Abstracts

Biochemistry and nutrition

EFFECT OF HYDROGENATION ON THE CHEMICAL COMPOSITION OF CANOLA OIL. J.D. Bansal and J.M. deMan (Dept. of Food Sci., Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1) *J. Food Sci. 47* (4):1338-1344 (1982). Canola oil was hydrogenated under selective and nonselective conditions with commercial nickel catalysts. The composition of the hydrogenated oils was characterized by iodine value, fatty acid composition, *trans*-isomer content and *cis,cis*-methylene interruped (CCMI) polyunsaturated fatty acids. Selectivity ratios and hydrogenation rates were calculated. Selective conditions resulted in high levels of *trans*-isomers. Loss of CCMI fatty acids occurred in the early stages of the hydrogenation process. Hydrogenation rates were higher under selective than under nonselective conditions.

EFFECT OF ANTIOXIDANTS ON MALONALDEHYDE PRODUCTION AND FATTY ACID COMPOSITION IN PIECES OF BOVINE MUSCLE AND ADIPOSE TISSUE STORED FRESH AND FROZEN. H.A. Caldironi and N.G. Bazan (Inst. de Invest. Biochem., Univ. Natl. del Sur, Consejo Natl. de Invest., Cientificas y Tecnicas, Gorriti 43-8000 Bahia Blanca, Argentina) *J. Food Sci. 47* (4):1329-1332 (1982). TBA-reactive material was produced in pieces of bovine semitendinosus muscle and adipose tissue during storage at 2 ± 2 C and -10 ± 2 C. The process was faster in muscle than in adipose tissue and the total content, higher at 2 C than at -10 C. The effect of sprying butylated hydroxytoluene and citric acid-EDTA-ascorbic acid mixture on the production of malonaldehyde was studied. Declines in both saturated and unsaturated fatty acid proportions of the polar lipids without increases in the content of free fatty acids suggest that enzymes involved in lipid catabolism remain active at low temperatures. Whereas lipid breakdown was unaffected, malonaldehyde production was inhibited by sprying antioxidants in early stages of the slaughtering process.

OSMOTIC SWELLING OF PHOSPHOLIPID VESICLES CAUSES THEM TO FUSE WITH A PLANAR PHOSPHOLIPID BILAYER MEMBRANE. F.S. Cohen, M.H. Akabas, and A. Finkelstein (Dept. of Physiol., Rush Med. Coll., Chicago, IL 61612) *Science 217* (4558):458-460 (1982). Fusion of phospholipid vesicles with planar bilayer membranes occurs if the vesicles that contact the planar membrane swell osmotically after the replacement in their medium of an impermeant solute by a permeant one. This finding directly demonstrates that osmotic swelling is a driving force for vesicle-planar membrane fusion. The method used to induce vesicle swelling and fusion may have relevance for biological systems.

ANTIOXIDANT PROPERTIES OF SYNTHETIC 5-HYDROXY-1, 3-BENZODIOXOLE DERIVATIVES. E.R. Cole, G. Crank, H. Minh (School of Chem., Univ. of New South Wales, Kensington, N.S.W., Australia, 2033) *J. Agric. Food Chem. 30*(4):719-724 (1982). The sesamol analogues 2,2-disubstituted-5-hydroxy-1,3-benzodioxoles all show greater antioxidant effects than the parent sesamol. Cycloalkyl-substituted compounds are the most efficient, but alkyl derivatives are also good antioxidants with optimum activity at C_7. Protection is shown to a variety of lipid substrates, with best effects observed for lard. The most effecient analogues are comparable in activity to butylated hydroxyanisole and propyl gallate.

SDS-GLYCEROL POLYACRYLAMIDE GEL ELECTROPHORESIS OF PLASMA APOLIPOPROTEINS. P.W. Connelly and A. Kuksis (Dept. of Biochem. and Banting and Best Dept. of Med. Res., Univ. of Toronto, Toronto, M5G 1L6 (Canada)) *Biochim. Biophys. Acta 711*(2):245-251 (1982). The apolipoproteins of plasma very low and high density lipoproteins were resolved in one dimension using SDS-glycerol polyacrylamide gel electrophoresis. Two major advantages of this method are the use of lipoprotein samples without prior delipidation over a wide range of sample volumes (up to 400 μl) and a high capacity, which is especially important in the resolution of large amounts (25 μg) of low molecular weight apolipoproteins.

CHOLERA TOXIN MEDIATED AGGLUTINATION OF GANGLIOSIDE G_{ml} CONTAINING PHOSPHOLIPID VESICLES AND G_{ml}-COATED POLYSTYRENE SPHERES. J.D. Dwyer and V.A. Bloomfield (Dept. of Biochem., Univ. of Minnesota, St. Paul, MN 55108) *Biochemistry 21*(13):3231-3234 (1982). Quasi-elastic laser light scattering is used to examine the cholera toxin mediated agglutination of ganglioside G_{ml} containing phospholipid vesicles and G_{ml}-coated polystyrene spheres. We find that the ability of cholera toxin to agglutinate G_{ml}-containing phospholipid vesicles depends markedly on the lipid composition of the vesicle, with only those composed of short-chain lipids (C14, C16) being appreciably agglutinated. This is interpreted as due to poor mixing of these lipids with the longer chain gangliosides, resulting in lateral separation of the gangliosides in the membrane bilayer. A simple quantitative model, a modification of that developed by von Schulthess et al. is developed to describe these agglutination processes. Application of this model to the agglutination of G_{ml}-coated polystyrene spheres by cholera toxin allows an estimate of a minimum value of 4.5×10^4 M^{-1} for the association constant of cholera toxin for its initial G_{ml} receptor.

ETHER-LINKED LIPIDS OF BALB/C3T3, SV3T3 AND CONCANAVALIN A-SELECTED SV3T3 REVERTANT CELLS. A. Fallani, M. Bracco, D. Tombaccini, G. Mugnai and S. Ruggieri (Inst. of Gen. Pathology, Univ. of Florence, Viale G.B. Morgagni, 50 Florence (Italy)) *Biochim. Biophys. Acta 711*(2):208-212 (1982). Ether-linked lipids were analyzed in Balb/c3T3, SV3T3 and Concanavalin A-selected SV3T3 revertant cells. The three cell lines were found to contain significant quantities of alk-1-enyl- and alkyl-linked phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and small amounts of alkyldiacylglycerols. Compared to 3T3 cells, SV3T3 cells contain a higher amount of alk-1-enyl-linked PC, while in SV3T3 revertant cells the concentrations of the various ether lipids are similar to those of 3T3 cells. The major difference in the composition of ether groups of SV3T3 cells, compared to 3T3 cells, is an increase of 18:0 accompanied by a decrease of 18:1 in the alk-1-enyl-linked PE and PC. Alk-1-enyl-linked PC of SV3T3 revertant cells also shows an increase of 18:0, while the decrease of 18:1 was not statistically significant.

FORMATION OF HYDROPEROXY BIS-EPIDIOXIDES IN SENSITIZED PHOTO-OXIDIZED METHYL LINOLENATE. E.N. Frankel, W.E. Neff, and D. Weisleder (Northern Regional Res. Center, Agric. Res. Service, U.S. Dept. of Agric., Peoria, Il 61604) Unique 10- and 15-hydroperoxides formed by sensitized photo-oxidation of methyl linolenate undergo serial 1,3-cyclization to produce hydroperoxy bis-epidoxides that may be separated into four diastereoisomeric pairs of enantiomers.

ISOLATION AND DETERMINATION OF CHOLESTEROL GLUCURONIDE IN HUMAN LIVER. A. Hara and T. Taketomi (Dept. of Biochem., Inst. of Adaptation Med., Shinshu Univ. Schl. of Med., Matsumoto 390, Japan) *Lipids 17*(8):515-518 (1982). Separation of the acidic lipid fraction from human liver led to the identification of cholesterol-β-glucuronide for the first time from this organ. Cholesterol glucuronide was purified by DEAE-Sephadex column chromatography and preparative silica gel thin-layer chromatography. The content in normal human liver was about 33nmol/g wet tissue. It must be emphasized that cholesterol glucuronide cannot be distinguished readily from ganglioside GM4 by thin-layer chromatography.

GLYCEROL KINASE ACTIVITY AND GLYCEROL METABOLISM OF RAT GRANULAR PNEUMOCYTES IN PRIMARY CULTURE. A.B. Fisher and A. Chander (Dept. of Physiol., Schl. of Med., Univ. of Penn., Philadelphia, PA 19104) *Biochim. Biophys. Acta 711*(1):128-133 (1982). Glycerol kinase activity and glycerol utilization by rat granular pneumocytes were determined in order to investigate the rate-limiting step for glycerol incorporation into lung lipids. Granular pneumocytes were isolated in primary culture following trypsinization of rat lungs. Glycerol kinase activity was 8.2 nmol/hr per 10^6 cells. Incorporation of [1,3-^{14}C] glycerol into total cell lipids was 0.29 nmol/hr per 10^6 cells. In the presence of saturating glycerol concentrations, production of ^3H_2O from [2-^{3}H] glycerol was 13 times greater than incorporation of [^{14}C] glycerol into lipids. Glycerol phosphate dehydrogenase activity in isolated cells was approximately 10 times glycerol kinase activity. In the presence of 5.6 mM glucose, glycerol incorporation into lipids was decreased 79% and detritiation of glycerol was decreased 34%. This effect of glucose was due to a 25% increase in cell glycerol 3-phosphate content, resulting in dilution of the precursor pool and possible inhibition of glycerol phosphorylation. These results indicate

that the relatively limited incorporation of glycerol into surfactant phospholipids by lung epithelial cells reflects the relatively high rate of glycerol 3-phosphate oxidation.

ISOLATION AND DETERMINATION OF CHOLESTEROL GLUCURONIDE IN HUMAN LIVER. A. Hara and T. Taketomi (Dept. of Biochemistry, Institute of Adaptation Medicine, Shinshu University School of Medicine, Matsumoto 390, Japan) *Lipids 17*(8): 515-518 (1982). Separation of the acidic lipid fraction from human liver led to the identification of cholesterol-β-glucuronide for the first time from this organ. Cholesterol glucuronide was purified by DEAE-Sephadex column chromatography and preparative silica gel thin-layer chromatography. The content in normal human liver was about 33 nmol/g wet tissue. It must be emphasized that cholesterol glucuronide cannot be distinguished readily from ganglioside GM4 by thin-layer chromatography.

A VIBRATIONAL STUDY OF THE CD_2 STRETCHING BANDS OF SELECTIVELY DEUTERATED PALMITIC AND STEARIC ACIDS. S.C. Hsi, A.P. Tulloch, H.H. Mantsch, and D.G. Cameron (National Research Council of Canada, Division of Chemistry, Ottawa, Ontario K1A 0R6, Canada) *Chem. Phys. Lipids 31*(1): 97-103 (1982). The C-D stretching regions of the infrared and Raman spectra of 14 different palmitic and stearic acids containing isolated CD_2 groups are reported. Anomalous behaviour is observed when substitution occurs near the terminal methyl group, which behaviour cannot be explained in terms of crystal field effects.

PHASE BEHAVIOR OF MIXED PHOSPHATIDYLGLYCEROL/PHOSPHATIDYLCHOLINE MULTILAMELLAR AND UNILAMELLAR VESICLES. B.R. Lentz, D.R. Alford, M. Hoechli, and F.A. Dombrose (Departments of Biochemistry, Anatomy, and Pathology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514) *Biochemistry 21*(18):4212-4219 (1982). The phase behavior of dipentadecanoylphosphatidylglycerol ($DC_{15}PG$)/dimyristoylphosphatidylcholine (DMPC) mixtures has been studied in both small, unilamellar vesicles and large, multilamellar vesicles. We have used both the steady-state fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and high-sensitivity differential scanning calorimetry to detect temperature-dependent changes in membrane structure. Electron microscopy has demonstrated different fracture face morphologies for large, multilamellar vesicles depending on sample composition and temperature. These data have been interpreted in terms of proposed phase diagrams for this lipid mixture. The shapes of the proposed phase diagrams have led us to conclude that DMPC and $DC_{15}PG$ mix freely in the plane of a lipid bilayer only at less than 50 mol % $DC_{15}PG$. At higher $DC_{15}PG$ content, the data have been interpreted as reflecting substantial compositional inhomogeneities in the plane of the bilayer, if not phase immiscibility, even in the fluid phase. In addition, small vesicles containing greater than 50 mol % $DC_{15}PG$ were unstable in the ordered phase and spontaneously converted to larger vesicles. Finally, the anisotropy of DPH fluorescence was found to be invariant with $DC_{15}PG$ content at temperatures just above the liquidus phase line in small, unilamellar vesicles. This demonstrated that inclusion of negatively charged phosphatidylglycerol does not noticeably affect the order within the acyl chain region of the bilayer, relative to phosphatidylcholine.

CHARACTERIZATION OF A GLYCOSPHINGOLIPID β-*N*-ACETYLGALACTOSAMINYL-TRANSFERASE ACTIVITY IN CULTURED HAMSTER (NIL) CELLS. M.W. Lockney and C.C. Sweeley (Department of Biochemistry, Michigan State University, East Lansing, MI 48824) *Biochim. Biophys. Acta 712*(2):234-241 (1982). The activity of a glycosphingolipid *N*-acetylgalactosaminyltransferase (GalNAc transferase) in cultured hamster fibroblasts (NIL-8) was characterized with respect to substrate binding, acceptor specificity, pH optimum and detergent requirements. Of the glycosphingolipid acceptors tested, transferase activity was observed only with globotriaosylceramide. The apparent K_m values for uridinediphosphate-*N*-acetylgalactosamine and globotriasylceramide were 0.14 and 0.42 mM, respectively. The enzyme required Mn^{2+} for maximum activity (4 mM), and Mg^{2+}. Of the detergents tested, sodium taurodeoxycholate gave the greatest activation of the enzyme at 1 mg/ml. A broad pH optimum (4.5-8.0) was obtained, with maximum activity at pH 6.0 in 2-(*N*-morpholino)ethanesulfonic acid. Globotetraosylceramide and II^3-α-*N*-acetylneuraminyl-lactosylceramide inhibited transferase activity with globotriaosylceramide as substrate, but lactosylceramide had no effect on the activity with this acceptor. The major product of the assay was shown to be a tetraglycosylceramide with a terminal β-*N*-acetylgalactosamine moiety by co-migration with authentic globotetraosylceramide on TLC plates and by cleavage of the labeled *N*-acetylgalactosamine from the product by jack bean β-hexosaminidase.

A SURFACE FILM STUDY OF THE LATERAL PACKING O- PHOSPHATIDYLCHOLINE AND CHOLESTEROL. B. Lundberg (Department of Biochemistry and Pharmacy, Åbe Akademi, SF-20500 Åbo 50, Finland) *Chem. Phys. Lipids 31*(1):23-32 (1982). The interaction between phosphatidylcholine (PC) and cholesterol (CHL) has been studied with equilibrium spreading pressure, and surface balance measurements. The results from the studies of mixed films composed of egg phosphatidylcholine (EPC), dipalmitoylphosphatidylcholine (DPPC) and CHL strongly indicate that all three components are miscible in the films and that CHL interacts randomly with the PCs. Starting from a hexagonal chain packing where two PC fatty acid chains are replaced by one CHL, critical proportions arise at CHL mole fractions of 0.20, 0.33, 0.50, and 1.00.

MIXED MONOLAYERS OF DIPALMITOYLGLYCEROPHOSPHOCHOLINE, DISTEAROYLGLYCEROPHOSPHOCHOLINE AND 1-PALMITOYLGLYCEROL. H. Matuo, K. Motomura, and R. Matuura (Department of Chemistry, Faculty of Science, Kyushu University 33, Fukuoka 812, Japan) *Chem. Phys. Lipids 31*(1):53-60 (1982). Mixed monolayer systems of dipalmitoylglycerophosphocholine (DPPC)-distearoylglycerophosphocholine (DSPC), palmitoylglycerol-DPPC, and palmitoylglycerol-DSPC have been investigated by a previously described thermodynamic treatment. The surface pressures of these systems were measured at various compositions and temperatures. The two-dimensional phase diagrams and the apparent molar entropy and energy changes were evaluated. It was found that the phase diagrams of the DPPC-DSPC, palmitoylglycerol-DSPC and palmitoylglycerol-DPPC systems are all of different types. According to our phase diagram classification, DPPC-DSPC, palmitoylglycerol-DSPC, and palmitoylglycerol-DPPC systems exhibited a modified cigar type, a eutectic type, and a negative azeotropic type, respectively.

PHOSPHORUS-31 AND CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES OF DIVALENT CATION BINDING TO PHOSPHATIDYLSERINE MEMBRANES: USE OF COBALT AS A PARAMAGNETIC PROBE. A.C. McLaughlin (Biology Dept., Brookhaven National Laboratory, Upton, NY 11970) *Biochemistry 21* (20):4879-4885 (1982). The paramagnetic divalent cation cobalt has large and well-understood effects on NMR signals from ligands bound in the first coordination sphere, i.e., inner-sphere ligands, and we have used these effects to identify divalent cation binding sites at the surface of phosphatidylserine membranes. ^{31}P NMR results show that 13% of the bound cobalt ions are involved in inner-sphere complexes with the phosphodiester group, while ^{13}C NMR results show that 54% of the bound cobalt ions are involved in unidentate inner sphere complexes with the carboxyl group. No evidence is found for cobalt binding to the carbonyl groups, but proton release studies suggest that 32% of the bound cobalt ions are involved in chelate complexes that contain both the carboxyl and amine groups. All (i.e., 13% + 54% + 32% = 99%) of the bound cobalt ions can thus be accounted for in terms of inner sphere complexes with the phosphodiester group or the carboxyl group. We suggest that the unidentate inner-sphere complex between cobalt and the carboxyl group of phosphatidylserine and the inner-sphere complex between cobalt and the phosphodiester group of phosphatidylserine provide reasonable models for complexes between alkaline earth cations and phosphatidylserine membranes.

EFFECT OF ALTERED STEROL COMPOSITION ON THE OSMOTIC BEHAVIOR OF SPHAEROPLASTS AND MITOCHONDRIA OF *SACCHAROMYCES CEREVISIAE*. C.A. Mclean-Bowen and L.W. Parks (Department of Microbiology, Oregon State University, Corvallis, OR 97331) *Lipids 17*(9):662-665, 1982. The effect of sterols on the osmotic stability of mitochondrial and plasma membranes of yeast wild-types and mutants that are defective in ergosterol biosynthesis has been studied. Incorporation of the nonfungal sterol, cholesterol, into yeast membranes reduces membrane elasticity which is observed as an increased susceptibility to osmotic lysis. However, the wild-type and nystatin-resistant strains which were examined indicate that qualitative alterations in endogenously generated sterols do not affect resistance to swelling. Although these strains exhibit differences in membrane fluidity, which is influenced by the sterol accumulated by the organisms, the membrane stretching capacity shows no distinct dependence on sterol structure or bilayer fluidity.

BRANCHED FATTY ACIDS FROM *MYCOBACTERIUM AURUM*. E. Rafidinarivo, A. Savagnac, C. Lacave and J.-C. Prome (Centre de Recherche de Biochimie et de Genetique Cellulaires du CNRS, 118, route de Narbonne, 31062 Toulouse Cedex France) *Biochim. Biophys. Acta 711*(2):266-271 (1982). New methyl-branched fatty acids were isolated from the lipids of *Mycobacterium aurum*, belonging to both saturated and non-saturated series. The most abundant

component of the former series was identified as a C_{22}-mycosanoic acid (2-L, 4-L-dimethyleicosanoic acid). The unsaturated fraction contained a mixture of 2-L, 4-L-dimethyl-11-eicosenoic acid and 2-L, 4-L-dimethyl-14-eicosenoic acid. The biosynthetic precursors of these, according to the hypothesis of elongation by propionate units, were found in the nonbranched hexadecenoic fraction. The lipidic fraction containing mycosanoic acid was a partially acylated oligosaccharide devoid of sulfate or phosphate groups.

SYNTHESIS AND PROPERTIES OF A NONEXCHANGEABLE RADIOIODINATED PHOSPHOLIPID. A.J. Schroit (The Cancer Metastasis and Treatment Laboratory, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701) *Biochemistry 21*(21): 5323-5328 (1982). An efficient method for the synthesis and purification of *N*-[3-(3-[^{125}I] iodo-4-hydroxybenzyl)propionyl]-phosphatidylethanolamine (^{125}I-phenylpropionyl-PE), a nonexchangeable iodinated lipid of high specific radioactivity, is described. The technique involves acylation of phosphatidylethanolamine with *N*-succinimidyl 3-(3-[^{125}I] iodo-4-hydroxyphenyl)propionate (monoiodinated Bolton-Hunter reagent) and purification by thin-layer chromatography. Quantitative incorporation of the iodinated lipid into vesicles prepared by a variety of techniques was observed to occur. With these vesicles, no transfer of the labeled lipid to other vesicles or cells occurred, irrespective of vesicle composition or the presence of other transferable lipids in the same bilayer membranes. This, ^{125}I-phenylpropionyl-PE appears to be an accurate liposome tracer and can be used in a variety of in vitro and in vivo assays that require high levels of sensitivity unobtainable with most other lipid-labeling techniques.

LIPID PEROXIDATION BY MICROSOMAL FRACTIONS ISOLATED FROM LIGHT AND DARK MUSCLES OF HERRING (*CLUPEA HARENGUS*). B.M. Slabyj and H.O. Hultin (Department of Food Science, Life Science & Agriculture Experiment Station, University of Maine, Orono, ME 04469) *J. Food Sci. 47*(5):1395-1398 (1982). Enzymic lipid peroxidation by light and dark muscle microsomes of herring (*Clupea harengus*) required ATP or ADP, NADH and Fe. NADPH could not effectively replace NADH. Inhibition was observed at high concentrations of ADP and NADH but not Fe. The optimal pH for the reaction of both types of microsomes was between 6 and 7. The average peroxidation rate was 362 and 1143 nmoles MDA per mg protein per hr at 6 C for the light and dark muscle microsomes, respectively. The energy of activation for the light and dark muscle microsomes was similar. The light muscle microsomes lost activity faster than the dark muscle microsomes when exposed to 35 C. Ferrous ion stimulated enzymic lipid peroxidation of light and dark muscle microsomes over that observed with ferric ion.

MEASUREMENT OF THE BINDING OF HUMAN COLIPASE TO HUMAN LIPASE AND LIPASE SUBSTRATES. B. Sternby and C. Erlandson-Albertsson (Department of Physiological Chemistry, University of Lund, P.O. Box 750, S-220 07 Lund, Sweden) *Biochim. Biophys. Acta 711*:193-195 (1982). Equilibrium partition in an aqueous two-phase system was the method used for quantitative determinations of the binding between human colipase and human lipase and three triacylglycerol substrates: Intralipid, tributyrin and triolein. The measurements were performed in a dextran/polyenthyleneglycol system at pH 7.0 in the presence of 4 mM sodium taurodeoxycholate and 150 mM NaCl. The binding of colipase to lipase has a dissociation constant $K_d = 4.8 \cdot 10^{-8}$ M. The dissociation constants for the binding of colipase to Intralipid, tributyrin and triolein were found to be $2 \cdot 10^{-7}$ M, $4.8 \cdot 10^{-8}$ M and $6.2 \cdot 10^{-8}$ M, respectively.

FILM CHARACTERISTICS OF LINSEED EPOXY ESTERS PREPARED FROM NOVOLAC-BASED POLYEPOXIDE RESINS. A.K. Vasishtha and D. Agrawal (Department of Oil and Paint Technology, Harcourt Butler Technological Institute, Kanpur – 208002, India.) *J. Oil Col. Chem. Assoc. 65*:276, 1982. Epoxy esters were prepared by reacting linseed oil fatty acids with polyepoxide resins based on phenol-formaldehyde novolac resins. Samples were prepared with polyepoxides of different epoxide equivalents and were compared with those based on bisphenol A; the films of novolac-based epoxy esters were found to have better resistance to alkali and acid. The water resistance of all the epoxy ester films was found to be good.

EFFECT OF DIETARY ERUCIC ACID ON THE METABOLIZABLE ENERGY VALUE OF THE DIET FOR POULTRY. Ayodhya Prasad and P.V. Rao (Central Avian Research Institute, Izatnagar, U.P. 243122) *Indian J. Nutr. Dietet. 18*:455, 1981. The role of dietary mustard erucic acid in depressing feed consumption and growth rate in chicks was examined in chicks fed rations containing pure erucic acid replacing groundnut oil in a control diet at 0.605, 1.210 and 1.815 percent. The metabolizable energy content of erucic acid diets was comparable statistically to that of the control, suggesting that the deleterious nutritional responses with the fatty acids were not mediated through a depression in the metabolizability of dietary energy.

RATIONALE FOR CHANGES IN THE DIETARY MANAGEMENT OF DIABETES. Barbara R.B. El-Beheri Burgess, M.P.H., R.D. (Westport, Connecticut) *J. Am. Dietet. Assn. 81*:258, 1982. Roughly three-quarters of Americans with diabetes die from atherosclerosis. Although the pathogenesis of cardiovascular disease in diabetes is not completely understood, diabetes is frequently associated with hyperlipidemia, often considered a major determinant of atherosclerosis, and with hyperglycemia, which may function as an independent risk factor. The new higher carbohydrate diets for management of diabetes facilitate reduction in the proportion of fat kilocalories. When total kilocalories are controlled, improvement in glucose tolerance also occurs in individuals with diabetes who have available endogenous or exogenous insulin.

A COMPARISON OF SEED PHOSPHATIDES AND SYNTHETIC COMPOUNDS AS ANTIOXIDANTS FOR COW AND BUFFALO GHEE (BUTTER FAT). Narinder Kaur, Pritam S. Sukhija and Iqbal S. Bhatia (Department of Biochemistry, Panjab Agricultural University, Ludhiana, India) *J. Sci. Food. Agric. 33*:576, 1982. The antioxidant capacity of seed phosphatides and synthetic antioxidants when compared in cow ghee was found to be in the order: phosphatidyl ethanolamine > propyl gallate > palmitoyl ascorbate > butylated hydroxy anisole > phosphatidyl choline. Phosphatidyl ethanolamine was found to be the most effective antioxidant. Cow ghee had less peroxide development than buffalo ghee. The ghee prepared at 100 C was more stable against peroxide development compared with that prepared at 50 C. These observations were supported by the analysis of ghee samples for peroxide values and for fatty acids. The phosphatides imparted more antilipolytic activity to ghee than to synthetic antioxidants.

THE ANALYSIS OF POLYUNSATURATED FATTY ACIDS IN MEAT BY CAPILLARY GAS-LIQUID CHROMATOGRAPHY. Andrew J. Sinclair, William J. Slattery and Kerin O'Dea (Department of Agriculture, Veterinary Research Institute, Park Drive, Parkville, Victoria 3052, Australia and Baker Medical Research Institute, Melbourne, Australia) *J. Sci. Food Agric. 33*:771, 1982. Polyunsaturated fatty acids (PUFA) of lean meat from domesticated and wild ruminants (cattle, sheep, goat, sambar deer and buffalo) and non-ruminants (pig, horse and kangaroo) have been examined by capillary gas-liquid chromatography. Ten different PUFA were found in all specimens with linoleic acid accounting for at least 50% of the total, and arachidonic and linolenic acids being the next most abundant. The total PUFA content for the ruminants ranged from 9% in beef to 31% in sambar deer and for the non-ruminants from 25% in pig to 43% in horse. In all species the meat phospholipids (PL) were rich in PUFA (range 24-46% of PL fatty acids), whereas the triglycerides were relatively more saturated (PUFA content range 2-17%). Overall, horse and kangaroo meat had the combination of lowest fat and highest PUFA content, while beef and sheep had the highest fat and lowest PUFA content. These results indicate that significant reductions in total fat intake and increases in the proportion of polyunsaturated fat in the diet could be achieved without necessarily requiring a diet low in meat.

THE EFFECT OF SUNFLOWER OIL ON THE FATTY ACID COMPOSITION OF THE MILK OF COWS FED EITHER A FAT-DEPRESSING DIET OR GRASS SILAGE. J.L. Clapperton (The Hannah Research Institute, Ayr KA6 5HL) *J. Sci. Food. Agric. 33*: 741, 1982. Two experiments have been carried out in which different forms of sunflower oil were added to the diet of Ayrshire heifers. In experiment 1, the animals were given a fat-depressing diet of dried, ground grass cubes and flakes maize. When either free sunflower oil or milled, unextracted sunflower seeds were added to this diet, the yield of milk fat and of all fatty acids up to 16:2 was decreased and the yield of all 18-carbon fatty acids was slightly increased. When the sunflower oil was protected by encapsulation in formaldehyde-treated casein, the yield of milk fat was increased, that of the fatty acids up to 16:1 was decreased and that of all the 18-carbon fatty acids, and in particular, of the polyunsaturated fatty acids was increased. In experiment 2, the animals were given a basal diet of grass silage and concentrates to which increasing amounts of protected sunflower oil were added. This intended to increase the yield of milk fat and to reduce that of the fatty acids up to 16:1. The yield of all the 18-carbon fatty acids increased and, in particular, that of the polyunsaturated fatty acids increased progressively as more protected oil was added. It is concluded that it should be possible to produce a milk with any desired proportion of polyunsaturated

fatty acids by adding a predetermined amount of protected oil to the diet of the cow.

EFFECT OF COFFEE AND TEA ON CHOLESTEROL AND TRIACYLGLYCEROL OF BLOOD SERUM OF HUMANS AND RATS. V. Srimathi, P.B. Seshagiri, R. Raju, and S. Ramakrishnan (Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, 6) *Indian J. Nutr. Dietet. 18*: 360, 1981. In vegetarian male non-smokers, drinking of four to five cups of coffee with milk and sugar did not elevate blood cholesterol and triglycerides. In albino rats, there was actually a decrease of serum cholesterol and triglycerides in rats force-fed with equivalent amounts of black coffee and tea for 20 days. The decrease was statistically significant in respect of tea only. Present work on humans and rats shows coffee drinking may not be harmful and tea is preferable to coffee.

