 7-fold higher increase in serum cholesterol in the hyperresponsive strain. On a low cholesterol commercial diet both strains did not differ with respect to the concentration of serum cholesterol and the output of fecal neutral steroids, despite

a 2-fold higher rate of whole body cholesterol synthesis in the hyporesponder. This suggests a higher rate of cholesterol turnover in the latter strain. Indeed, fecal excretion of bile acids and decay of specific radioactivity of serum cholesterol after intravenous administration of labelled cholesterol was twice as high in the hyporesponder as compared to the hyperresponder. Interestingly, the content of hepatic sterol carrier protein on the low-cholesterol diet was 786 ± 70 and 1066 ± 60 ng/mg 105,000 × *g* supernatant protein in the hyper- and hyporesponsive rats, respectively. Thus high levels of sterol carrier protein may be associated with high rates of bile acid synthesis rather than with cholesterol biosynthesis or esterification. Furthermore, the response to dietary cholesterol may be related to the sterol carrier protein. [1] Scallen, T. J., Schuster, M. W., and Dhar, A. K. (1971) J. Biol. Chem. 246, 224–230. [2] Gavey, K. L., Noland, B. J., and Scallen, T. J. (1981) J. Biol. Chem. 256, 2993–2999. [3] Van Heusden, G. P. H., Souren, J., Geelen, M. J. H., and Wirtz, K. W. A. (1985) Biochim. Biophys. Acta, in press. [4] Beynen, A. C., Boogaard, A., Van Laack, H. L. J. M., and Katan, M. B. (1984) J. Nutr. 114, 1640–1651.

14. REGULATION OF HMG-CoA REDUCTASE ACTIVITY BY CHANGES IN SUBSTRATE AVAILABILITY.

G. F. Gibbons and C. R. Pullinger. *Metabolic Res. Lab., Radcliffe Infirmary, Oxford OX2 6HE, England.*

An increasing number of cases are emerging in which changes in the activity of HMG-CoA reductase (HMGR) become uncoupled from changes in the rate of cholesterol synthesis. The stimuli which produce these changes appear to have little, if any, direct influence on the cell's supply of, or demand for, cholesterol. Three such instances are described below: (1) In isolated hepatocytes insulin stimulated HMGR activity and glucagon had the reverse effect. In neither case were these changes accompanied by corresponding variations in the rate of cholesterol synthesis. (2) During a 2-h period around the mid-point of the dark phase of the diurnal cycle, HMGR activity did not change significantly but the rate of cholesterol synthesis increased 2-fold. (3) The rate of hepatic cholesterogenesis is 10-fold higher than that in the lactating mammary gland. Despite this, HMGR activity in the latter tissue was considerably higher than that of the hepatic enzyme. These observations suggest that HMGR is not saturated with substrate in the intact cell. If this is the case, then in the absence of any other changes, carbon flux through this enzyme into cholesterol is dependent upon the concentration of HMG-CoA. This, itself, depends upon the availability of, and competition for, cytosolic acetyl-CoA (e.g., for lipogenesis). To compensate for these fluctuations in substrate availability, which result from changes in the cell's overall metabolic activity, cellular HMGR capacity changes in order to maintain a constant rate of mevalonate and, thus, cholesterol synthesis. The changing availability of acetyl-CoA and HMG-CoA may be signalled by a rapid change in the rate of synthesis of a regulatory pool of cholesterol or of a cholesterol precursor. This transient change would produce the appropriate response in HMGR capacity so that the original rate of cholesterol formation is maintained but at a different cellular concentration of HMG-CoA.

15. ENDOGENOUS FORMATION OF OXYSTEROLS IN THE REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE IN RAT INTESTINAL EPITHELIAL CELLS.

A. Gupta, R. C. Sexton, and H. Rudney. *Dept. of Biochemistry and Molecular Biology, Univ. of Cincinnati, Coll. of Med., Cincinnati, Ohio 45267-0522.*

Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase, reductase) activity was studied in cultured rat intestinal epithelial cells (IEC-6 cells) using ketoconazole, a known cytochrome P_{450} inhibitor of 14α-demethylation of lanosterol. Treatment of cells with ketoconazole (1–10 μM) caused a concentration-dependent inhibition of reductase activity. Further increase in the level of the drug (10–200 μM) paradoxically lessened the inhibition such that at a concentration of 200 μM, no inhibition of enzyme activity was observed. The drug had no effect on reductase activity in cell homogenates. Ketoconazole (1–200 μM) caused progressive inhibition of the incorporation of [^{3}H]mevalonolactone into cholesterol with a concomitant accumulation of radioactivity into methyl sterols and 24,25-epoxylanosterol. Interestingly, the incorporation of radioactivity into polar sterols showed a biphasic response to ketoconazole treatment which was inversely proportional to the biphasic response of reductase activity. Thus, at low concentrations of the drug (1–10 μM) incorporation of [^{3}H]mevalonolactone increased into polar sterols and at high concentrations (200 μM) decreased to control values. Incubation of cells with ketoconazole and [^{3}H]mevalonolactone followed by removal of the drug and radiolabel resulted in an inhibition of reductase activity and a redistribution of radiolabel from methyl sterols to cholesterol and polar sterols. This inhibition of reductase activity could not be prevented by U18666A (3β-[2-diethylaminoethoxy]androst-5-en-17-one), a 2,3-oxido squalene cyclase (EC 5.4.99.7) inhibitor, indicating that inhibitory polar sterols were not derived via squalene 2,3:22,23-dioxide. These results indicate that at low concentrations of the drug the inhibition of reductase activity was due to the formation of regulatory oxysterols from accumulating methyl sterols. Treatment of cells with 24,25-epoxylanosterol and 25-hydroxylanosterol caused an inhibition of reductase activity in a concentration-dependent manner. Ketoconazole prevented the inhibition by low concentrations of 24,25-epoxylanosterol but not by 25-hydroxylanosterol. This suggests that 24,25-epoxylanosterol is not an oxysterol suppressor of reductase per se but only an intermediate in the formation of a suppressor mediated via ketoconazole sensitive step(s). Treatment of cells with ketoconazole totally abolished the low density lipoprotein (LDL) suppression of reductase activity without affecting its cellular uptake and degradation. Although ketoconazole inhibited LDL-induced acyl-CoA:cholesterol acyltransferase (ACAT), with the aid of specific ACAT inhibitors we have found that ACAT is not involved in down-regulation of reductase activity by LDL (Fed. Proc. 44, 1786, 1985). Since ketoconazole could not prevent the action of preformed oxysterols on reductase but totally abolished LDL action, the possibility is raised that down-regulation of reductase by LDL is mediated via a cytochrome P_{450}-dependent formation of oxysterols generated from either endogenous precursors or from cholesterol carried on LDL. Supported by NIH NIADDK-12402 and NSF PCM 8204817.

