Abstracts

Biochemistry and nutrition

ARACHIDONIC ACID 15-LIPOXYGENASE PRODUCTS FROM HUMAN EOSINOPHILS. J. Turk, R.L. Maas, A.R. Brash, L.J. Roberts II, and J.A. Oates (Depts. of Med. and Pharmacology, Vanderbilt Univ. Schl. of Med., Nashville, TN 37232) *J. Biol. Chem. 257* (12):7068-7076 (1982). A series of six derivatives of arachidonic acid containing a conjugated triene system and hydroxyl groups at C-15 and at C-8 or C-14 (15-series leukotrienes) was isolated from eosinophil-rich human leukocyte preparations. Among classes of leukocytes in these preparations, eosinophils were the dominant source of the 15-series leukotrienes. The most abundant arachidonate metabolite recovered from human eosinophils was 15 (*S*)-hydroxyl-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid. This compound was synthesized by a lipoxygenase enzyme and is a reduction product of the precursor to the 15-series leukotrienes (15(*S*)-hydroperoxy-5,8,11-*cis*-13-*trans*-eicosatetraenoic acid).

FATTY ACID AMIDASES FROM *DICTYOSTELIUM DISCOIDEUM* THAT ACT ON LIPOPOLYSACCHARIDE AND DERIVATIVES. C.R. Verret, M.R. Rosner, and H.G. Khorana (Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139) *J. Biol. Chem. 257*(17):10222-10227 (1982). Two amidases have been partially purified from the slime mold *Dictyostelium discoideum*: these act sequentially on the β-hydroxymyristyl-amide groups present in the lipopolysaccharide derivative (4′-*O*-phosphoryl-*N*-β-hydroxymyristyl-D-glucosaminyl)-β-(1 → 6)-*N*-β-hydroxymyristyl-D-glucosamine-1-phosphate (III). Amidase-I, which specifically removes the myristyl chain near the 1-phosphate of compound III (apparent K_m, 3.7 μM), has been purified 110-fold from a lysate of *D. discoideum* NC4 cultivated on *Escherichia coli*. The partially purified enzyme contains no other amidase or phosphatase activities: however, an esterase activity can be detected. The second amidase has been purified about 12-fold from the extracellular fluid of *D. discoideum* AX3 cultured axenically. This amidase hydroxlyzes the distal amide linkage in III (apparent K_m, ~ 20 μM) only after prior deacylation of the first site by amidase-I. The preparation is free from phosphatases and glycosidases that can act on lipopolysaccharide. The differential expression of the amidases in *D. discoideum* and some of their kinetic properties have been described. The amidases should prove useful in strucutre-function studies of lipopolysaccharide.

FATTY ACYL AMIDASES FROM *DICTYOSTELIUM DISCOIDEUM* THAT ACT ON LIPOPOLYSACCHARIDE AND DERIVATIVES. II. ASPECTS OF SUBSTRATE SPECIFICITY. C.R. Verret, M.R. Rosner, and H. G. Khorana (Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139) *J. Biol. Chem. 257* (17):10228-10234 (1982). The substrate specificities of two fatty acyl amidases partially purified from the slime mold *Dictyostelium discoideum* have been studied. The amidases act on lipopolysaccharide derivatives, such as (4′-*O*-phosphoryl-*N*-β-hydroxymyristyl-D-glucosaminyl)-β-(1 → 6)-N-hydroxymyristyl-D-glucosamine-1-phosphate (III) in a sequential manner. Amidase-I removes the β-hydroxymyristyl residue present on the amino group adjacent to the 1-phosphate and the product formed is a substrate for amidase-II: the latter removes the remaining β-hydroxymyristyl residue from the distal amino group. Compound III itself is resistant to amidase-II. Removal of the C-1 of C-4 phosphate groups does not influence recognition by the amidases or their sequential action. Both amidases are specific for long chain fatty amide linkages. Thus, a formyl group on the glucosamine amino group adjacent to the C-1 phosphate is not hydrolyzed by amidase-I:however, this substituent does not hinder the action of amidase-II on the distal fatty acyl amide. The presence of the β-hydroxyl group in myristyl-amide residues is not required for hydrolysis. Further, while amidase-I requires disaccharide structures for its action, amidase-II acts on monosaccharides as well. Finally, the effects of a variety of substrate analogs and divalent ions on the activity of the enzymes are reported.

THE METABOLISM OF PRIMARY,7-OXO, AND 7β-HYDROXY BILE ACIDS BY *CLOSTRIDIUM ABSONUM*. J.D. Sutherland and I.A. Macdonald (Departments of Medicine and Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7) *J. Lipid Res. 23* (5):726-732 (1982). *Clostridium absonum* was shwon to metabolize primary bile acids to give rise to both 7-oxo bile acids and 7β-hydroxy (urso) bile acids. At relative low redox potential (Eh) values, high yields of urso bile acids were achieved. If, however, the Eh value of the culture was allowed to rise above approximately -100mv, the 7-oxo bile acid would tend to predominate and the 'death phase' was accelerated. Growth of *C. absonum* in sterile graduated cylinders instead of in conventional Erlenmeyer flasks was effective in delaying the rise in Eh value with time and in preserving a higher viable count of organisms. It is proposed that the formation of excess amounts of 7-oxo bile acid is a manifestation of oxygen toxicity and that it could be mediated by an increasing intracellular NADP:NADPH ratio. Additionally, the reaction: primary bile acid ⇋ oxo bile acid ⇋ urso bile acid was shown to be partially reversible. When the organisms were grown with [24-^{14}C] chenodeoxycholic, - cholic, or -7-keto-lithocholic acid, this reaction could be clearly demonstrated. The addition of an equimolar concentration of deoxycholic acid effectively enhanced the rate of bioconversion of cholate and 7-keto-lithocholic, but not chenodeoxycholate. When the organisms were grown with urso bile acids or the 7-keto-deoxycholic acid, very little metabolism occurred unless deoxycholic acid was added which induced formation of primary and keto bile acids. In all cases, formation of oxo bile acid from primary or urso bile acid occurred as the Eh value of the medium rose with time and could thus be delayed by the use of a cylinder instead of a flask of growing the culture.

UTILIZATION OF DISATURATED AND UNSATURATED PHOSPHATIDYLCHOLINE AND DIACYLGLYCEROLS BY CHOLINEPHOSPHOTRANSFERASE IN RAT LUNG MICROSOMES. G. P.H. Van Heusden and H. Van Den Bosch (Lab. of Biochem., State Univ. of Utrecht, Transirorium 3, Padualaan 8, 3584 CH Utrecht, Netherlands) *Biochim. Biophys. Acta 711* (2):361-368 (1982). 1. Cholinephosphotransferase catalyzes the conversion of diacylglycerol and CDPcholine into phosphatidylcholine and CMP. Incubation of rat lung microsomes containing phosphatidyl[*Me*-^{14}C] choline with CMP resulted in an increase in water-soluble radioactivity, suggesting that also in rat lung microsomes the cholinephosphotransferase reaction is reversible. 2. Microsomes containing ^{14}C-labled disaturated and ^{3}H-labeled monoenoic phosphatidylcholine were prepared by incubation of the organelles with [1-^{14}C] palmitate and [9,10-$^{3}H_2$] oleate in the presence of l-palmitoyl-*sn*-glycero-3-phosphocholine, ATP, coenzyme A and $MgCl_2$: Incubation of these microsomes with CMP resulted in an equal formation of ^{14}C- and ^{3}H-labeled diacylglycerols, indicating that disaturated and monoenioc phosphatidylcholines were used without preference by the backward reaction of the cholinephosphotransferase. When in a similar experiment the phosphatidylcholine was labeled with [9,10-$^{3}H_2$] palmitate and [1-^{14}C] linoleate, somewhat more ^{14}C- than ^{3}H-labled diacylglycerol was formed. 3. The backward reaction was used to generate membrane-bound mixtures of [1-^{14}C] palmitate- and [9,10-$^{3}H_2$] oleate- or of [9,10-$^{3}H_2$] palmitate- and [1-^{14}C] linoleate-labeled diacylglycerols. When the microsomes containing diacylglycerols were incubated with CDPcholine, both ^{3}H- and ^{14}C-labeled diacylglycerols were used for the formation of phosphatidylcholine, indicating that there is no absolute discrimination against disaturated diacylglycerols. This observation is in line with our previous findings and indicates that the CDPcholine pathway may contribute to dipalmitoylphosphatidylcholine synthesis in lung.

DIETARY CARBOHYDRATE AND ATHEROSCLEROSIS. J.A. Story (Dept. of Foods and Nutrition, Purdue Univ., West Lafayette, IN 47907) *Federation Proc. 41*: 2797-2800 (1982). Epidemiologic evidence indicated a relationship between refined sugar intake and increased serum cholesterol levels and atherosclerotic heart

disease, which resulted in a series of human and animal experiments examining this relationship. Sucrose and fructose were found to be more atherogenic in rabbits and baboons when fed as part of a semipurified diet. However, serum lipid levels were not always elevated when more severe atherosclerosis was present. Human studies generally observed increases in serum triglycerides and, less consistently, serum cholesterol in response to substitution of sucrose for starch or glucose. These differences in lipid levels and experimental atherosclerosis are thought to arise from 1) increased endogenous triglyceride synthesis, present in serum as very low-density lipoproteins; 2) impaired clearance of these lipoproteins; 3) slowed turnover of cholesterol into bile acids; and 4) possible changes in aortic connective tissue metabolism.

PLASMA LEVELS OF CHOLESTEROL AND TESTOSTERONE IN WHITE LEGHORN HENS THAT LAID SOFT-SHELLED AND SHELL-LESS EGGS. E. K. Wilson, P.Y. Hester, F.W. Pierson, I. Fabijanska (Dept. of Animal Sci., Purdue Univ., West Lafayette, IN 47907) *Poultry Science 61* (8): 1708-1712 (1982). Plasma concentrations of cholesterol and testosterone were determined in White Leghorn hens that had just laid soft-shelled (SS) or shell-less (SL) eggs and compared to those that laid hard-shelled (HS) eggs. Hens were bled at two different ages, at 22 to 34 and 66 to 74 weeks of age. Blood samples were collected in the morning hours (0600 to 1200 hr) for both age groups with an additional evening bleed (1500 to 1900 hr) for the 66 to 74-week-old hens. Both plasma constituents were higher in hens that laid SS or SL eggs when compared to those which laid HS eggs, but differences were statistically significant only for the 66 to 74-week-old hens bled in the morning hours. The results of this study indicate that elevated levels of testosterone and its precursor, cholesterol, may be related to the production of eggs with little or no shell calcification.

ANTIGENIC MAPPING OF HUMAN LOW DENSITY LIPOPROTEIN WITH MONOCLONAL ANTIBODIES. M.J. Tikkanen, R. Dargar, B. Pfleger, B. Gonen, J.M. Davie, and G. Schonfeld (Lipid Res. Center, Depts of Preventive Med., Med., Microbiology, and Immunology, Washington Univ. Schl. of Med., St. Louis, MO 63110) *J. Lipid Res. 23* (7): 1032-1038 (1982). Monoclonal anti-LDL antibodies were produced in a mouse spleen-myeloma system and purified by affinity chromatography on insolubilized low density lipoprotein (LDL). Five antibodies with different specificities could be distinguished by their immuno-reactivities with chemically modified LDL preparations, and by their competion for binding of ^{125}I-labeled LDL to the apoB,E receptors of cultured human fibroblasts. The same degree of inhibition was achieved using isolated Fab fragments. This antibody may bind to an antigenic site located near the cellular binding site of LDL-apoB.

ADAPTIVE EFFECTS OF DIETARY ETHANOL IN THE PIG: CHANGES IN PLASMA HIGH-DENSITY LIPOPROTEINS AND FECAL STEROID EXCRETION AND MUTAGENICITY. D.L. Topping, R.A. Weller, C.J. Nader, G.D. Calvert, and R. J. Illman (Division of Human Nutrition, Glenthorne Lab, O'Halloran Hill, South Australia 5158) *Am. J. Clin. Nutr. 36* (2): 245-250 (1982) Six young mature male pigs were maintained on a high fat low fiber 'Western' type diet. Substitution of ethanol for sucrose raised plasma total cholesterol, an increase that was solely due to a rise in high-density lipoproteins. Plasma triacylglycerols and apo-B concentrations were unchanged and although apo-A_1 rose with ethanol, this was not statistically significant. Ethanol did not alter total fecal steroids but both bile acids and the ratio of bile acids/neutral sterols were increased. In fecal extracts from these animals, mutagenic activity in the Ames bacterial test was also raised. The data are discussed in relation to the relationships between dietary ethanol and coronary heart disease and colorectal cancer.

METABOLISM OF SQUALENE IN HUMAN FAT CELLS. R. P.T. Kovanen, and T.A. Miettinen (2nd and 3rd Depts. of Med., Univ. of Helsinki, University Central Hospital, 00290 Helsinki 29, Finland) *J Biol. Chem. 257* (17): 10300-10305 (1982). Human adipose tissue has been shown to contain exceptionally high concentrations of squalene. In the present experiments, it was shown that most of adipose tissue squalene is located in the fat cells. Of this squalene, 80% is located in the central neutral lipid droplet and 20% is bound to the microsomal membranes. Upon incubation of isolated fat cells with radiolabeled substrates, both the microsomal and the droplet squalene became labeled. The specific activity of microsomal squalene increased faster than that of droplet squalene. In addition, the microsomal squalene quickly equilibrated to a maximal specific activity, whereas the droplet squalene showed a steady increase in specific activity. These observations indicate rapid turnover of microsomal squalene and slow turnover of droplet squalene. Moreover, they reflect intracellular transfer of labeled squalene from microsomes to the lipid droplet. During a 3-hour incubation of fat cells with labeled substrates, 90% of the newly formed labeled squalene was transferred to the lipid droplet and only 10% was converted into cholesterol. The results demonstrate that adipocyte squalene can be segregated anatomically and functionally into two distinct pools: a small metabolically active pool in the microsomal membranes where squalene is synthesized and a large metabolically inactive pool in the fat droplet where squalene is stored. The intracellular transfer of de novo synthesized squalene into the fat droplet of fat cells is one mechanism of squalene accumulation in adipose tissue.

DIETARY FIBER, LIPID METABOLISM, AND ATHEROSCLEROSIS. G.V. Vahouny (Dept. of Biochemistry, The George Washington University School of Medicine and Health Sciences, Washington, DC 20037) *Federation Proc. 41* : 2801-2806 (1982). Despite the physicochemical complexity of dietary fibers (plant cell walls) and their individual components, there is substantial epidemiologic, clinical and experimental evidence that these dietary components may have a role in modifying certain risk factors in coronary heart disease. Particulate fibers, such as wheat bran, do not appear to significantly alter plasma lipids or lipoprotein distributions in humans, or the atherogenicity of diets in experimental animals. Dietary fibers found in fruits, legumes, and vegetables, in contrast, show more definitive responses. Among the fiber isolates, the gelling and mucilaginois fibers, such as pectins and guar gum, predictably decrease circulating lipids in humans and animals and increase excretion of fecal metabolites of cholesterol, the bile acids. These fibers and fiber components can be shown to influence luminal solubility of lipids and the extent of lymphatic absorption of both cholesterol and triglyceride. In addition, these same fibers are able to reduce postprandial levels of glucose, insulin, and other hormones. These direct effects on lipid absorption, and secondary effects of glucose and insulin on hepatic and peripheral lipoprotein metabolism, can account for many of the hypolipidemic responses to specific dietary fibers or their components, and may be of long-term consequence in coronary heart disease.