ZINC CONTENT OF FATS AND OILS. Jayashree Pohit, Namita Pal and B. Pal (Department of Biochemistry, School of Tropical Medicine, Calcutta - 700 073) *Indian J. Nutr. Dietet. 18*: 341, 1981. Edible vegetable oils, butter fats and hydrogenated vegetable fats were analyzed for their zinc content. Vegetable oils appeared to be richer in zinc than butter fats. Values of zinc were the lowest in hydrogenated vegetable fats. It appears that the visible fat intake does not substantially contribute towards the dietary zinc.

ENERGY SOURCES OF PLANT ORIGIN IN MADAGASCAR: ETHYL ALCOHOL AND SEED OILS. A.M. Gaydou, L. Menet, G. Ravelojaona, P. Geneste, *Oléagineux* 37(3):135-141, 1982. Madagascar has many energy crops: sugar cane, cassava, castor, aleurites, curcas, etc. The conversion of the carbohydrates of sugar cane grown in a rural environment and of molasses extracted industrially would provide ethyl alcohol which could be used mixed with petrol (10-15%). The plantations of castor, aleurites and curcas could be revalorized with a view to producing industrial oils. The fatty acid composition of three tung oils (*Aleurites montana, A. moluccana* and *A. fordii*) and two physic nut oils (*Jatropha curcas* and *J. mahafalensis*) harvested in Madagascar shows the advantage of these oils, which could either be used as a source of fuel for diesel engines after refining or transesterification with ethyl alcohol, or else transformed by catalytic cracking to give hydrocarbon mixtures usable as substitute fuels or raw materials for the chemical industry.

ESTIMATES OF THE EFFECT OF TWO LIMITING FACTORS (DROUGHT AND NEMATODES) ON NITROGEN FIXATION (C_2H_2) BY GROUNDNUT AND SOYA. J. Meyer, G. Germani, B. Dreyfus, H. Saint-Macary, M. Boureau, F. Ganry, Y. Dommergues, *Oléagineux*, 1982, 37, No. 3, p. 127-134. An attempt has been made to quantify the effect of two limiting factors in groundnut and soya: drought and nematode attacks. On the basis of three postulates resulting from previous observations in the field, a simple model has been proposed making it possible to predict the effect of these two factors. On the agronomic plane, the results obtained confirm the unfavourable effect of drought on nitrogen fixation and show that nematode infestation slows down fixation, not only by reducing nodulation but also by diminishing the specific nitrogen-fixing activity of the nodules.

EFFECT OF CHLORINE DEFICIENCY ON THE GERMINATION GROWTH AND PHOTOSYNTHESIS OF COCONUT. J.M. Eschbach, D. Massimino, A.M.R. Mendoza, *Oléagineux* 37(3):115-125, 1982. The beneficial effect of good chlorine nutrition on the growth and yield of coconut has already been demonstrated. The most spectacular response was obtained in a fertilizer trial with or without chlorine on the Davao station of the Philippine Coconut Authority (PCA). It was considered that it would be interesting to try and find out the physiological mechanism of the chlorine effect. Because of the bulk of adult coconuts, trials were conducted on plants raised in a glasshouse in Montpellier and different characters of which were afterwards measured at Cadarache. The plants came from nuts harvested on trees either deficient in Cl (treatment KCl 0 in the PCA trial) or well provided with this element (treatment KCl 2). The effect of Cl was not very visible, either because when they start life the plants live on the reserves in the nut (which did not differ very much between treatments KCl 0 and KCl 2), or else because of poor vegetative development due to the difficult conditions of glasshouse growing. Nevertheless, this study showed that the coconut, like the oil palm, has all the characteristics of a plant in C3, with a low net photosynthesis rate, a high compensation point for CO_2, marked sensitivity of photorespiration to O_2 concentration, and photorespiration 3 or 4 times greater than night respiration. Trials studying the effect of Cl deficiency on leaf ionic balance are to be undertaken in the Ivory Coast in the dry season, a period when this deficiency manifests itself most strongly.

SYSTEM APPROACH TO THE CLIMATOLOGY OF OIL PALM. I. – IDENTIFICATION OF RAINFALL AND DRY SPELL ASPECTS. H.T. Ong. *Oléagineux* 37(3):93-105, 1982. EIA, and exploratory identification analysis, was introduced as a systematic and objective method of determining the relationships between the oil palm bunch yields and changes in rainfall and dry spells. Monthly oil palm bunch yields were studied for relationship to monthly rainfall or dry spell as far back as 42 months (or LAG 42) before harvest through a series of simple correlations (EIA-SC) and then re-evaluated through a series of partial correlations (EIA-PC). EIA in this paper identified the oil palm yields to be associated with rainfall at LAG *5-7, 16-18, 22-23, 28-30* and dry spell at LAG *5-6, 9-12, 16-18, 22-24* and 29-30; the italics being negative associations and the rest positive associations. Rainfall at LAG 16-18, 22-23 and dry spell at LAG 29-30 had associations with bunch yields which were partially independent but the other variables interacted completely with at least some of the others. It was found that while a month duration of the climatic factor would lead to association with bunch yields, very often a longer duration of the climatic factor at that stage would lead to greater association with bunch yields.

STATUS OF RESEARCH CONCERNING THE ANALYSIS OF SURFACTANT MIXTURE. SECOND PART. C. Demanze, *Rev. Franç. Corps Gras. 29*(5):211-217. French, RFCG 82-17. The analytical scheme for a full and accurate study of a surfactant complex mixture, for instance in a few sanitary or cleaning products is described from analytical processes developed during the past decade. Every phase of this analytical scheme is applicable to any active material whatever the composition may be; it gathers the usualest and/or the latest methods by means of a hundred bibliographical references and twenty figures.

PREPARATION OF A NEW COBALT CATALYST AND STUDY OF ITS PROPERTIES IN LIPID CHEMISTRY. E. Ucciani, G. Cecchi and A. Bonfand, *Rev. Franç. Corps Gras. 29*(5):219-224, 1982, French, RFCG 82-18. The thermal decomposition under hydrogen of $Co_2(CO)_8$ dissolved in a vegetable oil, leads to a suspension of metallic cobalt which exhibits catalytic properties for hydrogenation purposes. The formation of hydroformylation products is harmful to the catalyst stability and activity. These products can be avoided by using fully hydrogenated palm oil as solvent. Then the catalyst becomes stable. Such coated cobalt shows unexpected and interesting properties in the following reactions: partial hydrogenation of soybean and rapeseed oils, hydrogenolysis of saturated glycerides (production of alcohols and hydrocarbons), reduction of nitriles (production of primary and secondary amines). The main advantage of this cobalt catalyst lies in its ferromagnetic property which allows an easy separation from the reaction products with the help of a magnet.

ON THE REFINABILITY OF OILS. VIII. VERIFICATION ON INDUSTRIAL SAMPLES OF RELATIONS PROPOSED TO FORESEE IMMEDIATE TASTING SCORES OF SOYBEAN AND NEW RAPESEED OILS. M. Naudet, S. Biasini, J.M. Klein and M. Letouzey, *Rev. Franç. Corps Gras. 29*(5):225-228, 1982, RFCG 82-19. The validity of numerical relations proposed to foresee the immediate tasting scores of soybean and new rapeseed oils has been verified. For that, 25 samples of industrially refined soybean and new rapeseed oils have been analyzed in the conditions of manufacture continuous control. The foreseable scores deducted from analytical results agree satisfactorily with experimental scores assigned by tasting jury. Thus, the validity of postulated relations is verified, for organoleptically acceptable oils.

BIOCHEMICAL COMPONENTS OF OIL PALM YIELD. J.M. Eschbach, *Oléagineux* 37(4):159-168, 1982. From 1978 to 1981, the I.R.H.O. carried out a study comparing oil palm yield and the activities of certain enzymes like nitratereductase, glutamate dehydrogenase, glutamine synthetase, acid phosphatase, peroxidase and glucose 6 phosphate dehydrogenase in its Montpellier and Ivory Coast laboratories. Free fatty acid levels were also determined. It was first necessary to develop or adapt titration methods and define the sampling conditions. Relatively simple, quick methods to measure enzymatic activities were proposed at the end of this first part. In manuring experiments, the relations between mineral nutrition and these biochemical parameters were then studied. It was thus demonstrated that the levels of certain amino acids depend on potassic or nitrogenous nutrition, and that the activities of nitrate reductase and acid phosphatase are correlated to leaf mineral elements levels and the trees' bunch production. These results thus open new perspectives for better understanding and control of oil palm mineral nutrition, so long as the critical levels for enzymatic activities can first be de-

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fined. On the other hand, the third part of the study devoted to comparing variations in the biochemical parameters in function of yield for different types of planting material planted in the genetic trials, did not lead to precocious selection criteria being proposed, as the observations made did not point to constant relations between enzymatic activities and the yield from the various crosses under study.

LATOIA (PARASA) LEPIDA (Cramer) *LEPIDOPTERA LIMACODIDAE*, A COCONUT PEST IN INDONESIA. R. Desmier de Chenon, *Oléagineux* 37(4):177-183, 1982. The *Limacodidae Latoia (Parasa) lepida* (Cramer) is common in all South-West Asia, particularly in Indonesia, but although it is very polyphagous it attacks coconut for preference. The caterpillar with its pale blue median band and the adult with yellowish-green wings, dark brown at the base and light brown at the distal end, are easily recognized. The larval cycle lasts a little more than two months; incubation of the eggs takes 6 days, the larval stages 40 days, and pupation 22 days. The cycle may vary a lot depending on the climatic conditions. The time of reactivation always coincides with the start of the rains. The species is characterized by gregarity and high fertility, but the parasite complex, made up of many insect-eaters of which the most efficient are the braconidae *Apanteles parasae* Rohw, and the tachinid fly *Chaetexorista javana* Br. and B., both on larvae, usually keeps down the populations. In their absence and also because the parasites develop mainly on the later larval stages, damage is often spectacular and the young coconuts can be completely defoliated, only the midrib remaining on the rachis. On mature trees such defoliation causes very heavy yield losses for more than three years. Periodical checks are necessary, and the critical threshold is 10 caterpillars per frond in young palms and 20-25 in mature ones. When hand collection of the caterpillars and cocoons cannot be done on trees which are too tall, or if the attack is too severe, the most effective products are carbaryl and *Bacillus thuringiensis* for small foci and pyrethrinoids such as decamethrine or permethrine for larger areas. A preventive measure consisting in the elimination of preferential host plants such as *Metroxylon* round the edges of the plantations can avoid foci being kept in being.

USE OF SOAPS TO PRODUCE NUTRITIONAL YEASTS. F. Martinet, A. Ba, R. Ratomahenia, J. Graille and P. Galzy, *Oléagineux* 37(4):193-198, 1982. In the scope of valorization of by-products and wastes from vegetable oils refineries, it is proposed to grow food yeasts on soapstocks and on palm oil stearin converted into ammonium or sodium soaps. Growth parameters of different yeast strains have been determined. Results show that the studied strains are suitable for industrial production of single cell proteins from these substrates.

TRIGLYCERIDE STRUCTURE OF THE FATTY TISSUE OF RATS SUBJECTED TO A SHEA BUTTER DIET. K.A. Sawadago, J.A. Sawadago, J.A. Becard, *Oléagineux* 37(5):247-253, 1982. In this study were analyzed the triglycerides of adipose tissue from rats which were given a balanced diet containing 15% of shea butter for 4 months. Oleic acid, one major constituent of shea butter (45.6%) was found in high amount (63.0%), palmitic acid in lower amount (20.3%) and stearic acid, the other major constituent of the diet fat (44.3%), in very low proportion (5.6%). Oleic acid was found preferentially esterified in internal position of glycerol, palmitic acid in external position. The triglycerides were fractionated according to unsaturation into 7 classes of which the constituent fatty acids and triglycerides were analyzed by gas-liquid chromatography. From these data a precise composition in triglyceride types was mathematically determined (in a triglyceride type, the constituent three fatty acids are known, but not their positionning). The triglycerides of these 7 classes were submitted to rat pancreatic lipase for determination of the fatty acid esterified in the 2-position. From the fatty acid composition of the monoglycerides formed during lipolysis, plus, in some cases, that of one class of diglycerides, or two, the amount of 60 isomers were precisely determined (in an isomer, the constituent three fatty acids are known and also the fatty acid esterified in the 2-position). Sixteen isomers were in amount superior to 1%, together representing 88.1% of the total. The major triglyceride was triolein (28.0%), followed by 1,3-palmitooleo-2-olein (22.2%), that is together one half of the adipose tissue triglycerides. The percentages of these 16 major isomers, experimentally determined, did not deviate markedly from those calculated according to a random distribution.

MINERALISATION OF THE BUNCH IN THE HYBRID COCONUT PB-121 FROM THE FLOWER TO MATURITY. M. Ouvrier, *Oléagineux* 37(5):229-236, 1982. The author describes the results obtained in the Ivory Coast with the hybrid PB-121 (Malayan Yellow Dwarf X West African Tall). The major mineral elements (N, P, K, Ca, Mg, Na, Cl and S) were determined for the different bunch components (stalk, spikelets, husk, shell and albumen) from the flower to maturity to make a better approach to the plant's needs over a period of time. The total amount of each element increases considerably between bunches of rank 14 to 18, a time when there is strong mineralisation due to the rapid growth of the nut. The most important elements are potassium, chlorine and nitrogen. The rise in certain elements in the albumen while it is developing is partially compensated by their diminution in the husk. The results show that manuring cannot be estimated on the uncut crop because any fertilizer given will have no effect on bunches above rank 18, which account for 65 p. 100 of that crop. As for leaf analysis, it is a good approach to the problem.

FACTORS AFFECTING YIELD AND GROWTH OF OIL PALM *TENERA* IN WEST NEW BRITAIN. C.J. Breure, *Oléagineux* 37(5): 213-227, 1982. In a D X P progeny trial, testing nine *pisifera*, progenies differed significantly in yield, growth parameters, and leaf nutrient levels. Yield during the fourth to the sixth year of production was negatively correlated with vegetative growth ($r = -0.51$) and height ($r = -0.58$) while these parameters were less correlated with early yield. Progeny oil yields were positively correlated with leaf Mg-levels ($r = +0.70$). In a density X fertilizer experiment, comparing 110, 148, 186 palms per hectare, each with four fertilizer levels (split-plot), density and fertilizer affected yield and most growth parameters significantly. Increasing light competition reduced yield response to fertilizers, but except for leaf area, did not affect growth response. Effects of N, K, P, Mg, Mn and S were studied in a quarter replicate of a $4^2 \times 2^4$ design. Potassium chloride increased yield. Potash increased kernel-to-fruit at the expense of mesocarp. High negative correlations were found between leaf Cl-levels and oil-to-wet mesocarp ratio. Intensity of pollination differed between the experiments. Improving pollination increased yield and reduced growth. VDM has a high plasticity and seems, partly, replaceable by fruit dry matter through adequate pollination. The combination of less light-competitive progenies with high Mg-levels and adequate pollination without ablation could increase yield.

SEED COLONIZATION AND AFLATOXIN PRODUCTION IN GROUNDNUT GENOTYPES INOCULATED WITH DIFFERENT STRAINS OF *ASPERGILLUS FLAVUS*. V.K. Mehan, D. McDonald and R.W. Gibbons, *Oléagineux* 37(4):185-191, 1982. Nine groundnut genotypes were tested for resistance to seed colonization by five different strains of *A. flavus*. These genotypes, and the cultivar J 11 which has been shown to be resistant to seed colonization by *A. flavus*, were also checked for production of aflatoxin following infection of scarified surface-sterilized seeds by three aflatoxigenic strains of *A. flavus*. The genotypes PI 337394 F and PI 337409 showed significantly less seed colonization and internal invasion than the other gentotypes. The *A. flavus* strains differed significantly from one another in their ability to colonize seeds and produce internal infection. Strain NRRL 3000 was the least effective. Of the three strains used in the aflatoxin production tests, AF 8-3-2A produced the highest levels of aflatoxin B_1 on all genotypes while AFS-3 produced the least: NRRL 3000 being intermediate in this respect. Aflatoxin G_1 was produced on all genotypes by NRRL 3000, and on J 11 by AF 8-3-2A. There was no obvious correlation between seed resistance to *A. flavus* colonization and aflatoxin production when seeds were infected. Significantly higher amounts of aflatoxin B_1 were produced in the two genotypes resistant to *A. flavus* colonization than in the highly susceptible genotype FESR-11-P11-B2-B1.

THE RESPONSE OF THE GROUNDNUT (*ARACHIS HYPOGAEA* L.), VARIETY MH 383, TO CROP DENSITY AND FERTILIZER ON THE IRRIGATED, HEAVY CLAYS OF CENTRAL SUDAN. A.E.S. Ibrahim, A.M. Osman and M.O. Khidir, *Oléagineux* 37(5): 237-245, 1982. The effects of crop density and fertilizer on the vegetative growth, flowering, yield and yield components of the groundnut variety MH383 grown under irrigation in the heavy clay soils of central Sudan were investigated over two year-period. The number of leaflets per plant and their dry weights as well as those of the plant and stems increased significantly with the increase in spacing along the ridge. This increase was more prnounced at the later stages of growth. Wider spacing also increased the rate of flower production per plant which showed a bell-shaped distribution with time. At very high populations yield per unit area increased while that per plant decreased. Yield components related to plant size declined at hight populations but spacing has no effects on harvest index, shelling-out turn, 100-seed weight and oil and protein contents. Suggestions explaining the lack of response to fertilizer application are offered discussed. Most of the studied yield parameters were strongly and positively correlated with yield, however, yield associations with protein and oil contents were negative.

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CONSISTENCY OF FRACTIONATED MILK FAT AS MEASURED BY TWO PENETRATION METHODS. M. Hayakawa, J.M. deMan (Dept. of Food Sci., Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1) *J. Dairy Sci. 65*(7):1095-1101 (1982). Liquid and solid fractions of milk fat were prepared from the melt at each degree from 32 to 25 C. Consistency of solid and liquid fractions was measured at several temperatures with constant speed as well as cone penetrometers. Hardness of both liquid and solid fractions obtained with the constant speed penetrometer directly reflected trends in their triglyceride composition and solid fat content. Cone penetration methods are used widely to measure consistency of fat products. However, interpretation of the results causes difficulty. Comparison of the data by the two penetration methods indicated that hardness by the constant speed penetrometer was proportional to the mass of the cone assembly and inversely proportional to the penetration reading.

VARIATIONS IN COMPOSITION OF MOWRAH (*MADHUCA LATIFOLIA*) SEED. S.S. Gupta, M.M. Chakrabarty, and D.K. Bhattacharyya (Dept. of Applied Chem., Univ. Coll. of Sci. and Tech., 92, Acharyya Prafulla Chandra Road, Calcutta-700 009) *J. Oil Tech. Assoc. India* 13(4):139 (1981). Mowrah kernels collected from different locations contained 37.3 to 51.3% fat, 8.2 to 11.9% protein, 28.4 to 41.0% carbohydrate, 2.3 to 4.2% saponin, 2.4 to 4.1% ash and 3.0 to 7.2% crude fibre.

CAROTENE AND CHLOROPHYLL BLEACHING BY SOYBEANS WITH AND WITHOUT SEED LIPOXYGENASE-1. D.F. Hildebrand and T. Hymowitz (Dept. of Agronomy, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801) *J. Agric. Food Chem. 30*(4):705-708 (1982). Carotene and chlorophyll bleaching activities of whole mature seed extracts of two soybean genotypes that lack lipoxygenase-1 (L-1) activity were compared with two soybean genotypes with normal L-1 activity. Assays were conducted with three substrates: (1) methyl linoleate, pH 7.0; (2) linoleic acid, pH 7.0; (3) linoleic acid, pH 9.0. No carotene or chlorophyll bleaching occurred with substrate 2 or 3 although carotene and chlorophyll were bleached with substrate 2. The presence of L-1 in seed extracts stimulated carotene and chlorophyll bleaching some at pH 7.0 and much at pH 9.0. The presence of L-1 stimulated chlorophyll cooxidation more than carotene cooxidation. L-1 purified from soybean seeds by ammonium sulfate fractionation and ion-exchange chromatography bleached both carotene and chlorophyll. However, the cooxidation of carotene and chlorophyll relative to the peroxidation of linoleid acid (cooxidation potential) is lower for purified L-1 than for whole mature seed extracts. The relevance of this information to food processing is discussed.

POTENTIAL BILE ACID METABOLITES 6.[1] STEREOISOMERIC 3,7-DIHYDROXY-5β-CHOLANIC ACIDS. T. Iida and F.C. Chang (Dept. of Biochem., Coll. of Med., Univ. of South Alabama, Mobile, AL 36688) *J. Org. Chem. 47*(15):2966-2972 (1982). New synthetic routes to the four possible 3,7-dihydroxy acids are described. The principal reactions involved were inversions with DMF and Me_2SO-crown ether and reduction of 12-oxo tosylhydrazones. Inversion of 3α-tosylates by the Me_2SO-crown ether method succeeded but that of the corresponding mesylates did not. A table of ^1H NMR chemical shift reference data of monosubstituted methyl cholanates cholanates pertinent to bile acid characterization has been expanded.

POTENTIAL BILE ACID METABOLITES. 7. 3,7,12-TRIHYDROXY-5β-CHOLANIC ACIDS AND RELATED COMPOUNDS. T. Iida and F. C. Chang (Dept. of Biochem., Coll. of Med., Univ. of South Alabama, Mobile, AL 36688) *J. Org. Chem. 47*(15):2972-2978 (1982). With this work, the complete set of the eight possible stereoisomeric (5β)-3,7,12-trihydroxy acids are now know and characterized. The key intermediates methyl 3α,7β-dihydroxy-12-oxo- and 3β,7β-dihydroxy-12-oxocholanate have been synthesized, and their reductions by *tert*-butylamine-borane complex and by $NaBH_4$ are described.

CHARACTERISTICS AND COMPOSITION OF SEED AND FLOWER FATS OF *ADHATODA VASICA*. N. Kapoor, M.P. Jain, and K.L. Bedi (Reg. Res. Lab., Jammu) *J. Oil Tech. Assoc. India 13*(4):137-138 (1981). *Adhatada vasica* Nees (Vasaka, Acanthaceae), an abundant wild herb, is used against a number of ailments. The fatty acid composition (wt 0/0), as determined by gas chromatography, of the seed and flower fat, respectively, was lauric, -, 1.21; myristic, -, 2.37; palmitic, 4.25, 27.13; heptadecanoic -, 2.50; oleic 49.28, 16.58; linoleic 21.53, 13.13; linolenic 1.34, 5.53; arachidic 10.59, 6.99; eicosenoic, -, 3.33; eicosadienoic, -, 16.60; erucic 4.50, 4.63, lignoceric 8.56, -, The flower fat also contained traces of tridecanoic and pentadecanoic acids. The only alkaloids found in the defatted seeds and flowers are vasicine, vascinone and vasicinol.

DECORTICATION OF *XANTHIUM STRUMARIUM* SEED AND RECOVERY OF OIL. G.R. Krishna, D.A. Ramayya, G. Azeemoddin, and S.D.T. Rao (Oil Tech. Res. Inst., Anantapur, A. Pradesh) *J. Oil Tech. Assoc. India 13*(4):140-141 (1981). *Xanthium strumarium* (gokhru) seed (actually fruit) consisted of 68% hull and 32% kernel. The seed contained 11% fatty oil. On dehulling, a meats fraction (50% yield) rich in kernels was obtained. Expelling the meats gave an oil yield of 5% and a cake having 13.8% oil which could be extracted by n-hexane. Sulphuric acid-treatment of the seed was shown to be 100% effective in dehulling operation. The oil was easily refined and bleached in the laboratory to light colour.

INTERACTION OF OLEOYL COENZYME A WITH PHOSPHOLIPID BILAYERS. A.H. Lichtenstein, D.M. Small, and P. Brecher (Dept. of Med. and Biochem., Boston Univ. Schl. of Med., Boston, MA 02118) *Biochemistry 21*(9)2233-2241 (1982). The effect of oleoyl coenzyme A (CoA) on three phospholipid bilayer systems, human red blood cell ghosts, egg yolk lecithin dispersions, and unilamellar lecithin vesicles, was studied. Addition of oleoyl-CoA to sealed, right-side-out, human red blood cell ghosts resulted in a loss of latent NADH-cytochrome *c* oxidoreductase activity. The turbidity of lecithin desperisions decreased as a result of the addition of oleoyl-CoA in concentration-dependent manner. This decrease in turbidity was influenced by the mode of addition of oleoyl-CoA was dried together with the lecithin prior to resuspension in an aqueous solution. The presence of cholesterol (lecithin:cholesterol malar ratio 2:1) diminished the effect of oleoyl-CoA on the turbidity of the lecithin dispersions. Addition of oleoyl-CoA to unilamellar vesicles, which contained 5,6-carboxyfluorescein, increased the leakage of the dye from the vesicles in a concentration-dependent manner. This effect was diminished when cholesterol was incorporated into the vesicles (lecithin:cholesterol molar ratio 2:1).The interaction of oleoyl-CoA with lecithin was further studied by preparing mixtures where the lipids were dried together prior to sonication and had lecithin:oleoyl-CoA molar ratios of either 100:1 or 10:1. The resulting complexes were characterized by gel filtration and sucrose density gradient ultracentrifugation. Oleoyl-CoA was associated with particles having a size distinguishable from that of unilamellar vesicles. At the higher oleoyl concentration, the complex formed was readily detected by density gradient ultracentrifugation.

VARIATIONS IN CHARACTERISTICS AND FATTY ACID COMPOSITION OF SAL (*SHOREA ROBUSTA*) FATS. N.T. Mallika, S. Rajalakshmi, S. Vibhakar, M.N. Krishnamurthy, K.V. Nagaraja, and O.P. Kapoor (Analyt. Quality Control Lab., Central Food Tech. Res. Inst., Mysore-570 013) *J. Oil Tech. Assoc. India 13*(4):133-134 (1981). Fourteen seed samples were collected from Madhya Pradesh and Orissa, and extracted with hexane. The ranges in fat characteristics were: sap. value, 175.2-191.5; iodine value, 31.5-45.0; butyro-refractometer rading at 40 C, 45.5-54.0 C; free fatty acids, 1.0-23.0; stearic, 32.6-35.0; oleic, 31.0-52.5; linoleic, 0.3-5.0 and arachidic, 0.0-8.6.

MICELLAR COMPLEXES OF HUMAN APOLIPOPROTEIN A-I WITH PHOSPHATIDYLCHOLINES AND CHOLESTEROL PREPARED FROM CHOLATE-LIPID DISPERSIONS. C.E. Matz and A. Jonas (Dept. Biochem., Schl. of Basic Med. Sci. and Schl. of Chem. Sci., Univ. of Illinois, Urbana, IL 61801) *J. Biol. Chem. 257*(8):4535-4540 (1982), Micellar complexes of human apoliporotein A-I and phosphatidylcholine were prepared by adding apolipoprotein A-I (apo A-I) to sodium cholate-lipid mixtures. Cholate was removed by dialysis and the apo A-I·lipid complexes were isolated by gel filtration chromatography of by density gradient ultracentrifugation. The lipid mixtures consisted of dipalmitoylphosphatidylcholine or egg yolk ratios of cholesterol. The formation of complexes was examined at different phosphatidylcholine (PC)-to-apo A-I ratios, PC-to-cholate ratios, and cholate concentrations. Yields of complexes were maximal when incubation and dialysis were performed near the transition. temperature of the PC. Molecular weights were determined by sedimentation equilibrium; fluoresence polarization was used using the hydrophobic probe 1,6-diphenyl-1,3,5-hexatriene.

ANDROGENS ALTER THE TUNING OF ELECTRORECEPTORS. J.H. Meyer, H.H. Zakon (Scripps Institution of Oceanography, A002, La Jolla, CA 92093) *Science 217*:635-637 (1982). Weakly electric fish possess electroreceptors that are tuned to their individual electric organ discharge frequencies. One genus, *Sternopygus*, displays both ontogenetic and seasonal shifts in these frequencies, possibly because of endocrine influences. Systemic treatment with androgens lowers the discharge frequencies in these animals. Concomitant with these changes in electric organ discharge frequencies are decreases in electroreceptor best frequencies; hence the close match between discharge frequency and receptor tuning is main-

tained. These findings indicate that the tuning of electroreceptors is dynamic and that it parallels natural shifts in electric organ discharge frequency.

BIOSYNTHESIS AND LOCALIZATION OF GANGLIOSIDES IN CULTURED CELLS. H. Miller-Podraza, R.M. Bradley, and P.H. Fishman (Membrane Biochem. Sec., Development and Metabolic Neurology Branch, Natl. Inst. of Neurological and communicative Disorders and Stroke, Natl. Inst. of Health, Bethesda, MD 20205) *Biochemistry 21*(14):3206-3265 (1982). Mouse neuroblastoma N18 cells contain a homologous series of gangliosides (G_{M3}, G_{M2}, G_{M1}, and G_{Dla}) which constitute a biosynthetic pathway. When added to the culture medium, tritium-labeled palmitate, galactose, and *N*-acetylmannosamine were incorporated into these gangliosides. Incorporation of [^{3}H]galactose into all four gangliosides was detected by 5 min and continued at essentially linear rates for several hours. When the cells were treated with *Vibrio cholerae* neuraminidase, the amounts of G_{M3} and G_{Dla} were reduced from 72% to 85%; there was a severalfold increase in G_{M1} and no change in G_{M2}. In spite of these large alterations in cellular ganglioside composition, there was no change in the rate of [^{3}H]galactose incorporation into the ganglioside. A large proportion of G_{M3} and G_{Dla} also was accessible to neuraminidase in neuroblastoma NB-41A, Friend erythroleukemic, and rat glioma C6 cells. N18, NB-41A, and Friend cells bound large amounts of ^{125}I-labeled cholera toxin with high affinity. At saturation, the ratio of G_{M1} content to toxin bound for the three cell lines was between 5.5 and 7. When treated with neuraminidase, the cells bound more toxin in correspondence to the increase in G_{M1} content. As each toxin molecule has five binding sites, these results suggest that most of the G_{M1} in these cells in on the surface. Our results indicate that the sequential glycosylation of one ganglioside to form the next higher homolgue involves a very small pool of intermediates and that the bulk of the gangliosides are on the cell surface.