16. STUDIES ON CHANGES OF RATE OF SYNTHESIS AND RATE OF DEGRADATION OF HMG-CoA REDUCTASE IN ISOLATED RAT HEPATOCYTES.

D. Haro, P. Marrero, G. Asins, D. Serra, T. Royo, and F. G. Hegardt. *Dept. of Biochemistry, Univ. of Barcelona Sch. of Pharm., 08028 Barcelona, Spain.*

Rat hepatocytes isolated according to standard techniques were incubated with 35S methionine, pelleted, washed and then solubilized in buffer containing 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 1 mM leupeptin, 15 μg/ml aprotinin, 15 μg/ml antipain and 2 mM PMSF. The soluble fraction obtained after centrifugation at 100,000 × *g* for 60 min and containing nearly 100% of the TCA-precipitable radioactivity was immunoprecipitated with IgG anti-reductase (52 Kd) in rabbits; thereafter, pansorbin was added and incubated for 30 min. Polyacrylamide

gel electrophoresis in SDS and fluorography showed that antibody specifically precipitated two main polypeptides, one corresponding to 97 Kd, the other to 180 Kd. In addition to the 97 Kd, the polypeptide appearing to be 180 Kd seems to be HMG-CoA reductase according to the following criteria. (1) It is not immunoprecipitated by normal rabbit serum. (2) It is from microsomal origin as it also appears when the microsomal fraction is solubilized by the same buffer. (3) It disappears when rat liver hepatocytes are incubated in the presence of 10 mM mevalonate. (4) It is synthesized at a rate similar to that of polypeptide of 97 Kd. (5) A cyclic diurnal variation of its rate of synthesis similar to total reductase activity is observed. The ratio 180 Kd/97 Kd polypeptides is 85/15. Incubation of hepatocytes with 10 mM mevalonate produces a decrease of rate of synthesis of reductase independent of the time of the day; this effect is analogous in both the 97 Kd and its dimer of 180 Kd. However, mevalonate diminishes the half-life only of the 97 Kd polypeptide at D3, not producing any change in the half-life of the 180 Kd polypeptide not only in D3 but in any time of the diurnal cycle. On the other hand, mevalonate does not affect the rate of total cellular protein synthesis and degradation. When rat hepatocytes were incubated with ^{32}P and mevalonate there was a significant increase (70%) in the amount of ^{32}P associated with the 97 Kd reductase in 5 min with respect to a control without mevalonate; however, the incorporation of ^{32}P to the 180 Kd reductase did not change. It is also observed that phosphorylation of 180 Kd reductase is much less than that of 97 Kd. These data support the idea that phosphorylation is involved in the regulation of degradation of reductase.

17. INHIBITION OF HUMAN LEUKOCYTE HMG-CoA REDUCTASE ACTIVITY BY ASCORBIC ACID: AN EFFECT MEDIATED BY THE FREE RADICAL MONODEHYDROASCORBATE.

H. J. Harwood, Jr., Y. J. Greene, and P. W. Stacpoole. *Univ. of Florida, Coll. of Med., Gainesville, FL 32610.*

In man, doses of ascorbic acid (vitamin C) between 500 and 4,000 mg/day are reported to lower plasma total and LDL-cholesterol concentrations in both normal individuals and patients with hypercholesterolemia. One mechanism by which dietary ascorbic acid may influence circulating cholesterol levels is by altering endogenous rates of cholesterol biosynthesis. Indeed, hepatic cholesterolgenesis has been shown to be reduced in guinea pigs fed diets high in ascorbic acid. Recently we observed a pronounced transient, direct inhibition of HMG-CoA reductase activity by ascorbic acid when hepatic microsomes were incubated with the vitamin (Biochem. Biophys. Acta. 1985; 834: 134–138). In this study, we examined the mechanism of inhibition of HMG-CoA reductase by ascorbic acid. HMG-CoA reductase in microsomes isolated from cultured IM-9 cells or freshly isolated human leukocytes was markedly decreased by either ascorbic acid or its oxidized derivative, dehydroascorbate. Inhibition of IM-9 leukocyte HMG-CoA reductase activity was log linear between 0.01 and 10 mM ascorbic acid (25% and 81% inhibition, respectively) and 0.1 and 10 mM dehydroascorbate (5% and 75% inhibition, respectively). Inhibition was noncompetitive with respect to HMG-CoA (K_m = 10.2 μM (R,S); KiAA = 6.4 mM; KiDHA = 15 mM) and competitive with respect to NADPH (K_m = 16.3 μM; KiAA = 6.3 mM; KiDHA = 3.1 mM). Since ascorbic acid and dehydroascorbate may be interconverted through the free radical intermediate monodehydroascorbate, we asked whether HMG-CoA inhibition may be mediated by monodehydroascorbate. Reducing agents are required to convert dehydroascorbate to monodehydroascorbate, but prevent formation of the free radical from ascorbic acid. In microsomes from IM-9 cells, the reducing agent, dithiothreitol, abolished HMG-CoA reductase inhibition by ascorbic acid but enhanced inhibition by dehydroascorbate. In addition, the concentration of monodehydroascorbate present in ascorbic acid solutions increased with storage and was directly proportional to the degree of HMG-CoA reductase inhibition by 1.0 mM ascorbic acid. Fifty percent inhibition of enzyme activity occurred at a monodehydroascorbate concentration of 14 μM. These data indicate that monodehydroascorbate mediates inhibition of HMG-CoA reductase by both ascorbic acid and dehydroascorbate. The effect is not due to free radical-induced membrane lipid modification, however, since both ascorbic acid and dehydroascorbate also inhibited the protease-solubilized and partially purified human liver enzyme. Since inhibition of HMG-CoA reductase occurs at physiological concentrations of ascorbic acid in the human leukocyte (0.2–1.72 mM; average 1.26 mM), this vitamin may be important in the regulation of endogenous cholesterol synthesis in man.