PHOSPHOLIPID-SENSITIVE Ca^{2+}-DEPENDENT PROTEIN KINASE FROM HEART. I. PURIFICATION AND GENERAL PROPERTIES. B.C. Wise, R. L. Raynor, and J.F. Kuo (Dept. of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322) *J.Biol. Chem. 257*(14):8481-8488 (1982). A phospholipid-sensitive Ca^{2+}-dependent protein kinase was purified more than 15,000-fold from the bovine heart extract by the steps of ammonium sulfate fractionation, DEAE-cellulose chromatography, controlled-pore glass absorption, and Sephacryl S-200 and phosphatidylserine-Affi-Gel 102 chromatographies. The enzyme was about 80-95% homogeneous and had a M_r of 83,500, as estimated by sodium dodecyl sulfatepolyacrylamide gel electrophoresis. The enzyme had a frictional ratio (f/f_0) of 1.4, indicating an asymmetric nature of the molecule. The M_r of the enzyme was also approximated to be 99,500 based upon its sedimentation coefficient and Stokes radius, and 113,600 by gel filtration. The enzyme exhibited multi-isoelectric points of pH 5.2, 5.5, and 5.8, and a pH optimum of 6.5 for its reaction. The enzyme absolutely depended upon both phosphatidylserine and $Ca^{2+}$for its activity, 1,3-diolein decreased the corresponding values. Of several phospholipids tested, phosphatidylserine was the most effective in supporting the Ca^{2+}-dependent activity of the enzyme. Lysophosphatidylserine was totally ineffective and phosphatidylserine-Affi-Gel 102 was effective in this respect, suggesting that a hydrophobic interaction between the phospholipid cofactor and the enzyme may be crucial for activation of the enzyme of Ca^{2+}. Calmodulin was unable to substitute for phospholipids. Only Sr^{2+} and Ba^{2+} were among several divalent cations tested that could partially substitute for Ca^{2+}. The enzyme also required Mg^{2+} for its activity.

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LIPID-BOUND SACCHARIDES IN *RHIZOBIUM METLILOTI.* M.E. Tolmasky, R.J. Staneloni, and L.F. Leloir (Institute de Investigaciones Biozuinicas "Foundacion Campomar" and Faculdad de Ciencias Exactas y Naturales, Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina) *J. Biol. Chem* 257 (12): 6751-6757 (1982). The lipid-bound saccharides formed by incubation of uridine diphosphate glucose with a particulate enzyme of *Rhizobium meliloti* were studied. They behaved like polyprenyl diphosphate saccharides when treated with ammonia or hot phenol, when catalytically hydrogenated, and on DEAE-cellulose chromatography. The saccharide moieties obtained after heating at pH2 for 10 min at 100°C were separated with a gel filtration column. The following compounds were detected: galactose, glucosyl β1-3 galactose, and some octasaccharides (I). These were compared by paper electrophoresis, thin layer and paper chromatography with an octasaccharide obtained from *Alcaligenes Feacalis* var. myxogenes strain 22 (II). Furthermore, compounds I and II were compared with the exopolysaccharide of *Rhizobium meliloti* (III) by partial acid hydrolysis and methylation analysis. The results were consistent with the identity of the repeating unit of Compound III with Compounds I and II except for differences in the substitutents (acetyl or succinyl). Studies on the labeling of the lipid-bound saccharides have shown that the sequence is: first, galactose and glucosyl β1-3 glactose, then the rest of glucose residues, and finally, the substitutents (acetyl and pyruvic acid).

PHOSPHOLIPID-SENSITIVE Ca^{2+}-DEPENDENT PROTEIN KINASE FROM HEART. II. SUBSTRATE SPECIFICITY AND INHIBITION BY VARIOUS AGENTS. B.C. Wise, D.B. Glass, C.-H. Jen Chou, R.L. Raynor, N. Katoh, R.C. Schatzman, R.S. Turner, R. F. Kibler, and J.F. Kuo (Departments of Pharmacology and Neurology, Emory University School of Medicine, Atlanta, GA 30322) *J.Biol. Chem.* 257 (14):8489-8495 (1982). The specificity of substrates (including both phosphate donors and acceptors) for phospholipid-sensitive Ca^{2+}-dependent protein kinase purified (80-95% homogeneous) from bovine heart and inhibitors of its activity by various agents were investigated. Our findings indicate that (a) phospholipid-sensitive Ca^{2+}-dependent protein kinase had a protein substrate specificity distinguishable from that previously reported for cyclic nucleotide-dependent protein kinases and the calmodulin-sensitive species of Ca^{2+}-dependent protein kinases, such as myosin light chain kinase, and (b) trifluoperazine and *N*-(6-aminohexyl) 5chloro-1-naphthalenesulfonamide cannot be considered as selective inhibitors of calmodulin/Ca^{2+}-stimulated enzymes as commonly suggested.

3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE. THE DIFFERENCE IN THE MECHANISM OF THE IN VITRO MODULATION BY PHOSPHORYLATION AND DE-PHOSPHORYLATION TO MODULATION OF ENZYME ACTIVITY BY NON-ESTERIFIED CHOLESTEROL. S.Venkatesan and K.A. Mitropoulos (Med. Res. Council Lipid Metabolism Unit, Hammersmith Hosp., Ducane Road, London W12 OHS, U.K.) *Biochim. Biophys. Acta* 710 (3): 446-455 (1982). Incubation of rat liver microsomal fraction in the presence of increasing concentrations of a serum preparation and the re-isolation of the treated microsomal vesicles resulted in a progressive increase in the concentrations of non-esterified cholesterol, a progressive decrease in the activity of hydroxymethylglutaryl-CoA reductase and progressive changes in the characteristics of the Arrhenius plots of the enzyme. The microsomal fraction from rats fed on the standard, cholesterol or cholestyramine-supplemented diet showed considerable differences in the activity of hydroxymethylglutaryl-CoA reductase and differences in the characteristics of their Arrhenius plots. However, the incubation of the microsomal fraction from the rats in the three experimental conditions with ATP and Mg^{2+} and the futher incubation of the inactivated enzyme with a preparation of cytosolic phosphoprotein phosphatase resulted in Arrhenius plots with similar characteristics to those of the corresponding original microsomal fraction. These results suggest that changes in the concentration of non-esterified cholesterol in the endoplasmic reticular membrane are responsible for the differences in the activity of hydroxymethylglutaryl-CoA reductase in the microsomal fraction from the rats in these dietary conditions.

INCORPORATION OF [HYDROGEN-3] ALKYLGLYCEROL-ETHER AND [CARBON-14] HEXADECANOL INTO BOVINE COLOSTRUM AND MILK LIPIDS. L. Ahrne and D.L. Palmquist (Dept. of Animal Husbandry, Swedish Univ. of Agric. Sci., S-750 07 Uppsala, Sweden) *J. Dairy Sci.* 65(10):1905-1911 (1982). [Hydrogen-3] glycerol ether and [carbon-14] hexadecanol were infused into the mammary gland or jugular vein of cows in colostral or full phases of lactation to determine their relative rates of synthesis and degradation. Neutral alkylglycerols were both synthesized and cleaved in the bovine gland. Oxidation of fatty alcohols and cleaving of neutral alkylglycerol ethers was faster during the milk than the colostral phase of lactation. In the colostral phase, both increased rate of synthesis and decreased cleavage contributed to increased neutral alkylglycerol concentration; however, synthesis changes were greater. Disappearance of [carbon-14] fatty alcohol and [hydrogen-3] alkylglycerol ether from the blood was the magnitude of fatty acid disappearance in previous studies, but uptake by the mammary gland was slower. Alkylglycerols were taken up from the blood and secreted intact into both colostrum and milk.

CHOLECALCIFEROL REQUIREMENTS OF YOUNG TURKEYS UNDER NORMAL CONDITIONS AND DURING RECOVERY FROM RICKETS. A. Bar, S. Edelstein, U. Eisner, I. Ben-Gal, and S. Hurwitz (Biochemistry Department, The Weizmann Institute of Science, Rehovot, Israel) *J. Nutr.* 112(9):1779-1786 (1982). Day-old turkeys fed vitamin D-deficient diets became rachitic within 17-24 days. The symptoms included reductions in body weight, plasma calcium and inorganic phosphorus, plasma and intestinal calcium-binding protein (CaBP), plasma 25-hydroxycholecalciferol [25(OH)-D_3], bone ash, and kidney 25 (OH)D_3-24-hydroxylase and a rise in kidney 25(OH)D_3-1-hydroxylase activity. Supplementation of the diet with 12.5 μg cholecalciferol per kilogram was sufficient to promote maximal body weight and normal plasma calcium, plasma phosphorus and bone ash. Feeding diets containing 250 or 1250 μg cholecalciferol per kilogram resulted in a reduced body weight. An increase in the concentration was observed. Feeding vitamin D-deficient rachitic birds for 4 days a diet containing 50 μg cholecalciferol per kilogram restored plasma calcium and phosphorus and bond ash. Body weight remained lower than that of the control for an additional 6-day period. Additional cholecalciferol, 25(OH)D_3 or 1α-hydroxycholecalciferol in the diet, intramuscular injection of the vitamin D derivatives, or a high-calcium, high-phosphorus diet did not accelerate the recovery from the rachitic state.

POSSIBLE FUNCTIONS OF SHORT-CHAIN AND MEDIUM-CHAIN CARNITINE ACYLTRANSFERASES. L. Bieber, R. Emaus, K. Valkner, S. Farrell (Dept. of Biochem., Michigan State Univ., East Lansing, Michigan 48824) *Federation Proc.* 41(12):2858-2862 (1982). Several mammalian tissues contain water-soluble, branched chain acylcarnitines and other short-chain aliphatic acylcarnitines and also contain a broad spectrum of short-chain and medium-chain carnitine acyltransferase (CAT) activities. Although carnitine can stimulate the oxidation of branched chain α-ketoacids, it has not been established that carnitine is required for the oxidation of the α-ketoacids in the matrix of mitochondria. Rather it probably acts as a reversible sink for acyl residues, thereby generating CoASH, which can be used to maintain normal metabolism; thus carnitine would have a facilitative rather than an obligatory role. Microsomes and peroxisomes contain medium- and short-chain CATs. This occurrence of short- and medium-chain CATs in peroxisomes is consistent with carnitine's being involved in shuttling the chain-shortened products of β-osidation out of peroxisomes. Human urine contains a spectrum of short-chain acylcarnitines and data are presented that show a large amount of propionylcarnitine in the urine of the individual with a metabolic problem. The cumulative data are consistent with the conclusion that carnitine has multiple roles in mammalian metabolism, including the shuttling of β-oxidation chain-shortened products out of peroxisomes in liver, the modulation of the acyl-CoA/CoASH ratio in mammalian cells, and the translocation of acetyl units for selective synthesis in a yeast.

EVIDENCE FOR A COMPARTMENTATION OF BRAIN MICROSOMAL DIACYLGLYCEROL. L. Binaglia, R. Roberti, A. Vecchini, and G. Porcellati (Inst. of Biol. Chem., The Med. Schl., Univ. of

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Perugia, 06100 Perugia, Italy) *J. Lipid Res. 23*(7):955-961 (1982). Phosphatidylcholine synthesis from CDP-[methyl-^{14}C]choline and membrane-bound diacyl-[U-^{14}C]-*sn*-glycerol, formed through the glycerol phosphate pathway, has been examined in vitro in rat brain microsomes. When labeled diacylglycerol was incubated in the presence of unlabeled CDP-choline, the rate of phospholipid labeling looked very different from that measured in incubations of unlabeled diacylglycerol with CDP-[methyl-^{14}C] choline. Evidence is given that diacylglycerol formed through the glycerol phosphate pathway belongs to a metabolic pool separate from the bulk membrane diacylglycerol.

UPTAKE AND OXIDATION OF MALONALDEHYDE BY CULTURED MAMMALIAN CELLS. R.P. Bird and H.H. Draper (Dept. of Nutrition, College of Biological Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1) *Lipids 17*(8):519-523 (1982). Primary cultures of rat skin fibroblasts were used as a model system to investigate the cellular uptake and oxidation of malonaldehyde (MA). The cells were grown in a medium containing 10^{-5} M, 10^{-4} M, or 10^{-3} M concentrations of [1,3-^{14}C] MA. There was a limited, concentration-dependent uptake of MA by 24 hr (~4% at all concentrations). The uptake of [1,2-^{14}C] acetate by 24 hr was ~24%; 83-89% of the ^{14}C in the MA taken up was ozidized to $^{14}CO_2$ by 24 hr and ~5% was recovered in the major lipids. Despite its low uptake and rapid oxidation to CO_2, pretreatment of the cells with 10^{-3} M MA for 24 hr produced a latent inhibition of [^{14}C] glucose oxidation. Limited cellular uptake of MA may explain the tolerance of cells grown in culture to relatively high MA concentrations.

LOCALIZATION OF LIPID BINDING DOMAIN(S) ON SUBUNIT II OF BEEF HEART CYTOCHROME C OXIDASE. R. Bisson, G.C.M. Steffens, and G. Buse (Consiglio Nazionale delle Ricerche Center for Physiology of Mitochondria and Lab of Molecular Biol. and Pathology, Instituto di Patologia Generale, Univ. Padova, 35100 Padova, Italy) *J. Biol. Chem. 257*(12):6716-6720 (1982). Photoactivatable arylazido phospholipids have been used to study the arrangement in the membrane of beef heart cytochrome *c* oxidase subunit II. Fragmentation of the photocross-linked polypeptide showed that sequence 20-98 which contains two long stretches of uncharged amino acid residues, is in contact with lipids. Edman degradation of the labeled peptide indicated that His 26, Thr 27, and Met 29 are involved in the interaction with the polar head region of the bilayer. These data have been used to propose a scheme for the folding of subunit II in the mitochondrial membrane.

ORGANIZATION OF UNESTERIFIED CHOLESTEROL IN HIGH DENSITY LIPOPROTEINS PROBED BY FILIPIN. L. Blau, R. Bittman, P. Lagocki, R. Byrne, and A.M. Scanu (Department of Chemistry, Queens College of The City University of New York, Flushing NY 11367) *Biochim. Biophys. Acta 712*(3):437-443 (1982). The initial rate of filipin association with unesterified cholesterol in high density lipoproteins (HDL) was measured by stopped-flow spectrophotometry to assess the roles played by apolipoproteins and phospholipids in modulating the surface exposure of cholesterol. The initial rate of filipin-unesterified cholesterol association was enhanced upon hydrolysis of the glycerophospholipids of human HDL_3 by phospholipase A_2. Rate enhancements were also observed following trypsin-catalyzed hydrolysis of apolipoprotein A-I in canine HDL and of apolipoproteins A-I and A-II in human HDL_3. However, the initial rate of filipin-unesterified cholesterol association was not altered upon incubation of HDL_3 with polymorphonuclear cells, which causes hydrolysis of apolipoprotein A-II but leaves apo-lipoprotein A-I intact. These results are consistent with the general structural model of HDL in which unesterified cholesterol, apolipoproteins and glycerophospholipids are presumed to be localized at the surface of the HDL particle. From these studies and from results indicating that the initial rate of filipin-unesterified association was enhanced in canine HDL hybrids in which 50% of the apolipoprotien A-I had been replaced by apolipoprotein A-II, we also conclude that apolipoprotein A-I in HDL is in closer proximity to unesterified cholesterol than apolipoprotein A-II. Thus, it appears that rapid kinetic measurements of filipin-cholesterol association may be useful in assessing the organization of unesterified cholesterol in serum lipoproteins.