TRANSLOCATION OF NEWLY SYNTHESIZED GANGLIOSIDES TO THE CELL SURFACE. H. Miller-Podraza and P.H. Fishman (Membrane Biochem. Section, Developmental and Metabolic Neurology Branch, Natl. Inst. of Neurological and Communicative Disorders and Stroke, Natl. Institutes of Health, Bethesda, MD 20205) *Biochemistry 21*(14):3265-3270 (1982). A new method was developed to follow the translocation of gangliosides from their site of synthesis within the cell to the plasma membrane. Cultured mouse neuroblastoma N18 and rat glioma C6 cells were labeled for increasing times with D-[1-^{3}H] galactose and then subjected to milk oxidation with $NaIO_4$. Under the conditions chosen, oxidation was essentially restricted to cell-surface sialic acid residues, which were converted to derivatives with an aldehyde function. The labeled gangliosides were isolated from the cells and reacted with dinitrophenylhydrazine to from dinitrophenyl (DNP) derivatives of the oxidized gangliosides. The DNP-gangliosides then were separated from their unmodified counterparts by thin-layer chromatography. Thus, the rate of labeling of surface gangliosides was distinguished from the rate of labeling of total gangliosides. Our results indicated that the transfer of gangliosides from the site of synthesis to the cell surface required approximately 20 min and that newly synthesized gangliosides appeared to be transported to the plasma membrane at a constant rate. No essential differences were found in the rates of translocation of different ganglioside species by N18 cells or between gangliosides of N18 and C6 cells.

DIFFUSIONAL BAHAVIOR OF TRIPALMITIN IN FREEZE-DRIED MODEL SYSTEM AT DIFFERENT WATER ACTIVITIES. W. Naesens, G. Bresseleers, and P. Tobback (Lab. of Food Preservation, Catholic Univ. of Leuven, de Croylaan 42, 3030 Heverlee, Belgium) *J. Food Sci. 47*(4):1245-1249 (1982). The diffusional behavior of tripalmitin (TP) in low moisture model system composed of microcrystalline cellulose and gum arabic was found to be very dependent on the water activity (a_w), the temperature and the presence of paraffin oil (PO). The complex mechanism of mobilization of food components in dry systems, particularly that of TP in our system, has been discussed in detail. Such knowledge is especially important in relation to the reactions that occur in foodstuffs during dehydration and subsequent storage. As a typical example, the results on TP diffusion indicate that the restriction of enzymatic activity in dry systems may not solely be due to the diffusional limitations of the reactants.

SIZE ANALYSIS OF PHOSPHOLIPID VESICLE PREPARATIONS. Y. Nozaki, D.D. Lasic, C. Tanford, J.A. Reynolds (Dept. of Physiology, Duke Univ. Medical Center, Durhan, NC 27710) *Science 217* (4557):366-367 (1982). Gel exclusion chromatography based on the use of Sephacryl S-1000 provides a quick and convenient method for determining the average diameter of phospholipid vesicles and an approximate measure of size heterogeneity.

EFFECT OF EXTRACTABLE LIPID ON THE VISCOSITY CHARACTERISTICS OF YAM TUBER FLOURS. A.U. Osagie, A.O. Mologohme, and F.I. Opute (Depts. of Biochem. and Biol. Sci., Univ. of Benin, Benin City, Nigeria) *J. Food Sci. 47*(4):1378-1379 (1982). Extractable lipids comprised below 2% of the flours made from yam tubers. This small amount of lipid was best extracted by water-saturated-butanol compared to 80% methanol and chloroform-methanol (2:1) mixtures. The fatty acid composition in each lipid extract was not significantly affected by the method of extraction. Defatting yielded flours which gelatinized at higher temperatures and possessed increased overall viscosities compared with undefatted flours. It is suggested that the particular uses of the yam flours may be influenced by the lipids present.

AMINO ACID SEQUENCE OF AMYLOID-RELATED APOPROTEIN (APOSAA$_1$) FROM HUMAN HIGH-DENSITY LIPOPROTEIN. D.C. Parmelee, K. Titani, L.H. Ericsson, N. Eriksen, E.P. Benditt, and K.A. Walsh (Dept. of Biochem., Howard Hughes Med. Inst. Lab., and Dept. of Pathology, Univ. of Washingtion, Seattle, Washington 98195) *Biochemistry 21*(14):3298-3303 (1982). The amino acid sequence of apoprotein SAA from human high-density lipoprotein is derived by analysis of peptides isolated from enzymatic digests. This 104-residue sequence is 28 amino acids longer than the amyloid protein AA that accumulates in tissues during certain inflammatory conditions. Two species of protein, differing from each other at only two loci, were recognized and characterized.

SITES OF PHOTOLYTIC INTERMOLECULAR CROSS-LINKING BETWEEN FATTY ACYL CHAINS IN PHOSPHOLIPIDS CARRYING A PHOTOACTIVABLE CARBENE PRECURSOR. R.Radhakrishnan, C.E. Costello, H.G. Khorana (Depts. of Chem. and Biol., Massachusetts Inst. of Techn., Cambridge, MA 02139) *J. Am. Chem. Soc. 104*(14):3990-3997 (1982). A number of *sn*-glycero-3-phosphorylcholines containing the photosensitive ω-[*m*-(3*H*-diazirino) phenoxy] undecanoyl group in the *sn*-2 position and a dideuterated palmitic or stearic acid with both deuteriums on specific carbon atoms along the hydrocarbon chain in the *sn*-1 position were synthesized. Photolysis of either sonicated vesicles or multilamellar dispersions prepared from these synthetic phospholipids gave extensive intermolecularly cross-linked products. The distribution of the sites of cross-linking was determined by an analysis of cross-linked dimeric fatty esters by using low-resolution electron impact mass spectrometry. The predominance of the benzylic cleavage with a γ-hydrogen abstraction in the mass spectra of these diesters rendered such a quantitation relatively easy. Mass spectral analysis showed that there is broad distribution in the cross-linking positions along the deuterated *sn*-1 chain, with the amount of cross-linking increasing toward the hydrophobic core of the bilayer. These results are in agreement with the conformational mobility of the fatty acyl chains and the localization of the photosensitive diazirinophenoxy group in the middle of the bilayer.

QUANTITATIVE RING CLEAVAGE OF LONG CHAIN EPOXIDES BY CHLOROTRIMETHYLSILANE FOR CHLOROHYDRIN SYNTHESIS. A. Rauf, M.S. Ahmad Jr., and S.M. Osman (Dept. of Chem., Aligarh Muslim Univ., Aligarh-200 001, Uttar Pradesh) *J. Oil Tech. Assoc. India 13*(4):135-136 (1981). A rapid and clean method for preparing chlorohydrin from an epoxide by using chlorotrimethylsilane (ClMe3Si) as a ring opening reagent is described. Epoxy esters II and III furnished the isomeric mixture of chlorohydrin VI and VII respectively, while I gave only one isomer IV. It is pertinent to mention here that the chlorohydrin formation takes place within minutes in a quantitative yield.

LIPID ENVIRONMENT OF ACETYLCHOLINE RECEPTOR FROM *TORPEDO CALIFORNICA*. J.M. Gonzalez-Ros, M. Llanillo, A. Paraschos, and M. Martinez-Carrion (Dept. of Biochem., Med. Coll, of Virginia, Virginia Commonwealth Univ., Richmond, VA 23298) *Biochemistry 21*(14):3467-3474 (1982). The lipid matrix of both acetylcholine receptor (AcChR)-rich and AcChR-poor membranes from *Torpedo californica* electroplax has been chemically characterized. AcChR-rich membranes contained higher levels of free cholesterol and slightly higher proportions of polyunsaturated ethanolamine phosphoglycerides. Major fatty acid components in the total lipid extracts from either membrane fraction were 24% palmitic, 20% docosahexaenoic, and 20% oleic acids. The fatty acid composition from AcChR-rich and AcChR-poor membrane fractions was very similar. Native membranes and lipid vesicles were used for the determination of the temperature-dependent rotational relaxation time of two fluorophores: diphenylhexatriene (DPH) and its trimethylammonium derivative (TMA-DPH). In both domains, vesicles assembled with AcChR-rich membrane lipid exhibited a higher

degree of rigidity than vesicles composed of AcChR-poor membrane lipid. The difference can be partially explained by their different cholesterol levels. As judged by Arrhenius plots, lipid phase separations or transitions were absent within the temperature range used in every native lipid system studied. In all cases, the presence of protein apparently induced more restraint on the rotational motion of the flurorophores. Titration of the fluorescent pH probe 4-heptadecyl-7-hydroxycoumarin indicated that the apparent p*K* of the probe was influenced by the nature of the phospholipids and by phospholipids and bye the presence of protein. The p*K* value of the presence of protein. The p*K* value of coumarin was lower in vesicles formed with AcChR-rich membrane lipid than in the intact native AcChR-rich membranes.

DETERMINATION OF ALTERNATING CURRENT POLAROGRAPHIC BEHAVIOR OF SOME STEROIDS IN APROTIC ORGANIC SOLVENTS. J.C. Schaar and D.E. Smith (Dept. of Chem., Northwestern Univ., Evanston, IL 60201) *Anal. Chem. 54* (9):1589-1594 (1982). The work presented represents a continuation of our efforts to illustrate the superiority of aprotic organic solvent-electrolyte systems for the analysis of many organic drugs. Measurement of the peak in-phase component magnitude of the ac polarographic or faradalc admittance (FAM) response provides the assay observable. Investigations of five steroids show that three give essentially ideal responses, characterized by a facile one-electron heterogeneous charge transfer step and a stable radical anion product in aprotic solvent systems. Two show clear evidence of a second order following chemical reaction. Composite and single tablet assays are reported for two of the steroids.

ASYMMETRY OF LIPID DYNAMICS IN HUMAN ERYTHROCYTE MEMBRANES STUDIED WITH PERMEANT FLUOROPHORES. D. Schacter, U. Cogan and R.E. Abbott (Dept. of Physiology, Columbia Univ. Col. of Physicians and Surgeons, New York, NY 10032) *Biochemistry 21*(9):2146-2150 (1982). The fluorescence anisotropy and mean excited-state lifetime of 1,6-diphenyl-1, 3,5-hexatriene, 12-(9-anthroyloxy) stearate, 2-(9-anthroyloxy) stearate, and pyrenedecanoic acid in the membranes of intact human erythrocytes, lysate suspensions, and ghost membranes were compared. The excited-state lifetime of each lipid fluorophore, estimated by single photon counting, is significantly shorter in the intact erythrocytes as compared to the lysates, owing to nonradiative energy transfer from the lipid fluorophore donors in the membrane to heme acceptors at the endofacial surface of the intact cell. The fluorescence observed in intact cell suspensions is thus weighted in favor of outer leaflet fluorophores, and estimates of the fluorescence anisotropy by steady-state fluorescence polarization indicate that all four fluorescent probes experience greater motional freedom in the outer as compared to the inner membrane leaflet. The results are in accord with prior studies of impermeant pyrene derivatives, which also indicate that the outer leaflet lipids have greater motional freedom.

STYRENATED ALKYDS BASED ON TOBACCOSEED OIL. M.C. Shukla, M.S. Saxena and A.K. Visishtha (Dept. of Oil and Paint Technology, H.B. Technological Inst., Kanpur-2) *J. Oil Tech. Assoc. India 13*(4):130-132 (1981). Syrenated alkyd resins were prepared from tobaccoseed oil by pre-styrenation and post-styrenation processes and film properties of air dried and baked films of these alkyds were studied and compared. The post-styrenation process of making styrenated alkyds gave a product which had better film properties with respect to drying time, scratch hardness and resistance to water, acids and alkali than the alkyds obtained by the pre-styrenation process.

ADSORPTION OF BILE ACIDS BY COMPONENTS OF ALFALFA AND WHEAT BRAN IN VITRO. J.A. Story, A. White, and L.G. West (Dept. of Foods Nutr. Purdue Univ., West Lafayette, IN 47907) *J. Food Sci. 47*(4):1276-1279 (1982). Adsorption of 2.5 mM and 5.0 mM cholic acid and deoxycholic acid by plant materials high in dietary fiber (alfalfa and wheat bran) and fractions derived from sequential extraction of plant material to remove various components was measured. Results tend to support the hydrophobic nature of adsorption and indicate a lack of marcromolecular interactions between dietary fiber and bile acid micelles. Lignin appears to be an extremely important component in the interaction of dietary fiber with bile acids, but holocellulose also plays a significant role. Fractionation of dietary fiber sources may provide a method for study of the effects of specific components of dietary fiber which avoids some of the harsh isolaprocedures.

FUNCTIONAL PROPERTIES AND FOOD APPLICATIONS OF RAPESEED PROTEIN CONCENTRATE. L.U. Thompson, R.F.K. Liu, and J.D. Jones (Dept. of Nutr. and Food Sci., Univ. of Toronto, Toronto, Canada M5S 1A8) *J. Food Sci. 47*(4):1175-1180 (1982). Rapeseed protein concentrate (RC), prepared with 2% hexametaphosphate, was tested for its functionality and performance in some foods. The RC had good nitrogen solubility, fat absorption, emulsi-fication, and whipping capacities but poor water absorption and gelling properties. It increased the emulsion stability, and protein but lowered the fat content of wieners. It also increased the cooking yield, reduced the shrinkage and tenderized meat patties. Results were similar to soybean isolate except for the poorer color and flavor. The cooking yield of RC supplemented wieners was less than the all-meat control and soybean-supplemented wieners. A 9% RC dispersion mixed with an equal volume of eggwhite produced a meringue of comparable stability and texture to that of eggwhite alone.

INHIBITION OF STEROID GLYCOALKALOID ACCUMULATION BY ARACHIDONIC AND EICOSAPENTAENOIC ACIDS IN POTATO. E.C. Tjamos, J.A. Kuć (Dept. of Plant Pathol., Univ. of Kentucky, Lexington 40546) *Science 217*:542-543 (1982). Eicosapentaenoic and arachidonic acids extracted from the fungus *Phytophthora infestans* elicit the accumulation of fungitoxic sesquiterpenoid stress metabolites and inhibit the accumulation of steroid glycoalkaloids in potato tubers. This dual activity, which did not occur with other saturated and unsaturated fatty acids tested, corresponds to the activity of incompatible races of *Phytopthora infestans* and crude elicitor preparations from *Phytopthora infestans* that contain bound forms eicosapentaenoic and arachidonic acids. Arachidonic acid applied to potato slices, which had been aged for various time intervals, elicited the accumulation of sesquiterpenoid stress metabolites and concomitantly inhibited the accumulation of steroid glycoalkaloids.

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC ANALYSIS OF SEED OILS CONTAINING CONJUGATED UNSATURATED ACIDS. A.P. Tulloch (Natl. Res. Council of Canada, Prairie Regional Lab., Saskatoon, Saskatchewan, Canada S7N 0W9) *Lipids 17*(8):544-550 (1982). ^{13}C Nuclear magnetic resonance spectroscopy has been used in a nondestructive investigation of conjugated unsaturated acids in seed oil triacylglycerols. Spectra of seven seed oils, from *Punica granatum, Cucurbita palmata, Jacaranda mimosifolia, Centranthus ruber, Catalpa bignonioides, Chilopsis linearis and Calendula officinalis,* containing amount them six isomeric trienoic acids, *cis,trans, cis-* and *trans,trans,cis*-8,10,12-,*cis,trans,cis-, cis,trans,trans-, trans,trans,cis-* and *trans,trans,trans*-9,11,13-octadecatrienoic acids, and of the oil of *Impatiens balsamina* containing *cis,trans,trans,cis*-9,11,13,15-octadecatetraenoic acid, have been examined. Structures of component acids were derived from shifts of double bond carbons and of carbons close to the double bond systems. Compositions of the oils were obtained from signal intensities. Results were similar to those obtained by older methods. Only oil of *Centranthus ruber* contained more than one major conjugated acid; both *cis,trans,trans-* and *trans,trans,trans*-9,11,13-octadecatrienoic acids were found. The latter acid is now thought to occur naturally.

FORMATION OF ETHER LIPIDS AND WAX ESTERS IN MAMMALIAN CELLS. SPECIFICITY OF ENZYMES WITH REGARD TO CARBON CHAINS OF SUBSTRATES. N. Weber and I. Richter (Bundesanstalt für Fettforschung, Piusallee 68-76, D-4400 Münster F.R.G.) *Biochim. Biophys. Acta 711*(2):197-207 (1982). The pattern of incorporation of radioactivity from the substrates into alkyl and alk-l-enyl moieties of ether phospholipids and into alkyl moieties of wax esters reveals the following: 1) The enzymes catalyzing the biosynthesis of alkylacylglycerols, the common intermediates of cholinephospholipids and ethanolaminephospholipids, have no substrate specificity with regard to position of the double bond of either *cis-* or *trans*-octadecenols or of intermediate ether lipids derived therefrom. 2) CDPcholine: diradylglycerol cholinephosphotransferases exhibit a strong preference for alkylacylglycerols with *cis*-8, *cis*-9 and *cis*-10-octadecenyl moieties, but no preference for the double bond position in the *trans*-octadecenylacylglycerols. 3) CDP-ethanolamine: diradylglycerol ethanolaminephosphotransferases have have no substrate specificity with regard to position of the double bond in *cis-* or *trans*-octadecenyl moieties of alkylacylglycerols. 4) The enzyme ststems catalyzing the biosynthesis of alkylacylglycerophosphocholines and alkylacylglycerophosphoethanolamines exhibit substrate specificity with regard to chain-length of saturated alcohols and intermediate ether lipid derived therefrom. 5) Alkylacylglycerophosphoethanolamine desaturase and 6) Wax ester synthase are highly specific for alkylacylglycerolphosphoethanolamines and long-chain alcohols, respectively, with regard to chain-length of saturate alkyl moieties, but not with regard to position of double bonds of *cis-* or *trans*-octadecenyl moieties.

CARBON-13 NUCLEAR MAGNETIC RESONANCE INVESTIGA-

Abstracts

TIONS OF PHASE TRANSITIONS AND PHASE EQUILIBRIA IN PURE AND MIXED PHOSPHOLIPID BILAYERS. R.J. Wittebort, A. Blume, T.H. Huang, S.K. Das Gupta, R. G. Griffin (Francis Bitter Natl. Magnet Lab., Massachusetts Inst. of Tech., Cambridge, MN 02139) *Biochemistry 21*(14):3487-3502 (1982). Temperature dependence of the ^{13}C NMR spectra of dipalmitoylphosphatidylethanolamine (DPPE) and dimyristoyl-, dipalmitoyl-, and distearoylphosphatidylcholine, all ^{13}C-labeled at the *sn*-2 carbonyl, was studied. In the L_β or $L_{\beta'}$ phase, an axially symmetric powder pattern of about 100-ppm breadth is observed, and this transforms to an isotropic line at the main L_β ($L_{\beta'}$) → L_α phase transition. In the case of DPPE, this occurs precipitously and is shown to be due to a change in conformation at the *sn*-2 carbonyl. The ^{13}C=O *sn*-2 spectra of lecithins exhibit a gradual transformation, beginning at temperatures below the endothermic pretransition temperature. In the intermediate $P_{\beta'}$ phase, a temperature-dependent superposition of the $L_{\beta'}$-and L_α-like ^{13}C spectra is observed, suggesting that the $P_{\beta'}$ phase is structurally heterogeneous and exhibits properties of both $L_{\beta'}$ and L_α phases. phases. Simulation of the *sn*-2 ^{13}C=O powder patterns, provides excellent fits of the spectra. Addition of cholesterol to the four pure lipids results in a superposition of the L_β ($L_{\beta'}$) and L_α patterns, and simulation of the spectra provides a means to extract the fraction of L_α-like lipid as a function of temperature or cholesterol concentration. Similar results are observed for binary mixtures of dipalmitoylphosphatidylcholine and DPPE. The results suggest that the conformational change at the *sn*-2 C=O is general property of phospholipid phase transitions.

RAPID METHOD FOR QUANTITATIVE ANALYSIS OF INDIVIDUAL FREE FATTY ACIDS IN CHEDDAR CHEESE. A.H. Woo, R.C. Lindsay (Dept. of Food Sci., Univ. of Wisconsin-Madison, Madison, WI 53706) *J. Dairy Sci. 65*(7):1102-1109 (1982). A reliable method for routine quantification of carbon-4 to carbon 18:3 free fatty acids in Cheddar cheese was developed. The free fatty acids were isolated on a modified silicic acid-potassium hydroxide arrestant colum following removal of lactic acid with a partition precolum. Rapid separation and quantification of free fatty acids in formic acid-mobilized eluates was achieved by gas chromatographing on 5% diethylene glycol succinate (DEGS-PS) in packed, glass colums. Quantitative free fatty acid profiles of nonrancid and rancid Cheddar cheese samples were obtained.

EFFECTS OF SULFHYDRYL COMPOUNDS ON LIPID OXIDATIONS CATALYZED BY COPPER AND HEME. J. Yee and W Shipe (Department of Food Science, Cornell University, Ithaca, NY 14853) *J. Dairy Sci. 65*:1414-1420 (1982). Effects of cysteine and glutathione on copper- and heme-catalyzed oxidation of methyl linoleate were studied in a model emulsion system. In the copper-catalyzed system, cysteine exerted a strong prooxidative effect, and this prooxidative effect increased as the concentration of cysteine incereased. Furthermore, oxidized glutathione did not have a prooxidative effect, whereas reduced glutathione showed prooxidative activity. Blocking the sulfhydryl group by treatment of cysteine with iodoacetic acid completely eliminated the prooxidative activity of cysteine. In the heme-catalyzed system, cysteine showed an antioxidative effect that increased as concentration of cysteine increased. The antioxidative effect of cysteine was practically eliminated by treatment with iodoacetic acid. Reduced glutathione showed a greater antioxidative effect than oxidized glutathione. In the presence of sulfhydryl compounds, copper promoted superoxide anion generation whereas heme did not. The addition of cysteine (10 to 1000 M) to milk did not inhibit either copper or heme induced lipid oxidation.

EFFECT OF LIPID MIXING ON THE PERMEABILITY AND FUSION OF SATURATED LECITHIN MEMBRANES. A Nicolussi, S. Massari, and R. Colonna (C.N.R. Unit for the Study of Physiology of Mitochondria, Lab. of Biophys. and Molecular Biol., Inst. of General Pathology, Univ. of Padua, Padua, Italy) *Biochem. 21*(9):2134-2140 (1982). The effect of phospholipid mixing on the permeability properties of multilamellar lipid vesicles (MLV) was studied. In the solid state, dimyristoylphosphatidylcholine/dipalmitoylphosphatidylcholine (DMPC/DPPC) vesicles exhibit ideal lipid miscibility; dimyristoylphosphatidylcholine/distearoylphosphatidylcholine (DMPC/DSPC) vesicles exhibit nonideal lipid miscibility at low DSPC molar fractions; dimyristoylphosphatidylcholine/dibehenoylphosphatidylcholine (DMPC/DBPC) vesicles exhibit lipid immiscibility in a large range of DBPC molar fractions. The rates of K^+, ethylene glycol, and water diffusion from these vesicles in the solid state were measured by photometric and electrometric techniques. The following results were obtained: (1) The rate of solute diffusion, which is decreased monotonically, in DMPC/DPPC MLV, by increasing the molar fractions of DPPC, exhibits maxima at 0.2 molar fraction of DSPC in DMPC/DSPC MLV and at 0.4 molar fraction of DBPC in DMPC/DBPC MLV. (2) The activation energy of the solute diffusion process abruptly decreases in approximately the same range of lipid molar fractions where nonideal lipid miscibility is present. (3) The membrane pore radius is increased by increasing the lipid nonideal miscibility. The rate of vesicle size increase, measured by absorbance changes, is decreased monotonically in DMPC/DPPC monolamellar vesicles [small unilamellar lipid vesicles (SUV)] by increasing the molar fraction of DPPC. It, however, exhibits a maximum in DMPC/DSPC SUV at 0.15 molar fraction of DSPC. A model was suggested in which the solute diffusion and the membrane fusion processes are controlled by fractures. The average width of the fractures is increased by increasing the lipid immiscibility.

PHOSPHOLIPID VESICLE AGGREGATION: EFFECT OF MONOVALENT AND DIVALENT IONS. S. Ohki, N. Duzgunes, and K. Leonards (Dept. of Biophys. Sci., State Univ. of New York at Buffalo, Buffalo, NY 14214) *Biochemistry 21*(9):2127-2133 (1982). A study on cation-induced aggregation of unilamellar phospholipid vesicles was made by measuring the turbidity of vesicle suspensions. As the cation concentration was increased, the degree of aggregation of acidic phospholipid vesicles increased. The concentration at which the sharpest increase in turbidity was observed was defined as the "threshold" concentration. The order of threshold concentrations was the same for vesicles composed of mixtures of phosphatidylserine and phosphatidylcholine. A decrease in the monovalent ionic strength of the vesicle suspensionsolutions raised the threshold concentration for Mn^{2+} and Ca^{2+}. The order of effectiveness for monovalent cations to cause phosphatidylserine vesicle aggregation was $H^+ > Na^+ > Li^+ > K^+ > TMA^+$ (tetramethylammonium). The effect of pH on phosphatidylserine vesicle aggregation was studied in the presence of Na^+. The threshold pH for vesicle aggregation was about 2.6 in 100 mM NaCl. An attempt was made to explain vesicle aggregation in terms of surface potential, surface charge densities of the membranes, and the electrostatic repulsive interaction energy between two vesicles. It was proposed that the slope of the total interaction energy at the Debye distance is related to the degree of massive aggregation occurred among phosphatidylserine vesicles. Knowing the intrinsic binding constants of Ca^{2+} and Na^+ to a phosphatidylserine membrane and assuming that the slope of the interaction energy of two interacting membranes is the same at the Debye distance at the observed threshold concentrations of ions for vesicle aggregation, we calculated the binding constants of other metal ions.

THE EFFECTS OF TEMPERATURE ACCLIMATION ON MEMBRANE STEROLS AND PHOSPHOLIPIDS OF *NEUROSPORA CRASSA*. L.R. Aaronson, A.M. Johnston, and C.E. Martin (Dept. of Bio. Sci. and the Bureau of Bio. Res., Rutgers Univ., Douglass Campus, New Brunswick, NJ 08903) *Biochim. Biophys. Acta 713*(2):456-462 (1982). Experiments were conducted to examine the effects of temperature acclimation on sterol and phospholipid biosynthesis in *Neurospora crassa*. Cultures grown at high (37 C) and low (15 C) temperatures show significant differences in free and total sterol content, sterol/phospholipid ratios and distribution of major phospholipid species in total lipids and two functionally distinct membrane fractions. The ratio of free sterols to phospholipids in total cellular lipids from 15 C cultures was found to be about one-half that found at 37 C, whereas sterol/phospholipid ratios of mitochondrial and microsomal membranes were found to be higher at the low growth temperature. Total sterol and phospholipid biosynthetic rates showed parallel reductions in culture acclimating to a shift from 37 to 15 C growth conditions. Distribution of [^{14}C] acetate label into free sterols was significantly lower under these conditions, however; indicating an increase in the conversion rate of sterols to sterol esters at the lower temperature. Mitochondrial and microsomal membrane fractions showed distinct phospholipid distributions which also differed from total lipid distributions at the two growth temperatures. In each case there was a consistent decrease in phosphatidylcholine and a corresponding increase in phosphatidylethanolamine as growth temperatures were lowered.

LIPOPROTEIN LEVELS IN TISSUE LIPIDS IN FATTY-FIBROUS ATHEROSCLEROSIS INDUCED IN RABBITS BY TWO YEARS' CHOLESTEROL FEEDING AT A LOW LEVEL. C.W.M. Adams, N.E. Miller, R.S. Morgan, and S.N. Rao (Departments of Histopathology and Chemical Pathology, United Medical Schools of Guy's and St. Thomas's, London University, London, Great Britian) *Atherosclerosis 44*(1):1-8 (1982). A group of 12 young NZW rabbits of the same breeding strain were fed a diet enriched with 0.1% cholesterol by weight. The resulting modest hypercholesterolaemia resolved after 4-5 months. Two animals that died during this period showed no gross or microscopic atherosclerosis. After 6 months, the dietary cholesterol was increased to 0.2%. In some animals this resulted in moderate hypercholesterolaemia. One animal that died at this time showed no atherosclerosis with a mean serum cholesterol level of

224 mg/dl. Just after one year, dietary cholesterol was increased to 0.3%. This resulted in definite hypercholesterolaemia in some animals, but a few resisted the treatment with mean serum cholesterol levels around 40-60 mg/dl. In general, animals with established hypercholesterolaemia showed severe atherosclerosis, but often of a more fibrous and less cellular nature than is usual in the rabbit. Aortic wall cholesterol content (on a wet weight basis) correlated positively with serum cholesterol concentration ($r = +0.69$, $P \sim 0.05$) and negatively with the ratio of HDL cholesterol to (LDL plus VLDL) cholesterol (double log plot: $r = -0.79$, $P < 0.025$).

POPULATION-BASED REFERENCE VALUES FOR LECITHIN-CHOLESTEROL ACYLTRANSFERASE (LCAT). J.J. Albers, R.O. Bergelin, J.L. Adolphson and P.W. Wahl (Dept. of Med. and The Northwest Lipid Res. Clinic at Harborview Med. Center, Schl. of Med. and the Dept. of Biostatistics Schl. of Public Health and Community Med., Univ. of Washington, Seattle WA 98104) *Atherosclerosis 43*(2,3):369-379 (1982). Plasma unesterified cholesterol is converted to cholesteryl ester by the enzyme lecithin-cholesterol acyltransferase (LCAT). Plasma levels of LCAT were measured by a sensitive double antibody radioimmunoassay in a sample from an adult employee population, ages 20-59 years, in the Pacific Northwest. After adjusting for differences in relative body mass, women had significantly higher LCAT levels (5.90 ± 1.06, n = 154) than men (5.49 ± 0.89, n = 83). For ages 20-59 years, LCAT levels showed a slight association with age: r ≈ 0.13 for men and 0.29 for women. LCAT was positively correlated with relative body mass, total cholesterol, and LDL cholesterol. Men who smoked cigarettes had significantly lower LCAT mass than men who did not smoke cigarettes. No statistical differences in mean LCAT values were found between drinkers and nondrinkers. The 5th percentile LCAT value was 4.3 μg/ml for both men and women not using hormones. The 95gh percentile value was 7.3 μg/ml for men and 7.8 μg/ml for women regardless of hormone use. Subjects phenotypically LCAT-deficient by clinical criteria and by the absence or near absence of LCAT activity had levels of LCAT mass well below the reference values: 0.73 ± 0.70, range 0.10 μg/ml to 2.65 μg/ml, n = 20. Parents or chil children of LCAT-deficient subjects, i.e., obligate heterozygotes for familial LCAT deficiency, had reduced levels: 3.59 ± 0.69, range 2.59-4.61 μg/ml, n = 19.