18. THE CONTROL OF 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE ACTIVITY IN *SACCHAROMYCES CEREVISIAE*.

J. M. Haslam, D. J. Birch, and T. B. Crabbe. *Dept. of Biochemistry, Univ. of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.*

The activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and the sterol content of *S. cerevisiae* are considerably higher under catabolite-derepressed oxidative than under catabolite-repressed fermentative growth conditions. During diauxic growth on glucose (1%, w/v) medium, in which a change from fermentative to oxidative growth occurs, two large transient increases in the concentration of 3',5' cyclic AMP (cAMP) occur. The first of these coincides with a commitment to the derepression of HMG-CoA reductase and the second with the derepression of most other mitochondrial enzymes. Derepression is blocked by inhibition of protein synthesis, suggesting that de novo synthesis of HMG-CoA reductase occurs, but it is possible that cAMP also acts by modulation of enzyme activity. In addition to the previously reported mitochondrial location of HMG-CoA reductase, activity is also found in the endoplasmic reticulum. The two activities differ in their kinetic properties and in their sensitivity to compactin, and both increase during catabolite-derepression. Studies on a compactin-resistant clone containing multiple copies of a recombinant plasmid vector of a segment of the yeast genome, which confers an increase in total HMG-CoA reductase activity, indicate that a single genetic region causes the increase in both mitochondrial and endoplasmic reticulum activities under both repressed and derepressed conditions. Thus it is uncertain whether the two activities are isoenzymes or modified forms of the same enzyme.

19. MODE OF ACTION OF XENOBIOTICS ON THE REGULATION OF HYDROXYMETHYLGLUTARYL-CoA-REDUCTASE.

H-St. Jenke, M. Löwel, and J. Berndt. *GSF, Inst. Toxicol. and Biochemistry, 8042 Neuherberg, West Germany.*

Hydroxymethylglutaryl-CoA-reductase (HMGR), a transmembrane glycoprotein of the endoplasmic reticulum, is the rate-limiting enzyme for cholesterol biosynthesis in the liver. Polychlorinated biphenyls (PCB) industrially used as lubricants, insulators and as plasticizers, among others give rise to an altered lipid metabolism. γ-Hexachlorocyclohexane (HCH), a common insecticide used in forestry, is assumed to interfere with the lipid metabolism. Both xenobiotics have become widespread contaminants of the environment. We have been studying the influence of these xenobiotics on the cholesterogenesis in rat liver. Feeding 0.05% PCB to rats resulted in an increase of liver HMGR activity within 1–2 weeks followed by a decrease towards normal levels. PCB was incorporated into the microsomal membrane. There was a concomitant decrease in the cholesterol/phospholipid

ratio in the microsomal fraction (the locus of HMGR). Immunotitration studies suggest that PCB modulate preexisting HMGR activity by changing the lipid environment of the enzyme. Northern dot hybridization experiments showed an induction of the level of m-RNA coding for HMGR; this stimulation correlated with the increase in the activity of HMGR. In contrast, feeding 0.03% HCH to rats resulted in a decrease of liver HMGR activity within 2–3 weeks maintaining for at least 6 weeks. Again, HCH was incorporated into the microsomal membrane, but there was no concomitant alteration in the lipid status of the microsomal fraction. The catalytic activity was not modulated during the entire feeding time. Northern dot hybridization experiments showed an inhibition of the level of m-RNA coding for HMGR. Feeding a combination of 0.05% PCB and 0.03% HCH did not lead to an effect which was solely additive. The HCH effect appears to be more pronounced than that of PCB. These data indicate that PCB but not HCH act on the regulation of HMGR activity by enzyme-lipid-interaction. Both xenobiotics, however, act at the transcriptional level.

20. RAT HMG-CoA REDUCTASE cDNA: CLONING AND CHARACTERIZATION. S. A. Khan, S. Kabat, and P. J. Stambrook. *Univ. of Cincinnati, Coll. of Med., Cincinnati, OH 45267.*

Extensive biochemical evidence exists to support evidence that cholesterol inhibits HMG-CoA reductase activity, thereby regulating, in part, its own intracellular level. In order to extend this observation at the molecular level we have initiated studies on rat HMG-CoA reductase gene and its cDNA. The intent is to study how transcription of the reductase gene is modulated by extrinsic factors like LDL and oxysterols. As a first step towards achieving these goals, HMG-CoA reductase cDNA clones were identified by screening a rat liver cDNA library constructed in the expression vector, λ gt11. Of the four positive clones, one that had an insert size of 1.2 kb was subcloned into phage M13 MP10 and its nucleotide sequence was determined. Comparisons with Chinese hamster HMG-CoA reductase cDNA sequence revealed extensive homology in the coding region as well as in the 3'-untranslated region. However there is a 30 nucleotides stretch, encoding ten amino acids, in which nucleotide divergence is 80%. This divergence is reflected by a change in eight of the ten amino acids. Similarly, at the 3'-untranslated region, the rat cDNA contained an insertion of 38 nucleotide flanked by an inverted repeat. This insertion contains three repeat units of the nucleotide sequence "AAGCTG." The significance of the above differences is unknown at the present time; however, experiments are in progress to address a few possibilities. For example we have isolated rat genomic clones, determined their restriction patterns and are in the process of subcloning desired restriction fragments into appropriate vectors. (Supported by BRSG and AHA to SAK.)

21. FURTHER STUDIES ON THE EFFECT OF ACTH ON HAMSTER ADRENAL 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE (HMG-CoA) ACTIVITY. J-G. Lehoux, D. Bellabarba, S. Bélisle, and A. Lefebvre. *Dept. of Biochemistry, Faculty of Medicine, Univ. of Sherbrooke, Sherbrooke, Que., Canada J1H 5N4.*

Previous studies established that under normal conditions HMG-CoA reductase activity is higher in hamster adrenals than in those of rats and humans. The hamster reductase activity follows a diurnal rhythm corresponding to that of plasma ACTH and corticosteroids. ACTH treatments to hamsters increase reductase activity after a latency of 60 min; this enhancement can be prevented by the co-administration of cycloheximide. Immunotitration and immunoblotting studies revealed that ACTH caused an increase in reductase synthesis. Isolation procedures will facilitate the formation of S-S bonds between reductase molecules resulting in inactivation. Indeed when hamster adrenals were homogenized in the presence of Iodoacetamide, a single-band of enzyme was seen in the 97K area after SDS electrophoresis and Western blotting using antirat liver reductase antibody and a second antibody coupled to peroxydase. However, in non-reducing conditions and in the absence of Iodoacetamide, a higher molecular weight 175K–200K band was seen as well as the 97K and smaller forms (especially in the absence of proteolytic enzyme inhibitors). The higher molecular weight band was also observed when Iodoacetamide was added only after the isolation of microsomes. mRNA estimated using the plasmid p-Red 10 coding for the Chinese hamster ovary reductase revealed the presence of 12-fold more mRNA in a human carcinoma having a high HMG-CoA reductase activity (1146 pmol/mg protein/min) than in the normal human gland from a kidney donor (48.1 pmol/mg protein/min). The concentration of mRNA from hamster adrenal was also increased after ACTH stimulation. These results indicate that the level of HMG-CoA reductase activity might be controlled by the concentration of specific mRNA and de novo protein synthesis. This does not rule out the possibility that a part of the reductase activity might be also under the fine control of activation–inactivation processes. Acknowledgments: To the Medical Research Council of Canada; to Dr. G. C. Ness and Dr. M. S. Brown for their generous gift of rat liver antibody and the plasmid p-Red 10, respectively.