CMP-DEPENDENT INCORPORATION OF [^{14}C]GLYCEROL 3-PHOSPHATE INTO PHOSPHATIDYLGLYCEROL AND PHOSPHATIDYLGLYCEROL PHOSPHATE BY RABBIT LUNG MICROSOMES. J.E. Bleasdale, J.M. Johnston (The Cecil H. and Ida Green Center for Reproductive Biol. Sciences and the Dept. of Biochem. and ObGyn., Univ. of Texas Southwestern Med. Schl., Dallas, TX 75235) *Biochim. Biophys. Acta 710*(3):377-390 (1982). Rabbit lung microsomes catalyzed CMP-dependent incorporation of [^{14}C] glycerol 3-phosphate into a total lipid extract. The radioactively labeled products in the lipid extract were phosphatidylglycerol and phosphatidylglycerol phosphate. CMP-dependent incorporation of [^{14}C] glycerol 3-phosphate by lung microsomes was optimal at pH 7.4 and needed Mn^{2+}. K_m value for CMP in this reaction was 0.19 mM. Cytosine-β-D-arabinofuranoside-5′-monophosphate-dependent incorporation of [^{14}C] glycerol 3-phosphate was observed at pH 8.5 but not pH 6.8. CMP-dependent incorporation of [^{14}C] glycerol 3-phosphate by microsomes was inhibited by inositol. The optimal in vitro rates of CMP-dependent and CDPdiacylglycerol-dependent incorporation of [^{14}C] glycerol 3-phosphate into lipid were similar and were not additive. CMP-dependent and CDPdiacylglycerol-dependent incorporation of [^{14}C] glycerol 3-phosphate by lung microsomes appeared to involve CDPdiacylglycerol:glycerol-3-phosphate phosphatidyltransferase. The activity of this enzyme in a subcellular fraction did not relate directly to the extent of CMP-dependent [^{14}C] glycerol 3-phosphate incorporation. Preincubation of lung microsomes with 5 mM CMP plus 3 mM phosphatidylinositol increased CMP-dependent incorporation of [^{14}C] glycerol 3-phosphate. When lung microsomes were depleted of phosphatidylinositol, CMP-dependent incorporation lessened. These observations are consistent with a role for CMP in the regulation of the phosphatidylinositol and phosphatidylglycerol content of lung surfactant during lung maturation.

GLYCOSPHINGOLIPIDS OF A GREEN MONKEY KIDNEY CELL LINE (GMK AH-1). EVIDENCE FOR A NOVEL PENTAGLYCOSYLCERAMIDE BASED ON GLOBOTETRAOYLCERAMIDE. J. Blomberg, M.E. Breimer, and K-A. Karlsson (Department of Virology, University of Goteborg, Box 33031, S-40033 Goteborg, Sweden) *Biochim. Biophys. Acta 711*(3):466-477 (1982). Total non-acid glycolipid fractions have been isolated from GMK AH-1 cells grown in fetal calf serum and in horse serum. For comparison, glycolipids were also prepared from green monkey (*Cercopithecus aetiops*) kidney and from fetal calf serum. The major glycolipids from GMK AH-1 cells grown in fetal calf serum were isolated by silicic acid column chromatography and preparative thin-layer chromatography. These fractions were characterized mainly by thin-layer chromatography, mass spectrometry and gas chromatography. The structures of the glycolipids isolated were proposed as: Glc1→1Cer, GaL1→1Cer, GaL1→4Glc1→lCer, GaL1→4GaL1→4Glc1→1Cer, GalNAc1→3GaL1 →4GaL1→4Glc1→lCer. In addition, a novel pentaglycosylceramide with the probable structure Galβ1→3GalNAcβ1→3Galα1→4Galβ1→ 4Glcβ1→lCer was also present. The ceramides contained mainly dihydroxy 18:1 long-chain base in combination with non-hydroxy 16:0-24:0 fatty acids. Small amounts of trihydroxy 18:0 long-chain base and hydroxy 22:0-24:0 fatty acids were also present in the mono- and diglycosylceramide fractions. The glycolipid patterns of GMK AH-1 cells grown in fetal calf serum or horse serum were identical. The pentaglycosylceramide present in the cultured cells could not be detected with certainty in the kidney tissue. The uptake of this glycolipid from the culture medium is unlikely as it seems to be lacking in calf serum.

THE TRACING OF THE PATHWAY OF MEVALONATE'S METABOLISM TO OTHER THAN STEROLS. P.S. Brady, R.F. Scofield, W.C. Schumann, S. Ohgaku, K. Kumaran, J.M. Margolis, and B.R. Landau (Department of Medicine and Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH 44106) *J. Biol. Chem. 257*(18):10742-10746 (1982). Specifically ^{14}C-labeled mevalonic acids were administered to rats in diabetic ketosis, and the distribution of ^{14}C was determined in the hydroxybutyric acid each rat excreted. Also, the distributions of ^{14}C were determined in hydroxybutyric acid formed by slices of livers and kidneys from rats in diabetic ketosis and incubated with the specifically labeled mevalonic acids. The distributions found are in accord with the conversion of mevalonate to hydroxymethylglutaryl-CoA by the shunt pathway proposed by J. Edmond and G. Popjak. This is, carbon 5 of mevalonate was metabolized to form the carboxyl of acetyl-CoA and carbons 2 and 3 of mevalonate were converted in large measure to hydroxybutyric acid without acetyl-CoA as an intermediate, i.e. the bond between carbon 2 and 3 was not cleaved, while the bond between 1 and 2, traced with [1,2-^{14}C] mevalonate, was cleaved. Similar distributions of ^{14}C were found in hydroxybutyric acid excreted by rats in diabetic ketosis administered specifically ^{14}C-labeled isovaleric acids, isovaleric acid having in its metabolism intermediates common to those in the shunt pathway.

THE EFFECT OF TWO INTRAVENOUS FAT EMULSIONS AND THEIR COMPONENTS ON BILIRUBIN BINDING TO ALBUMIN. G.J. Burckart, P.F. Whitington, and R.A. Helms (University of

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Tennessee College of Pharmacy, 874 Union Avenue, Memphis, TN 38163) *Am. J. Clin. Nutr. 36*(3):521-526 (1982). The effect of two intravenous lipid emulsions on the binding of bilirubin to albumin was investigated in vitro. Various concentrations of a soybean (Intralipid) or a safflower (Liposyn) oil emulsion in 0.48 m*M* albumin were assayed for changes in bilirubin displacement using Sephadex G-25 gel filtration and for alterations of albumin reserve binding capacity by a novel difference spectroscopy technique. Two major components of the emulsions, glycerol and egg phosphatides, were also assayed by difference spectroscopy. A significant enhancement of the total reserve bilirubin binding capacity of albumin was noted with concentrations of 200 to 500 mg/100 ml lipid emulsion using difference spectroscopy. Using salicylate to block secondary albumin binding sites for bilirubin, reserve bilirubin binding capacity at nonsalicylate competitive sites was shown to have increased (maximum 61%) over the range of 50 to 1000 mg/100 ml lipid emulsion. Only changes in nonsalicylate competitive binding sites could be detected at concentrations normally achieved in vivo. Egg phosphatides had little effect and glycerol decreased reserve bilirubin binding capacity. Sephadex studies were unable to detect any significant change in bilirubin retained on the column with 50 or 500 mg/500 ml lipid emulsion at any bilirubin/albumin ratio. The enhancement of reserve bilirubin binding capacity caused by lipid emulsions is mediated through changes in nonsalicylate competitive albumin binding sites. Product differences demonstrated in vitro point out the complexity of their effects on bilirubin-albumin binding and the necessity for *in vivo investigations* with structurally different nutritional products designed for use in newborns.

5-DIMETHYLAMINONAPHTHALENE-1-SULFONYL 3-AMINOTYROSYL APOLIPOPROTEIN C-III. PREPARATION, CHARACTERIZATION, AND INTERACTION WITH PHOSPHOLIPID VESICLES. A.D. Cardin, R.L. Jackson, and J.D. Johnson (Div. of Lipoprotein Res., Depts. of Pharmacology and Cell Biophysics, Biological Chem. and Med., Univ. of Cincinnati College of Med., Cincinnati, OH 45267) *J. Biol. Chem. 257*(9):4987-4992 (1982). Human plasma apolipoprotein C-III was nitrated with tetranitromethane reduced with sodium hydrosulfite and labeled with 5-dimethylaminonaphthalene-1-sulfonyl chloride yielding fluorescently labeled apoC-III-Dns. The addition of sonicated vesicles of dipalmitoyl phosphatidylcholine (DPPC) to apoC-III-Dns resulted in complex formation as shown by gel filtration chromatography, a 2-fold increase in dansyl fluorescence intensity and a blue shift in the wavelength of the dansyl emission maximum. The addition of unlabeled apoC-I, C-II, C-III, or A-I to preformed complexes of apoC-III-Dns and DPPC vesicles resulted in displacement of apoC-III-Dns from the DPPC vesicle and a reversal of the original phospholipid-induced enhancement in dansyl fluorescence. The dissociation constants for these unlabeled apoproteins were in the micromolar range. The measured ΔG_A values were less negative than the free energy values calculated for the transfer of the hydrophobic residues of the amphiphatic helices from an aqueous to a hydrophobic environment. These findings suggest that these apoproteins experience an environment less hydrophobic than the lipid bilayer interior. Fluorescence stopped flow studies of the fluorescence increase produced by apoC-III-Dns binding to DPPC vesicles and of the reversal of this fluorescence increase produced by the interaction of native apoC-III with the apoC-III-Dns-DPPC complex indicate that the interaction between apoproteins and phospholipid vesicles is rapid, while the dissociation reactions are slow and that structural changes occur in apoC-III subsequent to its interaction with lipid.

HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS: EFFECTS OF DIETARY PROTEIN. K.K. Carroll (Dept. of Biochemistry, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1) *Federation Proc 41*:2792-2796 (1982). Hypercholesterolemia and atherosclerosis can be produced in rabbits by feeding cholesterol-free, semipurified diets containing proteins derived from animal sources, but these effects have not been observed with similar diets in which the protein is derived from plants. This difference appears to be largely caused by differences in amino acid composition of the dietary proteins. Epidemiologic data on human populations show a strong positive correlation between dietary animal proteins and mortality from cardiovascular disease. It has also been found that the level of plasma cholesterol in human subjects can be reduced significantly by substituting soybean protein for animal protein in the diet. Studies of the mechanism of action of dietary protein have shown that rabbits fed casein have a slower turnover of plasma cholesterol, excrete less neutral steroids and bile acids, and absorb cholesterol from the intestine more readily than rabbits fed soy protein in cholesterol-free, semipurified diets. The excess cholesterol in the plasma of casein-fed rabbits is carried mainly in the very low-density and intermediate-density lipoproteins. These lipo proteins also contain increased amounts of apoproteins corresponding to apo E and apo C, and show differences in turnover rates compared to the corresponding lipoproteins from rabbits fed soy protein.

REVERTANTS OF A CHINESE HAMSTER OVARY CELL MUTANT RESISTANT TO SUPPRESSION BY AN ANALOGUE OF CHOLESTEROL: ISOLATION AND PARTIAL BIOCHEMICAL CHARACTERIZATION. T-Y. Chang and C.C.Y. Chang (Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755) *Biochemistry 21*(21): 5316-5323 (1982). A highly efficient selection procedure was developed for isolating revertants of Chinese hamster ovary (CHO) cell mutants resistant to suppression by 25-hydroxycholesterol. The procedure is based on the fact that the specific polyene antibiotic amphotericin B caused a lethal porous complex formation with membrane cholesterol only in cholesterol-rich cells. The wild-type cells and the revertant cells swtiched to grow from fetal calf serum medium to delipidated fetal calf serum medium for approximately 1 day became deficient in cellular cholesterol content. These cells, unlike the cholesterol-rich mutant cells, became much less sensitive to amphotericin B cytotoxicity. The spontaneous reversion frequency of a previously reported 25-hydroxycholesterol-resistant cell clone, 25-RA was found to be approximately 3×10^{-6}, a frequency comparable to other single gene mutations of CHO cells. Biochemical analyses of three of these revertants showed that all defects manifested in 25-RA cells reverted back in parallel, a result suggesting that these observed defects in 25-RA cells are due to a single mutation event, thus supporting the hypothesis (Chang & Limanek, 1980) that a common controlling factor may be involved in mediating the suppressive action(s) of the cholesterol analogue on various cholesterogenic enzyme activities. The function of this common controlling factor is rendered abnormal in 25-RA cells by mutation.

DIETARY CELLULOSE, WHEAT BRAN, AND FISH MEAL IN RELATION TO HEPATIC LIPIDS, SERUM LIPIDS, AND LIPID EXCRETION IN LAYING HENS. J.A. Cherry and D.E. Jones (Poultry Science Department, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061) *Poultry Sci. 61*(9):1873-1878 (1982). Isonitrogenous diets supplemented with 10% cellulose, 10% wheat bran, or 10% fish meal were fed to White Leghorn hens from 245 to 322 days of age. Neither body weights nor egg production of the hens fed these diets differed significantly from those of controls fed a corn-soybean meal basal diet. Supplemental fish meal and wheat bran did not significantly affect liver weight or liver lipids, although serum lipids of the hens fed the diet containing fish meal were significantly lower than those of the hens fed the basal diet. The hens fed the cellulose-supplemented diet, however, exhibited significantly decreased liver weights, serum lipids, and liver lipids, which were accompanied by a significant increase in excreta lipid. It is speculated that lipid excretion may be involved in some dietary induced alterations in the hepatic lipid concentration of laying chickens.

CONTROL OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN MYOPATHIC HAMSTER HEARTS. P.C. Choy (Dept. of Biochem., Faculty of Medicine, Univ. of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3) *J. Biol. Chem. 257*(18):10928-10933 (1982). A previous report from this laboratory demonstrated that the majority of phosphatidylcholine in hamster heart was formed from choline via the CDP-choline pathway. In this study, phosphatidylcholine biosynthesis in myopathic hamster hearts was compared with date-matched controls. Upon perfusion with [*Me*-^{3}H] choline, a 22% increase in labeling of phosphatidylcholine was observed in the hearts of 150-200-day-old myopathic hamsters. However, total cardiac phosphatidylcholine remained unchanged. In order to elucidate the cause for the increase in labeling of phosphatidylcholine during cardiomyopathy, the intermediates for phosphatidylcholine formation in the myopathic hearts were analyzed. The labeling and pool size of CDP-choline in the myopathic hearts were found to be 72 and 60% of the controls. This uneven reduction caused a 20% increase in the specific radioactivity of CDP-choline. Since CDP-choline is the immediate precursor for phosphatidylcholine formation, it can be concluded that the increase in labeling of phosphatidylcholine in the myopathic heart was a direct reflection on the specific radioactivity of CDP-choline. Furthermore, the net amount of phosphatidylcholine synthesized was estimated to be similar between the normal and myopathic hearts. The reduction in CDP-choline formation was probably caused by an observed decrease in CTP concentration in cardiomyopathy. However, phosphocholine cytidylyltransferase activity was elevated. The enhanced enzyme activity is

regarded as one of the compensatory mechanism for the myopathic heart to maintain a minimum CDP-choline level, in order to prevent reduction of net phosphatidylcholine biosynthesis.