HEPATIC BILE ACID UPTAKE: EFFECT OF CONJUGATION, HYDROXYL AND KETO GROUPS, AND ALBUMIN BINDING. R. Aldini, A. Roda, A.M.M. Labate, G. Cappelleri, E. Roda, and L. Barbara (Clin. Med. III, Univ. Bologna, Policlinico S. Orsola, via Massarenti 9, Bologna, Italy) *J. Lipid Res. 23*(8):1167-1173 (1982). Hepatic extraction of trihydroxy (free, glyco- and tauro-conjugated), dihydroxy, and monohydroxy bile acids has been evaluated in single pass liver perfusion experiments in rats. The percentage of each bile acid bound to albumin was also evaluated by equilibrium dialysis. Conjugation increased bile acid liver extraction, without relevant differences in the percentage of bile acid bound to albumin. Among the free bile acids, trihydroxy bile acids were more efficiently cleared by the liver than the dihydroxy acids, and the latter more than the monohydroxy bile acids. 7-Keotlithocholic acid uptake was slightly less than that of cholic acid. Conversely, among dihydroxy bile acids, the percentage of the bile acid bound to albumin decreased from lithocholic acid to cholic acid. Decreasing the albumin concentration in the medium, and hence the fraction of the bile acid bound to albumin, resulted in an increase in bile acid liver extraction. Therefore, besides differences in the chemical structure of bile acids, the extent of bile acid-albumin binding may be a determinant in bile acid liver uptake.

VITAMIN E AND INFANT CHOLESTASIS. F. Alvarez (Unite de Recherche d'Hépatologie Infantile I.N.S.E.R.M. U 56 & Clinique de pediatrie, Univ. Paris-Sud, Hopital d'Enfants, F-94270 BICETRE) *Acta Vitaminol. Enzymol. 4*(3):253-258 (1982). The mechanisms and consequences of Vitamin E deficiency were studied in 12 children presenting with chornic cholestasis. Preliminary results indicate that: (1) Vitamin E serum levels are lowest in children with the long-lasting cholestasis and in children in whom fat malabsorption is deepest. (2) Signs of neurologic dysfunction involving peripheral nerves, cerebellum, eye movements and retina were present in 7 children. (3) In vitro study of RBC showed increased hemolysis with oxidating agents and in physiological saline. Degree of hemolysis was inversely related to the serum level of vitamin E. (4) Increased platelet aggregation was observed in 8 patients; 6 of these also had low levels of serum Vitamin E.

LYSOPHOSPHATIDYLCHOLINE & TRITON X-100 STIMULATED PALMITOYL-COA SYNTHETASE OF CHICKEN LIVER. S.G. Amur, B.R. Lokesh and S.K. Murthy (Dept. of Biochem., Indian Inst. of Sci., Bangalore 560 012) *Indian J. Biochem. Biophys. 19*(3): 180-185 (1982). The palmitoyl-CoA synthetase of chicken liver microsomes was activated by lysophosphatidylcholine (LPC) and Triton X1-100. Bile salts and a number of structural analogues of LPC did not activate the enzyme. Triton X-100 and LPC solubilise the microsomal palmitoyl-CoA synthetase and are essential for maximal activity. LPC increases the V_{max} of the reaction without affecting the K_m for palmitate whereas Triton X-100 increased the V_{max} with a simultaneous increase in K_m. The presence of both the compounds lead to increase in both the parameters.

ENERGY RESTRICTION AS A MEANS OF REDUCING FAT PADS IN BROILERS. A.S. Arafa, M.A. Boone, D.M. Janky, H.R. Wilson, R.D. Miles and R.H. Harms (Poultry Sci. Dept., Univ. Florida, Gainesville, FL 32611) *Poultry Sci. 62*(2):314-320 (1983). Two studies, 10 days each, were conducted to investigate the effect of energy restriction during the finishing period on abdominal fat in broilers. A total of 300 broilers, 52 days old, were used in the first study, and 512 broilers, 46 days old, were used in the second. Experimental treatments restricted energy intake but maintained full minimum daily requirements of all other nutrients. When this restriction was done, the nutrient density of the diets was increased to partially compensate for the decreased feed allowance. A graduated reduction of 21 to 32% and 16 to 24% in daily energy intake was obtained in Experiments 1 and 2, respectively. Fat pad weights of the broilers were decreased as the daily energy intake was decreased. Weight gain decreased, which resulted in reducing the final carcass weight. Energy restriction did not influence dressing percent and resulted in a significant reduction in the amount of feed required to produce a gram of cooked carcass.

15-HYDROXYLATION OF 5β-CHOLESTAN-3α-OL AND 24α-ETHYL-5β-CHOLESTAN-3α-OL IN RAT LIVER SUPERNATANTS (18,000 × *g*). L. Aringer (Res. and Development Lab., Dept. of Obstetrics and Gynecology, Karolinska Sjukhuset, S-104 01 Stockholm, Sweden) *J. Biol. Chem. 257*(22):13720-13725 (1982). 4-^{14}C-labeled-5β-cholestan-3α-ol and 24α-ethyl-5β-cholestan-3α-ol were incubated with rat liver 18,000 × *g* supernatant fractions fortified with NADPH. Among the metabolites formed were the 15α- and 15β-hydroxy derivatives of the two substrates. The identification of these metabolites with liquid chromatography, thin layer chromatography, and gas-liquid chromatography-mass spectrometry is described. The formation of 15β-hydroxylated metabolites exceeded that of 15α-hydroxylated ones. The total yields of 15-hydroxylated compounds formed was of the order 0.5-1.0%. The 15-hydroxylated metabolites could not be detected after incubations with rat liver mitochondria or a soluble liver fraction or after incubations of 5β-cholestan-3α-ol with soybean lipoxygenase and linoleic acid.

PATHWAYS FOR THE INCORPORATION OF ESTERIFIED CHOLESTEROL INTO VERY LOW DENSITY AND LOW DENSITY LIPOPROTEINS IN PLASMA INCUBATED IN VITRO. P.J. Barter, G.J. Hopkins, and G.D. Calvert (Clinical Biochem. Unit, Schl. of Med., Flinders Univ. of South Australia, Bedford Park, South Australia 5042) *Biochim. Biophys. Acta 713*(1):136-148 (1982). In vitro incubations of human or pig plasma containing a tracer amount of [^{3}H] cholesterol have been performed to determine which lipoprotein fractions are the immediate recipients of the esterified cholesterol formed in the reaction catalysed by lecithin:cholesterol acyltransferase. In pig plasma, which is deficient in activity of the protein which promotes transfer of esterfied cholesterol between different lipoprotein fractions 87-90% of the lecithin:cholesterol acyltransferase-derived esterified cholesterol was incorporated into the high density lipoprotein (HDL) fraction. In human plasma there was an initial recovery of more than 80% in HDL, although the proportion recovered in very low density lipoproteins (VLDL) and low density lipoproteins (LDL) became progressively greater with increasing duration of incubation, consistent with a transfer from an HDL-esterified cholesterol pool of increasing specific activity. Nevertheless, as in the pig plasma incubations, there was evidence that some 10-15% of the esterified cholesterol formed in the lecithin:cholesterol acyltransferase reaction was incorporated directly into human VLDL and LDL. In quantitative terms however, it was found that most of the esterified cholesterol delivered to human VLDL and LDL was the result of transfers from HDL rather than as a direct incorporation from its site of synthesis.

A UNIFIED MODEL OF ESTERIFIED CHOLESTEROL EXCHANGES BETWEEN HUMAN PLASMA LIPOPROTEINS. P. Barter, G. Hopkins, L. Forjatschko and M. Jones (Units of Clin. Biochem. and Human Morph., Schl. of Med., The Flinders Univ. of S. Australia, Bedford Park, S. Australia 5042) *Atherosclerosis 44*(1): 27-40 (1982). Exchanges of esterified cholesterol between human high density lipoproteins (HDL) and very low density lipoproteins (VLDL) and between VLDL and low density lipoproteins (LDL)

have been fitted to a mathemetical model previously developed to describe exchanges between human HDL and LDL. In all cases, the fit of the model predicted exchanges to those measured experimentally was extremely good, thus greatly increasing confidence in the validity of the model. The model assumes that esterified cholesterol exchanges are achieved by means of a transfer protein which interacts with lipoprotein particles from which it picks up and deposits esterified cholesterol. The values generated for the model constants indicated that, given equal concentrations of esterified cholesterol in each fraction, the relative probability that the transfer protein will pick up a molecule of esterified cholesterol in HDL vs VLDL vs LDL is the ratio 28.9:4.65:1. According to the model the transfer protein may "bind" to lipoproteins. The model predicts that, at physiological lipoprotein concentrations, the proportion of transfer protein bound to HDL will be more than double that which is unbound to lipoprotein and that bound to VLDL will be about one tenth that unbound. The model was unable to detect evidence of the transfer protein binding to LDL.

IMMUNOGLOBULIN AS THE MAJOR LOW DENSITY LIPOPROTEIN BINDING PROTEIN IN PLASMA. B.J. Bauer, K. Blashfield, R. Norris, D.A. Buthala and L.C. Ginsberg (Dept. of Biomedical Sci., Western Michigan Univ. and The Upjohn Co., Kalamazoo, MI 49008) *Atherosclerosis 44*(2):153-160 (1982). Plasma from normal humans and Chinese hamsters was shown to contain material which binds to low density lipoproteins (LDL). The binding capacity of these plasmas was demonstrated by passive hemagglutination against human LDL-coated red blood cells. The plasmas were fractionated by affinity chromatography, gel filtration and electrophoresis. Immunologic analyses of these fractions showed that IgM and IgA were the major plasma proteins responsible for the LDL binding titers of human and hamster plasmas. The titer of binding protein in diabetic and non-diabetic humans and hamsters was also determined.

HIGH CONTENT OF 22:6 (DOCOSAHEXAENOATE) AND ACTIVE [2-^{3}H]GLYCEROL METABOLISM OF PHOSPHATIDIC ACID FROM PHOTORECEPTOR MEMBRANES. N.G. Bazan, M.S. Di Fazio De Escalante, M.M. Careaga, H.E.P. Bazan and N.M. Giusto (Instituto de Investigaciones Bioquimicas, Universidad Nacional del Sur-Consejo Nacional de Investigaciones Cientificas y Tecnicas, Bahia Blanca, Argentina) *Biochim. Biophys. Acta 712*(3):702-706 (1982). This study describes the content, fatty acid composition and [2-^{3}H]glycerol metabolism of phosphatidic acid of rod outer segment membranes from vertebrate retinas. A relatively high content of phosphatidic acid was observed in rod outer segment membranes isolated from rat, toad and bovine retinas. In bovine retinas, about 65% of the acyl groups of phosphatidic acid were composed of docosahexaenoate. Arachidonate and docosapentaenoate represented about 4 and 5%, respectively, of the total, whereas stearate was the most common saturated acyl chain. An active [2-^{3}H]glycerol metabolism in the phosphatidic acid of these membranes was found when whole retinas were incubated with the precursor for short periods prior to subcellular fractionation. Our results suggested that the pool of phosphatidic acid enriched in docosahexaenoate may arise from de novo biosynthesis or from phospholipid degradation by a phospholipase D enzyme, and that it is not metabolically related, in any major fashion, to the diacylglycerols of rod outer segment membranes.

DEVELOPMENT OF HEPATIC AND ADIPOSE TISSUE LIPOGENIC ENZYMES AND INSULINEMIA DURING SUCKLING AND WEANING ON TO A HIGH-FAT DIET IN ZUCKER RATS. R. Bazin and M. Lavau (Unite de Recherches sur la Physiopathologie de la Nutrition, Inserm U 177, Institut Biomedical des Cordeliers, 15, rue de l'Ecole de Medecine, 75006 Paris, France) *J. Lipid Res. 23*(6):839-849 (1982). This study was designed to monitor the developmental changes in insulinemia and lipogenic enzyme activities in both inguinal adipose tissue and liver during suckling (7, 9, 14 and 17 days of age) and weaning (22 and 30 days of age) on to either a low-fat or a high-fat diet in lean (Fa/fa) and obese (fa/fa) rats. Tissues were removed through surgery and genotypes were retrospectively determined. Our results indicate that the increased adipose tissue capacity for lipogenesis, starting during the suckling period, could play an important etiologic role in the development and maintenance of obesity in the Zucker rat.

BLOOD CHANGES IN SEX STEROID HORMONE USERS. CIRCULATING IMMUNE COMPLEXES INDUCED BY ESTROGENS AND PROGESTOGENS AND THEIR RELATION TO VASCULAR THROMBOSIS. V. Beaumont, B. Delplanque, N. Lemort, and J.-L. Beaumont (Institut National de la Sante et de la Recherche Medicale, Unite de Recherches sur l'Atherosclerose) *Atherosclerosis 44*(3): 343-353 (1982). Oral contraceptives (OC) have been shown to induce in some women antiethinylestradiol antibodies which may be detected as circulating immune complexes by precipitation in ammonium sulphate at 25% saturation (CIC.AS). A reevaluation of the presence of CIC.AS in 644 women either receiving sex steroid hormones or not was made, and the respective role of estrogens and progestogens investigated, together with the influence of the dose. The study confirmed that CIC.AS levels were significantly different in controls (442 ± 246 μg/ml serum), healthy gonadal hormone users (754 ± 700 μg) and users with thrombosis (1331 ± 1099 μg/ml). These results indicated that: 1. CIC.AS could be induced by synthetic estrogens as well as progestogens, but not by non-synthetic hormones; 2. the inducation of CIC.AS seemed poorly dose-related, and 3. was not correlated with the duration of use; 4. in reactive women, high CIC.AS levels occurred as soon as 3 weeks after the beginning of synthetic gonadal hormones use, persisted throughout treatment and decreased slowly when discontinued; 5. in women with thrombosis CIC.AS were more frequently detected (64.7%) than in healthy users (32.2%) $P < 0.001$. The importance of the immunologic changes as a risk factor in thrombosis in OC users was evaluated in comparison with other predisposing factors and tobacco smoking.

VITAMIN B_{12}, E AND D CONTENT OF RAW AND COOKED BEEF. M.R. Bennink and K. Ono (Dept. of Food Sci. and Human Nutr., 106 Food Sci., Michigan St. Univ., East Lansing, MI 48824) *J. Food Sci. 47*(6):1786-1792 (1982). The relationship of carcass grade, primal cuts and cooking to vitamin B_{12}, E and D in separable lean beef was studied. The average vitamin B_{12} content in 471 samples of raw and cooked beef was 3.17 μg/100 g. The vitamin B_{12} content of raw and cooked beef was similar; however, on considering the moisture and fat losses during cooking, there was 27-33% loss of B_{12}. The vitamin E content of raw and cooked beef was similar and average 133 μg/100 g for 464 samples. From 33-44% of the original vitamin E in the meat was lost upon cooking. Raw and cooked beef contained 80-100 ng of vitamin D/100 g, with 35-42% of the original vitamin D content being lost upon cooking. The content of vitamins E and D in beef is low and of little nutritional importance; however, beef is an important dietary source of vitamin B_{12}.

LOW DENSITY LIPOPROTEIN METABOLISM BY CULTURED SKIN FIBROBLASTS FROM ATHEROSCLEROTIC PATIENTS. R.G. Behrman and V. Wynn (The Alexander Simpson Laboratory for Metabolic Research, St. Mary's Hospital, London, W2, Great Britain) *Atherosclerosis 42*(2,3):173-184 (1982). The aim of this study was to determine whether an abnormality in low density lipoprotein (LDL) metabolism could be demonstrated in fibroblasts cultured from normolipidaemic subjects with atherosclerosis. Seventeen male subjects aged 30-55 years with normal plasma lipid concentrations were divided into 2 groups on the basis of the presence or absence of proven coronary artery and/or peripheral vascular disease. LDL metabolism was assessed in cultured fibroblasts obtained from each of these subjects. After 6 h incubation with ^{125}I-labelled LDL, it was found that binding, uptake and degradation of the lipoprotein were all significantly higher in cells from the atherosclerotic group of subjects than the controls. Variations in cellular LDL metabolism were also correlated with 4 risk factors for cardiovascular disease. Plasma LDL concentration in the atherosclerotic subjects was found to be inversely related to LDL binding and degradation. Subject age was inversely related to LDL degradation in both groups of subjects. No association was demonstrated in either group of subjects between LDL metabolism and glucose intolerance, or between LDL metabolism and cigarette smoking. It is concluded from these results that cellular LDL binding may constitute a factor in determining the rate of atheroma formation, which is independent of other cardiovascular risk factors.

DETECTION OF CARBONYL FUNCTIONS IN PHOSPHOLIPIDS OF LIVER MICROSOMES IN CCl_4- AND $BrCCl_3$-POISONED RATS. A. Benedetti, R. Fulceri, M. Ferrali, L. Ciccoli, H. Esterbauer, M. Comporti (Istito di Patologia Generale dell'Universita di Siena, Via del Laterino 8, 53100 Siena, Italy) *Biochim. Biophys. Acta 712* (3):628-638 (1982). As the peroxidative cleavage of unsaturated fatty acids can result in the release of carboynl compounds or the formation of caronyl functions in the acyl residues, evidence for the presence of carboynl groups in liver microsomal phospholipids was searched for in in vivo conditions in which peroxidation of lipids of hepatic endoplasmic reticulum had been demonstrated. The spectrophotometric examination of 2,4-dinitrophenylhydrazine-treated phospholipids of liver microsomes from the intoxicated animals showed absorption spectra similar to those observed for the dinitrophenylhydrazones of various carbonyls. Similar spectra were also observed with 2,4-dinitrophenylhydrazine-treated phospholipids of liver microsomes peroxidized in the NADPH-Fe-dependent system.

Abstracts

The amount of 2,4-dinitrophenylhydrazine-reacting groups in phospholipids of liver microsomes increases with the incubation time and is correlated to the amount of malonic dialdehyde formed in the incubation mixture. The kinetics of the production of 4-hydroxynonenal was similar to that of malonic dialdehyde formation. In both in vivo conditions the amount of carbonyl functions in microsomal phospholipids, higher in the $BrCCl_3$-intoxicated animals as compared to the CCl_4-poisoned ones, was close to that in the vitro conddition in which lipid peroxidation is induced by 6 μM Fe^{2+}. The possible pathological significance of formation of carbonyl functions in membrane phospholipids is discussed.

EFFECTS OF DIABETES AND HIGH FAT-HIGH CHOLESTEROL DIET ON PLASMA LIPID LEVELS AND ON ERYTHROCYTE MEMBRANE COMPOSITION. R. Bhandaru, S.R. Srinivasan, B. Radhakrisnamurthy and G.S. Berenson (Dept. of Med. and Biochem., Louisiana State Univ. Med. Centre, New Orleans, LA 70112) *Atherosclerosis 43*(2,3):263-272 (1982). Erythrocyte membrane composition was studied in rats subjected to experimental hyperlipidemia and/or hyperglycemia by means of 6 weeks of high fat (40% w/w)-high cholesterol (5% w/w) diet with and without 8 weeks of streptozotocin-induced diabetes. High fat-high cholesterol diet lowered plasma glucose levels in control and in diabetic animals. While the atherogenic diet produced only hypercholesterolemia, the same diet fed to diabetic animals produced both hypercholesterolemia and hypertriglyceridemia. The membrane protein content was lower in diabetic rats than in controls, while the cholesterol and phospholipids were higher in diabetic rat erythrocyte membranes. Feeding the atherogenic ditet increased membrane lipid levels in only nondiabetic animals. The total carbohydrate content of the membranes was greater in diabetic animals than controls. Difference in relative proportion of individual sugars, e.g., galactose, mannose, glucose, and fucose of the membranes were observed between diabetic and control groups. These observations suggest that rat erythrocyte membrane composition is altered both in hyperglycemic and hyperlipidemic conditions, and may provide a useful model for evaluating lipidcarbohydrate abnormalities of membrane structures in diabetes mellitus.

TRIACYLGLYCEROL SECRETION IN RATS: EFFECTS OF ESSENTIAL FATTY ACIDS AND INFLUENCE OF DIETARY SUCROSE, GLUCOSE OR FRUCTOSE. M.I. Bird and M.A. Williams (Department of Nutritional Sciences, University of California, Berkeley, CA 94720) *J. Nutr. 112*(12):2267-2278 (1982). The rate of triacylglycerol (TG) secretion into plasma was determined in vivo in essential-fatty-acid(EFA)-adequate and EFA-deficient rats fed for 8-10 weeks on diets containing either sucrose, glucose or fructose (66.5% wt/wt) as the sole source of carbohydrate. The rate of TG secretion was increased in EFA-deficient rats, irrespective of the type of dietary carbohydrate. The secretion rate in EFA-deficient rats fed fructose was 50% higher than the rate in similar animals fed glucose or sucrose, although in the EFA-adequate rats, the secretion rate with fructose was not significantly higher than with sucrose or glucose. The concentrations of triacylglycerols and cholesteryl esters in plasma were generally reduced in EFA deficiency, whereas the concentrations of these lipids in liver were increased. Plasma free fatty acid concentrations were unchanged. The fatty acid composition of plasma and hepatic lipids was altered both by EFA deficiency and to a smaller extent by the type of dietary carbohydrate. The concentration of plasma glucagon was reduced in the deficient animals, although plasma insulin concentration was not affected. The resulting increase in the plasma insulin:glucagon molar ratio may explain some of the alterations in lipid concentrations and in lipid metabolism observed in EFA deficiency.

INDUCTION OF CALCIUM-BINDING PROTEIN BEFORE 1,25-DIHYDROXYVITAMIN D_3 STIMULATION OF DUODENAL CALCIUM UPTAKE. C.W. Bishop, N.C. Kendrick, and H.F. DeLuca (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison, Madison, WI 53706) *J. Bio. Chem. 258*(2): 1305-1310 (1983). Protein synthesis occurring before the onset of 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) stimulation of calcium uptake was examined in embryonic chick duodena by double label autoradiography. Duodena from 19-day-old embryos were cultured for 24 hr. Duodena exposed to a saturating concentration of 1,25-$(OH)_2D_3$ during the last 2, 4, or 6 H. were either cultured with [^{3}H] leucine for the final 2 hr or used for calcium uptake assays. Duodena not exposed to the hormone were either cultured with [^{14}C] leucine for the final 2 g or used for calcium uptake assays. Calcium uptake by non-radiolabeled duodena was increased ($p < 0.05$) only in tissues exposed to the hormone for 6 hr. Tissue uptakes of [^{3}H] leucine and [^{14}C] leucine were identical and linear with time. Soluble proteins were extracted from ^{3}H- and ^{14}C-labeled tissues, mixed, and simultaniously resolved by two-dimensional polyacrylamide gel electrophoresis. Analysis of fluorographs and autoradiographs prepared from the double-labeled gels revealed a protein induced by 1,25-$(OH)_2D_3$ within 2 hr or exposure to 1,25-$(OH)_2D_3$ and at least 2 hr before the calcium uptake response. The induced protein co-migrated with purified chick calcium-binding protein during two-dimensional electrophoresis. These results demonstrate that 1) this protein is the calcium-binding protein and 2) calcium-binding protein is clearly synthesized in the embryonic duodenum before the calcium uptake response to 1,25-$(OH)_2D_3$.

CHARACTERISTICS OF THE LIPOLYTIC β-ADRENERGIC RECEPTORS IN HAMSTER ADIPOCYTES. P. Bjorgell and P. Belfrage (Dept. of Physio. Chem., Univ. Lund, P.O. Box 750, S-220 07 Lund, Sweden) *Biochim. Biophys. Acta 713*(1):80-85 (1982). Hamster adipocyte β-adrenergic receptors were characterized by the effect on the lipolysis rate of several selective β-adrenergic agonists and antagonists. Prenalterol and procaterol, selective β_1- and β_2-agonists, respectively, both stimulated the lipolysis rate half-maximally at 1-2 μM, a concentration approximately 100-fold higher than that needed for haf-maximal stimulation by isoprenaline. The maximal procaterol effect was similar to that of isoprenaline, while the effect of prenalterol was 40% lower. Submaximally isoprenaline-stimulated lipolysis was half-maximally inhibited by propranolol at 0.2 μM and by atenolol and H 35/25, selective β_1- and β_2-antagonists, at 23 and 6 μM concentration, respectively. Highly specific β_1 and β_2 stimulation was induced by incubation with prenalterol or procaterol, with simulaneous maximal selective β_2 or β_1 inhibition, respectively. The maximal β_2 stimulation was approximately twice the corresponding β_1 effect under these conditions, the sum of both effects closely approaching that of maximal nonselective β-adrenergic stimulation with isoprenaline. Similar results were obtained with the selective β_2-antagonist, ICI 118,551, or the β_1-antagonists, pamatolol and practolol. The findings are most easily explained by the existence of a heterogeneous β_1- and β_2-adrenergic receptor population on hamster adipocytes.

DYNAMICS OF APOLIPOPROTEIN E METABOLISM IN HUMANS. C. Blum (Dept. Med. and the Arteriosclerosis Research Center, Columbia Univ., College of Physicians and Surgeons, New York, NY 10032) *J. Lipid Res. 23*(9):1308-1316 (1982). The dynamics of human apoE metabolism were explored by examining the effects of alimentary lipemia and postheparin lipolysis on the plasma level and lipoprotein distribution of apoE. In the studies of alimentary lipemia, fasting and postprandial plasma samples were obtained from five normal adult males, each of whom drank 100 g of corn oil. Although no change in the plasma concentration of apoE accompanied alimentary lipemia, a major redistribution of apoE among lipoproteins occurred. These studies provide evidence in intact humans for a dynamic traffic of apoE between triglyceride-rich lipoproteins and high density lipoprotein. This traffic is a prominent phenomenon of normal alimentary lipemia and of lipolysis. By modulating the lipoprotein distribution of apo E, it probably plays a key functional role in lipoprotein metabolism.

DEVELOPMENTAL CHANGES OF HEMATOSIDE OF RAT SMALL INTESTINE. D. Bouhours and J-F. Bouhours (Lab. de Biochimie des Membranes, Univ. Claude-Bernard, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France) *J. Biol. Chem. 258*(1):299-304 1983. The hematoside of rat intestine is analyzed from 1 day to 60 days of age. During the first 3 weeks of life, G_{M3} (N-acetylneuraminylgalactosylglucosylceramide) contains only nonhydroxylated fatty acids and accounts for 80-90% of the ganglioside sialic acid. Its concentration is maximum at 6 days (315 μg of NeuAc/g of intestine) and falls abruptly over the next 2 weeks. It reaches 45 μg of NeuAc/g of intestine at 60 days. Between 28 and 60 days, G_{m3} accounts for 72% of the total intestinal gangliosides. From 21 days on, strucutral modifications of G_{M3} are observed. N-Acetylneuraminic acid is replaced progressively by n-glycolylneuraminic acid and nonhydroxylated fatty acids are replaced by α-hydroxylated fatty acids. Both changes are interpreted as the result of hydroxylations of G_{M3} components which are triggered at the time of weaning. These hydroxylations take place chiefly in epithelial cells and to a much lesser extent in nonepithelial residue, as shown by the separate analysis of both compartments of rat intestine at 38 and 60 days. In epithelial cells, the highest percentage of α-hydroxylated fatty acids and of N-glycolylneuraminic acid is found at 60 days. In addition, 4 D-hydroxysphinganine is the major base of the G_{M3} of intestinal cells from birth to adulthood.

SERUM LIPOPROTEINS OF HEALTHY PERSONS FED A LOW-FAT DIET OR A POLYUNSATURATED FAT DIET FOR THREE MONTHS. J. Brussaard, M. Katan, P. Groot, L. Havekes, J. Hautvast (Dept. of Human Nutr., Agric. Univ., Wageningen, The Netherlands) *Atherosclerosis 42*(2,3):205-219 (1982). We have compared the

effects on some risk factors for atherosclerosis of 2 cholesterol-lowering diets, one low in total fat and in polyunsaturated fat (LO) and one moderate in total fat but high in polyunsaturated fat (MOD). Volunteers were given diet MOD during a 2.5-week control period. This diet provided 31% of the daily energy intake (energy%) as total fat; one-third of the fatty acids were polyunsaturated (PUFA). VLDL plus LDL cholesterol decreased by 0.66 mmol/l during the control period while the HDL cholesterol (HDL-C) level was unchanged. Total serum triglycerides decreased by 0.33 mmol/l. After the control period the subjects were randomized into 2 groups; one continued on diet MOD, the other received the low-fat diet LO, providing 21 energy% as total fat and 4 energy% as PUFA. Nutrient intakes were checked by 7-day records and by chemical analysis of double portions. The diets contained the same amounts of nutrients known to affect serum lipid levels. Total serum cholesterol increased by 0.21 ± 0.41 mmol/l on control diet MOD during the test period of 13 weeks. 0.09 ± 0.11 mmol/l of this was due to HDL-C. Total serum triglycerides remained constant during the test period in group MOD. Combination of these data with those from a preceding study leads to the following conclusions (1) Both diets lower total serum cholesterol levels when compared with the habitual diets of affluent communities. (2) The high-carbohydrate, low-fat diet causes lower HDL and higher fasting VLDL triglycerides levels than the recommended moderate-fat-high-PUFA diet.