22. PERINATAL DEVELOPMENT OF CHOLESTEROL SYNTHESIS AND HMG-CoA REDUCTASE REGULATION. S. Leoni, M. T. Mangiantini, S. Spagnuolo, L. Conti de Virgiliis, M. Valbonesi, and A. Trentalance. *Dept. of Cellular and Developmental Biology, Univ. of Rome I "La Sapienza", P. le A. Moro, 00185 Rome, Italy.*

During fetal development the liver undergoes a highly proliferative process committed to its growth and morphofunctional differentiation. Such anabolic state is joined with metabolic modifications as well as altered hormone levels and substrate availability. Although an increased requirement of cholesterol to build new cell structures is present, the cholesterol biosynthesis and the HMGCoA reductase (HMGCoAR) activity measured in microsomes prepared in the absence (−NaFR) or in the presence (+NaFR) of 50 mM NaF, show a discontinuous pattern, growing in fetal life, very low at the birth and rising again after weanling (Leoni et al., 1984). The activation state of the reductase (as revealed by the +NaFR/−NaFR ratio) changes during development showing the lowest expressed activity (+NaFR) at the birth, consistent with the nadir of cholesterol synthesis. We have already suggested that a short-term regulatory mechanism involving phosphorylation–dephosphorylation processes is working during development (Leoni et al., 1985), but its sensitivity to different modulators and the involvement of other regulatory mechanisms well established in the adult liver, is unknown. Therefore we studied the responsiveness of the phosphorylation–dephosphorylation mechanisms to various effectors besides the onset and development of the hormone (glucagon and insulin) and substrate (mevalonate and cholesterol) regulation of cholesterol synthesis and HMGCoAR activity in fetal and perinatal liver. The effect exerted by the in vitro stimulation of the phosphorylation–dephosphorylation mechanism on the HMGCoAR shows that the whole machinery appears early and keeps active until adult age. Mevalonate and cholesterol affect reductase activity only on the 19th–20th gestational day; just before the delivery the substrate effect is not more detectable, maybe

because of the higher lipid content of the liver. The hormone control on cholesterol synthesis and reductase activity appears on the 19th–20th day of fetal life: at this stage insulin and glucagon receptors are already present on the hepatocyte plasma membranes. The receptors are still detectable in the perinatal life, but at this time no hormone influence is observed, consistent with the existence of a perinatal resistance to these hormones. Leoni, S. et al., J. Cell. Physiol. 118, 62, 1984. Leoni, S. et al., Cell. Mol. Biol. 1985, in press.

23. 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY IN LM CELLS ISOLATED IN SPECIFIC PHASES OF THE CELL CYCLE BY CENTRIFUGAL ELUTRIATION. W. A. Maltese and K. M. Sheridan. *Div. of Pediatric Neurology, Coll. of P and S, Columbia Univ., 722 West 168th St., New York, NY 10032.*

Previous studies with synchronized cells have suggested that the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and the rate of sterol synthesis increase in conjunction with the entry of cells into the S-phase of the cell cycle. However, in these studies synchrony was obtained by stimulation of quiescent cells with lectins or growth factors, or by releasing cells from thymidine block. To determine whether cell cycle-dependent changes in HMG-CoA reductase activity and sterol synthesis occur in continuously proliferating cells, cultured LM cells in the exponential phase of growth were sorted according to their cell volume, using the technique of centrifugal elutriation. Cells were grown in serum-free medium, and the elutriation procedure involved sequential increases in the flow rate of the medium (16 ml/min–40 ml/min) while maintaining a constant rotor speed (2,000 rpm). Flow cytometric analysis of the DNA content of the cells eluted at each flow rate confirmed that populations enriched in G_1, S and G_2/M cells were obtained. When cells were pulse-labeled with [1-^{14}C]acetate (1 μCi/ml medium) for 30 min prior to elutriation, the incorporation of radioactivity into digitonin-precipitable sterols was similar in the G_1, S and G_2/M fractions. In separate experiments, the specific activities of cytosolic acetoacetyl-CoA thiolase, cytosolic HMG-CoA synthase and HMG-CoA reductase (preincubated and assayed in the presence of 50 mM NaF) also showed no consistent variations correlating with cell cycle stage. However, when HMG-CoA reductase was assayed after maximal activation by preincubation of cell homogenates or Kyro-EOB-solubilized extracts at 37°C without NaF, the specific activities were 30–60% higher in the G_1 cells compared to the S cells. This was due to an approximate 50% decline in the relative amount of latent enzyme activity in the S cells. Although further confirmation of this observation is required, the conversion of an increased proportion of latent enzyme to the 'active' form during S-phase could serve as a homeostatic mechanism to maintain a constant specific activity of HMG-CoA reductase in the face of a decrease in the concentration of enzyme protein relative to total cellular protein during this phase of the cell cycle. In any case, the data suggest that the activity of the de novo sterol biosynthetic pathway remains constant throughout the cell cycle in continuously proliferating cells. The previously reported increases in HMG-CoA reductase activity and sterol synthesis associated with late G_1 or early S-phase following stimulation of quiescent or arrested cell populations may represent a unique feature of cells undergoing the $G_0 \rightarrow G_1$ transition, rather than a true cyclic variation in enzyme activity. Additional studies are underway to test this hypothesis in non-transformed cells. Supported by PHS Grant R01 34569 from the National Cancer Institute.