STUDIES ON THE REGULATION OF ARACHIDONIC ACID SYNTHESIS IN ISOLATED RAT LIVER CELLS. B. Christophersen, T. Hagve, J. Norseth (Inst. of Clin. Biochem., Univ. of Oslo, Rikshospitalet, Oslo 1, Norway) *Biochim. Biophys. Acta 712*(2):305-314 (1982). Isolated liver cells from rats fed a diet deficient in essential fatty acids were used to study the oxidation, esterification and, especially, the desaturation and chain elongation of [1-^{14}C] linoleic acid. ^{14}C-labelled arachidonic acid (20:4) and smaller amounts of eicosatrienoic acid (20:3) were recovered mainly in the phospholipids, while γ-linolenic acid (18:3) was found in both the phospholipids and the triacylglycerol fraction. Lactate strongly increased the formation of arachidonic acid, which was found mainly in the phosphatidylcholine and the phosphatidylinositol fractions. Lactate reduced the amounts of γ-linolenic acid. Glucagon and (+)-deacnoylcarnitine reduced the formation of arachidonic acid, and (+)-decanoylcarnitine increased the incorporation of γ-linolenic acid, especially in the triacylglycerol fraction. Increasing concentrations of the [1-^{14}C] linoleic acid substrate increased the formation of arachidonic acid and of the other chain-elongated or desaturated fatty acids. Lactate also stimulated the formation of arachidonic acid in liver cells from animals fed adequate amounts of essential fatty acids. It is suggested that dietary and hormonal factors which can change the intracellular levels of malonyl-CoA may influence both the ratio of arachidonic acid/γ-linolenic acid formed and the total amounts of desaturated and chain-elongated fatty acids formed from linoleic acid.

LIPID CLASS ANALYSIS OF THE CENTRAL NERVOUS SYSTEM OF MYELIN-DEFICIENT WISTAR RATS. C.K. Csiza (Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201) *J. Lipid Res. 23*(5):720-725 (1982). Brains and spinal cords of myelin-deficient (md) and litter-mate control rats were analyzed serially for myelin lipids during the period from 13 to 32 days of age. The glycolipids of md rat brains were severely reduced and remained so during the period of study; brain cholesterol and phospholipids increased moderately but never reached the values for control brains. Deficiency of all three lipid classes was marked in the spinal cord and did not change with age. Among the glycolipids of md rats, deficiency was more severe in cerebrosides than sulfatides. The pronounced reduction of cerebrosides in the early stages of myelination suggests that abnormal synthesis of these glycolipids may be the most important biochemical anomaly responsible for myelin deficiency.

THE EFFECT OF BILE ACID STRUCTURE ON THE ACTIVITY OF BILE ACID-CoA:GLYCINE/TAURINE-*N*-ACYLTRANSFERASE. B. Czuba and D.A. Vessey (Liver Studies Unit, Dept. of Med., Veterans Admin. Med. Center, San Francisco, CA 94121) *J. Biol. Chem. 257*(15):8761-8765 (1982). Bile acid-CoA:glycine/taurine *N*-acyl-transferase from bovine liver was analyzed for its ability to conjugate a variety of different bile acid-CoA analogues. A complete steady state bisubstrate kinetic analysis was conducted for each analogue. The enzyme demonstrated strict specificity for the normal 4-substituted pentanoic acid side chain; shortening the side chain by 1 methylene group (norcholyl-CoA) completely eliminates enzymatic activity, and extending the side chain by 1 methylene group (homocholyl-CoA) causes a 30-fold decrease in activity at V_{max}. These effects of side chain modification were not related to decreased binding affinity as much as to decreases in the rates of the bond-breaking and bond-making steps. Bile acid-CoA analogues with a variety of ring substitutions involving keto and hydroxyl groups were also examined. Varying the position of substitution and the nature of the substituent had major effects on both the K_m and V_{max} terms. The analogues with the highest activities at V_{max} were 7-dehydrocholyl-CoA and the "allo" bile acid 5α,6-ketolithocholyl-CoA. However, in both cases, the high activity is obtained at the expense of binding energy. The most efficient substrates were 7-ketolithocholyl-CoA and 3-dehydrocholyl-CoA. The more common analogue, chenodeoxycholyl-CoA, was a surprisingly inefficient substrate. The relative rates of formation of glycine vs taurine conjugates were also found to vary with changes in structure. This indicates that certain bile acids are more likely to be conjugated with taurine, and others with glycine.

PRESENCE OF 25-HYDROXYVITAMIN D_3 AND 1,25-DIHYDROXYVITAMIN D_3 24-HYDROXYLASE IN VITAMIN D TARGET CELLS OF RAT YOLK SAC. J.-L. Danan, A-C. Delorme, and H. Mathieu (Unite de Recherches sur le Metabolisme Hydro-Mineral, Institut National de la Sante et de la Recherche Medicale, U.120, 78110 Le Vesinet, France) *J. Biol. Chem. 257*(18):10715-10721 (1982). In the pregnant rat, the yolk sac, which possesses true placental functions, is a vitamin D target organ. We tested its ability to hydroxylate 25-hydroxy- and 1,25-dihydroxyvitamin D_3 (25-OHD$_3$ and 1,25-(OH)$_2$D$_3$). 24,25-Dihydroxy- and 1,24,25-trihydroxyvitamin D_3 were produced by rat yolk sac homogenates incubated with tritiated 25-OHD$_3$ and 1,25-(OH)$_2$D$_3$. Rat yolk sac homogenates also formed small amounts of 25,26-dihydroxy-vitamin D_3. These newly synthesized metabolites were isolated and identified by Sephadex LH-20 chromatography, high performance liquid chromatography, and periodate cleavage. Yolk sac 25-OHD$_3$- and 1,25-(OH)$_2$D$_3$-24-hydroxylases were present in mitochondria and were of a mixed function oxidase nature. They were detected in the yolk sac as early as day 12 in the embryonic period and until the end of gestation. No hydroxylation occurred in maternal liver, amnion, fetal brain, or skin homogenates. Both 24-hydroxylases were detected in pure isolated rat yolk sac endodermal cells. This may be of physiological importance, since they are the 1,25-(OH)$_2$D$_3$ target cells in the yolk sac. Injection of 1,25-(OH)$_2$ [^{3}H] D$_3$ into rat yolk sac vitelline veins strongly suggested that the yolk sac produced 1,24,25-(OH)$_3$D$_3$ in vivo. We conclude that the yolk sac and more precisely its endodermal cells may help to control vitamin D metabolism within the feto-placental unit.

ON THE MECHANISM OF THE DISPLACEMENT OF APOLIPOPROTEIN A-I BY APOLIPOPROTEIN A-II FROM THE HIGH DENSITY LIPOPROTEIN SURFACE. C. Edelstein, M. Halari, A.M. Scanu (Depts. of Med. and Biochem., Univ. of Chicago, Pritzker Schl. of Med, Chicago, IL 60637) *J. Biol. Chem. 257*(12):7189-7195 (1982). Based on our original observation that native human apolipoprotein A-II (double chain) displaces apo-A-I from the surface of canine serum high density lipoproteins, we have compared the behavior of human native apo-A-II with that of single chain apo-A-II either naturally occurring or obtained by reduction and alkylation of human apo-A-II. Both human RCM apo-A-II and rhesus apo-A-II displaced apo-A-I from canine HDL as a linear function of their mass. The replacement of apo-A-I by apo-A-II was attended by a slight increase in lipoprotein mass due to the replacement of one chain of apo-A-I by a total of four chains of apo-A-II. The excess apo-A-II (double or single chain) in the aqueous phase formed a complex with the displaced apo-A-I. Incubation of this complex with fresh canine HDL resulted in the transfer of apo-A-II from the solution phase onto the HDL surface and in a mass-dependent displacement of apo-A-I. Oligomers of apo-A-I were readily displaced by either double or single chain apo-A-II. When apo-A-I and apo-A-II present in HDL hybrids were cross-linked, the former was not displaced by the addition of free apo-A-II. The similarity in behavior between double and single chain apo-A-II suggests that the disulfide bridge does not play an important role in its interaction with the HDL surface. Our results suggest that, whereas apo-A-I and apo-A-II form mixed associations in solution, these do not occur on the native HDL particle. This is explained on the basis of the greater affinity of apo-A-II for the HLD surface than for apo-A-I in solution.

HUMAN PLASMA LIPID EXCHANGE PROTEIN(S): A METHOD FOR SEPARATION OF DONOR AND ACCEPTOR LIPOPROTEINS BY HEPARIN-SEPHAROSE CHROMATOGRAPHY. J.L. Ellsworth, L. McVittie, R.L. Jackson (Div. of Lipoprotein Res., Dept. of Pharmacology and Cell Biophysics, Biol. Chem., and Med., Univ. of Cincinnati Med. Center, Cincinnati, OH 45267) *J. Lipid Res. 23*(4):653-659 (1982). The transfer or exchange of cholesteryl esters, triglycerides, and phospholipids between plasma very low (VLDL), low (LDL), and high (HDL) density lipoproteins is facilitated by specific lipid transfer proteins. The present report describes a method to separate donor and acceptor lipoprotein pools used in assays for lipid exchange activities. The method is based on the differential binding of lipoproteins to immobilized heparin. At 50 mM NaCl concentration, VLDL and LDL bind to heparin-Sepharose whereas >85% of HDL is unretained; VLDL and LDL are then eluted with 300 mM NaCl, 2% sodium dodecyl sulfate with a recovery ≥85%. The procedure is rapid and quantitative, as judged by a comparison to ultracentrifugation.

VITAMIN D, HYDROXYAPATITE, AND CALCIUM GLUCONATE IN TREATMENT OF CORTICAL BONE THINNING IN POST-MENOPAUSAL WOMEN WITH PRIMARY BILIARY CIRRHOSIS. W. Epstein, Y. Kato, R. Dick, and S. Sherlock (Royal Free Hospital, Pond Street, Hampstead, London NW3 2QG, England) *Am. J. Clin. Nutr. 36*(3):426-430 (1982). Women with primary biliary cirrhosis malabsorb calcium, phosphate and vitamin D, and develop accelerated cortical bone thinning. We have assessed the

value of parenteral vitamin D, oral hydroxyapatite (HA), and calcium gluconate (CG) in the treatment of cortical bone thinning in primary biliary cirrhosis. Sixty-four postmenopausal women with primary biliary cirrhosis were assigned randomly into three groups: one group receiving no mineral supplements (control), one group receiving HA, and one group receiving CG. All patients received parenteral vitamin D_2 (100,000 IU monthly). Eleven patients withdrew from the study and 10 withdrew due to poor compliance (six HA, four CG). Over a 14-month follow-up period, none of the groups showed a significant change in serum calcium or inorganic phosphate levels. Pre- and posttreatment hand radiographs were used to assess changes in metacarpal cortical thickness using the technique of caliper radiogrammetry. Cortical bone loss occurred in the control group ($p<0.01$). The HA group showed a significant gain in cortical bone thickness ($p<0.01$), while no significant change occurred in the CG group. This study indicates that vitamin D_2 does not halt metacarpal cortical bone thinning in primary biliary cirrhosis. The addition of CG prevents bone thinning and HA promotes positive cortical bone balance.

STRUCTURAL AND METABOLIC HETEROGENEITY OF β-VERY LOW DENSITY LIPOPROTEINS FROM CHOLESTEROL-FED DOGS AND FROM HUMANS WITH TYPE III HYPERLIPOPROTEINEMIA. M. Fainaru, R.W. Mahley, R.L. Hamilton, and T.L. Innerarity (Gladstone Foundation Laboratories for Cardiovascular Disease, Cardiovascular Research Institute and Depts. of Anatomy & Pathology, Univ. of California, San Francisco, San Francisco, CA) *J. Lipid Res.* *23*(5):702-714 (1982). Cholesteryl ester-rich β-very low density lipoproteins (β-VLDL) are β-migrating lipoproteins that accumulate in the d<1.006 g/ml fraction of plasma from cholesterol-fed animals and from patients with Type III hyperlipoproteinemia. They can be separated from pre-β-migrating very low density lipoproteins in the d 1.006 g/ml fraction by Geon-Pevikon block electrophoresis. The β-VLDL have a general property of stimulating cholesteryl ester synthesis and accumulation in macrophages. In the present study, we demonstrated that β-VLDL obtained from the cholesterol-fed dogs fasted for 16 hr were heterogeneous and that two subpopulations of particles, referred to as Fractions I and II, could be isolated from the whole β-VLDL fraction using gel filtration chromatography. These fractions of β-VLDL were similar in that both were cholesteryl ester rich, had β-electrophoretic mobility on Geon-Pevikon electrophoresis, and possessed the B and L apoprotein as major constituents. However, Fractions I and II differed in size, shape, electrophoretic mobility, chemical composition, and apoprotein B type. Furthermore, Fraction I was 3- to 15-fold more active than Fraction II in stimulating cholesteryl ester formation in mouse peritoneal macrophages. The concentration of Fraction I, but not Fraction II, was diminished in plasma by prolonged fasting, and Fraction I transported more intestinal-absorbed retinol than Fraction II.

CA^{2+}-DEPENDENT AND CA^{2+}-INDEPENDENT EFFECTS OF PANCREATIC SECRETAGOGUES ON PHOSPHATIDYLINOSITOL METABOLISM. R.V. Farese, R.E. Larson, M.A. Sabir (James A. Haley Veterans Hospital and Dept. of Med., Coll. of Med., Univ. of South Florida, Tampa, FL 33612) *Biochim. Biophys. Acta 710* (3):391-399 (1982). We tested the effects of secretagogues on $[^{32}P]$ phosphate incorporation into, and net changes in the concentrations of, phosphatidic acid and phosphatidylinositol during incubation of pancreatic tissue fragments in vitro. All tested secretagogues caused a 30-40% decrease in the concentration of phosphatidylinositol and a 50-100% increase in the concentration of phosphatidic acid. The effects of prostaglandins E_1 and E_2 appeared to be due to enhanced insulin secretion, since blockade of the latter by epinephrine selectively abolished the effects of prostaglandins on phospholipid metabolism. Secretagogue-induced amylase secretion and changes in the concentrations of phosphatidic acid and phosphatidylinositol were abolished by Ca^{2+} deficiency. The increases in phosphatidic acid concentration were inhibited by cycloheximide. Decreases in phosphatidylinositol concentration correlated reasonably well with increases in amylase secretion in all tested experimental conditions. The increases in $[^{32}P]$phosphate incorporation into phosphatidic acid and phosphatidylinositol provoked by carbachol and cholecystokinin were not influenced by either Ca^{2+} deficiency or cycloheximide treatment. This Ca^{2+} independent effect on $[^{32}P]$phosphate incorporation may reflect the rapid turnover of a small pool of phosphatidylinositol occurring prior to increases in cytosolic Ca^{2+} in the action of carbachol, cholecystokinin and other related peptides. The large-scale, Ca^{2+}-dependent decrease in phosphatidylinositol appears to be a "post-second messenger" event. The latter may contribute to exocytosis.