INCREASING THE LEVELS OF ETHER-LINKED LIPIDS IN L-M CELLS BY GLYCERYL ETHER SUPPLEMENTATION DEPRESSES GROWTH AND CHOLINE UTILIZATION. M.C. Cabot, C.J. Welsh and F. Snyder (Medical & Health Sciences Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37830) *Biochim. Biophys. Acta 713*(1):16-22 (1982). Hexadecylglycerol was added to medium required for the growth of L-M cells in culture. L-M fibroblasts cultured through several generations in the presence of hexadecylglycerol grow at a reduced rate. Experimental cells at their sixth passage, with 2 μg supplement/ml, double at 50% the rate of control cell populations. Hexadecylglycerol added 1 day after cell passage does not retard growth; however, within 1 hr it decreases the incorporation of choline into the choline glycerophosphatide fraction. Inhibition is specific for choline; ethanolamine incorporation is not affected. The inhibition of choline utilization by hexadecylglycerol-treated cells is dose-dpendent and reaches a maximum 12 hr after supplementation. Cellular uptake of choline is reduced but not as much as the incorporation of choline into the phospholipids. The assimilation of ether lipid precursor into cellular phospholipids was followed by incubating cells with (1-^{14}C) hexadecylglycerol. Incorporation of radioactivity into cellular phospholipids begins to plateau after 24 hr, whereas the interference of hexadecylglycerol with choline metabolism could be detected as early as 1 hr. The majority of the radioactivity recovered from cells incubated with labeled hexadecylglycerol is localized in the microsomal fraction where the label was distributed as free hexdecylglycerol, alkylacylphospholipids and alkyldiacylglycerols. The supplementation of a glycerol ether to L-M fibroblast growth media selectively inhibits the utilization of choline for choline glycerophospholipid biosynthesis and causes a reduction in cell growth rate when cells are continually passaged in the presence of the glyceryl ether.

SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN CHINESE HEALTHY SUBJECTS AND PATIENTS WITH CERTAIN DISEASES. H.-J. Cai, Z.-X. Li and S.-M. Yang (Pathophysiology Dept., Nanjing Med. College, Nanjing) *Atherosclerosis 43*(2,3): 197-207 (1982). Serum high density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglycerides (TG) were determined, and values of low density lipoprotein cholesterol (LDL-C), HDL-C/TC (%), and the LDL-C/HDL-C (ratio) were calculated in 1101 healthy Chinese men and women, 77 patients with coronary heart disease (CHD), 70 patients with cerebral vascular disease (CVD), 64 patients with diabetes mellitus (DM), 40 bilaterally oophorectomized women and 95 women using oral contraceptives. Serum HDL-C levels seemed higher and LDL-C levels lower in the healthy Chinese population as compared with those previously reported from European and American whites. Serum HDL-C was significantly higher in fertile females than in males of comparable ages. We failed to demonstrate any sharp fall in HDL-C after the menopause or bilateral oophorectomy. Serum HDL-C levels were significantly lower in both CHD and CVD patients than in healthy subjects of comparable sex and age. Concomitant increases in serum TC, LDL-C and TC, however, were found in CHD patients but not in CVD patients. No abnormality in the mean serum HDL-C level was found in DM patients. However, those complicated with CHD had significantly lower HDL-C than those without CHD. A striking serum HDL-C lowering effect was found with some kinds of oral contraceptives.

GLYCINE- AND FATTY-ACID-INDUCED RESTRICTION OF FEED INTAKE: EFFECTS ON BODY WEIGHTS AND HATCHING EGG PRODUCTION OF BROILER BREEDERS RESTRICTED FROM DAY OF HATCHING. N.A. Cave (Animal Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6) *Poultry Sci. 62*(1):125-132 (1982). In two experiments, broiler breeder replacement stocks were subjected to voluntary feed restriction from day of hatching by 1) inclusion of 30 g glycine per kilogram diet for 21 days followed by temporal skip-a-day restriction or b) by inclusion of 50 g coconut fatty acids (CFA) per kilogram diet for 21 or 35 days followed by quantitative skip-a-day restriction regimen. Feed intake and body weight were reduced by 14 to 17% during consumption of experimental diets compared with an unsupplemented starter diet. Following skip-a-day feeding during growing period, body weights were not different at 140 days of age in each experiment. In Experiment 1, higher egg production (hen housed %) was associated with glycine-induced restriction of intake of starter diet, but there was no difference in egg weight. The difference was attributable, in part, to reduced mortality during the early laying period. In Experiment 2, no differences were observed in egg production, grams feed per egg, or egg weight due to glycine- or CFA-induced restriction from 1 to 21 days followed by quantitative restriction. Egg production was lower with a lower level of CFA feed restriction from 22 to 35 days, indicating that restricted feeding prior to 35 days increased production of hatching eggs.

RESOLUTION INTO TWO DIFFERENT FORMS AND STUDY OF THE PROPERTIES OF PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C FROM HUMAN PLATELET CYTOSOL. L-Y. Chau and H-H. Tai (Dept. of Biochem., North Texas State Univ. and Texas College of Osteopathic Med., Denton, TX 76203) *Biochim. Biophys. Acta 713*(2):344-351 (1982). Two forms of phosphatidylinositol-specific phospholipase C from human platelet cytosol were resolved by DEAE-cellulose chromatography and purified further by hydrophobic chromatography. Both forms utilized phosphatidylinositol as the best substrate. However, the enzyme did not distinguish 2-arachidonylphosphatidylinositol from 2-oleoylphosphatidylinositol although the former substrate was known to be predominant species in human platelets. Both forms exhibited pH optimum at 7.0. Both activities were inhibited completely by 1 mM EDTA and the inhibited preparations could be restored to full activity or to 60% by free Ca^{2+} or Co^{2+}, respectively, at 100 μM. Higher concentrations of either ion were inhibitory. Other metal ions were ineffective. Addition of calmodulin in the presence of Ca^{2+} did not show any additional effect. Both forms were inhibited comparably by various phospholipids, fatty acids and detergents, suggesting that phosphatidylinositol in membranes might be a poor substrate for the enzyme. Initiation of phosphatidylinositol breakdown through the phospholipase C pathway may require additional activator(s). A variety of anti-platelet drugs, including phenylthiazines, local anesthetics and mepacrine, were found to be potent inhibitors of the enzyme, suggesting that these drugs might inhibit platelet function by inhibiting the early phase of arachidonate release.

SOME CHARACTERISTICS AND CELLULAR DISTRIBUTION OF PHOSPHOLIPASES A_1 AND A_2 OF RAT TESTIS. L. Chaudhary (Div. of Biochem. & Food Sci., Indian Vet. Res. Inst., Izatnagar, U.P. 243122, India) *Acta Vitaminol. Enzymol. 4*(4):325-335 (1982). The subcellular distribution and some properties of different phospholipases A of rat testis were studied using exogenous 1-acyl-2-[1-^{14}C]oleoyl-*sn*-glycero-3-phosphocholine as substrate. The presence of distinct phospholipases A:two phospholipases A_1 with pH optimum at 3.0 and 7.4, respectively and a phospholipase A_2 with pH optimum at 7.4 has been shown. The neutral phospholipases A_1 and A_2 have been further studied. The activities of phospholipases A_1 and A_2 with pH optimum of 7.4 were greatly stimulated by sodium deoxycholate but were not affected by Triton X-100. The enzyme activities were slightly stimulated by Ca^{2+} but inhibited by EDTA at higher concentration. Studies using various effecters showed that the phospholipase A_1 and A_2 were sensitive to sulfhydryl reagents and heat, insensitive to DIFP and cyclic nucleotides and completely inhibited by sodium dodecyl sulfate. Subcellular fractionation of testis homogenates and the subcellular distribution patterns of markers, marker enzymes and phospholipases A indicated that the phospholipases A_1 and A_2 with highest specific and relative specific activities were mainly localized in microsomal fractions.

INHIBITION OF PROTEIN SYNTHESIS BLOCKS THE RESPONSE TO 25-HYDROXYCHOLESTEROL BY INHIBITING DEGRADATION OF HYDROXYMETHYLGLUTARYL-COA REDUCTASE. H.W. Chen, B.A. Richards and A.A. Kandutsch (The Jackson Lab., Bar Harbor, ME 04609, U.S.A.) *Biochim. Biophys. Acta 712*(3): 484-489 (1982). The activity of the rate-limiting enzyme of the cholesterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase in Chinese hamster ovary (CHO) cells decreased more

rapidly in cells treated with 25-hydroxycholesterol alone ($t_{1/2}$ = 1.5 hr) than in those incubated with cycloheximide alone ($t_{1/2}$ = 5 hr). The inhibitory action of 25-hydroxycholesterol on reductase activity was reduced when the sterol and cycloheximide were added together, and was totally abolished when cells were preincubated with cycloheximide for 30 min before the addition of 25-hydroxycholesterol. The effect of puromycin was similar to that of cycloheximide. Treatment of cells with an inhibitor of RNA synthesis, i.e., actinomycin D or cordycepin, had little effect on hydroxymethylglutaryl-CoA reductase activity; however, preincubation of cells with these reagents also decreased the ability of 25-hydroxycholesterol to suppress the reductase activity. These data are consistent with a model which suggests (a) that 25-hydroxycholesterol inhibits the activity of hydroxymethylglutaryl-CoA reductase by repressing its synthesis, (b) that cycloheximide and puromycin affect hydroxymethylglutaryl-CoA reductase activity by blocking the de novo synthesis of the enzyme and by reducing the degradation of the preexisting enzyme, (c) that actinomycin D and cordycepin affect the supply of message for the continuous synthesis of at least one component of a system which degrades hydroxymethylglutaryl-CoA reductase, and (d) that one component of the degradative system has a half-life shorter than 0.5 hr.

GLUCOCORTICOID REGULATION OF 1,25(OH)$_2$-VITAMIN D$_3$ RECEPTORS IN CULTURED MOUSE BONE CELLS. T.L. Chen, C.M. Cone, E. Morey-Holton and D. Feldman (Dept. Med., Stanford Univ. Schl. Med., Stanford, CA 94305) *J. Biol. Chem. 257*(22): 13564-13569 (1982). The ability of glucocorticoids to modulate 1,25(OH)$_2$-Vitamin D$_3$ receptors was investigated in primary cultures of mouse osteoblast-like bone cells. The concentration of receptors fluctuated during the culture cycle. High levels were found during log phase growth and low levels at confluence. Because of these dynamic changes in receptor concentration, effects of glucocorticoids were examined throughout the culture cycle. During early log phase, dexamethasone reduced both the concentration of receptors and the rate of [^{14}C] thymidine incorporation to 60-70% of control levels. In late log phase, dexamethasone-treated cells transiently exhibited a higher receptor number than control cells. This relative increase in receptors appeared to result from a dexamethasone-mediated delay in reaching confluence, and, therefore, a slower decline in receptors than exhbited by control cells. After confluence, dexamethasone decreased the receptor content to the same extent as at the early log phase, but at this stage there was only a slight effect on [^{14}C] thymidine incorporation and no change in DNA content. The dexamethasone inhibitory effects at both growth phases were dose-dependent, glucocorticoid-specific, and involved a selective change in receptor number without a change in receptor affinity. The relationship of the receptor level to the rate of cell proliferation was tested using epidermal growth factor. In early log phase, epidermal growth factor flocked the inhibition of cell growth caused by dexamethasone and reversed the dexamethasone-mediated decline in receptor levels. The inhibitory effect of dexamethasone at confluence was independent of its effects on cell proliferation.

VITAMIN A PALMITATE DECREASES INTRAVENOUS GLUCOSE TOLERANCE IN MAN. B.S. Chertow, W.I. Sivitz, N.G. Baranetsky, T.B. Styer, B.J. Sorensen, C.H. Schikman, R.M. Norris and B.A. Ozuk (Section of Endocrinology, Depts. of Med. and Nuclear Med., Huntington Veterans Administration Med. Center; Marshall Univ. Schl. of Med., Huntington, WV) *Acta Vitaminol. Enzymol. 4* (4):291-298 (1982). We tested retinyl palmitate for in vivo effects in man and in vitro effects on the IM-9 lymphocyte insulin receptor. Intravenous glucose tolerance tests (IVGTT) with 25 g glucose were performed on 10 healthy subjects before and after two intramuscular injections of retinyl palmitate (25,000 IU) 18 hours apart. In 9 of 10 subjects, glucose disposition was impaired after treatment with retinyl palmitate. In vitro, retinyl palmitate 10^{-4} - 10^{-6} M did not affect the binding or displacement of insulin ^{125}I from lymphocyte receptors. We conclude that retinyl palmitate decreases glucose tolerance without demonstrable effects on insulin release or insulin binding to receptors.

EFFECTS OF GARLIC PRODUCTS ON LIPID METABOLISM IN CHOLESTEROL-FED RATS. M.S. Chi (Department of Home Economics, Alcorn State University, Lorman, Mississippi 30906) *Proc. Soc. Exper. Biol. Med. 171*(2):174-178 (1982). The effect of garlic prepared in several forms on lipid metabolism was studied in male rats fed a diet containing 1% cholesterol. Garlic was supplemented at 2% of the diet as fresh garlic in forms of ethanol extracted garlic residue, ethanol extract of garlic, whole garlic, and autoclaved garlic. Diets were fed for 4 weeks from 6 weeks of age. The supplementation of garlic products except ethanol-extracted garlic residue reduced plasma and liver cholesterol levels. The reduction in the plasma cholesterol by feeding garlic products was in very low density lipoprotein and low-density lipoprotein cholesterol fractions. Animals fed diets supplemented with garlic decreased liver glucose-6-phosphate dehydrogenase and malic enzyme activities and also reduced the liver weight, inquinal adipose tissue weight, liver total lipids, and plasma triglycerides. The hypocholesterolemic activity of garlic was contained in the ethanol extract and stable when autoclaved at 120° for 1 hr.

EFFECT OF DIETARY VITAMIN E ON IMMUNE RESPONSES OF CALVES. J.E. Cipriano, J.L. Morrill, and N.V. Anderson (Dept. of Animal Sci. and Industry and Surgery and Med., Kansas State Univ., Manhattan, KA 66506) *J. Dairy Sci. 65*(12):2357-2365 (1982). The effect of vitamin E on humoral and cell-mediated immune responses in calves was determined, and plasma vitamin E and immunological status of calves under normal herd management were studied. Twelve newborn calves were fed skimmed colostrum for 2 days and thereafter skimmed milk plus vitamin E-stripped lard and emulsifying agents. Six calves each orally received 0, and six each orally received 1 g of DL-α-tocopherol acetate daily. Rations were supplemented with trace minerals and vitamins A and D. Twenty calves were fed colostrum for 3 days and thereafter milk and dry feed. At 6 wk, mean plasma vitamin E concentrations (μg/100 ml) for groups were 71, 639, and 155, respectively; and mean serum glutamic oxalacetic transaminase concentrations (IU/liter) were 310, 61, and 43, respectively. Mean serum immunoglobulins concentrations (mg/100 ml) were: G1, 1079, 1168, and 1315; G2, 588, 562, and 432; A, 37, 53, and 85; M, 151, 118, and 110. Mean lymphocyte stimulation indexes were 76, 220, and 152, respectively. At 6 wk there were large but nonsignificant differences in mean indexes among groups.

CHARACTERIZATION OF MONOHYDROXYLATED LIPOXYGENASE METABOLITES OF ARACHIDONIC AND LINOLEIC ACID IN RABBIT PERITONEAL TISSUE. M. Claeys, M.-C. Coene, A.G. Herman, G.H. Jouvenaz and D.H. Nugteren (Division of Pharmacology, Dept. of Pharmaceutical Sci., Univ. of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium) *Biochim. Biophys. Acta 713* (1):160-169 (1982). Rabbit peritoneal tissue contains a lipoxygenase which converts arachidonic acid preferentially into 15-hydroxy-5,8,11,13-eicosatetraenoic acid. Stereochemical analysis of the menthyloxycarbonyl derivative of this metabolite means of a high-pressure liquid chromatography method, involving the use of a Ag^+-loaded cation-exchange column, indicated that it has mainly the 15-L$_S$-hydroxy configuration. The biosynthesis of 15-hydroxy-5,8,11,13-eicosatetraenoic acid could be confirmed during examination of the monohydroxy acids obtained without addition of fatty acids, thus formed from endogenously released substrate. However, the 9- and 13-hydroxy derivatives of linoleic acid were also formed and in quantities exceeding those of 15-hydroxy-5,8,11,13-eicosatetraenoic acid.

THE SELECTIVE LIPID-LOWERING EFFECT OF VEGETARIANISM ON LOW DENSITY LIPOPROTEINS IN A CROSS-OVER EXPERIMENT. R.S. Cooper, R.B. Goldberg, M. Trevisan, Y. Tsong, K. Liu, J. Stamler, A. Rubestein, and A.M. Scanu (Department of Community Health and Preventive Medicine, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611) *Atherosclerosis 44*(3):293-305 (1982). In a cross-over experiment the effect of short-term vegetarianism on serum lipids, lipoproteins and apoproteins was studied. The experimental diet was free of animal products, with the exception of skim milk, and consequently low in saturated fat and cholesterol. Fifteen free-living individuals were randomly assigned to 3-week periods on either the experimental diet or a control diet which closely approximated the usual intake in the U.S.A. Significant reductions in total cholesterol (12.5%), low density lipoprotein cholesterol (14.7%), and apo B (13.2%) were observed, accompanied by a non-significant decrease in high density lipoprotein cholesterol (10%), apo A-I (3%) and a non-significant increase in apo-II (4%). These data suggest that a fat-modified diet low in total fat, saturated fat and cholesterol, and moderate (not high) in polyunsaturated fat may not lower HDL-C or its apoproteins as much as a diet high in polyunsaturated fat, while having similar effects on LDL-C, and would therefore be preferable as the basis for primary prevention of atherosclerosis.

THE EFFECTS OF PROBUCOL ON PLASMA LIPOPROTEINS IN POLYGENIC AND FAMILIAL HYPERCHOLESTEROLAEMIA. C. Cortese, C.B. Marenah, N.E. Miller, and B. Lewis (Department of Chemical Pathology and Metabolic Disorders, St. Thomas' Hospital Medical School, London, Great Britain) *Atherosclerosis 44*(3):319-325 (1982). During treatment with probucol at the dose of 1 g per day, the mean reduction in low density lipoprotein (LDL) cholesterol concentration was 11.2% in polygenic hypercholesterolaemia (n=9) and 9.4% in heterozygous familial hypercholesterolaemia (n=6).

However, there was marked heterogeneity of response: in seven of the patients with polygenic hypercholesterolaemia who had in comcommon moderate elevation of LDL cholesterol (5.3-6.4 mmol/l), th reduction ranged from 13 to 40% (mean, 23%). In two of this group the change in LDL concentration was associated with a decrease in LDL apolipoprotein B synthetic rate. Of the patients with familial hypercholesterolaemia one showed a 33% reduction in LDL cholesterol, and one a 13% reduction. Total high density lipoprotein (HDL) cholesterol concentration tended to decrease during treatment. This reflected a reduction of the cholesterol concentration in the HDL_3 subclass; HDL_2 cholesterol remaining unchanged. Plasma triglyceride and very low density lipoprotein cholesterol were unaffected by probucol. The drug was well tolerated with only one patient complaining of severe diarrhoea, and two of mild and transient diarrhoea. No clinically significant changes occurred in serial resting electrocardiograms. Thus, probucol appears to be a useful drug for the treatment of most patients with polygenic hypercholesterolaemia, and of some patients with heterozygous familial hypercholesterolaemia.

STUDIES OF THE EFFECTS OF ESSENTIAL FATTY ACID DEFICIENCY IN THE RAT. J.W. Cox, G.W. Rutecki, L.L. Francisco, and T.F. Ferris (Department of Internal Medicine, University of Minnesota, School of Medicine, Minneapolis, MN) *Circ. Res. 51*(6): 694-702 (1982). We report a model of prostaglandin depletion induced in rats by fasting for 11 days, followed by institution of an essential fatty acid-deficient diet. Urinary prostaglandin E E was 22 ± 2 ng/24 hours compared to 113 ± 8.5 ng/24 hours in controls. There was no difference in 24-hour urine volume or solute excretion. Five hours after administration of NaCl, 10 mM/kg, essential fatty acid-deficient diet rats excreted 1.85 ± 0.78 ml urine compared to 6.42 ± 2.26 ml in control with Na^+ excretion 447 ± 273 μEq in essenfatty acid-deficient rats vs 1483 ± 366 μEq in control. Intravenous isotonic NaCl, 1.5% body weight, resulted in increased urine flow rate in control rats from 8.3 ± 2 μl/min to 28.7 ± 8.8 μl/min, with sodium excretion increasing from 0.19 ± 0.2 to 3.3 ± 0.9 μEq/min. In the essential fatty acid-deficient diet animals, there was no significant change in flow rate or sodium excretion after saline infusion. There was no difference in the glomerular filtration rate or plasma aldosterone in the two groups after the salt load. When given a water load, 3 ml/100 g body weight, essential fatty acid-deficient diet rats excreted 2.5 ± 0.7 ml in 5 hours compared to 6.3 ± 1.4 ml in controls. When isotonic saline was substituted for drinking water, there was an increase in systolic blood pressure in essential fatty acid-deficient diet rats. The administration of linoleic acid for 4 days increased urinary prostaglandin E excretion and the alterations in the ability to excrete a sodium and water load were reversed. In essential fatty acid-deficient diet animals made hypertensive by 9 days of saline drinking, the institution of linoleic acid to the diet normalized the blood pressure despite the continued administration of saline.

TRUE METABOLIZABLE ENERGY OF FATS AT LOW LEVEL DIETARY INCLUSION. N.M. Dale and H.L. Fuller (Department o of Poultry Science, University of Georgia, Athens, GA 30602) *Poultry Sci. 61*(12):2415-2420 (1982). When assayed at a 2.5% level of inclusion in a corn-soybean meal basal diet with 40 replications per treatment, no significant differences could be demonstrated between the true metabolizable energies (TME) of corn oil and two samples of tallow varying in stearic acid (18:0) content. All values were in excess of the gross energy of fat, suggesting an improvement in the absorption of other dietary constituents. When assayed at a 15% level in a purified basal diet, the TME of corn oil was significantly higher than that of the tallows; all values were below the gross energy of fat. Use of the practical corn-soybean meal basal improved the TME of the fats by 22.4% (corn oil), 34.9% (low 18:0 tallow), and 43.0% (high 18:0 tallow). A portion of the improvement in the TME of the tallows is presumed to be accounted for by an interaction with fatty acids in the practical basal ingredients that may be obscured at higher levels of inclusion. It is concluded that with adequate replication it is feasible to assay the TME of fats at low levels of dietary inclusion. A comparison of fatty acid absorption in Single Comb White Leghorn roosters during a TME study and in 8 to 9 week broilers on a full-feed regimen suggests that the conditions im imposed during a TME assay provide a satisfactory model for evaluating the fat absorption of chickens reared under normal conditions.

ABNORMAL HIGH DENSITY LIPOPROTEINS OF ABETALIPOPROTEINEMIA: RELEVANCE TO NORMAL HDL METABOLISM. R. Deckelbaum, S. Eisenberg, Y. Oschry, M. Cooper, C. Blum (Depts. of Gastroenterology and Pediatrics, Lipid Res. Lab., Dept. of Med. B, Hadassah Univ. Hosp., Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel) *J. Lipid Res. 23*(9):1274-1282 (1982). We investigated high density lipoprotein (HDL) subfractions in abetalipoproteinemia (ABL) using rate zonal ultracentrifugation. In ABL, HDL_2 is the major subfraction, 65% of total mass compared to less than 10% in normal subjects with similar HDL levels. HDL_2 and HDL_3 in ABL (n=3) are larger and lighter than in normals (n=3), with mean diameters of 136 ± 19 Å and 100 ± 12 Å, respectively (as compared to 113 ± 12 Å and 86 ± 11 Å), and contained more apoprotein E. ABL-HDL_2 and HDL_3 particles contain 2- to 2.5-fold more cholesteryl ester molecules than normals. ABL-HDL can be modified towards normal HDL by allowing VLDL triglycerides to exchange for ABL-HDL cholesterol esters, followed by addition of lipoprotein lipase and hydrolysis of the triglycerides. In addition, ABL plasma contains a previously undescribed small and spherical (61 ± 8 Å) protein-rich (63% by weight) HDL fraction, which we call ABL-HDL_4.

DISCRIMINATIVE VALUE OF LIPIDS AND APOPROTEINS IN CORONARY HEART DISEASE. G. De Backer, M. Rosseneu, J.P. Deslypere (National Fund for Scientific Research, Dept. of Cardiology, Brugge, Belgium) *Atherosclerosis 42*(2,3):197-203 (1982). Serum cholesterol, HDL cholesterol (HDL-C), and apoproteins, A1, A2 and B were determined in 70 male survivors of myocardial infarction and in an equal number of healthy controls, matched for age, sex and body masss index. In univariate analyses, the Apo B/ Apo A1 ration discriminated the best between cases and controls, giving a 72% exact classification. In a multivariate analysis, the Apo B/Apo A1 ratio, HDL-C and the Apo A2/ Apo A1 ratio contributed independently to the discrimination of cases from controls while the overall exact classification was 82%. These promising results were comparable in younger and older subgroups. Thus, the determination of apoproteins yielded complementary information in this cross-sectional survey and warrants further study in a prospective setting.

VERY SMALL FAT CELL POPULATIONS: MAMMALIAN OCCURRENCE AND EFFECT OF AGE. F.D. DeMartinis and A. Francedese (Dept. of Physiology and Biochem., the Med. College of Pennsylvania, Philadelphia, PA) *J. Lipid Reş. 23*(8):1107-1120 (1982). A normal Gaussian distribution of fat cell diameters has been found in the adipose depots of most animals and man. In our early work we observed very small fat cells in isolated cell preparations of normal adult rat epididymal pads and frequency distributions of measured fat cell diameters were bimodal, indiacting the existence of a separate population of very small fat cells in addition to the normal adipocyte population. We investigated in detail the age- and weight gain-associated changed in adipocyte size distribution from a unimodal distribution in very young animals to a bimodal distribution in maturing heavier animals. Bimodality in the epididymal depot was fully established by 14 weeks of life and persisted without detectable change to 28 months of age. Several other depots representing deep abdominal and subcutaneous adipose tissue in adult rats and in the adult form of male guinea pigs, female C57 BL/6J lean mice, rabbits, and cats also have been found to contain bimodal adipocyte populations. Our results show that in normal adipose depots of several mammalian species a separate population of very small fat cells exists in addition to the usual adipocyte population and we conclude this is a characteristic morphologic feature of adult mammalian adipose tissue.

INFLUENCE OF UNREFINED POTATO STARCH ON CECAL FERMENTATIONS AND VOLATILE FATTY ACID ABSORPTION IN RATS. C. Demigne and C. Remesy (Laboratoire des Maladies Métaboliques, Institut National de la Recherche Agronomique, Theix, 63110 Beaumont, France) *J. Nutr. 112*(12):2227-2234 (1982). The effects of uncooked potato starch in the diet on cecal fermentation and absorption of volatile fatty acids and on changes in the digestive supply of nutrients were investigated. Dietary potato starch (25%) markedly increased cecal size and the cecal pool of volatile fatty acids. These were maximal in the postabsorptive state, whereas the most acidic cecal pH readings were observed during the late absorptive period. Compared to the basal diet, feeding potato starch did not change arterial concentrations of glucose, lactate of alanine. Nevertheless, blood acetate was increased, whereas plasma triglycerides and cholesterol were reduced. The digestive balance of nutrients was determined by measurements of portal blood flow and arteriovenous differences across the digestive tract: the digestive supply of glucose was maximal during the absorptive period, but was lower in rats fed the potato starch diet. The absorption of volatile fatty acids was strongly increased by feeding potato starch and was maximal in the postabsorptive state, whereas glucose absorption was finished. The volatile fatty acids then constituted the main source of energetic fuels coming from the digestive tract. These results indicate that the effect of potato starch on digestion and the metabolism is similar to that of dietary fibers, and that the regular supply of large amounts of volatile fatty acids, particularly during the postabsorptive period, could bring about substantial changes in

liver metabolsim.

CONFORMATIONAL TRANSITIONS IN SERUM HIGH DENSITY APOPROTEINS OF HYPERCHOLESTEROLEMIC MONKEYS. S. Dhawan, S. Nityanand, N.K. Kapoor, S. Singh (Central Drug Res. Inst., Lucknow, India) *Atherosclerosis 41*:81-86 (1982). Conformational transitions in serum high density apolipoproteins of normal and hypercholesterolemic monkeys have been studied by circular dichroism. This study has revealed that under hypercholesterolemic conditions the secondary structure of apolipoproteins suffers permanent changes which could be observed even after a lengthy procedure of siolation, purification and delipidation of high density lipoprotein.

THE MODULATING INFLUENCE OF VITAMIN E IN BIOLOGICAL MEMBRANE UNSATURATED PHOSPHOLIPID METABOLISM. A.T. Diplock (Dept. of Biochem., Guy's Hosp. Med. Schl., Univ. of London, London SE1 9RT, UK) *Acta Vitaminol. Enzymol. 4*(4):303-309 (1982). A tissue culture system in which the composition of the medium, with respect to vitamin E, linoleic acid, and cholesterol, can ge manipulated at will, was used to study the effect of vitamin E on the fatty acid profiles of fibroblast membrane phospholipids. The effect was studied of α-tocopherol, and of butylated hydroxytoluene, on the uptake of isotopically labeled linoleic acid and cholesterol, and of the effect of these antioxidants on the metabolic interconversion of linoleic acid with other unsaturated fatty acids. Butylated hydroxytoluene was without effect on any of the parameters measured, α-tocopherol caused a large enhancement in the content and radioactivity of the arachidonyl residues of phosphatidyl choline, phosphatidyl serine, and phosphatidyl ethanolamine, generally at the expense of linoleic acid in the same phospholipids. There was no effect of α-tocopherol on the unsaturated fatty acids of the neutral lipids, suggesting that there was no general effect on the chain elongation and desaturation of linoleic acid. The results are thought to demonstrate a specific effect of α-tocopherol upon the architecture of membrane phospholipids by controlling the profiles of their unsaturated fatty acid components. The uptake of radioactive cholesterol, the content of cholesterol and cholesteryl-esters in cultured cells was also significantly increased by the presence of α-tocopherol in the medium.