24. ACTH INDUCTION OF THE SYNTHESIS AND ACTIVITY OF HMG-CoA REDUCTASE IN ADRENOCORTICAL CELLS. J. I. Mason, R. Magness, and W. E. Rainey. *Cecil H. and Ida Green Ctr. for Reproductive Biology Sci., Dept. of Biochemistry, Pediatrics, and Obstetrics and Gynecology, Univ. of Texas Hlth. Sci. Ctr., Dallas, TX 75235.*

Adrenal cells respond to stimulation by adrenocorticotropin (ACTH) with an increase in steroidogenesis. Cholesterol, which acts as substrate for steroid biosynthesis, may originate from either exogenous serum cholesterol or from intracellular de novo synthesis. 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is a primary rate-limiting enzyme in the synthesis of cholesterol. Thus understanding the regulation of this enzyme is important in the study of cholesterol biosynthesis and consequently steroid production. In the present study, we examined the effect of ACTH on the regulation of HMG-CoA reductase levels in sheep adrenocortical (SAC) cells. Primary cultures of SAC cells were stimulated by ACTH (10 nM) in medium containing ovine low density lipoprotein (oLDL 25 μg/ml), ovine high density lipoprotein (oHDL; 100 μg/ml), and in medium depleted of lipoproteins. Regardless of lipoprotein content of the medium, ACTH stimulated similar levels of cortisol release, suggesting that exogenous cholesterol was not necessary for maximal steroidogenesis by these cells. In order to monitor cellular levels of de novo synthesis of cholesterol, we examined the activity of HMG-CoA reductase during the first 24 h of ACTH stimulation. ACTH increased reductase activity 5–10-fold above basal levels with maximal activity seen after 12 h of stimulation in media depleted of lipoproteins. If oLDL (25 μg/ml) was added to the medium, the increase in reductase activity was not observed. Polyclonal IgG antibodies against rat liver HMG-CoA reductase were used to examine the amount of total reductase protein (by protein blotting) and the rate of reductase synthesis (by immunoprecipitation from cells pulse labeled with ^{35}S methionine). Within 12 h, ACTH treatment of SAC cells resulted in the accumulation of a 97 KD protein (mass and radiolabeled immunoprecipitates) which corresponded to the size of reductase in other tissues. Therefore, the increased reductase activity in response to ACTH was probably the result of increased synthesis and accumulation of the enzyme and not merely activation. In order to determine if steroidogenesis was necessary for the increase in reductase activity, aminoglutethimide was used to inhibit cholesterol conversion to pregnenolone. When cells were stimulated with ACTH in the presence of this drug, the increase in reductase activity was attenuated ($\sim$75% decrease). These results lead us to suggest that the ACTH-induced increase in HMG-CoA reductase within SAC cells is regulated by the movement of cholesterol into steroid. The depletion of the cellular cholesterol stores leads to an increase in reductase synthesis and accumulation within the SAC cell.

25. HORMONAL REGULATION OF THE EXPRESSION OF HMG-CoA REDUCTASE OCCURS AT TRANSCRIPTIONAL AND POSTTRANSLATIONAL LEVELS. G. C. Ness, C. E. Sample, and L. C. Pendleton. *Dept. of Biochemistry, Coll. of Med., Univ. of South Florida, Tampa, FL 33612.*

Hypophysectomized and hypophysectomized-diabetic rats were used as animal models to study the regulation of hepatic HMG-CoA reductase by thyroid hormones, glucocorticoids and insulin. Reductase activity, immunoreactive 100,000 M_r protein and hybridizable reductase mRNA were all reduced essentially to undetectable levels in these animals. Administration of L-triiodothyronine (T_3) to hypophysectomized rats caused about a

500-fold increase in reductase activity, at least a 100-fold increase in hybridizable RNA and an increase from undetectable to detectable levels of immunoreactive protein. Maximal levels were reached at 48 hours after T_3 administration and were maintained for another 36 to 48 hrs. Surprisingly, feeding these hypophysectomized rats diets containing the bile acid sequesterant, Colestipol, and the potent reductase inhibitor, Mevinolin, mimicked these effects of T_3. Administration of dexamethasome effectively prevented the T_3-mediated stimulation of reductase activity and immunoreactive 100,000 M_r protein but did not affect hybridizable RNA levels. Administration of hydrocortisone or dexamethasone at 48 hrs after T_3 treatment resulted in a rapid decline in reductase activity which could be prevented by giving cycloheximide. This suggests that glucocorticoids may exert their effect by enhancing reductase degradation by promoting the synthesis of a protein required in degradation, while T_3 acts by promoting transcription of the reductase gene. In hypophysectomized-diabetic rats, administration of both T_3 and insulin was required to obtain full reductase activity. Administration of T_3 alone caused a small increase in reductase activity but full increases in hybridizable RNA and immunoreactive 100,000 M_r protein. Reductase activity in liver microsomes from such animals could be fully restored in vitro by increasing the ratio of dithiothreitol to microsomal protein. When reductase activity was assayed at physiological levels of glutathione and NADPH, activity was very low. These findings suggest that thyroid hormones regulate expression of the reductase gene at the level of transcription while glucocorticoids and insulin exert their effects at posttranslational levels. (Supported by NIH grant HL 18094).

26. ENHANCEMENT OF HMG-CoA REDUCTASE DEGRADATION BY MEVALONATE OR 25-HYDROXYCHOLESTEROL IN HEPATOCYTES OR BY PHOSPHORYLATION IN VITRO.

R. A. Parker, S. J. Miller, and D. M. Gibson. *Dept. of Biochemistry, Indiana Univ. Sch. of Med., Indianapolis, IN 46223; and Bristol-Myers Pharm. Res. Div., Evansville, IN 47721.*

Intracellular degradation of HMG-CoA reductase (HMGR) was probed in cholestyramine-fed rat hepatocytes isolated at 9 hours-dark in the diurnal cycle. We monitored both total microsomal HMGR activity and levels of immunoreactive enzyme mass assessed by quantitative SDS-urea-PAGE/immunoblot analysis of DTT and iodoacetamide-treated subcellular fractions. In this model HMGR total activity and the levels of 97–100 kDa HMGR diminished at identical rates, with an apparent degradative rate constant for HMGR (Kd') of 0.31/hr ($t_{1/2}$ = 2.2 hr). Cycloheximide had no effect on this value, suggesting that HMGR de novo synthesis was very low. Several inhibitors of lysosomal functions, viz, methylamine, propylamine, amino acid mixtures, the Na^+ ionophore monensin, or insulin, each decreased Kd' by 40–50% compared to controls. Incubation of cells with mevalonolactone (1 mM, R,S) produced a rapid but transient phosphatase-reversible inhibition of activity (net phosphorylation) within 5–10 min, followed by a 2.3-fold increase in Kd' (loss of both total activity and 97–100 kDa protein). Propylamine or monensin suppressed the mevalonolactone enhancement of Kd' by 100% or 70%, respectively, compared to controls with propylamine or monensin alone. Cell incubation with 25-hydroxycholesterol (12 μM) increased Kd' by 2.1-fold, but in contrast to mevalonolactone the effect of the oxysterol was blocked only 40% by propylamine or monensin. The following conclusions are drawn: (1) degradation of HMGR in hepatocytes involves acidic subcellular compartments; (2) differential inhibitor sensitivity of mevalonate- vs. oxysterol-stimulated degradation implicates a non-oxysterol product of mevalonate as a feedback effector in HMGR degradation; (3) increased phosphorylation of HMGR precedes the enhancement of HMGR degradation produced by exogenous mevalonate. An in vitro model was developed for analysis of the effect of phosphorylation of HMGR upon its susceptibility to proteolysis. Microsomal 97–100 kDa HMGR prepared in its two extremes of phosphorylation state was subjected to limited proteolysis by the Ca^{2+}-dependent thiol protease calpain-II from rat liver. Calpain-II generated two HMGR cleavage products of ~62 kDa and 52–56 kDa as judged by immunoblot analysis. The yield of the soluble 52–56 kDa species was up to 6-fold greater in the phosphorylated series compared to dephosphorylated controls, reflected in both total activity and immunoblot densities. The 62 kDa form, membrane-bound at low salt, appeared to be a precursor of the 52–56 kDa form. These results are consistent with the proposal that phosphorylation of HMGR increases the rate of its degradation in the hepatocyte by increasing the susceptibility of native 97–100 kDa HMGR to proteolytic cleavage.