EFFECT OF PHOSPHOLIPIDS ON α-1,2 MANNOSIDASE ACTIVITY. W.T. Forsee, J.D. Springfield, and J.S. Schutzbach (Department of Microbiology, the Diabetes Research and Training Center, The University of Alabama in Birmingham, Birmingham, Alabama 35294) *J. Biol. Chem. 257*(17):9963-9967 (1982). An α-1,2-mannosidase has been solubilized and partially purified from rabbit liver microsomes. The partially purified enzyme was activated by the addition of zwitterionic phospholipids but maximal activity was found to be relatively independent of acyl chain length or degree of unsaturation. Titration of the enzyme with increasing concentrations of water-soluble and long acyl chain phospholipids demonstrated that an ordered lipid structure of either micelles or bilayers was required for α-mannosidase activity. Mixed micelles of Triton X-100 and zwitterionic phospholipids also activated the enzyme. The concentration of phospholipid in the mixed micelles required for activation was dependent upon acyl chain length, but maximal activity was unaffected by this parameter. The addition of negatively charged phospholipids not only failed to activate the enzyme but also inhibited α-mannosidase activity in the presence of zwitterionic phospholipids. Inhibition by negatively charged phospholipids was pH dependent with maximal inhibition at pH values of 6.0 or lower. These results suggest that the activity of the α-1,2-mannosidase could be subject to modulation by the composition and structure of the microsomal membranes.

HIGH DENSITY LIPOPROTEIN-3 HETEROGENEITY IN SUBJECTS WITH THE APO-AI_{Milano} VARIANT. G. Franceschini, T.G. Frosi, C. Manzoni, G. Gianfranceschi, and C.R. Sirtori (Center E. Grossi Paoletti, University of Milan, 20129 Milan, Italy) *J. Biol. Chem. 257*(17):9926-9930 (1982). The structure of high density lipoproteins (HDL) isolated from subjects with the AI_{Milano} (AI_M) apoprotein variant was studied by the use of the cross-linking reagent dimethylsuberimidate. The HDL_2 subfraction is markedly reduced, as compared to control subjects; the HDL_3 subfraction, on the other hand, shows a marked heterogeneity, being characterized by at least three particle subpopulations, identified as HDL_3-I, HDL_3-II, and HDL_3-III. The HDL_3 fraction purified from the AI_{Milano} subjects eluted as a symmetrical peak from a 6% agarose column, corresponding to a unimodal particle size distribution. The content of the different HDL_3 particles, detected by cross-linking, varied widely along the elution profile, the tail of the peak being enriched in anomalous particles of very small size (HDL_3-III). Apoprotein compositional studies indicated that these small HDL_3-III may be enriched in the AI_M monomer, the larger particles containing more AI_M-AII complexes and AI_M dimers. All the anomalous HDL_3 particles are triglyceride enriched, with a decreased cholesterol ester content. They may be an intermediate product in the cholesterol transfer chain between HDL and very low density lipoproteins, or be generated during interconversion of HDL. These particles may have a functional role in tissue cholesterol homeostasis; their unusual compositonal changes may help explain the protection of the studied subjects from tissue cholesterol deposition, in spite of the marked decrease of the total HDL fraction.

EVIDENCE THAT TWO SYNTHETIC PATHWAYS CONTRIBUTE TO THE APOLIPOPROTEIN B POOL OF THE LOW DENSITY LIPOPROTEIN FRACTION OF RABBIT PLASMA. G. Ghiselli (Instituto di Farmacologia e Farmacognosia, Universita'degli Studi di Milano, Milan, Italy) *Biochim. Biophys. Acta 711*(2):311-315 (1982). The plasma specific activity of apolipoprotein B was determined in normal rabbit injected with homologous radioiodinated very low and intermediate density lipoproteins (VLDL and IDL). The specific activity was followed in the same density range of the injected lipoproteins and in two low density lipoprotein subfractions (LDL_1 and LDL_2). VLDL B ($d<1.006$ g/ml) is cleared from plasma mainly in IDL (d=1.006-1.019 g/ml), with minor radioactivity recovery in LDL_1 (d=1.019-1.040 g/ml) and LDL_2 (d=1.040-1.063 g/ml) fractions. The main catabolic product of IDL B is, on the other hand, LDL_1 B. LDL_2B, which represent more than 20% of the whole LDL B plasma pool in this animal species, is not derived to a significant extent from either VLDL or IDL and, conceivably, is synthesized independently.

STUDIES OF THE CA^{2+} TRANSPORT MECHANISM OF HUMAN ERYTHROCYTE INSIDE-OUT MEMBRANE VESICLES. Evidence for the development of a positive interior membrane potential. J.M. Gimble, D.M. Waisman, M. Gustin, D.B.P. Goodman, and H. Rasmussen (Depts. of Cell Biology and Internal Medicine, Yale Univ. School of Medicine, New Haven, CN 06510) *J. Biol. Chem. 257*(18): 10781-10788 (1982). Previous observations on the effects of permeant anions on ATP-dependent calcium transport in inside-out vesicles prepared from human erythrocytes suggested that the cal-

cium pump is electrogenic, generating a positive interior membrane potential. The present work demonstrates the development of a positive interior membrane potential across inside-out vesicles membranes during calcium transport in the absence of permeant anions. Several membrane potential probes, 1-anilino-8-naphthalene-sulfonate, 3,3'-dipropylthiodicarbocyanine iodide, and an electron paramagnetic resonant triphenyl-phosphonium derivative, provdie qualitative evidence for the development of a membrane potential. Moreover, a number of parallels are observed between the changes in the membrane potential measured by the probes and calcium transport. These include enhancement by calmodulin, time course of change, similar kinetic properties, and the requirement for intact vesicle membranes. Quantitative measurements of the membrane potential shows a positive interior membrane potential of 26-37 mV using radiolabeled permeant anion distribution and 38-57 mV using 3,3'-dipropylthiodicarbocyanine iodide fluorescence changes. These membrane potentials are of a similar magnitude to those reported for the sarcoplasmic reticulum calcium pump.

CHANGES IN CHOLESTEROL AND TRIGLYCERIDE AS PREDICTORS OF ISCHEMIC HEART DISEASE IN MEN. R.J. Glynn, B. Rosner, and J.E. Silbert (Normative Aging Study, Veterans Administration Outpatient Clinic, and the Dept. of Preventive and Social Medicine, Harvard Med. Schl. and Affiliated Hosp., Boston, MA 02108) *Circulation 66*(4):724-731 (1982). We examined the relation of longitudinal changes in cholesterol and triglyceride to the subsequent development of heart diesase. The data were from 1437 participants of the Normative Aging Study, a prospective study of men from the Boston area who were free of ischemic heart disease on two examinations approximately 5 years apart. Forty-four had symptoms or ECG findings of ischemic heart disease after their second but before their third examination, a period of 3-5 years. The risk of heart disease was studied using a multiple logistic risk model that took into account smoking and other risk factors. Changes in cholesterol and triglyceride levels between Exams 1 and 2, when corrected for regression to the mean, were better predictors of heart disease incurred between Exams 2 and 3 than initial levels of cholesterol, triglyceride or systolic blood pressure. When two age groups (28-52 years and 53-85 years) were considered, changes were important predictors in each age group. These findings suggest the importance of monitoring lipid changes over time.

MINERAL HOMEOSTASIS DURING LACTATION – RELATIONSHIP TO SERUM 1,25-DIHYDROXYVITAMIN D, 25-HYDROXYVITAMIN D, PARATHYROID HORMONE, AND CALCITONIN. F.R. Greer, R.C. Tsang, J.E. Searcy, R.S. Levin, and J.J. Steichen (Department of Pediatrics, Clinical Sciences Center, 600 Highland Avenue, Madison, WI 53792) *Am. J. Clin. Nutr. 36*(3):431-437 (1982). During lactation maternal losses of calcium and phosphorus through human milk average 220 to 340 and 110 to 170 mg/day, respectively. The present study reports maternal serum concentrations of vitamin D metabolites, parathyroid hormone, calcitonin, calcium, magnesium, and phosphorus during the first 6 months of lactation. Serum calcium and magnesium concentrations increased during the first 6 months of lcatation. Serum 1,25-$(OH)_2$ vitamin D was increased at 6 months of lactation compared to values in nonpregnant nonlactating controls. During this same period, serum parathyroid hormone decreased slightly and serum calcitonin remained unchanged. Our data do not support the observation that lactation represents a state of physiological hyperparathyroidism. On the contrary, our results suggest that lactating women are able to adequately compensate for the losses of calcium and phosphorus during the early months of lactation, although increased serum 1,25-$(OH)_2$ vitamin D concentrations may be necessary to maintain calcium homeostasis with lactation beyond 6 months.

SECRETION OF NASCENT LIPOPROTEINS AND APOLIPOPROTEINS BY PERFUSED LIVER OF NORMAL AND CHOLESTEROL-FED GUINEA PIGS. L.S.S. Guo, R.L. Hamilton, R. Ostwald, and R.J. Havel (Cardiovascular Research Institute, University of California, San Francisco, CA 94143) *J. Lipid Res. 23*(4):543-555 (1982). Triglyceride-rich very low density lipoproteins (VLDL) are the major lipoprotein in perfusates of normal guinea pig livers. Their component apoprotein B is mainly B-100 together with some B-95. This apoprotein is actively synthesized, as are C apoproteins and small amounts of apoprotein E. Only trace amounts of intermediate density lipoproteins (IDL) are found in perfusates but appreciable amounts of low density lipoproteins (LDL) accumulate. These LDL are not newly synthesized. High density lipoproteins (HDL) also accumulate, which contain newly synthesized apoproteins A-I, E and C. Fatty livers of guinea pigs fed cholesterol secrete less VLDL and more IDL than normals. These lipoproteins contain newly synthesized apoprotein B, are enriched in cholesteryl esters and newly synthesized apoprotein E, and have reduced electrophoretic mobilities, making them resemble remnants. Large amounts of LDL also accumulate in perfusates of livers from cholesterol-fed animals and it does not appear to be newly synthesized. However, the LDL fraction is complex and includes particles that contain newly synthesized apoprotein B. Thus, these livers appear to secrete a spectrum of cholesteryl ester-rich particles, containing newly synthesized apoproteins B and E that span the density range of VLDL, IDL, and LDL. Livers of cholesterol-fed guinea pigs secrete large amounts of discoidal HDL. Accumulation of protein in HDL is increased 25-fold over from normal guinea pig livers.

REGRESSION OF ABDOMINAL FAT IN BROILERS ON ABDOMINAL FAT IN SPENT PARENTS. N.R. Gyles, A. Maeza, and T.L. Goodwin (Department of Animal Sciences, University of Arkansas, Fayetteville, AR 72701) *Poultry Sci. 61*(9):1809-1814 (1982). A total of 261 pedigreed broilers out of 10 sires and 27 dams from a high fat line and 378 pedigreed broilers out of 11 sires and 34 dams from a low fat line were probed in the vent at 8 weeks of age as an indirect measure of abdominal fat. The broilers were weighed alive, slaughtered, and eviscerated. Abdominal fat, eviscerated carcass without giblets, and carcass shell weights were taken for each chicken. The correlations between caliper measurements and abdominal fat were below +.2. The sires and dams of the two lines were weighed alive at 14 months of age, slaughtered, eviscerated, and the same carcass measurements taken as for their broiler progeny. The coefficient of variation for abdominal fat as a percentage of live weight of dams was 28%. Regressions of daughters and sons on midpoint of parents for abdominal fat as a percentage of live weight was +.305 and +.204, respectively, and were significant ($P \leqslant .05$). The regression of daughters on dams and of sons on dams for abdominal fat as a percentage of live weight was +.186 and +.154, respectively, and both were significant ($P \leqslant .01$).

ROLE OF LIPID PHASE SEPARATIONS AND MEMBRANE HYDRATION IN PHOSPHOLIPID VESICLE FUSION. D. Hoekstra (Dept. of Embryology, Carnegie Inst. of Washington, Baltimore, MD 21210) *Biochemistry 21*(12):2833-2840 (1982). The relationship between lipid phase separation and fusion of small unilamellar phosphatidylserine-containing vesicles was investigated. The kinetics of phase separation were monitored by following the increase of self-quenching of the fluorescent phospholipid analogue *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine, which occurs when the local concentration of the probe increases upon Ca^{2+}-induced phase separation in phosphatidylserine (PS) bilayers. Fusion was determined by using the resonance energy transfer fusion assay, which monitors the mixing of fluorescent lipid donor and acceptor molecules, resulting in an increase in energy transfer efficiency. The results show that in the presence of Ca^{2+}, fusion proceeds much more rapidly ($t_{1/2} < 5s$ than the process of phase separation $t_{1/2} \cong 1$ min). Mg^{2+} also induced fusion, albeit at higher concentrations than Ca^{2+}. Mg^{2+}-induced phase separations were not detected however. Subthreshold concentrations of Ca^{2+} (0.5 mM) or Mg^{2+} (2 mM) induced extensive fusion of PS-containing vesicles in poly(ethylene glycol)-containing media. This effect did not appear to be due to a poly (ethylene glycol)-facilitated enhancement of cation binding to the bilayer, and consequently Ca^{2+}-induced phase separation was not observed. The results suggest that macroscopic phase separation may facilitate but does not induce the fusion process and is, therefore, not directly involved in the actual fusion mechanism. The fusion experiments performed in the presence of poly(ethylene glycol) suggest that the degree of bilayer dehydration and the creation of "point defects" in the bilayer without rigorous structural rearrangements in the membrane are dominant factors in the initial fusion events.

CHANGES IN HIGH DENSITY LIPOPROTEIN CONTENT FOLLOWING ENDOTOXIN ADMINISTRATION IN THE MOUSE. FORMATION OF SERUM AMYLOID PROTEIN-RICH SUBFRACTIONS. J.S. Hoffman and E.P. Benditt (Department of Pathology, University of Washington, Seattle, WA 98195) *J. Biol. Chem. 257*(17):10510-10517 (1982). Bacterial endotoxin is a potent inducer of the serum amyloid protein (apo-SAA), a high density lipoprotein (HDL) apoprotein. In a study of the induction of apo-SAA and the structure of apo-SAA-rich lipoprotein particles in mice, we have observed that, following intraperitoneal administration of *Salmonella typhosa* lipopolysaccharide (50 μg), plasma apo-SAA levels rose from base-line levels of less than 1% to greater than 20% of the HDL protein content at 20 h postinjection. No changes in the relative content of other HDL apoproteins were noted; analysis of apo-SAA-rich HDL lipid content indicated a significant decrease (10%) in

phospholipid content relative to that of control HDL. Two major apo-SAA isotypes, apo-SAA_1 and apo-SAA_2, were identified, having apparent molecular weights of 12,600 and 11,800, respectively, and isoelectric points of 6.35 and 6.20, respectively. Quantitative immunoprecipitation experiments indicated that essentially all of the apo-SAA was bound to lipoprotein particles containing apo-A-I. Apo-SAA was distributed among higher density HDL subfractions than were other HDL apoproteins following density gradient centrifugation, and subfractions having apo-SAA:apo-A-I molar ratios of greater than 2:1 were identified. These results indicate the formation of a subset of apo-SAA-rich HDL particles following apo-SAA induction by endotoxin.