SECRETION OF SURFACTANT BY PRIMARY CULTURES OF ALVEOLAR TYPE II CELLS ISOLATED FROM RATS. I.G. Dobbs, R.J. Mason, M.c. Williams, B.J. Benson and K. Sueishi (Cardiovascular Research Institute, University of California, San Francisco, CA 94143) *Biochim. Biophys. Acta 713*(1):118-127 (1982). Pulmonary surfactant is prepared from material obtained by endobronchial lavage. Although it has been assumed that the components of surfactant are secreted by alveolar type II cells, direct proof of this assumption has not been available. It is possible that the final material obtained by lavage has been modified after secretion or altered during the isolation procedure. Type II cells, after 1 day in primary culture, secrete saturated phosphatidylcholine, one of the lipid components of surfactant. Because saturated phosphatidylcholine is not unique to surfactant and because type II cells in culture lose differentiated characteristics over the first several days in culture, it was not known how closely the secretory products of cultures of type II cells resemble surfactant as obtained by endobronchial lavage. We therefore studied the morphologic, physical and chemical characteristics of the material that type II cells secrete under basal conditions and after stimulation with terbutaline or 12-*O*-tetradecanoyl-13-phorbol acetate. The secreted material resembled surfactant obtained by lavage; it was similar morphologically to the lamellar material and tubular myelin seen in the fluid-filled alveoli of fetal rats, it lowered surface tension to 5mN per meter, and it contained the 72000 dalton apolipoprotein of surfactant. When cells were incubated for 22 hr with [1-^{14}C] acetate, the sitribution of radioactivity in the secreted material was very similar to the phospholipid composition of rat surfactant. This material secreted by alveolar type II cells after 1 day in primary culture is similar to surfactant obtained by endobronchial lavage.

RATE OF LCAT-MEDIATED CHOLESTEROL ESTERIFICATION AND SERUM LIPIDS DURING ETIROXATE THERAPY IN HYPERLIPOPROTEINEMIA. M. Dobiášová, K. Vondra, V. Matoušek and J. Válek (Inst. of Nuclear Bio. and Radiochem., Czechoslovak Academy of Sci. and Inst. for Clinical and Experimental Med., Prague) *Atherosclerosis 42*(2,3):251-261 (1982). Changes in the rate of the plasma cholesterol ester production mediated by lecithin: cholesterol acyltransferase (LCAT, E.C. 2.3.1.43) were examined in 15 patients suffering from types II and IV HLP who had been treated for 14 weeks with etiroxate. Whereas the plasma cholesterol concentration decreased significantly only in the initial phase of the therapy, the rate of cholesterol esterification increased gradually and attained at the end of the study a value exceeding by 50% the initial level. The final fractional turnover rate nearly equalled that characteristic for the control group of healthy subjects, in spite of the fact that the concentration of plasma cholesterol in the diseased subjects was higher by 50-100%. Triglyceride concentration decreased only transitorily in the course of the therapy with etiroxate. It is concluded that etiroxate is likely to normalize the rate of cholesterol turnover in the endogenous pool.

THIOL-DISULFIDE-DEPENDENT INTERCONVERSION OF ACTIVE AND LATENT FORMS OF RAT HEPATIC +-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE. I. Dotan and I. Schechter (Dept. Biochem., George S. Wise Facul. of Life Sci., Tel Aviv Univ., Ramat Aviv, Tel Aviv, 69978 Israel) *Biochim. Biophys. Acta 713*:427-434 (1982). The activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (hydroxymethylglutaryl-CoA reductase, EC 1.1.1.34) in preparations of thil-deficient rat liver microsomes containing thiols have been compared. Unlike microsomes containing thiols, which possess an active hydroxymethylglutaryl-CoA reductase(E_a), thiol-deficient microsomes contain an inactive, latent enzyme (E_i) which can be activated by addition of thiols. E_a can be converted to E_i by dialysis. The degree of the inhibition of E_a by GSSG is proportional to the ratio GSSG/thiol in the reaction. Our results indicate that thiols are necessary cofactors for hydroxymethylglutaryl-CoA reductase reaction. Their effect on the activation of E_i is not caused by change in the state of aggregation of the enzyme. Rather, the reversible change of the enzyme from E_i to E_a is affected by increasing the affinity of the enzyme to the substate hydroxymethylglutaryl-CoA.

PREFERENTIAL UPTAKE OF LIPIDS BY MYCOPLASMA MEMBRANES FROM HUMAN PLASMA LOW-DENSITY LIPOPROTEINS. H. Efrati, Y. Oschry, S. Eisenberg, S. Razin (Dept. of Membrane and Ultrastructure Res., Hebrew Univ.-Hadassah Med. Schl. (H.E. and S.R.), Jerusalem, Israel) *Biochemistry 21*(25):6477-6482 (1982). The binding and transfer of low-eensity lipoprotein (LDL) constituents to mycoplasma membranes were examined. *Mycoplasma capricolum* was found to bind more ^{125}I-labeled LDL than *Acholeplasma laidlawii*. Free and esterified cholesterol uptake was 3-4 times higher in *M. capricolum* than in *A. laidlawii*. Cholesterol transfer to the membranes of both organisms far exceeded the amounts of cholesterol expected according to LDL protein found associated with the membranes. Trypsin digestion of the membranes prior to incubation with LDL decreased the binding of LDL and the transfer of free cholesterol to *M. capricolum* membranes but did not affect these processes with *A. laidlawii* membranes. These findings suggest the existence of protease-sensitive receptors on *M. capricolum* cell surface responsible for tighter contact with LDL. Further observations lead us to conclude that both free and esterified cholesterols are transferable from LDL to the membranes by a simple exchange process. Similar mechanisms of free and esterified cholesterol transfer may also operate in vivo and contribute to the process of cholesterol exit from the plasma.

LYMPHATIC TRANSPORT OF BILE ACIDS IN MAN. S. Ewerth, I. Björkhem, K. Einarsson, and L. Öst (Dept. of Surgery, Clinical Chem., and Med., Karolinska Institutet at Huddinge Univ. Hospital, Stockholm, Sweden) *J. Lipid Res. 23*(8):1183-1186 (1982). The possibility of a lymphatic transport of bile acids in man was investigated. Four patients were studied 2-4 weeks after renal transplantation. As a part of the postoperative immunosuppressive treatment they all had a thoracic duct fistula for lymphatic drainage. After a standardized meal, lymph and peripheral blood samples were simultaneously collected at 30-minute intervals for 210 minutes. The bile acids, cholic acid, chenodeoxycholic acid, and deoxycholic acid were assayed by a gas-liquid chromatography-mass spectrometry method using deuterium-labeled internal standards. The concentration of cholic acid was about the same in lymph and serum. The concentrations of chenodeoxycholic and deoxycholic acids were 2-3 times higher in lymph than in serum, both in the fasting state and postprandially. These results are explained by a more efficient passive absorption of the less polar dihydroxy bile acids. The transport of bile acids in the lymph was calculated to be only about 0.2% of that in the portal vein.

EFFECT OF HIGH FAT AND IRON LEVELS ON THE GROWTH AND MORTALITY OF CHICKENS. G. Farrow, A.S. Glassman, P. Vohra, and F.H. Kratzer (Department of Avian Sciences, University of California, Davis, California 95616) *Poultry Sci. 62*(1):85-90 (1982). Four experiments were conducted with chicks to determine whether the heating of vegetable oil and its contamination with iron might have detrimental effects upon its use in chicken diets. Soybean oil and safflower oil heated at 230 C for 51 hr and linseed oil heated at 240 C for 24 hr gave reduced growth when fed to chickens at 20

percent of the diet. Heating soybean oil at 175 C for 51 hr had no detrimental effect. However, iron at 5000 ppm depressed growth significantly when fed in a low fat diet. The depression was greatly reduced when soybean oil was fed at 10 or 20% of the diet. A different lot of soybean oil was found to cause skin dermatitis and high mortality when fed at 20% of the diet due to its physical contamination on the skin.

DIETARY DETERMINANTS OF LIPOPROTEINS, TOTAL CHOLESTEROL, VISCOSITY, FIBRINOGEN, AND BLOOD PRESSURE. A.M. Fehily, J.E. Milbank, J.W.G. Yarnell, T.M. Hayes, A.J. Kubiki, and R.D. Eastham (Medical Research Council Epidemology Unit, South Wales, 4 Richmond Road, Cardiff, CF2 3AS, Wales) *Am. J. Clin. Nutr. 36*(5):890-896 (1982). High-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, viscosity, fibrinogen, and blood pressure were determined in 117 men aged 44 to 60 yr selected from the general population who also completed 7-day weighed dietary records. Associations between these measurements and a number of dietary factors were assessed by multiple regression analysis, allowing where necessary for the effects of age, body mass index, and smoking habit. High-density lipoprotein cholesterol was associated positively with both alcohol and fish consumption and negatively with saturated fat intake. High-density lipoprotein cholesterol expressed as a percentage of total cholesterol was associated negatively with the percentage of energy from fat and positively with fish consumption. Low-density lipoprotein cholesterol was associated positively with the percentage of energy from fat and negatively with fish consumption. Fibrinogen and systolic blood pressure were inversely related to cereal fiber intake.

EFFECT OF DIETARY FAT SATURATION, CHOLESTEROL AND CHOLESTYRAMINE ON ACYL-COA: CHOLESTEROL ACYLTRANSFERASE ACTIVITY IN RABBIT INTESTINAL MICROSOMES. F.J. Field and R.G. Salome (Dept. of Med., Univ. of Iowa Schl. of Med., Iowa City, IA 52242) *Biochimica et Biophysica Acta 712*(3):557-570 (1982). The regulation of intestinal acyl-CoA: cholesterol acyltransferase was investigated by dietary manipulation. Rabbits were fed the following diets: normal rabbit chow, 10% safflower oil, safflower oil plus 1% cholesterol, coconut oil plus 1% cholesterol, or cholestyramine. It is concluded that dietary manipulation can alter microsomal lipid content. Microsomal fat saturation, independent of microsomal cholesterol content, regulates intestinal acyl-CoA: cholesterol acyltransferase and modifies the stimulatory effect of exogenous cholesterol on this enzyme.

GENETIC MEDIATION OF *LIPOPROTEIN* CHOLESTEROL AND APOPROTEIN CONCENTRATIONS IN THE BABOON (*PAPIO SP.*). B.L. Flow, G.E. Mott, and J.L. Kelley (Dept. of Pathology, The Univ. of Texas Health Science Center at San Antonio and the Southwest Foundation for Res. and Education, San Antonio, TX 78284) *Atherosclerosis 43*(1):83-94 (1982). Genetic effects on serum lipoprotein and apoprotein concentrations were investigated using a paternal half-sib design with 79 progeny of 6 sires. Significant differences ($P < 0.05$) were observed among sire progeny groups at 4-6 years of age for serum cholesterol, HDL cholesterol and apoA-I concentrations. A sire effect ($P < 0.10$) also was observed for VLDL + LDL cholesterol concentrations, but differences were not observed ($P > 0.10$) among sire progeny groups for apoB concentrations. Estimates of heritability were 0.54 for serum cholesterol, 0.32 for VLDL + LDL cholesterol, 0.78 for HDL cholestero, 0.56 for apoA-I, and 0.20 for apoB concentration. Genetic (r_g) and environmental (r_e) correlations among serum cholesterol, lipoprotein cholesterol, and apoprotein concentrations were positive except for the negative genetic relationships of apoA-I with apoB and with VLDL + LDL cholesterol. Phenotypic correlation of VLDL + LDL and HDL cholesterol was due to the genetic contribution since the environmental contribution was zero. The positive genetic relationship ($r_g = 0.62$) of VLDL + LDL cholesterol and HDL cholesterol may be due to biochemical mechanisms controlling cholesterol turnover since the genetic correlations of VLDL + LDL cholesterol and HDL cholesterol with cholesterol turnover rate were both negative (-0.43 and -0.68, respectively). A strong negative genetic correlation ($r_g = -0.95$) was observed between apoA-I and cholesterol turnover, a finding that suggests a close physiologic relationship between these variables.

METABOLISM OF 6-KETOPROSTAGLANDIN $F_{1\alpha}$ AND PROSTACYCLIN TO 6,15-DIKETO-13,14-DIHYDROPROSTAGLANDIN $F_{1\alpha}$-LIKE MATERIAL IN CATS AND RABBITS. U. Förstermann, B. Neufang and G. Hertting (Dept. of Pharmacology, Univ. of Freiburg, Hermann-Herder-Strasse 5, D-7800 Freiburg, F.R.G.) *Biochim. Biophys. Acta 712*(3):684-691 (1982). Using previously described radioimmunoassays for 6-ketoprostaglandin $F_{1\alpha}$ and 6,15-diketo-13,14-dihydroprostaglandin $F_{1\alpha}$, plasma levels of these two products of degradation of prostacyclin (prostaglandin I_2) were monitored in cats and rabbits. It was shown that both exogenous prostaglandin I_2 and 6-ketoprostaglandin $F_{1\alpha}$ could be rapidly converted to 6,15-diketo-13,14-dihydroprostaglandin $F_{1\alpha}$ immunoreactivity, 6-ketoprostaglandin $F_{1\alpha}$ to an even somewhat larger extent. This difference in conversion could be explained by competing mechanisms which could delay or hinder the access of prostaglandin I_2 to the sites of metabolism by 15-hydroxyprostaglandin dehydrogenase. We conclude that, under in vivo conditions in the two species investigated, 6-ketoprostaglandin $F_{1\alpha}$ can be rapidly and effectively metabolized by 15-hydroxyprostaglandin dehydrogenase.

HIGH DE NOVO SYNTHESIS OF GLYCEROLIPIDS COMPARED TO DEACYLATION-REACYLATION IN RAT LIVER MICROSOMES. P.L. Fox and D.B. Zilversmit (Div. of Nutr. Sci. and Section of Biochem., Molecular and Cell Bio., Div. of Bio. Sci., Cornell Univ., Ithaca, NY 14853) *Biochim. Biophys. Acta 712*(3):605-615 (1982). A microsomal system characterized by high flux through the entire de novo pathway from glycerol phosphate to phosphatidylcholine and triacylglycerol has been developed. Optimum synthesis of phosphatidylcholine requires CDPcholine, Mg^{2+}, KCl and palmitoyl-CoA-generating system containing palmitic acid, ATP and CoA. Incorporation of [^{14}C] glycerol phosphate into phosphatidylcholine is greater than its incorporation into triacylglycerol at all levels of added palmitate, but the phosphatidylcholine/triacylglycerol synthesis ratio decreases as palmitate is increased. Phosphatidylcholine synthesis from glycerol phosphate is stimulated more by palmitate than by other saturated fatty acids; phosphatidylcholine synthesis increases with increasing unsaturation of the added fatty acids. The ratio of incorporation of [^{3}H] palmitate to [^{14}C] glycerol phosphate was determined for phosphatidic acid, diacylglycerol, phosphatidylcholine and triacylglycerol. This ratio is approximately 2 for all diacylglycerolipids and 3 for triacylglycerol. In our system, incorporation of palmitate into microsomal glycerolipid proceeds primarily by the de novo pathway, with minimal fatty acid recycling via deacylation-reacylation.

FECAL CHOLESTEROL EXCRETION STUDIES IN TYPE II HYPERCHOLESTEROLEMIC PATIENTS TREATED WITH THE SOYBEAN PROTEIN DIET. R. Fumagalli, L. Soleri, R. Farina, R. Musanti, O. Mantero, G. Noseda, E. Gatti and C.R. Sirtori (Inst. of Pharmacology and Pharmacognosy, Univ. of Milan, Milan Italy) *Atherosclerosis 43*(2,3):341-353 (1982). The fecal steroid elimination profile was studied in 7 type II hyperlipoproteinemic patients given a low-lipid diet with textured soybean proteins, in order to define the mechanism of the hypocholesterolemic activity of this new dietary regimen. Four of the patients followed a 3- +3-week cross-over protocol, comparing the soybean diet with a reference low-lipid diet with animal proteins. In these, fecal neutral steroids and bile acids were analyzed by chromatography during the two dietary periods. In spite of the clear hypocholesterolemic effect, no significant differences in steroid output were noted between the two dietary periods. In the 3 remaining patients, a chromatographic + isotopic method (by injecting ^{14}C-labelled cholesterol i.v. 4-6 weeks prior to the dietary study) was employed. Again, no marked changes were noted in the fecal neutral steroid and bile acid outputs and the slope of the decay curve of the plasma cholesterol-specific activity was not changed by the experimental diet, in spite of the remarkable decrease in plasma cholesterol. The reported results do not provide a definitive contribution to the mode of action of the soybean protein diet. They suggest, however, that it is not an effect mediated by undigestible dietary components. The possibility of a cholesterol redistribution from plasma to tissue pools should be considered.

REQUIREMENTS OF Δ^9 and Δ^{12} FATTY ACID DESATURATION IN *NEUROSPORA*. C. Gabrielides, A.L. Hamill and W.A. Scott (The Rockefeller Univ., New York, NY 10021) *Biochimica et Biophysica Acta 712*(3):505-514 (1982). Microsomes prepared from the wild-type strain and lipid auxotrophs of *Neurospora* were analyzed for Δ^9-(stearoyl-CoA) and Δ^{12}-(olcoyl-CoA) desaturase activities. The wild-type Δ^9-desaturase was found to have a 20-fold higher specific activity and 2-fold lower activation energy than the Δ^{12}-desaturase. In addition, Δ^{12}-desaturase had higher $K_{m\ app}$ values for oleoyl-CoA and for NADH than the equivalent values for Δ^9-desaturase. These properties were correlated with a rate-limiting role of Δ^{12}-desaturase in the production of 18:2, the major fatty acid of *Neurospora*. The Δ^{12}-desaturase also exhibited a higher tolerance to pH changes and to cyanide than did the Δ^9-desaturase. Both activities could be measured in the same reaction mixture using stearoyl-CoA as the substrate, indicating a coupling of the two enzymes. Enrichment of cellular membranes of the wild-type *Neurospora* with 18:0 and 18:1, 18:2, 18:3 fatty acids led to the conclusion that the presence of excess substrate in the membrane induces activation of the appropriate desaturase. These experiments also suggested that

the membrane fluidity, as determined by the degree of unsaturation of membrane fatty acids, may influence the activities of the desaturating enzymes. Perturbation of the polar head groups of the membrane phospholipids indicated that the correct composition of anionic phospholipids is an absolute requirement for the function of both desaturases. These studies show that the Δ^{12}-desaturase is subjected to less stringent controls than the Δ^{9}-desaturase.

BIOLOGICAL ACTIONS AND POSSIBLE USES OF VITAMIN E. C. Galli and A. Socini (Inst. of Pharmacology and Pharmacognosy, Univ. of Milan, Via A. Del Sarto 21, Milan (Italy)) *Acta Vitaminol. Enzymol. 4*(3):245-252 (1982). Symptoms of Vitamin E deficiency can be generally attributed to derangement of processes depending upon the integrity of cellular and subcellular membranes, following the formation of tissue-damaging products of lipid peroxidation. Antioxidants modulate also the formation of products derived from long-chain polyunsaturated fatty acids, such as arachidonic acid – which are structural components of biological membranes – through oxidative reactions involving the cyclooxygenase and lipoxygenase systems. Vitamin E inhibits the aggregatory responses of blood platelets to aggregating agents (in vitro), after a preincubation period required for the uptake of the compound by the cells. The antiaggregatory activity of α-tocopherol, however, does not appear to be strictly dependent upon inhibition of the formation of thromboxane, the proaggregatory compound derived from arachidonic acid through be cyclooxygenase system. The effects of Vitamin E on platelet function may be of relevance in the control of thromboemolic processes of clinical importance.

PALMITATE OXIDATION BY INTACT PREPARATIONS OF SKELETAL MUSCLE. J.F.C. Glatz and J.H. Veerkamp (Dept. of Biochem., Univ. of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen The Netherlands) *Biochim. Biophys. Acta 713*(2):230-239 (1982). 1. The palmitate oxidation by intact preparations of rat hemidiaphragm, m.soleus and m.flexor digitorum brevis and by teased fibers of human m.pectoralis was studied. 2. The structural and metabolic viability of the in vitro preparations was shown by a low leakage of soluble creatin kinase, a constant rate of palmitate oxidation and only a small stimulatory effect of L-carnitine. 3. With hemidiaphragm the palmitate oxidation rate increases with both the palmitate concentration (0-3 mM) and the palmitate/albumin molar ratio (0.5-5.0). 4. The apparent K_m for palmitate oxidation was about 1.5 mM at 0.1 and 0.2 mM albumin and about 2.7 mM at 0.4 and 0.6 mM albumin, which correlates with the higher affinity of albumin for palmitate at lower palmitate/albumin molar ratios. 5. After prolonged starvation in the apparent K_m at 0.4 mM albumin is markedly lower. In whole homogenates of diaphragm the apparent K_m at 0.4 mM albumin is only about 370 μM. 6. The calculated maximal oxidation rate was not significantly different for all albumin concentrations examined (23-32 nmol/min per g), did not change after starvation and appears to be of the same order of magnitude as the rate of endogenous fatty acid consumption (30-40 nmol/min per g). 7. Results suggest that substrate availability is a main factor for the oxidation rate of exogenous palmitate by hemidiaphragm in vitro and that the magnitude of the apparent K_m is largely dependent upon the degree of label dilution with fatty acids of endogenous origin.

SOYBEAN PROTEIN INDEPENDENTLY LOWERS PLASMA CHOLESTEROL LEVELS IN PRIMARY HYPERCHOLESTEROLEMIA. A.P. Goldberg, A. Lim, J.B. Kolar, J.J. Grundhauser, F.H. Steinke, and G. Schonfeld (Lipid Res. Center, Dept. Prev. Med. and Med., Washington Univ. Schl. of Med., St. Louis, MO) *Atherosclerosis 43*(2,3):355-368 (1982). The effects of isocaloric substitution of soy for animal protein on plasma lipoprotein lipid and apoprotein levels was examined in 12 outpatients with primary hypercholesterolemia and 4 normals. The soy (LDL) cholesterol and apoprotein B levels in the hypercholesterolemic patients were significantly below levels achieved on the basal diets. Since the two experimental diets differed only in their protein constituents, the independent effect of soy protein on plasma lipid and apoprotein concentrations could be determined. We conclude from our results that diets containing soy proteins lower plasma LDL cholesterol more effectively in patients with hypercholesterolemia than do diets containing animal proteins.

ESSENTIAL FATTY ACID DEFICIENCY AFTER HEPATIC PORTOENTEROSTOMY FOR BILIARY ATRESIA. G.R. Gourley, P.M. Farrel, G.B. Odell (Univ. of Wisconsin Schl. of Med., Dept. of Pediatrics, Medison, WI) *Am. J. Clin. Nutr. 36*(6):1194-1199 (1982). Gas chromatography was used to determine the fatty acid composition of total lipids extracted from plasma and erythrocytes of five patients who had received an hepatic portoenterostomy for treatment of extrahepatic biliary atresia. Three patients, including one with successful surgery, demonstrated evidence of essential fatty acid deficiency, including decreased levels of linoleic and arachidonic acids with concomitant increases in palmitoleic and oleic acids. In two of these patients, the ratio of 5,8,11-eicosatrienoic acid to arachidonic acid ("triene/tetraene") exceeded 0.3, diagnostic of essential fatty acid deficiency. Even patients with successful hepatic portoenterostomy are at risk to develop essential fatty acid deficiency.

PROFILES OF PROSTAGLANDIN METABOLITES IN THE HUMAN CIRCULATION. IDENTIFICATION OF LATE-APPEARING, LONG-LIVED PRODUCTS. E. Granstrom, H. Kindahl, and M-L. Swahn (Dept. of Physiological Chemistry, Karolinski Institute, S-104 01 Stockholm, Sweden) *Biochim. Biophys. Acta 713*(1):46-60 (1982). The pattern of metabolites appearing in the circulation after intravenous injection of [9β-^{3}H] prostaglandin $F_{2\alpha}$ was investigated in the human. Analysis of profiles of products was performed by two-dimensional TLC and autoradiography. Identification of labeled metabolites was accomplished by comparing their chromatographic behavior with reference compounds in several chromatographic systems. After injection of [9β-^{3}H] prostaglandin $F_{2\alpha}$ the initially formed metabolite was 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$. However, this compound only dominated the spectrum of metabolites during the first few minutes, and several more polar products soon appeared. About 20 min after the injection the most prominent metabolite was 5α,7α-dihydroxy-11-ketotetranorprostane-1,16-dioic acid, which remained the dominating plasma compound and was also the major metabolite in urine. Several other highly oxidized products were also identified in plasma. Also these metabolites appeared later and remained longer in the circulation than the initially formed 15-ketodihydro metabolite. Our findings suggested that the more degraded metabolites might serve as more reliable plasma parameters for monitoring prostaglandin production than the traditional parameter, 15-ketodihydroprostaglandin $F_{2\alpha}$. This hypothesis was supported by radioimmunoassay of metabolite levels in plasma appearing after either exogenous (intravenous administration) or endogenous prostaglandin $F_{2\alpha}$ (late human pregnancy and parturition). In all cases studied, the tetranor metabolites remained elevated in the circulation for several hours, in contrast to their precursor, 15-ketodihydroprostaglandin $F_{2\alpha}$, which disappeared rapidly.

DIETARY PHOSPHATE DEPRIVATION INCREASES 1,25-DIHYDROXYVITAMIN D_3 SYNTHESIS IN RAT KIDNEY IN VITRO. R.W. Gray and J.L. Napoli (Depts. of Med. and Biochem. and the Clinical Res. Center, Med. College of Wisconsin, Milwaukee, WI 53225) *J. Biol. Chem. 258*(2):1152-1155 (1983). A sensitive radioreceptor assay has been used to measure in vitro 1,25-dihydroxyvitamin D_3 ($1,25\text{-}(OH)_2D_3$) synthesis in vitamin D-replete rats. Incubation of kidney cortical slices with 25-hydroxyvitamin D_3 produced a product which co-migrated on high performance liquid chromatography with authentic $1,25(OH)_2D_3$ in two different solvent systems and displaced $1,25(OH)_2D_3$ from its intestinal receptor. In addition, mass spectral analysis of the product produced a mass fragmentation consistent with that of authentic $1,25(OH)_2D_3$. Endogenous renal cortical $1,25(OH)_2D_3$ pmol/g (n=11), which was significantly greater than the renal cortical $1,25(OH)_2D_3$ content of age-matched rats eating a normal diet which average 0.44 ± 0.21 pmol/g (n=8, $< p$ 0.001). After incubation, net $1,25(OH)_2D_3$ synthesis in renal slices from phosphate-deprived rats averaged 51 pmol/g/h, about 13-fold greater than the mean of 3.8 pmol/g/h observed in renal slices from rats eating the normal diet. These results indicate that the elevated plasma $1,25(OH)_2D_3$ levels observed in rats during dietary phosphate deprivation are due to increased renal synthesis of the hormone.

SEPARATE MONOACYLGLYCEROL AND DIACYLGLYCEROL ACYLTRANSFERASES FUNCTION IN INTESTINAL TRIACYLGLYCEROL SYNTHESIS. M.R. Grigor and R.M. Bell (Dept. of Biochem., Univ. of Otago, Dunedin, New Zealand) *Biochim. Biophys. Acta 712*(3):464-472 (1982). The monoacylglycerol and diacylglycerol acyltransferases in microsomes from the rat small intestinal epithelium have been examined in order to: (1) differentiate between the monoacylglycerol acyltransferase and diacylglycerol acyltransferase and (2) establish whether there is more than one diacylglycerol acyltransferase involved in the two pathways of triacylglycerol synthesis. Assays were established for the monoacylglycerol acyltransferase using *sn*-2-monoolein as substrate and for the diacylglycerol acyltransferase using three different substrates: exogenous *sn*-2-diolein; diacylglycerol synthesized endogenously from monoolein; or diacylglycerol synthesized endogenously from glycerol phosphate as substrate. Compared to the diacylglycerol acyltransferase, the monoacylglycerol acyltransferase was distributed widely throughout the intestine and was insensitive to inhibition by mercuric chloride. Exogenous diolein did not inhibit the monoacylglycerol acyltransferase activity. The distribution of the diacylglycerol acyltransferase

activity assayed either with exogenous diolein or diacylglycerol produced from monoolein was similar in different sections of the intestine. The diacylglycerol acyltransferase activity was inhibited by mercuric chloride with all three assays. However, the activity assayed with exogenous substrate was selectively inhibited by dithiothreitol and the non-ionic detergents, Triton X-100 and Tergitol NP-40. It is concluded that these reagents interfere with the association of exogenous diolein with the microsoma membrane and that there is only one diacylglycerol acyltransferase active in the small intestinal epithelium.

ACROLEIN: A POTENT MODULATOR OF LUNG MACROPHAGE ARACHIDONIC ACID METABOLISM. C.C. Grundfest, J. Chang and D. Newcombe (Dept. Env. Health Sci., The Johns Hopkins University, Baltimore, MD 21205) *Biochim. Biophys. Acta 713*(1):149-159 (1982). Resting rat pulmonary alvelolar macrophages exposed to acrolein were stimulated to synthesize and release thromboxane B_2 and prostaglandin E_2 in a dose-dependent manner. Our results suggest that acrolein selectively inhibited the enzyme, prostaglandin endoperoxide E isomerase, necessary for the conversion of the endoperoxide to prostaglandin E_2. Sulhydryl reagents such as *N*-ethylmaleimide and 5,5'-dithiobis (2-nitrobenzoic acid) mimicked acrolein's effects, and reduced glutathione afforded protection against th effects of acrolein. These results indicated the possible involvement of acrolein's sulhydryl reactivity in the inhibition of the isomerase enzyme. Propionaldehyde had no effect on macrophage arachidonic acid metabolsim whereas crotonaldehyde mimicked the effects of acrolein. Pulmonary macrophages were unable to reverse the acrolein effects on arachidonate metabolite synthesis after 6 hr in an acrolein-free environment. These data indicated the necessity of the unsaturated carbon bond for the acrolein effects on arachidonic acid metabolism and the relative irreversibility of acrolein's reaction with the macrophage.