27. TRANSLATIONAL CONTROL OF HMG-CoA REDUCTASE BY A NON-STEROL MEVALONATE-DERIVED PRODUCT.

D. Peffley and M. Sinensky. *Eleanor Roosevelt Inst. for Cancer Res., Denver, CO, 80262.*

The activity rates of synthesis and mRNA levels of HMG-CoA reductase were measured in a somatic cell mutant (Mev-1) of the CHO-K1 cell auxotrophic for mevalonate due to a lack of detectable HMG-CoA synthase activity. Incubation of the Mev-1 cell in medium free of sterol results in a level of HMG-CoA reductase activity which is 5–10-fold higher than that of the wild-type cell. Treatment of Mev-1 with 25-hydroxycholesterol under conditions which bring about maximal inhibition of HMG-CoA reductase activity in wild-type cells produces only partial inhibition in the Mev-1 mutant. The decrease in enzyme activity under these conditions (2–5-fold) correlates well with the reduction in the rate of enzyme synthesis and mRNA levels for HMG-CoA reductase. Treatment of Mev-1 with a combination of 25-hydroxycholesterol and mevalonate produces a substantially greater inhibition of enzyme activity (nearly 100-fold) and synthesis than treatment with 25-hydroxycholesterol alone. However, under these conditions, the level of HMG-CoA reductase mRNA is similar to that observed upon treatment with 25-hydroxycholesterol alone. These observations suggest a specific and quantitatively significant effect of mevalonate on HMG-CoA reductase synthesis mediated by a translational control process. ERICR contribution #587. Supported by NIH grant #'s HD02080 and HL27877.

28. EVIDENCE FOR ACTIVATION OF HMG-CoA REDUCTASE BY LIMITED PROTEOLYSIS.

B. Preiss and M. Delisle. *Dept. de Biochimie, Univ. de Sherbrooke, Sherbrooke, Que., Canada J1H 5N4.*

HMG-CoA reductase in hamster adrenal microsomes undergoes an increase of about 2-fold in specific activity during preincubation for 30 minutes at 37°C in a Tris-EDTA-KCl-DTT medium. This activation is not inhibited by NaF. Reductase activity is assayed in the presence of 3 millimolar $NADP^+$ and a NADPH-generating system. When 5 mM EGTA, 50 μM leupeptin and 1 mM phenylmethylsulfonyl fluoride are included during homogenization, isolation, preincubation and assay, the specific activity attained is not much higher than that of control microsomes from the same animal prior to preincubation. Microsomes prepared and assayed in the presence of the protease inhibitors have about half the specific activity of control microsomes, in both cases without preincubation. In microsomes

isolated in the presence of the protease inhibitors, preincubated and assayed in their absence, the increase in specific activity is almost fully restored to control values. The reaction product with and without preincubation was identified by GLC as mevalonolactone. No HMG-CoA lyase was detected in the microsomal preparations. These results support the conclusion that HMG-CoA reductase in this system is activated by limited proteolysis. The protease inhibitors appear to block proteolysis only partially under the experimental conditions. Hamster adrenal reductase appears to differ from the rat liver enzyme which was reported (Roitelman and Shechter (1984) JBC 259 14029) to show a maximal increase in specific activity upon apparent limited proteolysis when assayed in the presence of 60 μM pyridine nucleotide and no increase with levels above 0.4 mM. (Supported by grant MA-8742 of the Medical Research Council of Canada).

29. STUDY AND PRELIMINARY CHARACTERIZATION OF A NOVEL SYSTEM FOR THE IN VITRO RECONSTITUTION OF SOLUBLE 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE IN SYNTHETIC LIPOSOMES.

H. Ramirez, F. Rodriguez, C. Marco, and E. Garcia-Peregrin. *Dept. Bioquimica, Univ. de Granada, Spain.*

During the last three years, concluding evidence has been accumulated regarding two main characteristics of reductase. (1) It is an integral glycoprotein attached to the endoplasmic reticulum in the cell. (2) Reported purification of HR really corresponds to a 53 Kd dalton proteolytic fragment, fully active but incapable of interaction with the lipidic bilayer. As a consequence of these relatively new features, partial review of those aspects concerning lipid–protein interactions is required. We feel that reconstitution in vitro of integral membrane proteins, and particularly of HR, in synthetic liposomes of known composition should be a suitable model to investigate possible lipid–reductase interactions. This experimental strategy has been highly successful in a number of studies on membrane proteins. With this in mind we have developed an original method of in vitro reconstitution of soluble, hepatic HR in artificial liposomes with prefixed composition. The new method includes previous solubilization of microsomal samples in 1% Triton X-100 buffer followed by exhaustive dialysis of the 105,000 $\times$ *g* nonprecipitable material against free detergent buffer and in the presence of liposomal suspension. The procedure is based on Racker's method for Ca^{2+}-ATPase reconstitution, widely extended in other labs. However, significant differences have been included. Dialyzed recombinant particles were finally exposed to a high ionic strength and diluted, before sedimentation at 105 *Kg*, 90 min, 4°C. Recombinant particles exhibited a specific HR activity that represents more than 90% of the total initial activity. Furthermore, partial characterization of particles and preliminary studies on lipid-HR interactions have been accomplished.