THE RELATIVE DEACYLATION OF DIFFERENT MOLECULAR SPECIES OF ENDOGENOUS PHOSPHATIDYLETHANOLAMINE IN RAT LIVER MICROSOMES BY PHOSPHOLIPASE ACTIVITY. B.J. Holub (Dept. of Nutr., Univ. of Guelph, Guelph, Ontario, N1G 2W1, Canada) *Biochim. Biophys. Acta 711*(2):305-310 (1982). The relative deacylation of the 1-palmitoyl and 1-stearoyl homologues of different molecular species of endogenous phosphatidylethanolamine in rat liver microsomes via phospholipase activity was studied. For this purpose, the various molecular species of microsomal phosphatidylethanolamine were labelled specifically in the 1-position by incubation of rat liver microsomes with [^{14}C] palmitoyl-CoA or [^{14}C] stearoyl-CoA plus 2-acyl-*sn*-glycero-3-phosphorylethanolamine containing 18:1, 18:2, 20:4, 22:6, etc. followed by resuspension of the microsomal pellet. The loss of radioactivity from the total 1-[^{14}C] palmitoyl and 1-[^{14}C] stearoyl homologues of phosphatidylethanolamine amounted to 31 and 29%, respectively, when these microsomal preparations were incubated for 1 h in 50 mM Tris-HCl buffer (pH 8.5) containing 10 mM Ca^{2+}. Regardless of whether palmitate or stearate resided in the 1-position, the susceptibility of the phosphatidylethanolamines to deacylation was not influenced significantly by the nature of the unsaturated fatty acid in the 2-position, as judged by selectivity indices for the relative disappearance of radioactivity from the individual classes (monoenoic, dienoic, trienoic, tetraenoic, pentaenoic and hexaenoic). A moderate discrimination against 1-stearoyl 2-saturated species was indicated. The findings indicate that fatty acid selectivity in the microsomal deacylation of phosphatidylethanolamine cannot account for the unique fatty acid and molecular species composition of this phospholipid in rat liver.

CARNITINE AND CARNITINE PALMITOYLTRANSFERASE IN FATTY ACID OXIDATION AND KETOSIS. C.L. Hoppel (Veterans Administration Medical Center, Department of Pharmacology and Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio, 44106) *Federation Proc. 41*(12):2853-2857 (1982). Carnitine is an essential factor in long-chain fatty acid oxidation. Carnitine acts as a carrier of fatty acyl groups from the cytoplasm to the mitochondrion. Long-chain acyl-CoA derivatives do not penetrate the mitochondrial inner membrane. Carnitine palmitoyl-transferase A (CPT-A), located on the external surface of the inner membrane, catalyzes the conversion of cytoplasmic long-chain acyl-CoA and carnitine into acylcarnitine. The acylcarnitine is reconverted to intramitochondrial acyl-CoA by the action of carnitine palmitoyltransferase B located in the inner membrane. Now, the acyl-CoA is available for β-oxidation in the matrix. An inner membrane carnitine-acylcarnitine translocase exchanges carnitine and acylcarnitine across the inner membrane but its role in long-chain acyl transfer has not been established. The tissue concentration of carnitine is important; liver carnitine is correlated with the rate of hepatic ketoacid production. In liver, malonyl-CoA, an intermediate in fatty acid synthesis, is proposed to regulate the activity of CPT-A. Studies using various purified transferases have not provided an answer to the question of whether the two activities expressed in mitochondria are separate enzymes. The absence of only CPT-A activity in isolated skeletal muscle mitochondria obtained from a patient with a lipid-storage myopathy suggests two separate activities.

RATE-LIMITING, DIURNAL ACTIVITY OF HEPATIC MICROSOMAL CHOLESTEROL-7α-HYDROXYLASE IN PIGEONS WITH HIGH SERUM CHOLESTEROL. F.H. Hulcher and R.D. Margolis (Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103) *Biochim. Biophys. Acta. 712*(2):242-249 (1982). Efficiency of regulating serum cholesterol by cholesterol-7α-hydroxylase was studied in pigeon strains hypo-(SR-39) and hypercholesterolemic (SR-37) with respect to dietary cholesterol. Diurnal hydroxylase activity in SR-37 was 10% of that in strain SR-39 adapted to a light-dark cycle and fed a non-cholesterol diet. Acrophase (6 pm) activity was 54-fold greater in Sr-39 than in SR-37 pigeons. Dietary cholesterol elevated enzyme activity 2.8-fold in SR-37 pigeons. Dietary cholestyramine plus cholesterol increased hydroxylase activity 21-fold in SR-37 and 3-fold in SR-39 strain; yet, activity remained greater in SR-39. Cholestyramine feeding prevented elevated cholesterol levels in both groups. The circadian rhythms of hydroxylase and serum corticosterone were determined. The dirunal activity in SR-37 was 10% of that in SR-39 and acrophase activity was 34-fold greater in SR-39. Hormone levels were comparable. Programmed acrophase was asynchronous between strains. Hydroxylase activity was positively correlated with corticosterone levels and inversely correlated with serum cholesterol. A defect in the up-regulation of cholesterol-7α-hydroxylase is proposed which limits the catabolism of cholesterol in strain SR-37.

PLASMA PROTEIN-FACILITATED COUPLED EXCHANGE OF PHOSPHATIDYLCHOLINE AND CHOLESTERYL ESTER IN THE ABSENCE OF CHOLESTEROL ESTERIFICATION. J. Ihm, J.L. Ellsworth, B. Chataing, and J.A.K. Harmony (Dept. of Biol. Chem., Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267) *J. Biol. Chem. 257*(9):4818-4827 (1982). A protein(s) which catalyzes the exchange of phosphatidylcholine and cholesteryl ester between plasma lipoproteins has been purified 10,000-fold from lipoprotein-free human plasma. The molecular weight of the protein of the active fraction is approximately 61,000; when electrophoresed the protein appears as a doublet of molecular weights 58,000 and 63,000. The active material is a glycoprotein which binds to concanavalin A. Human LTC is a lipid-protein complex with phospholipid, cholesterol, cholesteryl ester, and glyceride comprising 7% of the total mass. A similar glycoprotein exists in rat plasma. The rat preparation enhances exchange of phosphatidylcholine, but does not enhance exchange of cholesteryl ester. Partially purified LTC exists in a complex with lecithin:cholesterol acyltransferase. Active lecithin:cholesterol acyltransferase is not required for exchange of phosphatidylcholine or cholesteryl ester facilitated by human LTC. The rats of exchange of phosphatidylcholine and cholesteryl ester are equal. Coupled lipid exchange occurs at all stages of LTC purification. Phosphatidylcholine and cholesteryl ester are exchanged with 1:1 stoichiometry in the presence of thiol group reagents. Both lipid exchange activities are resistant to elevated temperatures. Coupled exchange of phospholipid and neutral lipid is not dictated by the nature of the lipoprotein donor and acceptor substrates: bovine liver phospholipid exchange protein catalyzes exchange of phosphatidylcholine but no cholesteryl ester between low and high density lipoproteins under conditions identical with those in which human LTC facilitates exchange of both lipids.

LOW DOSE INTRARENAL INFUSIONS OF PGE_2, PGI_2, and 6-KETO-PGE_1 VASODILATE THE IN VIVO RAT KIDNEY. E.K. Jacksons, H.T. Heidemann, R.A. Branch, and J.F. Gerkens (Dept. of Pharmacology, Div. of Clinical Pharmacology, Vanderbilt Univ. Schl. of Med., Nashville, TN) *Circ. Res. 51*(1):67-72 (1982). The renal vascular effects of prostaglandin E_2(PGE_2), 6-keto-PGE_1, and PGI_2 were investigated in indomethacin-pretreated rats. These prostanoids were infused directly into the left renal artery at rates ranging from 0.01 to 1.0 μg/min, while renal blood flow and mean arterial blood pressure was constantly monitored. PGE_2, 6-keto-PGE_1, and PGI_2 produced reductions in mean arterial blood pressure with threshold doses of 1.0, 0.3, and 0.03 μg/min ($P<0.01$), respectively, and maximal vasodepressor responses of 18.9±4.3, 37.0±7.8, and 58.7±8.2 mm Hg ($P<0.01$), respectively. In addition, all three prostanoids caused a dose-related reduction in renal vascular resistance with a threshold dose of 0.01 μg/min ($P<0.05$). The maximal reductions in renal vascular resistance were 2.59 ±0.52, 4.41±1.20, and 5.29±1.06 mm Hg/ (ml per min) for PGE_2, 6-keto-PGE_1, and PGI_2 ($P<0.01$), respectively. Whereas PGE_2 and 6-keto-PGE_1 produced dose-dependent increases in renal blood flow (maximal increases of 1.5±0.3 and 1.0 ±0.3 ml/min, respectively ($P<0.01$), PGI_2 nonsignificantly increased renal blood flow at low doses and decreased renal blood flow at low doses and decreased renal blood flow at higher infusion rates ($P < 0.01$). These data indicate that the in vivo rat kidney, similar to the kidneys of other species, is vasodilated by low doses of PGE_2, PGI_2, and 6-keto-PGE_1.

CHOLESTEROL TURNOVER IN LIPID PHASES OF HUMAN ATHEROSCLEROTIC PLAQUE. S.S. Katz, D.M. Small, F.R. Smith, R.B. Dell, and D.S. Goodman (Department of Medicine, Royal Victoria Hospital, Montreal, Quebec H3A 1A1, Canada) *J. Lipid Res. 23*(5):733-737 (1982). The turnover of free cholesterol in atheromatous plaque lipid phases was studied in a patient undergoing peripheral vascular surgery. [^{14}C] Cholesterol was injected intravenously 139 days prior to surgery, and [^{3}H] cholesterol was injected 12 days pre-op. The plasma cholesterol specific radioactivity decay curves were determined from the times of isotope injection until surgery. At surgery, atheroma, skin, muscle, and tendon were obtained. Lipid phases of plaque homogenate were isolated by density gradient centrifugation. The top layer of the gradient, layer 1,

contained the cholesteryl ester oil droplet phase, layer 2 was enriched in phospholipid bilayer phase, layer 3 contained cholesterol monohydrate crystals and the pellet, layer 4 had more dense plaque components such as collagen and elastin. Thus, plaque atheroma, which contains physically distinct forms of cholesterol, had correspondingly different rates of cholesterol turnover. Cholesterol solubilized in liquid oil droplets (layer 1) and liquid crystalline phospholipid bilayers (layer 2) had specific radioactivity values similar to those of tendon cholesterol, and represented tissue cholesterol that was undergoing slow equilibration with the plasma cholesterol pool. Pellet cholesterol (layer 4), which is probably connective tissue-associated, had lower specific radioactivity values, well below those of plasma cholesterol even after 5 months. Crystalline cholesterol (layer 3) had the lowest specific radioactivity values of all tissues and plaque fractions. Therefore, cholesterol in the crystalline state is relatively inert. Since crystalline cholesterol can account for over 40% of plaque free cholesterol, resistance to mobilization of this lipid may be an important obstacle to plaque regression.

ISOLATION AND CHARACTERIZATION OF SIMPLE AND COMPLEX LIPOPROTEINS CONTAINING APOLIPOPROTEIN F FROM HUMAN PLASMA. E. Koren, W.J. McConathy, and P. Alaupovic (Laboratory of Lipid and Lipoprotein Studies, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104) *Biochemistry 21*(21):5347-5351 (1982). Apolipoprotein F (ApoF), one of the minor apolipoproteins in human plasma, has been recently isolated and partially characterized. In the present work, the interaction of ApoF with other apolipoproteins and lipids in human plasma was studied. By the successive use of immunosorbers specific for ApoF, apolipoprotein A-II (ApoA-II) and apolipoprotein A-I (ApoA-I), three different ApoF-containing lipoproteins were isolated from normolipidemic fasting human plasma. Their apolipoprotein content was determined by double immunodiffusion against monospecific antisera to all known serum apolipoproteins, electroimmunoassay, crossed immunoelectrophoresis, and polyacrylamide gel electrophoresis. Their lipid composition was determined by thin-layer chromatography. The three ApoF-containing lipoproteins were identified as LpF:A-I:A-II (lipoprotein containing ApoF, ApoA-I, and ApoA:II), LpF:A-I(lipoprotein containing ApoF and ApoA-I), and LpF (lipoprotein containing only ApoF). LpF:A-I:A-II was found to contain ApoF, ApoA-I, and ApoA-II in an apparent 2:1:1 molar ratio. Its lipid moiety was characterized by cholesterol ester (45%) and free cholesterol (28%) as the predominant lipids. LpF contained only ApoF, and in its major lipid components were also cholesterol esters (63%) and free cholesterol (21%). It is suggested that ApoF-containing lipoproteins may be involved in transport and/or esterification of cholesterol.

EFFECT OF MEMBRANE CHOLESTEROL ON PHOSPHOLIPID METABOLISM IN THROMBIN-STIMULATED PLATELETS. R.M. Kramer, et. al. (Boston Veterans Admin. Med. Center and the Dept. of Biochem., Boston Univ. Schl. of Medicine, Boston, MA 02130) *J. Biol. Chem. 257*(12):6844-6849 (1982). The cholesterol to phospholipid mole ratio (C/PL) of human platelets was increased 1.3-fold or maintained at a normal value by incubating platelets with sonicated dispersions of cholesterol and phosphatidylcholine (PC) (C/PL = 3 or 1, respectively). Thrombin-induced mobilization of [^{3}H] arachidonic acid from prelabeled phospholipids and subsequent formation of labeled cyclo-oxygenase and lipoxygenase products were increased in cholesterol-enriched platelets as a function of thrombin concentration. Elevated platelet cholesterol content affected thrombin-induced changes in platelet phospholipids: (a) hydrolysis of PC was more sensitive to thrombin and was markedly enhanced over a wide range of thrombin concentrations (0.1-2 units/ml); (b) hydrolysis of phosphatidylinositol (PI) was increased at thrombin concentrations $\geq$0.2 unit/ml. Increased metabolism of [^{3}H] arachidonic acid in stimulated cholesterol-enriched platelets was due to loss of [^{3}H] arachidonate from PC at 0.1 unit/ml of thrombin. At higher thrombin concentrations (0.2-2 units/ml) it reflected enhanced hydrolysis of predominantly PC, but also PI. We conclude that cholesterol, possibly through its effect on platelet lipid organization, influences arachidonic acid metabolism in stimulated platelets by promoting enhanced activity of platelet phospholipase(s) for liberation of arachidonic acid.

FURTHER CHARACTERIZATION OF THE CHANGES OCCURRING IN THE PLASMA LIPOPROTEIN SPECTRUM IN THE EUROPEAN BADGER (*Meles meles* L.) DURING WINTER. P.M. Laplaud, L. Beaubatie, and D. Maurel (Laboratoire de Biochimie medicale, Faculte de medecine et de pharmacie, 2 rue du Dr. Marcland, 87032 Linoges Cedex, France) *Biochim. Biophys. Acta 711* (2):213-223 (1982). The plasma lipoprotein pattern in the European badger has been shown previously to undergo marked and complex quantitative and qualitative seasonal modifications. However, the conventional ultracentrifugal techniques then in use in our laboratory were of insufficient discriminating power with regard to the numerous lipoprotein fractions whose presence was suggested by our analyses. In the present study, a new density gradient ultracentrifugation procedure was applied to the more detailed determination of the distribution of plasma lipoproteins. The first series of analyses was performed in early December and the second in March. The fractions thus obtained, each of which corresponded to a narrow density interval, were analyzed subsequently for chemical composition, appearance upon polyacrylamide gel electrophoresis, and for their content of tetramethylurea-soluble apolipoproteins in alkaline-urea gels. Changes occurring from December to March included a large decrease in the plasma concentration of the 1.015-1.065 g/ml lipoproteins, chemical analysis of this material being compatible with the presence of at least two lipoprotein populations. On the other hand, high-density lipoproteins appeared less variable in chemical composition, although the proportion of those with lower density decreased considerably in early spring. Polyacrylamide gel electrophoresis of the native fractions showed multiple bands in most of them; the tetramethylurea-soluble apoprotein profile remained similar at the two dates considered with an apolipoprotein A-I-like component present in large amounts throughout the entire low- and high-density ranges.