ENDOTHELIAL MORPHOLOGY AND PLASMA TOTAL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL CHANGES IN HYPOTHALAMICALLY STIMULATED SQUIRREL MONKEY FED A MODIFIED ATHEROGENIC DIET. W.H. Gutstein, P. Andversa, L. Korcek, J.E. Harrison, G.K. Turi, G. Kiu (Dept. of Pathol., New York Med. Coll., Valhalla, NY 10595) *Atherosclerosis 41*(1): 41-51 (1982). Experimental animals fed atherogenic diets show endothelial damage, impairment of endothelial regeneration and plasma lipid changes characterized by elevation of LDL and decrease of HDL cholesterol concentrations. Previous studies in this laboratory disclosed that chronic electrical stimulation of the lateral hypothalamus was associated with electron-microscopic evidence of endothelial injury in rats and squirrel monkeys maintained on basal (low fat/cholesterol-free) diets. In the present investigation squirrel monkeys fed similar diets supplemented with "modest" amounts of caloric fat and cholesterol were subjected to chronic lateral hypothalamic stimulation for periods as long as 20 months with the expectation that endothelial injury would be greater than in the absence of the supplements. These expectations were not substantiated. Endothelium was found to be surprisingly intact by electron microscopy and similar to that of implanted nonstimulated controls. A further observation of interest was the cholesterolemic response, notably in the HDL fraction, observed in both groups, but more striking in experimental animals. The data suggest that an interaction between a modified lipid/cholesterol diet and hypothalamic stimulation may lead to elevation of plasma HDL cholesterol concentration and preservation of endothelial integrity. Further investigation is required to determine whether these two events are causally related.

PLASMA TOCOPHEROL LEVELS AND VITAMIN E/β-LIPOPROTEIN RELATIONSHIPS DURING PREGNANCY AND IN CORD BLOOD. P. Haga, J. Ek, and S. Kran (Pediatric Res. Inst., Natl. Hospital of Norway, and Dept. of Pediatrics, Ullevål Hospital, Oslo, Norway) *Am. J. Clin. Nutr. 36*(6):1200-1204 (1982). Plasma tocopherol, β-lipoprotein concentrations, and tocopherol/β-lipoprotein ratios were studied during 40 normal pregnancies. The levels in 36 cord blood samples from the newborn of these pregnancies and in 25 normal nonpregnant women were also determined. In agreement with earlier studies plasma tocopherol levels rose gradually and significantly during pregnancy, which the levels in cord blood were much lower. β-Lipoprotein concentrations showed similar changes as for tocopherol, rendering the tocopherol/β-lipoprotein ratio unchanged during gestation. The ratios in cord blood and in nonpregnant women were similar to those of pregnant women. A significant positive correlation ($r=0.84$, $p < 0.001$) was found between tocopherol and β-lipoprotein concentrations. The results indicate that the increased plasma tocopherol levels during pregnancy and the low levels in cord blood result from differences in plasma transport capacity.

LIPID ALLEVIATES FATTY LIVER HEMORRHAGIC SYNDROME. F. Haghighi-Rad and D. Polin (Department of Animal Science, Michigan State University, East Lansing, MI 48824) *Poultry Sci. 61*(12):2465-2472 (1982). At 135% of control, Single Comb White Leghorn laying hens were force fed for 3 weeks, diets based on corn or wheat, the latter made isocaloric to the corn-based diet with either corn oil, corn starch, or wheat starch. The hens fed *ad libitum* received a corn-based diet. Force feeding the corn-based diet produced fatty liver hemorrhagic syndrome (FLHS) with scores averaging 3.3 where 1 = no hemorrhages and 5 = 25 or more hemorrhagic points per liver. Force feeding the wheat-based diet with corn starch, wheat starch, or corn oil resulted in scores of 3.1, 2.7, and 1.9, respectively. Only the latter score was significantly different from the score produced by force feeding the corn-based diet, which when fed *ad libitum* resulted in an average score of 1.3. Based on these data and the criteria of retained energy, weight gain, percent fat in liver, and plasma estradiol concentrations, we concluded that wheat-based diets with corn- or wheat-starch produce FLHS equivalent to that caused by diets based on corn. Lipid at 4% of the diet had an alleviating effect on FLHS.

ARTERIAL NEUTRAL CHOLESTERYL ESTERASE. D.P. Hajjar, C.R. Minick, and S. Fowler (Dept. of Biochem. and Pathology, Cornell Univ. Med. College and the Lab. of Biochem. Cytology, The Rockefeller Univ. of New York, New York 10021) *J. Biol. Chem. 258*(1):192-198 (1983). We describe here an activable neutral cholesteryl esterase (EC 3.1.1.13) in arteries similar to the hormone-sensitive lipase of adipose tissue and adrenal cortex. Maximum enzyme activity in rabbit aorta was given by cholesteryl ester substrates disperses as a mixed micelle with phosphatidylcholine and Na taurocholate (molar ratio 1:4:2). A quantitative assay of enzymic activity was obtained with the following component concentrations: 6.0 μM cholesteryl [1-^{14}C] oleate, 23.7 μM phosphatidylcholine, 12.5 μM Na tuarocholate, .04% serum albumin, and 85 mM K phosphate buffer, pH 7.0. The enzymic activity in aortic homogenates was stimulated 2-fold by addition of 5 μM glucagon or 100 μM dibutyryl cAMP. This activation was Mg-ATP dependent. Addition of 50 μg/ml of exogenous protein kinase could reverse the action of protein kinase inhibitor on dibutyryl cAMP activation of the neutral cholesteryl esterase. In addition to activation by cAMP-dependent protein kinase, the enzyme could be distinguished from the more active arterial lysosomal cholesteryl esterase by its pH 7.0 optimum, relative stability to preincubation at elevated temperatures, and exclusive localization in the cell cytosol. Subcellular fractionation of lipid-laden arterial foam cells revealed a significant portion of the neutral cholesteryl esterase bound to cytoplasmic cholesteryl ester-rich lipid droplets. Our results suggest that the breakdown of cytoplasmic cholesteryl ester droplets in arterial cells may be under hormonal regulation.

THE EFFECT OF ENZYME THERAPY ON PLASMA LIPID LEVELS IN THE ELDERLY. D.A. Hall, A.R. Zajac, R. Cox, and J. Spanswick (Dept. of Med., Univ. of Leeds, General Infirmary, Leeds LS1 3EX, Great Britain) *Atherosclerosis 43*(2,3):209-215 (1982). (1) An enzyme preparation, Vasolastine, administered intramuscularly, to a group of elderly subjects (mean age 81.7) reduced fasting plasma cholesterol and triglyceride levels by amounts which were proportional to their initial concentration. (2) Triglyceride levels 3 hr after a fat-rich meal were also directly dependent on the initial fasting level. This relationship is lowered significantly after 25 days of Vasolastine treatment. (3) It is suggested that these observations, taken in conjunction with the results of in vitro studies, confirm that Vasolastine acts synergistically with endogenous lipoprotein lipase.

A SATURABLE, HIGH-AFFINITY BINDING SITE FOR HUMAN LOW DENSITY LIPOPROTEIN ON FRESHLY ISOLATED RAT HEPATOCYTES. L. Harkes and T.J.C. Van Berkel (Dept. of Biochem. I, Faculty of Med., Erasmus Univ., P.O. Box 1738, 3000 DR Rotterdam, The Netherlands) *Biochim. Biophys. Acta 712*(3):677-683 (1982). Freshly isolated rat hepatocytes bind the solely apolipoprotein B-containing human low density lipoprotein (LDL) with a highly-affinity component. After 1 hr of incubation less than 30% of the cell-associated human LDL is internalized and no evidence for any subsequent high-affinity degradation was obtained. Scatchard analysis of the binding data for human ^{125}I-labeled LDL indicates that the high-affinity receptor for human LDL on rat hepatocytes possesses a K_d of $2.6 \cdot 10^{-8}$ M, while the binding is dependent on the extracellular Ca^{2+} concentration. Competition experiments indicate that both the apolipoprotein B-containing lipoproteins (human LDL and rat LDL) as well as the apolipoprotein E-containing lipoproteins (human HDL and rat HDL) do compete for the same surface receptor. It is concluded that hepatocytes freshly isolated from untreated

rats do contain, in addition to the earlier described rat lipoprotein receptor which does not interact with human apolipoprotein B-containing LDL, a high-affinity receptor which interacts both with solely apolipoprotein B-containing human LDL and apolipoprotein E-containing lipoproteins.

CHARACTERIZATION AND PURIFICATION OF FATTY ACID-BINDING PROTEIN IN RAT AND HUMAN ADIPOSE TISSUE. R-U. Haq, L. Christodoulides, B. Ketterer, and E. Shrago (Dept. of Med. and Nutr. Sci., Univ. of Wisconsin, Madison, WI 53706 U.S.A. and Courtauld Inst. of Biochem., Middlesex Hospital Med. Schl., London U.K.) *Biochim. Biophys. Acta 713*(2):193-198 (1982). A protein with properties similar to fatty acid-binding protein has been isolated from rat and human adipose tissue. Comparison of fatty acid-binding protein from rat liver and adipose tissue and human adipose tissue shows that all have approximately similar molecular weights. Immunologically, rat liver fatty acid-binding protein is similar to the protein characterized from rat adipose tissue. In isolated rat fat cells the fatty acid-binding protein was demonstrated to be involved in the uptake and esterification of long-chain fatty acids. These observations constitute evidence for a potential role of this protein in the fatty acid metabolism of adipocytes.

ENHANCEMENT OF THE 7α-DEHYDROXYLASE ACTIVITY OF GRAM-POSITIVE INTESTINAL ANAEROBE BY *BACTERIODES* AND ITS SIGNIFICANCE IN THE 7-DEHYDROXYLATION OF URSODEOXYCHOLIC ACID. S. Hirano and N. Masuda (Dept. of Bacteriology, Faculty of Med., Kagoshima Univ., 1208 Usuki, Kagoshima, 890, Japan) *J. Lipid Res. 23*(8):1152-1158 (1982). The 7α-dehydroxylation of chenodeoxycholic acid (CDCA) and cholic acid (CA) by a *Eubacterium lentum*-like intestinal anaerobe was specifically enhanced by the bacteroides present in mixed cultures and also by the addition to the growth medium of cell extracts from the bacteroides. The 7α-dehydroxylating organism also possessed 7α-hydroxysteroid dehydrogenase activity, and, in collaboration with a 7β-dehydrogenating organism, converted ursodeoxycholic acid (UDCA) into CDCA. Large quantities of lithocholic acids were produced from UDCA as well as CDCA in in vitro cocultures of these three kinds of microorganisms.

CORONARY ARTERY DISEASE IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA. K. Hirobe, Y. Matsuzawa, K. Ishikawa, S. Tarui, A. Yamamoto, S. Nambu and K. Fujimoto (Second Dept. of Internal Med., Osaka Univ. Med. Schl., Res. Inst. Osaka (Japan)) *Atherosclerosis 44*(2):201-210 (1982). Serum lipids, lipoproteins and Achilles tendon thickness in 52 patients with heterozygous familial hypercholesterolemia (FH) were investigated in order to clarify what are the important factors for the development of coronary artery disease (CAD) in heterozygous FH patients. There were no significant differences in the average concentration of total cholesterol and triglyceride between the patients with and those without CAD. The HDL cholesterol (HDL-C) level was significantly lower in patients with CAD than in those without, and the HDL-C value was within the normal range in most of the patients with heterozygous FH, if not associated with CAD. Although most of the males aged over 50 years had CAD and a decreased level of HDL-C, many of the aged females were without signs of CAD. The HDL-C value of heterozygous FH patients with CAD was significantly lower compared with the age-matched group without CAD. The Achilles tendon was thicker in patients with CAD than in those without CAD, both for males and females, although it was less closely correlated with the incidence of CAD than HDL-C or the atherogenic index. A forecast concerning the development of CAD in heterozygous FH may be possible if we consider multiple parameters, such as HDL-C, atherogenic index, Achilles tendon thickness, etc.

TRANSBILAYER REDISTRIBUTION OF PHOSPHATIDYLETHANOLAMINE DURING FUSION OF PHOSPHOLIPID VESICLES. DEPENDENCE ON FUSION RATE, LIPID PHASE SEPARATION, AND FORMATION, AND FORMATION OF NONBILAYER STRUCTURES. D. Hockstra and O. Martin (Dept. Embryol., Carnegie, Inst. of Wash., Baltimore, MA 21210) *Biochemistry 21*(24):6097-6103 (1982). The effect of membrane fusion on the transbilayer distribution of dioleoyl- and dipalmitoylphosphatidylethanolamine (DOPE and DPPE) in phosphatidylserine (PS) vesicles was investigated. A 7-fold increase in the external pool of DOPE, determined by labeling the vesicle surface with 2,4,6-trinitrobenzenesulfonic acid, was observed when multilamellar vesicles (MLV) consisting of PS and DOPE were incubated with small unilamellar vesicles (SUV) of PS in the presence of Ca^{2+}, but no significant redistribution of DPPE was seen when similar experiments were performed by using PS bilayers that contained DPPE instead of DOPE. Redistribution of neither DOPF nor DPPE could be detected during SUV-SUV fusion. Using the resonance energy transfer fusion assay for mixing membrane lipids, it was demonstrated that fusion between SUV and MLV had occurred. The results suggested that (partial) fusion of internal bilayers within the multilamellar system occurred. Although Mg^{2+}-induced fusion between SUV and MLV was observed, no redistribution of DOPE was seen. The observed translocation of DOPE during fusion was probably mediated via inverted micellar structures, which were formed when the lipid was converted to the hexagonal (H_{II}) phase resulting from lipid phase separation between PS and DOPE. Induction of the hexagonal phase in the absence of fusion did not cause substantial transbilayer redistribution of DOPE, suggesting fusion was intimately involved. We suggest that fusion represents the "driving force" for transbilayer DOPE redistribution, requiring a (partial) overlap between the kinetics of phase separation and fusion.

ACCUMULATION OF LIPOPROTEINS CONTAINING ApoB IN THE AORTA OF CHOLESTEROL-FED CYNOMOLGUS MONKEYS. H.F. Hoff and M.G. Bond (Dept. Atherosclerosis and Thrombosis, Res. Div., Cleveland Clinic Found., Celveland, OH 44106) *Atherosclerosis 43*(2,3):329-339 (1982). Accumulation of lipoproteins containing apoB in the aortic arch of cholesterol-fed cynomolgus monkeys was determined using an electroimmunoassay (EIA) to quantitate apoB. A loosely-bound fraction of apoB was measured. A tightly-bound apoB fraction was measured. These two fractions of apo B were determined in aortas of 7 animals fed an atherogenic diet for 24 months, 4 animals fed a control diet for 24 months, 4 animals fed an atherogenic diet for 30 months, and 2 animals fed a control chow. The apoB values were compared to plasma cholesterol and HDL cholesterol concentrations. The extent and severity of atherosclerosis in the aortic arch was estimated. Our results suggest that the diet-induced hypercholesterolemia in cynomolgus monkeys results in accumulation of lipoproteins containing apoB (probably LDL) in the aorta in both loosely- and tightly-bound forms, and that total lipoprotein accumulation increases with further time on this diet, probably as a result of further development of atherosclerotic lesions.

HIGH-DENSITY LIPOPROTEIN-CHOLESTEROL AND DIET IN A HEALTHY ELDERLY POPULATION. P.L. Hooper, P.J. Gary, J.S. Goodwin, E.M. Hooper, A.G. Leonard (Departments of Pathology and Medicine, University of New Mexico School of Medicine, and Veterans Administration Medical Center, Albuquerque, NM) *J. Am. College Nutr. 1*(4):337-343 (1982). This study examined how high-density lipoprotein-cholesterol (HDL-C) correlated with a 3-day food record of fat, protein, carbohydrate, and alcohol consumption in a group of 270 healthy subjects over age 60. HDL-C concentrations correlated with alcohol consumption (expressed as grams/day) ($r = +.25$, $P < .001$), and inversely with total carbohydrate ($r = -.18$, $P < .01$) and refined carbohydrate ($r = -.17$, $P < .01$) ingestion (expressed as a percent of total caloric intake). Subjects consuming diets low in either total carbohydrate or refined carbohydrate had 10 to 20% higher HDL-C levels that did those consuming diets high in these food substances. The relationships between HDL-C levels and alcohol and carbohydrate ingestion were independent of other variables which correlated with HDL-C levels. Dietary fat (total fat, saturated fat, unsaturated fat, and cholesterol) did not correlate with HDL-C. LDL-cholesterol and triglyceride levels did not correlate with any dietary variable measured.

THE EFFECT OF EXCESS (ACYL)CARNITINE ON LIPID METABOLISM IN RAT HEART. W.C. Hülsmann, H. Stam, and F. Maccari (Department of Biochemistry I, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands) *Biochim. Biophys. Acta 713*(1):39-45 (1982). During Langendorff perfusion of rat hearts with Intralipid, the resulting fat accumulation in the hearts can be inhibited by the addition of 5 mM L-carnitine to the perfusion medium. The mechanism of this phenomenon is probably the inhibition of lipid accumulation in the heart by acylcarnitine rather than stimulation of fatty acid oxidation by excess carnitine addition. Palmitoylcarnitine was found to stimulate trioleoylglycerol hydrolysis at neutral pH in heart homogenates, when it was tested in the presence of relatively much protein. At higher palmitoylcarnitine:protein ratios, however, lipolysis was inhibited. Inhibition of lipolysis was also observed in lipid-enriched hearts during retrograde perfusion by the addition of 5 mM carnitine suggesting that also in intact heart long-chain acylcarnitine excess may inhibit lipolytic activity.

EFFECT OF RANCIDITY ON THE FEEDING VALUE OF RICE BRAN FOR CHICKENS. A.S. Hussein and F.H. Kratzer (Department of Avian Sciences, University of California, Davis, CA 95616) *Poultry Sci. 61*(12):2450-2455 (1982). Rice bran that was allowed to become rancid and was fed to chicks at 60% of the diet reduced growth more than fresh rice bran or a stored sample in which rancidity was retarded by the addition of ethylenediaminetetraacetate (EDTA). The EDTA had no effect on the rice bran that was already

rancid or in which the lipase activity had been destroyed by autoclaving. There was no significant difference in the taste of the thigh or skin samples of fryers fed stock mash or the diets in which the rice bran was fresh, rancid, or stored with EDTA added. Thiobarbituric acid reactive compounds were also similar in the fat of the skin and adipose tissue of these birds. Metabolizable energy values of fresh and rancid rice bran were similar as determined with adult male chickens.

DEGRADATION OF HUMAN MYELIN PHOSPHOLIPIDS BY PHOSPHOLIPASE-ENRICHED CULTURE MEDIA OF PATHOGENIC *NAEGLERIA FOWLERI*. R.M. Hysmith and R.C. Franson (Dept. of Pathology and Biochem., Med. College of Virginia, Virginia Commonwealth Univ., Richmond, VA 23298) *Biochim. Biophys. Acta 712*(3):698-701 (1982). Cell-free media from cultures of virulent *Naegleria fowleri* were tested for phospholipase activities and their ability to degrade phospholipids of human myelin. Virulent *N. folweri* selectively released lipolytic enzymes into the media at various times during growth and hydrolyzed the phospholipids of human myelin, while media from virulent-attenuated and nonpathogenic *Naegleria* spp. were almost totally inactive. Hydrolysis of myelin phospholipid increased concomitantly with amebal growth, and the relative rate of breakdown at pH 7.5 was sphingomyelin > phosphatidylcholine > phosphatidylethanolamine. Elevated levels of lysophosphatidylcholine and lysophosphatidylethanolamine were also noted.

COMPARISON OF RAT HEPATIC CHOLESTEROL BIOSYNTHESIS DURING SKIM MILK VERSUS WHEY PERMEATE INGESTION. N.L. Keim, J.A. Marlett, C.H. Amundson, and L.D. Hagemann (Dept. of Nutr. Sci., Univ. of Wisconsin, Madison, WI 53706) *J. Dairy Sci 65*(12):2274-2280 (1982). Whey permeate is an ultrafiltrate of whey that is devoid of protein but contains lactose, salts, and other soluble low molecular weight compounds. These experiments compared cholesterol concentrations of blood plasma, hepatic lipids, and hepatic cholesterol biosynthesis of rats ingesting skim milk powder versus whey permeat powder. Groups of young male rats weighing 90 to 92 g were fed a casein-based diet into which skim milk powder or whey permeate powder was incorporated isocalorically. No effects of skim milk or whey permeate on plasma cholesterol concentrations were observed at any time during 5-wk of feeding. However, 3-hydroxy-3-methylglutaryl co-enzyme A reductase activity was increased by either skim milk or whey permeate feeding. Hepatic cholesterol, triglyceride, and phospholipid concentrations at wk 5 were unchanged. Plasma and hepatic cholesterol responses of rats to whey permeate ingestion are similar to those that occur with skim milk consumption, and plasma and hepatic cholesterol concentrations do not reflect necessarily an increase in hepatic cholesterol biosynthesis.

CIPROFIBRATE IN THE THERAPY OF TYPE II HYPERCHOLESTEROLEMIA A DOUBLE-BLIND TRIAL. D.R. Illingworth, G.D. Olsen, S.F. Cook, G.J. Sexton, H.A. Wendel, and W.E. Connor (Div. of Endocrinology-Metabolism-Clinical Nutr., Dept. of Med. and Dept. of Pharmacology, Oregon Health Sci. Univ., Portland, OR 97201) *Atherosclerosis 44*(2):211-221 (1982). The hypolipidemic efficacy of ciprofibrate was evaluated in patients with type II hypercholesterolemia. Patients were randomized to placebo or ciprofibrate (50 mg or 100 mg/day) and, after a 6-week baseline period, received medication for a period of 12 weeks. Blood samples were analyzed every 2 weeks. Twenty patients completed the study (4 on placebo, 7 on 50 mg/day, and 9 on 100 mg/day ciprofibrate). The drug was well tolerated in all patients. Lipid values in the patients on active drug decreased and attained stable values after 4 weeks of treatment. Compared to baseline values, total and LDL cholesterol decreased 11% and 13% on the 50-mg baseline values, total and LDL cholesterol decreased 11% and 13% on the 50-mg dose whereas HDL increased 8%. Plasma triglyceride fell by 22%. In patients receiving 100 mg ciprofibrate, total and LDL cholesterol fell by 20% (334 → 269 mg/dl) and 24 (262 → 198 mg/dl), respectively. HDL increased 9.8% (51 → 56 mg/dl) and triglyceride decreased by 30% (102 → 69 mg/dl). Values in the placebo group remained stable. We conclude that once daily therapy with 100 mg ciprofibrate is effective in reducing LDL levels in patients with type II hypercholesterolemia (mainly heterozygous FH) and that this decrease is paralleled by small rises in HDL.

ACTIVATION OF CATALASE AND OTHER ENZYMES BY CORN OIL INTAKE. N. Iritani and Y. Ikeda (Tezukayama Gakuin College, Sumiyoshi-ku, Osaka 558, Japan) *J. Nutr. 112*(12):2235-2239 (1982). The effects of linoleic acid intake on catalase and other enzymes were investigated by feeding 0, 1, 5, or 10% corn oil diet to rats previously fed a fat-free diet. Rats fed more than 1% corn oil for 2 weeks showed significant increases of glutathione peroxidase and superoxide dismutase in liver cytosol when compared to the controls fed no corn oil. Peroxisomal catalase activity especially was increased. The catalase activity was markedly increased also by ethyl linoleate intubation. Thus, it was demonstrated that the peroxide elimination mechanisms were activated by linoleic acid intake. The elevation of peroxisomal catalase by linoleic acid intake might be related to hypolipidemic effect, similar to the possible relation between peroxisome induction and the hypolipidemic effect of many hypolipidemic compounds, which has been reported.

INDUCTION OF CHOLINE KINASE BY POLYCYCLIC AROMATIC HYDROCARBONS IN RAT LIVER. I. A COMPARISON OF CHOLINE KINASES FROM NORMAL AND 3-METHYLCHOLANTHRENE-INDUCED RAT LIVER CYTOSOL. K. Ishidate, M. Kihara, E. Tadokoro and Y. Nakazawa (Med. Res. Inst., Tokyo Med. and Dental Univ., 2-3-10 Kandasurugadai, Chiyoda-ku, Tokyo 101 (Japan)) *Biochim. Biophys. Acta 713*(1):94-102 (1982). Choline kinase in rat liver has been shown to be induced up to 2-fold by the administration of polycyclic aromatic hydrocarbon carcinogens such as 3-methylcholanthrene and 3,4-benzo(*a*)pyrene. In order to characterize the nature of choline kinase induction by these carcinogens, the 3-methylcholanthrene-induced form as well as the normal form of choline kinase were partially purified from rat liver cytosol through acid treatment, $(NH_4)_2SO_4$ precipitation and DEAE-cellulose column chromotography with linear KCL-gradient elution, and the catalytic properties were compared between the two preparations. Both enzyme activities were purified about 17-fold and there appeared no detectable difference in the elution pattern from either DEAE-cellulose column of Sephadex G-200 gel filtration. However, some differences were observed in catalytic properties between the two enzyme preparations; (1) the induced form showed a higher apparent K_m value for choline when compared to the normal form and (2) the addition of polyamines caused a considerable increase in the maximum reaction velocity for the normal form whereas no remarkable change for the induced form, when the activities were plotted as a function of choline concentration. The results suggest that the 3-methylcholanthrene-induced form of choline kinase in rat liver could be different from the normal form, or that there exist several isoenzymes of choline kinase in rat liver, and one or some of them are inducible by the administration of polycyclic aromatic hydrocarbons.

INDUCTION OF CHOLINE KINASE BY POLYCYCLIC AROMATIC HYDROCARBONS IN RAT LIVER. II. ITS RELATION TO NET PHOSPHATIDYLCHOLINE BIOSYNTHESIS. K. Ishidata, M. Tsuruoka and Y. Nakazawa (Med. Res. Inst. Tokyo Med. and Dental Univ., 2-3-10 Kandasurugadai, Chiyoda-ku, Tokyo 101 (Japan)) *Biochim. Biophys. Acta 713*(1):103-111 (1982). The effect of a single dose of 3-methylcholanthrene on de novo phosphatidylcholine biosynthetic activities in rat liver was studied both in a cell-free system and with slice experiments. 3-methylcholanthrene caused a significant depression of either [methyl-^{14}C] choline or [2-^{3}H] glycerol incorporation into phosphatidylcholine when the precursor was incubated with liver slices. At the same time, there occurred a significant accumulation of radioactivity in either cholinephosphate or diacylglycerol molecule from [^{14}C] choline or [^{3}H] glycerol, respectively, suggesting that 3-methylcholanthrene could cause an inhibitory effect on hepatic phosphatidylcholine synthesis at the cholinephosphotransferase or/and cholinephosphate cytidylyltransferase step. Subsequent studies demonstrated that the cholinephosphotransferase step could be the site of inhibition by 3-methylcholanthrene. On the other hand, 3-methylcholanthrene caused a significant induction of choline kinase activity in a time-dependent manner and, at the same time, the cholinephosphate pool size in liver cytosol was enlarged 2-3-fold when compared to the respective control. The overall results suggested strongly that 3-methylcholanthrene causes the counteractive effects on the de novo phosphatidylcholine biosynthesis, induction of choline kinase activity and inhibition of choline-phosphotransferase activity, both of which could participate in a concomitant increase in cholinephosphate pool size in rat liver.

A NEW MODEL FOR ARTERIOSCLEROSIS – AN ELECTRON-MICROSCOPIC STUDY OF THE LESIONS INDUCED BY I.V. ADMINISTERED FAT. H. Jellinek, J. Hársing, and Sz. Füzesi (2nd Dept. of Pathology, Semmelweis Med. Univ., Ü11öi út 93, 1091 Budapest, Hungary) *Artherosclerosis 43*(1):7-18 (1982). Lesions in the arterial wall induced in rats by means of intravenous injection of Lipofundin-S* were studied by electron microscopy. The morphological changes in this model are the enlargement of the subendothelial space, proliferation of smooth muscle and accumulation of basement membrane-like material. These changes are a closer model for the fibro-proliferative aspects of human atherosclerosis than those seen in short-term (2-3 month) cholesterol feeding. Moreover, such

changes are produced in 8 days rather than 2-3 month. These features indicate that the Lipofundin-S model may be of value in testing anti-atherosclerotic drugs.

EFFECT OF INFANT FORMULAS ON BLOOD AND TISSUE CHOLESTEROL, BONE CALCIUM, AND BODY COMPOSITION IN WEANLING PIGS. A.D. Julius, K.D. Wiggers, and M.J. Richard (Dept. of Animal Sci., Nutr. Physiology Section, Iowa State Univ., Ames, IA 50011) *J. Nutr. 112*(12):2240-2249 (1982). Weanling pigs were fed four commercial infant formulas to determine effects on blood and tissue cholesterol parameters and on body composition. Two milk protein (MP)-based formulas and two soy protein isolate (SPI)-based isocaloric formulas were fed in concentrated liquid form for 32 days. A commercial cow's milk replacer fed to a fifth group of pigs served as a control diet. Pigs fed SPI-based formula had significantly less cholesterol in the plasma than did pigs fed MP-based formulas. Whole-body and adipose tissue cholesterol concentrations were greatest in pigs fed formulas containing a relatively high concentration of polyunsaturated fatty acids (PUFA). Liver cholesterol concentration was inversely related to plasma cholesterol concentrations. Bone calcium, measured as percentage of dry, fat-free bone (femur), and whole-carcass ash were significantly less in pigs fed SPI-based formula than in pigs fed MP-based formula. Similar growth and development were observed in pigs fed SPI-based or MP-based formulas; however, pigs fed Spi-based formulas had significantly less bone calcium.