30. INVOLVEMENT OF CYTOCHROME P-450 IN THE DOWN-REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG-CoA) REDUCTASE ACTIVITY BY LOW DENSITY LIPOPROTEINS IN CULTURED HUMAN SKIN FIBROBLASTS AND RAT EPITHELIAL CELLS.

R. Sexton, A. Gupta, H. Shertzer, and H. Rudney. *Dept. of Biochemistry and Molecular Biology, Dept. of Environmental Health, Univ. of Cincinnati, Cincinnati, Ohio 45267-0522.*

Much evidence exists that supports the hypothesis of Kandutsch et al. (Science 201, 498–501, 1978) that oxidation products of sterols (oxysterols) some of which are intermediates in the biosynthesis of bile acids or steroid hormones may be involved in the regulation of cholesterol biosynthesis by suppressing the activity of HMG-CoA reductase. Formation of such intermediates is known to involve cytochrome P-450-dependent reactions. It was therefore of interest to determine if low density lipoprotein (LDL) suppression of HMG-CoA reductase activity in extrahepatic nonsteroidogenic cells involved a cytochrome P-450-dependent oxidation of cholesterol. Because human skin fibroblasts (GM-43) and rat intestinal epithelial cells (IEC-6) do not oxidize cholesterol to bile acids or steroid hormones and have significant HMG-CoA reductase activity, they were chosen for this study. Incubation of cells for 6h or 24h with ketoconazole and zu-clomiphene, known cytochrome P-450 inhibitors, prevented the suppression of HMG-CoA reductase activity by LDL. Neither compound inhibited the rate of uptake and degradation of ^{125}I-LDL nor prevented the increase in cellular free cholesterol induced by LDL. Previously, we have reported that U18666A (3β-[2-diethylaminoethoxy] androst-5-en-7-one) and progesterone prevented LDL suppression of HMG-CoA reductase activity without inhibiting LDL uptake or degradation. With the aid of specific inhibitors of acyl-CoA:cholesterol acyltransferase (ACAT) we also showed that the prevention of LDL suppression of HMG-CoA reductase activity by U18666A and progesterone was not related to their ability to inhibit ACAT activity, nor do they affect the intracellular transport of cholesterol (Fed. Proc. 44, 1786, 1985). To determine if these compounds were also cytochrome P-450 inhibitors and thereby prevented the action of LDL cholesterol on HMG-CoA reductase activity, we examined the effects of U18666A and progesterone on the cytochrome P-450 dependent oxidation of three substrates (benzphetamine, aminopyrine, and aniline) by liver microsomes from phenobarbital-treated rats. Both U18666A and progesterone strongly inhibited the oxidation of all three substrates. These results are highly suggestive that cytochrome P-450-mediated formation of inhibitory oxysterols, from either endogenous precursors or from LDL-derived cholesterol, could be a general mechanism for the regulation of HMG-CoA reductase activity and thereby cholesterol synthesis. Supported by NIH-NIADDK-AM-12402 and NSF-PCM 8404817.

31. ORGANIZATION AND REGULATED EXPRESSION OF RAT LIVER HMG-CoA REDUCTASE GENES.

D. J. Shapiro, L. Gould, G. Martin, D. Williams, and R. Wolski. *Dept. of Biochemistry, Univ. of Illinois, 1209 W. California St., Urbana, IL 61801.*

Genomic and cDNA clones encompassing rat liver HMG-CoA reductase have been isolated. Surprisingly, the 5' portion of these genes exhibits rather poor homology to hamster HMG-CoA reductase genes. The 3' portion of the genes is strongly conserved between the two species. We have developed a simple model system for investigation of the regulation of HMG-CoA reductase gene expression in cultured rat liver cells (RLC) using the water soluble cholesterol derivative methoxypolyoxyethylated (MPOE) cholesterol. Preliminary experiments indicated that MPOE cholesterol regulates the level of HMG-CoA reductase, and does not inhibit reductase, alter membrane fluidity or change the phosphorylation state of the enzyme. Current studies focus on analyzing the site at which MPOE cholesterol regulates reductase mRNA levels in wild type and MPOE resistant cells. Rat liver reductase mRNA consists of two species which differ in size by approximately 500 nucleotides. The possibility that these mRNAs code for distinct polypeptides which are independently targeted to the peroxisomes and endoplasmic reticulum (which both contain HMG-CoA reductase), is currently under investigation. The existence of sequence elements in the 5'-flanking region of HMG-CoA reductase which resemble those of other

cell cycle regulated genes led us to examine cell cycle regulation of HMG-CoA reductase. HeLa cells were synchronized with aphidicolin and thymidine and HGM-CoA reductase activity and reductase mRNA levels examined throughout the cell cycle.

32. DEFECTIVE REGULATION OF HMG-CoA REDUCTASE BY LDL IN A SOMATIC CELL MUTANT DEFECTIVE IN SQUALENE SYNTHESIS. M. Sinensky and J. Logel. *Eleanor Roosevelt Inst. for Cancer Res., Denver, Colorado, 80262.*

A somatic cell mutant (CA5) has been isolated which is auxotrophic for lanosterol and exhibits a greater than 10-fold deficit in the conversion of acetate to lanosterol. Nutritional and precursor incorporation data suggest that the major defect in this mutant lies in squalene synthase. We have examined regulation of HMG-CoA reductase by human low density lipoprotein (LDL), 25-hydroxycholesterol and mevalonate in this mutant. We find that 25-hydroxycholesterol and mevalonate regulate HMG-CoA reductase activity normally in this cell but that LDL does not. Studies with ^{125}I-LDL show that the LDL pathway is intact in CA5. These results suggest that endogenous sterol synthesis is required in cultured fibroblasts in order for LDL to act as a regulator of HMG-CoA reductase and that cholesterol delivered to cells through the LDL pathway does not directly regulate HMG-CoA reductase. ERICR contribution #588. Supported by NIH grant #'s HD02080 and HL27877.