THE MODIFICATION OF LIPID COMPOSITION IN L-M CULTURED CELLS SUPPLEMENTED WITH ELAIDATE. INCREASED FORMATION OF FATTY ALCOHOLS. T.-C. Lee, N. Stephens (Med. and Health Sciences Div., Oak Ridge Assoc. Univ., P.O. Box 117, Oak Ridge, TN 37830) *Biochim. Biophys. Acta 712*(2)299-304 (1982). Supplementation of culture medium with elaidic acid (40 μg/ml) resulted in the incorporation of this acid into 50% of the acyl groups of phospholipids in L-M cells; elaidate was esterified at both the *sn*-1 and the *sn*-2 positions of phosphatidylcholine and phosphatidylethanolamine. In addition, elaidate supplementation of L-M cells induces the accumulation of free fatty alcohols, alkyldiacylglycerols and wax esters/cholesterol esters. The concentration of intracellular free fatty alcohols increased as a function of the concentration of elaidic acid in the growth medium and the duration of exposure. However, the concentration of alcohol-containing lipids, total alkyl and alk-1-enyl lipids, was only slightly decreased. This decrease was much less than the increase in the level of free fatty alcohols. Therefore, we conclude that when elaidic acid is supplemented to L-M cells in culture, a net increase in the production of free fatty alcohols occurs.

NEUTRAL GLYCOSPHINGOLIPIDS OF HUMAN ACUTE LEUKEMIAS. W.M.F. Lee, M.A. Westrick, and B.A. Macher (Department of Medicine, Cancer Research Institute, University of California, San Francisco, CA 94143) *J. Biol. Chem. 257*(17):10090-10095 (1982). Neutral glycosphingolipids were isolated from the maglignant cells of several patients with different types of acute leukemia. Analyses were performed by high performance liquid chromatography combined with enzymatic hydrolysis of glycosphingolipids using glycosidases (*Escherichia freundii* endo-β-galactosidase, jack bean β-galactosidase, and beef kidney β-hexosaminidase). We found that acute leukemia cells contain very little or none of the more complex neutral glycosphingolipids that are found in normal leukocytes or chronic leukemia cells. Lymphoblasts, in particular, are rich in neutral glycosphingolipids with only 1 or 2 carbohydrate units. The most significant finding of our study was that, in contrast to normal leukocytes and chronic leukemia cells which have a single predominant tetraosylceramide species, acute leukemia cells (9 out of 10 patients analyzed) were found to have significant amounts of both globo and neolactotetraosylceramide. These results indicate that the composition of neutral glycosphingolipids in acute leukemia cells differs significantly from that found in normal or chronic leukemia cells.

STIMULATION OF PHOSPHOLIPID AND CHOLESTEROL ESTER SYNTHESIS BY PLATELET-DERIVED GROWTH FACTOR IN NORMAL AND HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA HUMAN SKIN FIBROBLASTS. C.C. Leslie, H.N. Antoniades, and R.P. Geyer (Dept. of Nutr. Harvard Schl. of Public Health, 665 Huntington Ave., Boston, MA 02115) *Biochim. Biophys. Acta 711*(2):290-304 (1982). Pure human platelet-derived growth factor at nanogram levels stimulates cholesterol ester, phospholipid and DNA synthesis in normal and familial hypercholesterolemia mutant human skin fibroblasts. The stimulation of [^{3}H] oleic acid incorporation into cholesterol ester by platelet-derived growth factor was inhibited in both normal and FH mutant skin fibroblasts by progesterone, an inhibitor of acyl-CoA:cholesterol acyltransferase. The rate of cholesterol ester synthesis in the normal cells increased as the concentration of platelet-poor plasma or low density lipoprotein

(LDL) was increased, especially in the presence of platelet-derived growth factor. Linearization of the LDL dose-response curve indicated that platelet derived growth factor increased the rate rather than the affinity of the overall cholesterol esterification system. The rate of cholesterol esterification in the FH mutant cells was highest in the absence of LDL or at low levels of platelet-poor plasma. Consequently, platelet-derived growth factor can stimulate cholesterol ester synthesis by LDL- and non-LDL-mediated processes.

FORMATION OF A NOVEL DIHYDROXY ACID FROM ARACHIDONIC ACID BY LIPOXYGENASE-CATALYZED DOUBLE OXYGENATION IN RAT MONONUCLEAR CELLS AND HUMAN LEUKOCYTES. R.L. Maas, et. al. (Depts. of Pharmacology and Med., Vanderbilt Univ. Schl. of Med., Nashville, TN 37232) *J. Biol. Chem.* 257(12):7056-7067 (1982). Elicited rat peritoneal mononuclear cells converted arachidonic acid to a new dihydroxy acid, 5(S), 15(S)-dihydroxy-6,13-*trans*-8,11-*cis*-eicosatetraenoic acid (5,15-DiHETE). In this system, the amount of 5,15-DiHETE formed was about 20% that of leukotriene B_4. The structure of the compound was determined by ultraviolet and mass spectrometric analysis, and comparison to a reference compound prepared by incubation of synthetic 5(*R,S*)-hydroxy- or hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5(*R,S*)-HETE or HPETE) with soybean lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.13). Cell incubations performed under an atmosphere of $^{18}O_2$ demonstrated that both hydroxyl groups in the cell product derived from molecular oxygen and that the oxygen atoms were from different oxygen molecules. Steric analysis indicated that each hydroxyl group had the *S*-configuration. The structural data thus indicate that 5,15-DiHETE is formed by an enzymatic double oxygenation of arachidonic acid catalyzed by both C-5 and C-15 lipoxygenases. Incubations with [$^3H_8$] 5(*S*)-HETE and [$^3H_8$] 5(*S*)-HETE revealed that both compounds could be converted to the product. When [$^3H_8$]-5(*S*), 15(*S*)-DiHPETE was added to cells, the majority of the substrate was reduced to 5,15-DiHETE. Leukocytes obtained from three human donors with peripheral blood eosinophilia also synthesized 5,15-DiHETE. Formation of compound occurred in both eosinophils and neutrophils from these donors.

A RE-EXAMINATION OF THE FATE OF GLYCERIDE-GLYCEROL IN NEUTRAL LIPID ABSORPTION AND TRANSPORT. C.M. Mansbach II and S. Parthasarathy (Veterans Administration Hospital and Dept. of Med., Div. of Gastroenterology, Duke Univ. Med. Center, Durham, NC 27710) *J. Lipid Res.* 23(7):1009-1019 (1982). Conventional ideas concerning the unidirectional movement of triacylglycerol from intestinal lumen to lymph with *sn*-2-monoacylglycerol being the major glyceride-glycerol precursor were challenged by our finding that steady state specific activities of radiolabeled triacylglycerol (glyceryl moiety) in the intestinal mucosa and lumen were greatly reduced as compared to the specific activity of intracuodenally infused triacylglycerol. Investigation of the point at which the radio-label was diluted was performed in mesenteric lymph duct-cannulated rats with a duodenal cannula through which trioleoyl[^{3}H]glycerol was constantly infused. Both within the bowel lumen and in the intestinal mucosa, monoacylglycerol, diacylglycerol, and triacylglycerol specific activities were 31% or less of the specific activity of the infusate; chylomicron triacylglycerol specific activity was 75%. Efflux of neutral lipid from the mucosa into the bowel lumen was directly demonstrated by finding that when ^{3}H glucose was injected intraperitoneally during triolein infusion, luminal triacylglycerol had a higher specific activity than was present in the mucosa.

STUDIES ON THERMAL ADAPTATION IN *TETRAHYMENA* MEMBRANE LIPIDS. MODIFICATION OF POSITIONAL DISTRITUTION OF PHOSPHOLIPID ACYL CHAINS IN PLASMA MEMBRANES, MITOCHONDRIA AND MICROSOMES. H. Maruyama, Y. Banno, T. Watanabe, and Y. Nozawa (Dept. of Biochem., Gifu Univ. Schl. of Med., Tsukasamachi-40, Gifu, Japan) Biochim. Biophys. Acta 711(2):229-244 (1982). The positional distribution of fatty acyl chains in major glycerophospholipids of three membrane fractions of the thermotolerant *Tetrahymena pyriformis* NT-1 cells was analyzed at various time intervals within 10 h after a temperature shift from 39 to 15 C. There were no changes in both the total phospholipid content and its proportional composition. At the 1-position, the content of palmitate in phosphatidylethanolamine, diacylphosphatidylcholine, and diacyl-(2-aminoethyl)phosphonolipid was decreased progressively after temperature-shift, γ-linolenate increased in a complementary fashion, in mitochondria and microsomes. The increase in the percentage of linoleate was compensated by the decrease in oleate at the 2-position of two 1,2-diacylphospholipids. As for 1-alkyl-2-acyl-phospholipids, a marked increment in γ-linolenate occurred, with a decline of oleate and linoleate at the 2-position of 1-alkyl-2-acyl-phosphatidylcholine, but no significant alterations were seen at the 2-position of 1-alkyl-2-acyl-(2-aminoethyl)phosphonolipid. The newly modified phospholipid molecular species such as 1-γ-linolenoyl-2-linoleoyl-phosphatidylethanolamine and 1-hexadecyl-2-γ-linolenoyl-phosphatidylcholine disseminate rapidly to other cell compartments and would play a pivotal role in the adaptive amelioration of altered membrane physical states during the cold acclimation.

EFFECT OF LIPOSOME COMPOSITION ON THE ACTIVITY OF DETERGENT-SOLUBILIZED ACYLCOENZYME A:CHOLESTEROL ACYLTRANSFERASE. S.N. Mathur and A.A. Spector (Dept. of Biochem., Univ. of Iowa, Iowa City, IA 52242) *J. Lipid Res.* 23(5):692-701 (1982). Acylcoenzyme A:cholesterol acyltransferase (ACAT) was solubilized from Ehrlich ascites cell microsomes with Triton X-100. After removal of the detergent, ACAT activity per mg protein was reduced by 50 to 65% as compared with untreated microsomes. When this microsomal extract was combined with liposomes composed of cholesterol and egg phosphatidylcholine, the ACAT activity increased 5.4- to 6.7-fold. Under these conditions sucrose density gradient centrifugation indicated that more than 50% of the added lipid was incorporated into vesicles having the same density as the ACAT activity, suggesting the formation of a complex. ACAT activity increased 2.9-fold when the phosphatidylcholine content of the liposomes were raised from 0.5 to 5.0 μmole/mg microsomal protein. By contrast, the ACAT activity increased only 42% when the cholesterol content of the lipsomes was raised from 0.17 to 0.57 μmol/mg microsomal protein. Addition of phosphatidylethanolamine to the liposomes produced little change in ACAT activity, whereas the activity was reduced by 25 and 50%, respectively, when sphingomyelin or phosphatidylserine was added. ACAT activity was five times higher when the liposomes were prepared from dioleoylphosphatidylcholine than from saturated phosphatidylcholines, including hydrogenated egg yolk, dimyristoyl or dipalmitoyl phosphatidylcholine. Likewise, the ACAT activity with liposomes made from soybean or egg yolk phosphatidylcholine was almost 3.5-fold greater than with those prepared from the saturated phosphatidylcholines.

PHOSPHOLIPID BIOSYNTHESIS IN HUMAN PLATELETS. FORMULATION OF PHOSPHATIDYLCHOLINE FROM 1-ACYL LYSOPHOSPHATIDYLCHOLINE BY ACYL-CoA: 1-ACYL-*SN*-GLYCERO-3-PHOSPHOCHOLINE ACYLTRANSFERASE. M.L. McKean, J.B. Smith, and M.J. Silver (Cardeza Foundation and the Department of Pharmacology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107) *J. Biol. Chem.* 257(19):11278-11283 (1982). Arachidonic acid and other fatty acids are taken up by human platelets from plasma and incorporated into membrane phospholipids. However, little is known about the mechanism and specificity of the various steps of fatty acid insertion into phospholipid. Previous findings from this laboratory have shown that the incorporation of radioactive C_{20}-unsaturated fatty acids (arachidonic, 8,11,14-eicosatrienoic, and 5,8,11,14,17-eicosapentaenoic) into the phospholipids of resting platelets is more rapid than that of the radioactive C_{16}- and C_{18}-saturated and unsaturated fatty acids. We now provide evidence that human platelet microsomes contain acyl-CoA: 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase. The enzyme preparation has a pH optimum of 7.0. Activity is insensitive to 1 mM EDTA and is inhibited 37% by 1 mM Ca^{2+} and 20% by 1 mM Mg^{2+}. Maximal activity is observed at 100 μM 1-acyl lysophosphatidylcholine as fatty acyl group acceptor. Comparison of the apparent V_{max} values showed that unsaturated CoA esters were transferred more rapidly to 1-acyl lysophosphatidylcholine than saturated CoA esters. Oleate, linoleate, and arachidonate, the major unsaturated fatty acids in platelet phosphatidylcholine, were transferred at similar rates. 8,11,14-eicosatrienoate was transferred about two times faster than there three fatty acyl groups. The data indicates that the incorporation of unsaturated fatty acids into phosphatidylcholine by human platelets occurs via reacylation of 1-acyl lysophosphatidylcholine.

CHOLESTEROL HOMEOSTATSIS IN RATS FED A PURIFIED DIET. D.J. McNamara, A. Proia and K.D.G. Edwards (Rockefeller Univ. and Cornell Univ. Med. Col./Memorial Sloan-Kettering Cancer Center, New York, NY 10021) *Biochim. Biophys. Acta* 711(2): 252-260 (1982). The rate of whole body cholesterol synthesis was measured in male Sprague-Dawley rats fed either a standard chow, cereal-based diet or a semi-synthetic purified diet consisting of casein, sucrose and lard. The purified diet significantly decreased daily fecal excretion of neutral and acidic sterols, the specific activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase, the bile acid pool size, and total daily cholesterol synthesis in the rat, while increasing plasma cholesterol concentrations and the total body con-

tent of cholesterol. The increased body content of cholesterol occurred primarily in muscle and connective tissue and not in the liver. The data demonstrate the importance of quantitating the net tissue accumulation of cholesterol for accurate measurement of daily sterol synthesis in growing animals when sterol balance measurements are used. Tissue accumulation accounted for 7% of total daily cholesterol synthesis in rats fed the cereal diet, and 20% of daily synthesis in animals fed the purified diet.

TRACE MINERALS AND ATHEROSCLEROSIS. W. Mertz (Beltsville Human Nutrition Research Center, Science and Education Administration, Human Nutrition, U.S. Department of Agriculture, Beltsville, MD 20705) *Federation Proc. 41*:2807-2812 (1982). Although there is no evidence for a direct cause-effect relationship between mineral and trace element status and atherosclerosis in humans, many elements exert a strong influence on individual risk factors for cardiovascular disease, such as disorders of blood lipids, blood pressure, coagulation, glucose tolerance, and circulating insulin. Studies in humans and animals have shown that optimal intakes of elements such as sodium, magnesium, calcium, chromium, copper, zinc, and iodine can reduce individual risk factors; some of these studies are consistent with the results of epidemiologic correlations. Influences of local geochemical environment and of dietary practices can result in mineral and trace element imbalances; deficiencies of chromium, iron, copper, zinc, selenium, and iodine are well defined. Detection and correction of such imbalances in populations, through diminishing individual risk factors, might ultimately reduce the incidence of atherosclerotic heart disease.