33. SUPPRESSION OF MONONUCLEAR LEUKOCYTE HYDROXYMETHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY IN FAMILIAL HYPERCHOLESTEROLEMIA. P. W. Stacpoole, D. Bridge, and H. J. Harwood, Jr. *Univ. of Florida, Coll. of Med., Gainesville, FL 32610.*

Microsomal hydroxymethylglutaryl coenzyme A reductase (reductase) is the rate-controlling enzyme of cholesterol biosynthesis. Studies with cultured cells have indicated that enzyme activity in normal subjects and patients with heterozygous familial hypercholesterolemia (hFH) is suppressed by means of a receptor-mediated process for uptake of LDL cholesterol but is abnormally increased in patients with homozygous familial hypercholesterolemia (HFH), in whom receptor-mediated uptake of cholesterol is very low or absent. To investigate the in vivo regulation of reductase, we measured its activity in microsomes of freshly isolated mononuclear leukocytes from 30 healthy subjects and nine patients with hFH or HFH. Basal reductase activity in patients was decreased 47% ($P < 0.001$), compared to normals. Decreased reductase activity in FH was not associated with decreased enzyme protein levels, as determined by immunoblotting. Among the entire study population, serum total and LDL-cholesterol levels correlated inversely ($r = -0.51$; $P < 0.001$) with enzyme activity. Plasmapheresis in two normals, a patient with hFH and a patient with HFH led to a rapid increase in reductase activity in freshly isolated monocytes but not in lymphocytes. Enzyme activity in monocytes was inversely correlated ($r = -0.82$; $P < 0.001$) with LDL-cholesterol concentration. We conclude that reductase activity in freshly isolated mononuclear leukocytes is regulated, in part, by circulating LDL-cholesterol. In addition, enzyme activity but not enzyme protein is decreased in most patients with FH, despite the diminished LDL receptor status of these subjects. The marked increase in reductase activity in monocytes (and, perhaps, other cells) following plasmapheresis indicates that stimulation of endogenous cholesterol synthesis may contribute to the rapid restoration of circulating cholesterol levels in patients with FH who undergo periodic therapeutic plasma exchange.

34. REVERSIBLE COVALENT MODIFICATION OF HMG-CoA REDUCTASE OF RAT INTESTINAL EPITHELIAL CELLS. M. Sugano, H. Oku, and T. Ide. *Lab. of Nutrition Chemistry, Kyushu Univ. Sch. of Agriculture, Fukuoka 812, Japan.*

Short-term regulation of intestinal HMG-CoA reductase activity by reversible phosphorylation and dephosphorylation was further studied. Intestinal epithelial cells were isolated by the modified dual-buffer method of Weiser and the cell homogenate was used as an enzyme source. Intestinal reductase was, like liver enzyme, inactivated by the ATP-Mg. The inactivation reaction was in no way influenced by the trapping pool of mevalonate. In addition, it was confirmed that the reaction was not mediated by the nonenzymatic action of ATP-Mg by heat-inactivating the reductase kinase present in the soluble fraction of the epithelial cell homogenate; when the homogenate was heated (50°C, 15 min), the reductase activity was raised markedly while the response to ATP-Mg vanished. When the soluble cell fraction of the epithelial homogenate was added to the heated homogenate, the reductase activity was reduced by ATP-Mg, the extent of reduction being the same as that observed when the soluble fraction from the liver was added. Only ATP was the effective phosphate donor; virtually all other common nucleotides failed to inactivate the reductase. The phosphorylated inactive enzyme was re-activated by the 35–60% acetone-precipitable fraction of the soluble fraction of the mucosa as effectively as by the corresponding fraction from the liver. This fraction also re-activated the hepatic microsomal reductase. The activation by the soluble activator disappeared in the presence of phosphatase inhibitor such as NaF in incubation media. In contrast to the liver enzyme, no evidence that supports the cyclic AMP-dependent regulation of the reductase was obtained; the addition of cAMP or cGMP to the mucosal homogenate, even at the relatively high concentration, neither inactivated the enzyme nor stimulated the effect of ATP. Furthermore, incubation of intact epithelial cells with dibutyryl-cAMP or dibutyryl-cGMP did not cause a detectable reduction of the reductase activity. More than one-half of intestinal reductase existed in an active form and the extent of the activation state was rapidly and characteristically modified by the dietary manipulations. The response sometimes differed in the proximal and distal intestine. These results suggest a specific feature of regulation of intestinal HMG-CoA reductase that is, in some respects, in contrast with the liver enzyme.

35. REGULATION OF HMG-CoA REDUCTASE ACTIVITY BY CATECHOLAMINES AND INSULIN IN ISOLATED RAT HEPATOCYTES. R. Devery and G. Tomkin. *Dept. of Biochemistry, Trinity College Dublin, and Dept. of Metabolic Medicine, The Adelaide Hospital, Dublin, Ireland.*

Both short and long term regulatory mechanisms have been reported in the control of cholesterogenesis and of the activity of HMG-CoA reductase. However the mechanism regulating the changes observed in rat liver HMG-CoA reductase activity in diabetes mellitus has not yet been established. The poorly controlled diabetic rat has a lower hepatic HMG-CoA reductase activity than the well controlled diabetic rat. (Scott and Tomkin 1982 Diabetologia 23: 14). We have investigated the effect of insulin, noradrenaline (α_1, β_1, agonist), isoprenaline (β_1, β_2, agonist) and the combination of either catecholamine and insulin on the activity of microsomal HMG-CoA reductase in isolated rat hepatocytes maintained in culture for 3 h. Insulin (ImU/ml) alone produced a 4-fold increase in the activity of HMG-CoA reductase, while both noradrenaline and isoprenaline (5.5×10^{-5} M) produced a 2.5-fold increase in enzyme activity. Insulin in the presence of either catecholamine also produced a

2.5-fold increase in the activity of HMG-CoA reductase. It is possible that insulin can exert a short term regulation of hepatic cholesterol synthesis by decreasing the level of cAMP, and thereby activating HMG-CoA reductase by decreasing the activity of the phosphatase inhibitor protein and the activity of the cAMP-dependent reductase kinase. (Beg and Brewer 1982 Federation Proc. 41: 2634). By contrast, an increase in enzyme synthesis may be responsible for the stimulatory effect of catecholamines on HMG-CoA reductase. The increase in HMG-CoA reductase brought about by the combination of either catecholamine and insulin may illustrate a possible antagonistic action of catecholamines on insulin. It remains to be established if insulin can lower the increased cAMP levels produced by catecholamines and thereby inactivate HMG-CoA reductase by a phosphorylation cascade system. It is of interest that in the poorly controlled diabetic, catecholamine levels are reported to be higher compared to the well controlled diabetic. (Christensen 1978 Diabetologia 16: 211). Thus increased sympathetic activity in the presence of hypoinsulinaemia in the rat animal model we previously examined may be correlated with a lower rate of hepatic cholesterol synthesis compared to that found in the insulin-treated rat. Insulin administration, known to normalise catecholamine levels in the poorly controlled diabetic rat could, on the basis of its ability to enhance HMG-CoA reductase, increase total liver cholesterol synthesis. (This work was supported by the Medical Research Council of Ireland).