UPTAKE AND METABOLISM OF FREE FATTY ACIDS BY THE MORRIS 7777 HEPATOMA AND HOST RAT LIVER. R.E. Morton, M. Waite, V.L. King and H.P. Morris (Dept. of Biochemistry, Bowman Gray School of Medicine, Winston Salem, NC 27103) *Lipids 17*(8):529-537 (1982). The relative capacity of Morris 7777 hepatomas and livers of tumor-bearing rats to take up and subsequently metabolize intravenously injected radiolabeled free fatty acids was investigated. The objective was to determine differences in lipid metabolism which may affect the lipid composition previously observed in this tumor. Both tissues demonstrated comparably selectivity in the uptake of palmitate, linoleate and arachidonate from blood, although the hepatoma took up one-tenth as much free fatty acid per g wet wt as liver. A much greater percentage of fatty acid taken up by the hepatoma was converted to aqueous soluble radioactivity, perhaps the result of oxidation. In the hepatoma, palmitate was incorporated into phospholipid molecular species in a pattern similar to that observed for diglyceride, which suggested that phospholipid synthesis occurred predominantly de novo. On the other hand, in liver, a large percentage of palmitate was incorporated into polyunsaturated phospholipid molecular species that were not present in the diglyceride pool, which suggested significant incorporation by the acylation of monoacyl phosphoglycerides. These studies indicate that the specificity for the uptake of fatty acids was not different in the two tissues; however, the subsequent metabolic processes are markedly different.

Fats and oils

FLUORESCENCE STOPPED-FLOW STUDY OF THE INTERACTION OF ALKYLPYRIDINIUM SALTS AND PYRENE-SUBSTITUTED FATTY ACIDS WITH LECITHIN VESICLES. M. Almgren and S. Swarup (Physical Chemistry 1, Chemical Center, University of Lund, P.O. Box 740, S-220 07 Lund 7, Sweden) *Chem. Phys. Lipids 31*(1):13-22 (1982). The migration of alkylpyridinium surfactants and pyrene-substituted fatty acids between vesicles has been studied. The results are in accord with the assumption of diffusion-controlled processes. Changes in relaxation time with charge type, degree of ionization, pH, and alkyl chain length of the migrating species are investigated and discussed. The presence of a slower relaxation type, apparently due to a transmembrane transport was established in the case of the pyridinium surfactants, but could not be found for pyrene-substituted fatty acids. Fluorescence-quenching studies indicate that these are present at both vesicle interfaces.

HALO LIPIDS. V. SYNTHESIS, NUCLEAR MAGNETIC RESONANCE SPECTRA AND CYTOSTATIC PROPERTIES OF HALO ANALOGUES OF ALKYLLYSOPHOSPHOLIPIDS. H. Brachwitz, P. Langen, R. Hintsche, and J. Schildt (Academy of Sciences of the German Democratic Republic, Central Institute of Molecular Biology, Department of Cell Kinetics, Lindenberger Weg 70, DDR-1115 Berlin, West Germany) *Chem. Phys. Lipids 31*(1):33-52 (1982). A series of fluorine- and chlorine-containing analogues of alkyllysophospholipids has been synthesized as potential antimetabolites of phospholipids. These compounds include various structural isomers of racemic long-chain alkyldeoxyhaloglycerophosphocholines and *N,N*-dimethylethanolamines, alkyldeoxyhaloglycerophosphoric acids, and alkyl esters. ^{1}H- and ^{19}F-NMR spectroscopic data are presented and analyzed. Alkyldeoxyhaloglycerophosphocholines were found to exhibit a strong inhibitory effect on the proliferation of Ehrlich ascites carcinoma cells in vitro.

CHOLESTEROL OXIDATION AND THE BEHAVIOR OF 5-α-HYDROPEROXYCHOLESTEROL AT THE AIR/WATER INTERFACE. D.A. Cadenhead, B.M.J. Kellner, and D.M. Balthasar (Department of Chemistry, State University of New York at Buffalo, Buffalo, New York 14214) *Chem. Phys. Lipids 31*(1):87-96 (1982). The oxidation of cholesterol and the behavior of an oxidized sterol, 5-α-hydroperoxycholesterol (5-AHC), have been investigated. It is demonstrated that previous work is correct in observing that cholesterol oxidation does take place at the air/water interface, but predicts initial effects and rates that are much too large. The oxidation of cholesterol is found to be autocatalytic as long as the oxidized sterol compounds (OSC) remain miscible with the cholesterol. The OSC are postulated to adopt tilted conformations with respect to the air/water interface when oxidized at or about the sterol-5,6-positions, and to segregate out when saturation OSC levels in cholesterol are reached. Pure films of 5-AHC are found to be more expanded, more compressible and less stable than those of cholesterol. In mixed films with other selected lipids, 5-AHC behaves as a greater impurity than does cholesterol when the second component is more condensed, and as a poorer condensing agent when the second component is more expanded.

SYNTHESIS OF ENZYME-INHIBITORY PHOSPHOLIPID ANALOGS. III. A FACILE SYNTHESIS OF *N*-ACYLAMINOETHYLPHOSPHORYLCHOLINES. N.S. Chandrakumar, V.L. Boyd and J. Hajdu (Dept. of Chem., Boston Col., Chestnut Hill, MA 02167) *Biochim. Biophys. Acta 711*(2):357-360 (1982). A facile and efficient synthesis of *N*-acylaminoethylphosphorylcholines, a series of inhibitory substrate analogs of phospholipase A_2, is described. The procedure consists of a three-step sequence including: (1) *N*-acylation of ethanolamine with fatty acid chloride, followed by (2) phosphorylation of the alcohol function using 2-chloro-2-oxo-1,3,2-dioxaphospholane and (3) nucleophilic ring opening of the cyclic phosphate triester (IV) with anhydrous trimethylamine. The resulting isosteric amide analogs of glycol-lecithins have been isolated in high yields. The synthesis is illustrated by the preparation of the compounds containing palmitoyl, stearoyl and lauroyl fatty acid side-chains. The *N*-acylaminoethylphosphorylcholines have been shown to function as reversible phospholipase A_2 inhibitors. They are likely to become a new series of useful substrate analogs and an attractive replacement for the *n*-alkylphosphorylcholines commonly used as single-chain-carrying phospholipase inhibitors.

IONIZATION BEHAVIOR OF AQUEOUS SHORT-CHAIN CARBOXYLIC ACIDS: A CARBON-13 NMR STUDY. D.P. Cistola, D.M. Small, and J.A. Hamilton (Biophysics Inst., Depts. of Med. and Biochem., Boston Univ. Schl. of Med., Boston, MA 02118) *J. Lipid Res. 23*(5):795-799 (1982). The ^{13}C chemical shift of each carbon of aqueous acetic, propionic, and butyric acids has been measured as a function of pH or of added equivalents of base. A plot of chemical shifts for the carboxyl, α, and β carbons as a function of pH is sigmoidal and yields pK_a values that agree closely with values obtained by potentiometric titration. In contrast, a plot of chemical shift as a function of added equivalents of base is linear and has a sharp break at the equivalence point. Based on this result, we propose that the local (microscopic) ionization state of the carboxyl group can be determined directly by NMR without need for pH or pK determinations. In addition to titration curves, the effects of concentration, ionic strength, and temperature upon fatty acid chemical shifts are reported. For aqueous acids, changes in ionic strength and temperature have no effect on chemical shifts. However, changes in concentration do affect chemical shifts, probably as a result of changes in the relative degree of acid-acid and acid-water hydrogen bonding. Our results provide necessary background data for ^{13}C NMR studies of higher fatty acids in lipid-lipid and lipid-protein systems.

INFLUENCE OF PHORBOL ESTERS ON IONOPHORE-MEDIATED CALCIUM EXCHANGE-DIFFUSION IN LIPOSOMES. M. Deleers and W.J. Malaisse (Laboratory of Experimental Medicine, Brussels University, Brussels, B-1000, Belgium) *Chem. Phys. Lipids 31*:1-11 (1982). The interference of phorbol esters upon the process of A23187-mediated calcium exchange diffusion was examined in multilamellar liposomes formed of different types of lipids and incu-

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bated at variable temperatures. Phorbol esters facilitated the process of calcium ionophoresis in liposomes formed of dipalmitoylphosphatidylcholine (DPPC) or dimyristoylphosphatidylcholine (DMPC) and incubated below transition temperature. The magnitude of this facilitating action was negatively correlated with the tumor-promoting capacity of the phorbol esters. The phorbol esters also facilitated calcium ionophoresis in liposomes formed of a mixture of DPPC and cholesterol, provided that the temperature exceeded 34 C. The magnitude of the latter facilitating action was positively correlated with both the temperature and the tumor-promoting potency of the phorbol esters. Thus, the existence of a parallelism between the biological potency of phorbol esters and their biophysical effect in this artificial system tightly depended on such factors as the lipid composition of the liposomal matrix and the ambient temperature.

LONG-CHAIN PHENOLS: XX. SYNTHESIS OF OXIDATIVE DEGRADATION PRODUCTS FROM THE METHYLATED COMPONENT PHENOLS OF *ANACARDIUM OCCIDENTALE* AND OTHER PHENOLIC LIPIDS: CONFIRMATION OF THE STRUCTURE OF THE PARENT PHENOLS AND OF A RELATED MATERIAL. A.A. Durrani, G.C. Sun, and J.H.P. Tyman (Schl. of Chem. Brunel Univ., Uxbridge, Middlesex UB8 3PH, England) *Lipids 17*(8): 561-569 (1982). A general procedure for the determination of the first double bond position in the side-chain of a phenolic lipid has been investigated and, in the first place, the phenols of natural cashew nut-shell liquid (*Anacardium occidentale*) have been examined. An improved oxidative degradation procedure has been applied consisting of methylation by the phase transfer procedure, hydroxylation with performic acid and oxidation of the mixture of vicinal diols with periodic acid (Malaprade reaction) followed by reduction of the aldehyde fragments with sodium borohydride.

IDENTIFICATION OF A "URINARY-TYPE" METABOLISM OF PROSTAGLANDIN $F_{2\alpha}$ IN THE RAT CIRCULATION. N.S. Edwards and C.R. Pace-Asciak. (Res. Inst., The Hosp. for Sick Children, 555 Univ. Ave., Toronto, M5G 1X8, Canada) *Biochim. Biophys. Acta 711*(2):369-371 (1982). During constant slow intravenous infusion of tritiated prostaglandin $F_{2\alpha}$ into male adult rats, a major portion of radioactivity in blood appeared as a new metabolite, identified as *tetranor*-15-keot-13,14-dihydroprostaglandin $F_{2\alpha}$ (18.8 ± 4.2%, n = 7). The previously recognized blood metabolite, 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$, was also observed (15.1 ± 5.1%, n = 7). 15-keto-13,14-Dihydroprostaglandin $F_{2\alpha}$ disappeared quickly from the circulation while *tetranor*-15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ was still detected (8.6 ± 2.8%, n = 3) 2 hr after infusion was stopped. These results indicate that *tetranor*-15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$, because of its slow disappearance from the circulation, may provide a better indicator than 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ of prostaglandin $F_{2\alpha}$ synthesis in vivo.

PHOSPHOLIPASE ACTIVITIES OF RAT BRAIN CYTOSOL. OCCURRENCE OF PHOSPHOLIPASE C ACTIVITY WITH PHOSPHATIDYLCHOLINE. A.D. Edgar and L. Freysz (Centre de Neurochimie du CNRS, 5 rue Blaise Pascal, 67084 Strasbourg Cedex (France)) *Biochim. et Biophys. Acta 711*(2):224-228 (1982). 21-day-old rat brain contains a soluble phospholipase C with the ability to hydrolyse phosphatidylcholine. This enzyme has an alkaline pH optima. The results of the DEAE-cellulose fractionation, the pH profile and the Ca^{2+}-dependency suggest that the enzyme may be the same as that responsible for phosphatidylinositol hydrolysis. The activity of the phospholipase C is associated closely with a diacylglycerol lipase. The two enzyme activities could be separated by DEAE-cellulose fractionation, resulting in greater than 100% apparent recovery of the phospholipase C activity. This phospholipase C activity is not the result of the back reaction of choline phosphotransferase.

MORPHOLOGY OF GEL STATE PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE LIPOSOMES: A NEGATIVE STAIN ELECTRON MICROSCOPIC STUDY. B. Egerdie and M. Singer (Department of Medicine, Queen's University, Kingston, Ontario K7L 3N6, Canada) *Chem. Phys. Lipids 31*(1):75-85 (1982). The capture volumes (internal aqueous spaces) of liposomes prepared from a series of saturated phosphatidylcholines (PC) and saturated phosphatidylethanolamines (PE) had previously been found to be a function of lipid structure. PE vesicles have larger internal aqueous spaces than PC vesicles and for lipids with the same head group, capture volume increases with lengthening of the fatty acyl chains. Capture volume is determined by vesicle size, number of lamellae, and interlamellar distance. In this study, liposomes were formed from a saturated PC or PE and their morphology studied in the gel state using the technique of negative staining transmission electron microscopy. The measured interlamellar distances were quite similar among these various lipids while the number of lamellae was found to decrease as the fatty acyl chain length increased. In general PEs form fewer lamellae than PCs and in particular mono- and di-methylated dipalmitoyl-PE form only unilamellar vesicles. The number of lamellae then appears to bear a relationship to the size of the capture volume in that liposomes with larger capture volumes have fewer lamellae.

ENRICHMENT OF THE INTRACELLULAR DOLICHOL POOL IN ISOLATED LIVER CELLS. T. Ekström, T. Chojnacki, and G. Dallner (Dept. of Biochem., Arrhenius Lab., Univ. of Stockholm and Dept. of Pathology at Huddinge Hospital, Karolinska Inst., Stockholm, Sweden, and Inst. of Biochem. and Biophys., Polish Academy of Sci., Warszaw, Poland) *J. Lipid Res. 23*(7):972-983 (1982). Isolated hepatocytes were incubated with egg lecithin liposomes containing dolichol (C55), dolichol (C95), and dolichol phosphate (C55) in order to enrich intracellular membranes with these polyprenols. After incubation, the lipids were recovered from various membrane fractions and from the supernatant. The highest concentration was found in the microsomes. A part of the dolichol in microsomes, as well as in other fractions, was phosphorylated. This phosphorylation is mediated by the CTP-specific kinase that is present only on the outer surface of the microsomes and uses α-saturated polyprenols as substrates. The isolated microsomes enriched with dolichol in vivo exhibited increased lipid and protein glycosylation upon incubation with nucleotide sugars and it was demonstrated that the increased lipoid glycosylation was due to transfer of the sugar to the exogenous incorporated dolichol.

INFLUENCE OF HYDROTHERMIC ACTION ON SUNFLOWER PROTEIN COMPOSITION. V.T. Zolotchevski et al., *Pishtch. Tekhnol. 1*:110-112 (1982). Sunflower seed proteins are very heat stable. Major change and mutual transfers appear over 100 C. Protein composition changes in optimal running show that the extraction cake has highly nutritive properties. The percentage of soluble proteins is more than 68-70%. The one of hydrosoluble nonproteic nitrogen is more than 1%.

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