EFFECTS OF DIETARY CARBOHYDRATE AND FAT ON PLASMA LIPOPROTEINS AND APOLIPOPROTEINS C-II AND C-III IN HEALTHY MEN. M.L. Kashyap, R.L. Barnhart, L.S. Srivastava, G. Perisutti, P. Vink, C. Allen, E. Hogg, D. Brady, C.J. Glueck, and R.L. Jackson (Depts. of Med., Pharmacology and Cell Biophys., Biol. Chem., and the General Clinical Res. and CLINFO Centers, Univ. of Cincinnati Med. Center, Cincinnati, OH 45267) *J. Lipid Res. 23*(6):877-886 (1982). Effects of isocaloric changes in dietary fat and carbohydrate on plasma apolipoproteins (apo) C-II, C-III, and lipoproteins were assessed in nine healthy men. After a 1-week basal diet (40% of calories from carbohydrate), the subjects received either a high (65% of calories) or low (15% of calories) carbohydrate diet for 3 weeks; subsequently the diets were switched, those initially on high carbohydrate going on to low carbohydrate, and vice versa, and the new diets were maintained for 3 weeks. ApoC-II, C-III, and triglycerides initially rose and then declined during the high carbohydrate diet period; high density lipoprotein cholesterol (HDL-C) decreased. Comparing results after 3 weeks of high carbohydrate diet to those after 3 weeks on low carbohydrate, we observed the following significant differences: 1) total plasma apoC-II and C-III were higher; the apoC-III/C-II ratio in very low density lipoproteins (VLDL) and in the lighter HDL subfraction (HDL_2) was lower indicating net lipoprotein enrichment with apoC-II than with apoC-III; 2) unsialylated $apoC\text{-}III_0$ comprised a higher percent of total VLDL apoC-III mass; 3) HDL_2 and HDL_2/HDL_3 ratio were lower. Isocaloric changes in dietary carbohydrate and fat cause significant alterations in plasma levels of VLDL and HDL_2, the two major lipoproteins that transport apoC-III and apoC-II. Diet-induced changes in circulating apoC-III and C-II may, in part, play a role in regulation of plasma triglycerides in man.

REDUCTION OF CASEIN-INDUCED HYPERCHOLESTEROLAEMIA AND ATHEROSCLEROSIS IN RABBITS AND RATS BY DIETARY GLYCINE, ARGININE AND ALANINE. M.B. Katan, L.H.M. Vroomen and R.J.J. Hermus (Dept. Human Nutrition, Agricultural Univ., De Dreijen 12, 6703 BC Wageningen, The Netherlands) *Atherosclerosis 43*(2,3):381-391 (1982). We have studied the effect of amino acid supplementation on hypercholesterolaemia and atherosclerosis caused by cholesterol-free casein-containing diets in rabbits, and on the hypercholesterolaemia caused in rats by diets containing casein plus added cholesterol. We conclude that casein-induced hypercholesterolaemia and its sequelae are partly relieved by addition of glycine to the diet, while supplementation of casein-based rations with arginine and/or alanine may be necessary for rabbits. However, the beneficial effects of these amino acids could just as well be explained by the presence of an excess of other amino acids in casein (amino acid imbalance) as by an absolute shortage of glycine, arginine or alanine.

ISOLATION OF AN UNUSUAL "LIPID A" TYPE GLYCOLIPID FROM *PSEUDOMONAS PAUCIMOBILIS*. K. Kawahara, K. Uchida and K. Aida (The Inst. of Applied Microbio., Univ. of Tokyo, Hongo, Tokyo 113, Japan) *Biochim. Biophys. Acta 712*(3):571-575 (1982). A new glycolipid was isolated from defatted cells *Pseudomonas paucimobilis* IAM 12576, and called "bound lipid." The "bound lipid" could not be extracted by hot phenol extraction, but could be extracted with hot chloroform/methanol after hydrolysis with 5% trichloroacetic acid. The "bound lipid" was purified by thin-layer chromatography on silica gel plates using the solvent mixture $CHCL_3/CH_3OH/CH_3COOOH/H_2O$ (25:15:4:2, v/v). It consisted of glucosamine, 2-hydroxy mysristic acid, galactose, mannose and uronic acid with ratios of 1.0:0.75:0.77:0.44:1.5, respectively, and other fatty acids besides 2-hydroxy myristic acid was probably bound to glucosamine residues by amide linkage, because milk alkali treatment did not liberate the fatty acid. From these results, we discussed the possibility that the "bound lipid" was some kind of lipid A of this bacteria.

LOCALIZATION OF RECEPTORS FOR 1,25-DIHYDROXYVITAMIN D_3 ALONG THE RAT NEPHRON. H. Kawashima, and K. Kurokawa (The Nephrology Section, Med. and Res. Services, Veterans Administration Wadsworth Med. Center, Los Angeles, CA 90024) *J. Biol. Chem. 257*(22):13428-13432 (1982). We recently demonstrated that 25-hydroxyvitamin D_3-24-hydroxylase, which can be further induced by 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), is localized exclusively in the proximal nephron of the vitamin D-replete rat kidney. These data and the proposed mode of action of $1,25(OH)_2D_3$ predict the presence of a receptor for $1,25(OH)_2D_3$ in the proximal nephron. However, autoradiographic studies failed to detect significant nuclear uptake of $1,25(OH)_2D_3(^3H)D_3$ in the proximal nephron. To localize and characterize receptors for $1,25(OH)_2D_3$ along the nephron, preparations of both microdissected defined nephron segments and isolated tubules from the whole kidney of vitamin D-deficient rats were incubated with $1,25(OH)_2(^3H)D_3$, and specific uptake of the sterol was assessed in the cytosol preparation of tubular cells sonicated in a hypertonic solution. The preparation of isolated tubules incubated for 1 hr at 37 C accumulated $1,25(OH)_2(^3H)D_3$ with high affinity (K_d = 0.54 nM), and the receptors exhibited a sedimentation constant of 3.7 S in a hypertonic sucrose gradient. The uptake of $1,25(OH)_2(^3H)D_3$ was also examined in three defined single nephron segments: proximal convoluted tubules, medullary thick ascending limb of Henle's loop, and collecting tubules. Both proximal convoluted tubules and medullary thick ascending limb of Henle's loop showed a significant uptake of $1,25(OH)_2(^3H)D_3$, 29.9 ± 8.1 and 20.8 ± 4.5 fmol/1000-mm tubule length, respectively, while collecting tubules showed no significant uptake. The receptors in both segments sedimented at 3.7 S in sucrose gradient. These data demonstrate the presence of $1,25(OH)_2D_3$ receptors in both proximal and distal nephron.

LIPOPROTEIN LIPASE ACTIVITY IN TURKEY AORTA. J.L. Kelley, C-S. Wang, H.B. Bass, R.H. Thayer (Lab. of Lipid and Lipoprotein Studies, Oklahoma Med. Res. Found., Oklahoma City, OK 73104) *Artery 10*(6):379-394 (1982). Lipoprotein lipase activity was measured in heparin extracts of aortic intima and adventitia from normal and cholesterol-fed turkeys. This lipolytic activity showed typical characteristics of lipoprotein lipase i.e., requirement of serum for activity and a 92% inhibition by protamine sulfate. The highest lipoprotein lipase activity was found in the adventitia of the abdominal aorta. Lipoprotein lipase activity was greater in the intima of the thoracic aorta than in the intima of the abdominal aorta. This higher activity of thoracic intima was not correlated with development of fibrous plaques which were found only in abdominal aorta. Cholesterol-feeding resulted in plasma very low density lipoproteins enriched in cholesterol-ester but had no effect on the level of lipoprotein lipase activity of aortic intima. Cholesterol-feeding, although altering lipoprotein composition, did not increase aortic intima lipoprotein lipase activity.

BILE ACIDS AND LIPIDS IN ISOLATED RAT HEPATOCYTES: CONTENT, SYNTHESIS, AND RELEASE, AS EFFECTED BY CHOLESTYRAMINE TREATMENT OF THE DONOR RATS. H.H.M. Kempen, M.P.M. Vos-Van Holstein, and J. de Lange (Gaubius Inst. TNO, Herenstraat 5d, 2313 Ad Leiden, The Netherlands) *J. Lipid Res. 23*(6):823-830 (1982). Contents of bile acids and lipids, as well as rates of triglyceride synthesis, were determined in isolated hepatocytes from control or cholestyramine-fed rats (denoted below as "control" or "treated" hepatocytes, respectively). During a 3-hr incubation period, total bile acid production was markedly higher in "treated" cells than in "control" cells. With both kinds of cells a marked fall in production rate occurred after the first hour of incubation. Newly produced bile acids appeared in the conjugated form with both kinds of hepatocytes. "Control" cells produced only taurine-conjugated, while "treated" cells made both taurine-conjugated and glycine-conjugated bile acids. However, with exogenous taurine (0.5 mM), the latter cells also produced taurine-conjugated bile acids only. With both kinds of cells, cholic and β-muricholic acids, but not dihydroxylated bile acids, appeared as newly formed species during the incubation. Addition of dialyzed rat serum to the incubation did not result in a stimulation of bile acid production, with either kind of hepatocytes. "Treated" cells had a slightly higher content of free

cholesterol than control cells; contents of other lipids were not different. Fractional release of bile acids and lipids into the medium did not differ between the two kinds of cells. Triglyceride synthesis from added [^{14}C] palmitate (0.5 mM) was 1.8-fold higher in "treated" than in "control" hepatocytes.

RESPONSE TO THE RENAL VITAMIN D ENDOCRINE SYSTEM TO OXIDIZED PARATHYROID HORMONE. A.D. Kenney and P.K.T. Pang (Department of Pharmacology and Therapeutics, Texas Tech University Health Service Center, Lubbock, Texas 79430) *Proc. Soc. Exper. Biol. Med. 171*(2):191-195 (1982). Two preparations of bovine parathyroid hormone (bPTH), the natural bPTH (1-84) and the synthetic bPTH (1-34) fragment, were treated with hydrogen peroxide and assayed for the effect of such treatment on the renal vitamin D endocrine system in Japanese quail. The oxidized and untreated preparations were injected intramuscularly into 4-week-old male Japanese quail, 12 hr after which the kidneys were removed and homogenized. The kidney homogenates were incubated with tritiated 25-hydroxyvitamin D_3 [25-(OH)D_3] and the production rates of 1,25-$(OH)_2D_3$ and of 24,25$(OH)_2D_3$ were determined as indices of 25-(OH)-D_3-1-hydroxylase and 25-(OH)D_3-24-hydroxylase activities, respectively. Both untreated bPTH (1-34) and untreated bPTH (1-84) stimulated 1-hydroxylase and suppressed 24-hydroxylase activities. Oxidation of either bPTH (1-34) or bPTH (1-84) did not eliminate these responses. The importance of these findings is heightened when viewed in the context of our previous reports that oxidation of bPTH (1-34) leaves the hypercalcemic and hypocalciuric responses intact while partially or possibly totally inactivating all other major responses studied to date. It may be concluded that the mechanisms involved in effecting the hypercalcemic, hypocalciuric, and renal 25-(OH)D_3-1-hydroxylase responses to bPTH (1-34) demand structural requirements in the peptide molecule which are different from those needed to effect the hyperphosphaturic, hypophosphatemic, hypotensive, smooth muscle relaxing, and renal adenylate cyclase responses.

LOCALIZATION OF GLYCEROPHOSPHATE ACYLTRANSFERASE IN *ESCHERICHIA COLI*. J.M.M. Kessels, R.P.G.M. Van Den Grekel, G. Schrakamp, and H. Van Den Bosch. (Lab. of Biochem., State Univ. of Utrecht, Transitorium III. Padualaan 8, NL-3584 CH Utrecht The Netherlands) *Biochim. Biophys. Acta 713*(2):285-291 (1982). *sn*-Glycero-3-phosphate acyltransferase (EC 2.31.15), the first enzyme involved in phospholipid biosynthesis, is known to be associated with the cytoplasmic membrane of *Escherichia coli*. The localization of this enzyme in the transverse plane of the membrane was investigated by proteolysis of intact and lysed spheroplasts and by inhibition of glycerol 3-phosphate transport into intact cells in the presence of azide. Glycerophosphate acyltransferase was found to be resistent to proteolysis by trypsin in intact spheroplasts, whereas its enzymatic activity could be destroyed completely by trypsin in lysed spheroplasts. These results are in line with a localization of the acyltransferase at the inner aspect of the cytoplasmic membrane. Sodium azide was shown to have no inhibitory effect on glycerophosphate acyltransferase activity. Lack of incorporation of glycero phosphate into the phospholipids of glycerol phosphate transport-negative cells and inhibition of this incorporation in wild-type and glycerol 3-phosphate transport-constitutive cells by azide support a cytoplasmic-oriented localization of the glycerophosphate acyltransferase.

PREPARATIVE ISOLATION OF POLYPHOSPHOINOSITIDE FRACTIONS FROM OX BRAIN. G.V. Kiseleve (Lab. of Brain Metabolism, Pavlov Inst. of Physiology of the Academy of Sci. of the U.S.S.R., Nab. Makarova, 6, Leningrad 199164, U.S.S.R.) *Biochim. Biophys. Acta 712*(3):719-721 (1982). A simple preparative method for chromatographic isolation of pure fractions of di- and triphosphoinositides (1-phosphatidylinositol 4-phosphate and 1-phosphatidylinositol 4,5-bisphosphate) from ox brain is described. Polyphosphoinositide fractions have been obtained by ion-exchange chromatography of the lipid extract using gradient elution with 0-0.6M ammonium acetate in chloroform/methanol/water (20:9:1) from a DEAE-cellulose column. Before chromatography, divalent metal ions were removed from the lipid extract by passing through a Dowex-50 (H^+) column and lipids were converted to the sodium salt by neutralisation with sodium hydroxide in methanol solution. After chromatography, fractions of di- and triphosphoinositides were precipitated in methanol/water mixture (1:1) by evaporation in a vacuum to a final concentration of about 4 M ammonium acetate. Necessary salts of di- and triphosphoinositides were obtained by passing the ammonium salts of the lipids through Dowex-50 (H^+) and neutralising with corresponding base in methanol solution. About 0.35 mmol of diphosphoinositide and 0.63 mmol of triphosphoinositide were obtained from 1 kg of wet ox brain tissue.

LONG-LIVED LABELING OF PHAGOCYTIC CELLS WITH ANALOGS OF ATHEROMA LIPIDS. Y.Kleinman, G. Halperin, O. Stein, and Y. Stein (Lipid Research Laboratory, Dept. of Med. B, Hadassah Univ. Hospital, and Dept. of Experimental Med. and Cancer Res., Hebrew Univ.-Hadassah Med. Schl., Jerusalem, Israel) *Atherosclerosis 43*(1):1-6 (1982). [^{3}H] Trioleyl glyceryl ether and [^{3}H] cholesteryl linoleyl ether were bound to Intralipid and injected intraperitoneally into mice. About 20% of the injected label was recovered from peritoneal macrophages up to 2 weeks after injection, and there was a gradual appearance of the label in the liver. Following injection of acetylated low density lipoprotein, labeled with [^{3}H] trioleyl glyceryl ether, into rats, about 90% of the label appeared in the liver shortly after injection and all label was retained up to 73 days. These findings indicate that [^{3}H] cholesteryl linoleyl ether and [^{3}H] trioleyl glycerol can serve as nondegradable analogs of atheroma lipids, which are readily taken up by macrophages when presented in the form of appropriate substrates. These preliminary results serve as basis for experiments designed to study the role of macrophages in the transport of atheroma lipids.

THE CONCENTRATION OF CHOLESTEROL IN SERUM AND IN VARIOUS SERUM LIPOPROTEINS IN MACROBIOTIC, VEGETARIAN AND NON-VEGETARIAN MEN AND BOYS. J.T. Knuiman and C.E. West (Dept. of Human Nutrition, Agr. Univ., De Dreijen 12, 6703 BC Wageningen, The Netherlands) *Atherosclerosis 43*(1): 71-82 (1982). The concentrations of total and high density lipoprotein (HDL)-cholesterol and the ratio of HDL-cholesterol to total cholesterol have been examined in groups of non-vegetarian, semi-lactovegetarian, lactovegetarian and macrobiotic men aged 30-39 years and boys aged 6-11 years. In the men, the concentration of total cholesterol ranged from 3.8 mmol/l in the macrobiotics to 5.5 mmol/l in the non-vegetarians, while the concentration of HDL-cholesterol varied between 1.2 mmol/l and 1.4 mmol/l. The ratio of HDL-cholesterol/total cholesterol varied from 0.23 in the non-vegetarian men to 0.31 in the macrobiotics and it was negatively related to the body mass index (ratio of weight to height^2). In the boys the concentration of total cholesterol ranged from 3.4 mmol/l in the macrobiotics to 4.3 mmol/l in the semi-lactovegetarians, while the concentration of HDL-cholesterol varied between 1.2 mmol/l to 1.4 mmol/l. The ratio of HDL-cholesterol/total cholesterol was similar in the four groups (0.33-0.35). The concentration of cholesterol in various lipoprotein fractions separated by ultracentrifugation was also estimated in subsamples of the population. The variation between groups in the concentration of HDL-cholesterol appeared to be largely due to variations in the concentration of cholesterol in the HDL_2 fraction ($1.063 < \rho_{,20} < 1.125$).

GANGLIOSIDES WITH SIALIC ACID BOUND TO N-ACETYLGALACTOSAMINE FROM HEPATOPANCREAS OF THE STARFISH, *EVASTERIAS RETIFERA* AND *ASTERIAS AMURENSIS*. N.K. Kochetkov, G.P. Smirnova and I.S. Glukhoded (Inst. for Organic Chem., Acad. of Sci. of USSR, Moscow, USSR) *Biochim. Biophys. Acta 712*(3):650-658 (1982) Sialoglycolipids containing glucose, galactose, N-acetylgalactosamine and sialic acids were isolated from the total lipid extracts of hepatopancreas of the starfish, *Evasterias retifera* and *Asterias amurensis*, by partition dialysis, DEAE-cellulose column chromatography and preparative silica gel TLC. The structures of the sialoglycolipids were established by total and partial acid hydrolysis, total and partial methanolysis, methylation analysis, periodate oxidation, neuraminidase treatment and chromium trioxide oxidation. The sialoglycolipid from *E. retifera* was identified as N-acetylneuraminosyl-α-(2 → 9)-N-acetylneuraminosyl-α-(2 → 3)-N-acetylgalactosaminyl-β-(1 → 3)-galactosyl-β-(1 → 4)-glucosyl-β-(1 → 1)-ceramide and that from *A. amurensis* was identified as 8-*O*-methyl-N-glucoloylneuraminosyl-(2 → 3)-8-*O*-methyl-N-glycoloylneuraminosyl-(2 → 6)-N-acetylgalactosaminyl-β-(1 → 3)-galactosyl-β-(1 → 4)-glucosyl-β-(1 → 1)-ceramide. The long-chain bases of both sialoglycolipids were found to be mixtures of phytosphingosines with both branched and linear structures and the fatty acids were shown to be mixtures of normal and α-hydroxy fatty acids. The composition of the lipid moieties of the sialoglycolipids was determined by GLC and GLC-MS.

THE INFLUENCE OF TEMPERATURE AND STRUCTURE OF PALLADIUM SURFACES ON THE ADSORPTION OF ETHYLENE AND HYDROGENATION OF ADSORPTION COMPLEXES BY MOLECULAR AND ATOMIC HYDROGEN. J. Kopeštanský (J. Heyrovský Inst. of Physical Chem and Electrochemistry, Czechoslovak Academy of Sci., 121 38 Prague 2) *Collection Czechoslovák Chem. Commun. 47*(9):2307-2322 (1982). A measurement of the work-function change, combined with the volumetric method and gas product analysis were used for investigation of the influence of temperature and palladium surface structure on the adsorption of

ethylene and hydrogenation of its adsorption complexes by molecular and atomic hydrogen. It was verified that on palladium the highest activity for the C–H bond splitting of hydrocarbons is found on the adsorption sites corresponding to low-coordination surface atoms. The activation energy of the C–H bond dissociation is very low ($E_D \approx 2$ kJ/mol); as a result, in the early stages of surface coverage, hydrogen appears on the surface – together with the formation of surface adsorption complexes. The presence of hydrogen is the main reason for the non-linearity of the work-function changes, observed for ethylene adsorption in the low-coverage region. Stable dehydrogenated surface particles are formed also by self-hydrogenation of ethylene in the higher-coverage region. With increasing temperature, the extent of dehydrogenation of the adsorbed complexes also increases – while the influence of molecular hydrogen on the work-function of the surface with pre-adsorbed ethylene becomes less significant. The interaction of atomic hydrogen with ethylene adsorption-complexes caused in all cases irreversible changes of the surface work-function.

PHOSPHOLIPID COMPOSITION OF SUBCELLULAR FRACTIONS AND PHOSPHOLIPID-EXCHANGE ACTIVITY IN CHICKEN LIVER AND MC-29 HEPATOMA. K. Koumanov, A. Boyanov, T. Neicheva, T. Markovska, A. Momchilova, E. Gavazova, and H. Chelibonova-Lorer (Central Laboratory of Biophysics and Institute of General and Comparative Pathology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria) *Biochim. Biophys. Acta 713*(1):23-28 (1982). The phospholipid composition of mitochondria, microsomes and plasma membranes from liver and MC-29 hepatoma from White Leghorn chickens has been investigated. It was established that all mitochondria and microsome phospholipid fractions obtained from MC-29 hepatoma are increased strongly compared to those from liver. The sphingomyelin augmentation was particularly great. In hepatoma plasma membranes only the sphingomyelin quantity was increased. Sphingomyelin- and phosphatidylcholine-exchange activities were observed in avian liver for the first time. These two activities were increased in MC-29 hepatoma cells. Three phospholipid exchange proteins have been established in chicken liver 105000 ×*g* supernatant. One of them specifically transports phosphatidylcholine, the second one is non-specific for phosphatidylcholine and sphingomyelin and the third one is specific only for sphingomeylin. In hepatoma cells only a non-specific phosphatidylcholine- and sphingomyelin-exchange protein was found.

STUDIES ON THE ETIOLOGY OF INCREASED TISSUE CHOLESTEROL CONCENTRATION IN CHOLESTEROL-FED HYPOTHYROID RATS. P.M. Kris-Etherton, M.A. Fosmire, D.J. Mela, and T.D. Etherton (Nutritional Program and Department of Dairy and Animal Science, The Pennsylvania State University, University Park, PA 16802) *J. Nutr. 112*(12):2324-2332 (1982). Hypothyroid rats fed an atherogenic diet (A) for 3 weeks developed a marked hyperlipidemia characterized by elevated very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol. Cholesterol concentrations of adipose tissue, liver, carcass and soleus muscle were significantly increased in rats fed the A diet versus rats fed a control diet (C). After 5 months on the A diet, cholesterol concentrations of adipose tissue, carcass and soleus muscle were not different from those measured in rats fed the A diet for 3 weeks; however, liver cholesterol concentration was 20-fold higher. To study the mechanisms by which the A diet increased adipocyte cholesterol content, in vitro binding studies were conducted with normal (N) and cholesterol enriched (CH) ^{125}I-labeled VLDL. The inability of unlabeled N and CH VLDL to displace ^{125}I-labeled VLDL supports the concept that VLDL was not specifically bound by rat adipocytes. The observation that adipocyte and other tissue cholesterol levels were similar at 3 weeks and 5 months suggests regulation of tissue cholesterol concentrations. The mechanism of regulation of adipocyte cholesterol was not related to VLDL binding or differential binding rates between N and CH VLDL.

INFLUENCE OF NATIVE AND RANDOMIZED PEANUT OIL ON LIPID METABOLISM AND AORTIC SUDANOPHILIA IN THE VERVET MONKEY. D. Kritchevsky, L.M. Davidson, M. Weight, N.P.J. Kriek, and J.P. du Plessis (The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA 19104) *Atherosclerosis 42*(1):53-58 (1982). Vervet monkeys (*Cercopithecus aethiops pygerethrus*) were fed cholesterol-free, semipurified diets containing 40% sucrose, 25% casein, 15% cellulose and 14% peanut oil (PNO), randomized peanut oil (RPNO) or corn oil (CO). After 4 months, serum cholesterol and triglyceride levels, serum lecithin-cholesterol acyl transferase (LCAT) activity and plasma lipoprotein lipase (LPL) activity were similar in all groups. Livers of monkeys fed CO converted 156% more acetate and 24% more mevalonate to cholesterol than those of monkeys fed RPNO. Cholesterogenesis in RPNO-fed monkeys was enhanced compared to PNO (68% from acetate; 62% from mevalonate). Incidence of atherosclerosis was 33% in monkeys fed RPNO, 80% in those fed CO and 90% in those fed PNO. Extent of sudanophilia was lowest in aortas of monkeys fed RPNO. Incidence of arteriosclerosis was 40% in monkeys fed CO, 56% in those fed RPNO and 70% in those fed PNO. Extent of aortic surface showing arteriosclerosis was highest in monkeys fed RPNO.

THE EFFECTS OF MEVINOLIN ON SERUM CHOLESTEROL LEVELS OF RABBITS WITH ENDOGENOUS HYPERCHOLESTEROLEMIA. P. Kroon, K. Hand, J. Huff and A. Alberts (Merck Inst. for Therapeutic Res., Merck, Sharp and Dohme Res. Labs., Rahway, NJ 07065) *Atherosclerosis 44*(1):41-48 (1982). Mevinolin, a fungal metabolite isolated from cultures of *Aspergillus terreus*, is a potent competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase, the rate-controlling enzyme in cholesterol biosynthesis. In the current studies we demonstrate that mevinolin significantly lowers serum cholesterol in rabbits fed a cholesterol free, low-fat semi-synthetic diet. Rabbits maintained on this diet developed endogenous hypercholesterolemia with average cholesterol concentrations of 310 mg/dl over a 66-day period. Treatment with mevinolin for 39 days at a dose of 2 mg/kg per day lowered serum cholesterol levels by an average of 37% ($P < 0.05$), while a dose of 6 mg/kg per day resulted in a 48% ($P < 0.05$) decrease when compared with the control groups. When the administration of mevinolin was discontinued, serum cholesterol levels of the 6 mg/kg per day group increased significantly to a maximum post-treatment value of 319 mg/dl ($P < 0.0001$). The results of this study demonstrate that rabbits with endogenous hypercholesterolemia are a useful animal model for the study of cholesterol biosynthesis inhibitors like mevinolin.

ORGANIZATION OF THE CORE LIPIDS OF LIPOPROTEINS FROM NORMAL AND CHOLESTEROL-FED RABBITS. A PROTON NUCLEAR MAGNETIC RESONANCE STUDY. P. Kroon and J. Seidenberg (Merck Inst. for Therap. Res., Merck Sharp & Dohm Res. Labs., Rahway, New Jersey 07065) *Biochemistry 21*(25): 6483-6488 (1982). Rabbits fed a diet supplemented with cholesterol have increased plasma cholesterol levels and develop atherosclerosis. Most plasma cholesterol exists as cholesteryl esters in very low density lipoproteins and intermediate-density lipoproteins. The triglyceride content of VLDL decreases from 74% to 5% during cholesterol feeding; the cholesteryl ester content increases from 26% to 95%. The IDL and low-density (LDL) fractions have a triglyceride content of 2% or less in their cores. The mobility of the core cholesteryl esters was studied. Changes in the mobility were assessed by measuring the temperature dependence of the amplitude of the methylene resonances. The decrease in spectral amplitute for VLDL, IDL, and LDL from cholesterol-fed rabbits between 55 and 15 C shows that the mobility of the core cholesteryl esters is temperature dependent and that the cholesteryl esters display thermal order-disorder transitions with midpoints of 42, 40, and 38 C. At physiological temperatures, the core cholesteryl esters of lipoproteins from cholesterol-fed rabbits exist in a partially ordered state. The core cholesteryl esters of VLDL, IDL, and LDL from normal rabbits show no evidence for an order-disorder transition. This is consistent with their high core triglyceride content which precludes the existence of an ordered cholesteryl ester phase within the core. The core cholesteryl esters of normal rabbit lipoproteins exist in a liquid state at physiological temperatures. High-density lipoproteins from normal and cholesterol-fed rabbits fail to display an order-disorder transition due to the constraints imposed by the small HDL core diameter which prevents the existence of an ordered arrangement of cholesteryl esters, irrespective of the core triglyceride content.

CLEARANCE OF SUBCUTANEOUS IMPLANTS OF CHOLESTEROL IN THE RAT PROMOTED BY OXIDATION PRODUCTS OF CHOLESTEROL – A POSTULATED ROLE FOR OXYSTEROLS IN PREVENTING ATHEROSCLEROSIS. L.H. Krut (Dept. of Med., Baragwanath Hospital, and the Univ. of the Witwatersrand P.O. Bertsham 2013, Johannesburg, South Africa) *Atherosclerosis 43*(1): 105-118 (1982). Cholesterol in crystalline form cannot be readily cleared from tissue and in this form it is sclerogenic. Phospholipids can solubilise cholesterol, promote its clearance and reduce the sclerosis. The phospholipids accompanying cholesterol deposited in atherogenesis are not adequate to solubilise all the cholesterol. It has been found that some oxidation products of cholesterol act synergistically with phosphatidylcholine to enhance the solubility of cholesterol in vitro. The effect of these oxysterols on solubilisation and clearance of cholesterol in vivo was examined in rats by implanting subcutaneously tablets made of cholesterol, cholesterol plus oxysterols and both with phosphatidylcholine. Tablets containing oxysterols went into solution rapidly, were cleared completely and allowed regression of the initial fibrosis promoted by the sterol

Abstracts

without needing exogenous phospholipid. Solubilisation and clearance seem to have been affected by endogenous phospholipid, possibly high density lipoproteins, and by macrophages. Tablets without oxysterols showed no clearance at all but were cleared in part when phosphatidylcholine was added. Oxidation products of cholesterol form readily in foods of animal origin when suitably exposed to light and air. It is suggested that technology designed to prevent spoilage of foods has inadvertently resulted in the elimination from the Western diet of compounds which prevent the accumulation of cholesterol in the arterial wall.

SOLUBILITY OF CHOLESTEROL IN VITRO PROMOTED BY OXIDATION PRODUCTS OF CHOLESTEROL. L.H. Krut (Dept. of Med., Baragwanath Hospital, and the Univ. of the Witwatersrand, P.O. Bertsham 2013, Johannesburg, South Africa) *Atherosclerosis 43*(1):95-104 (1982). Compounds promoting the solubility of cholesterol could have a role in preventing its crystallisation and accumulation in tissue and in body fluids. A small quantity of phosphatidylcholine added to a supersaturated solution of cholesterol in a triglyceride oil has limited capacity to maintain solubility of cholesterol. Small quantities of oxidation products of cholesterol (oxysterols) have no material effect on the solubility of cholesterol in this system. However, the combination of phosphatidylcholine and oxysterols effectively maintains cholesterol in stable solution. In aqueous medium, the capacity of phosphatidylcholine to solubilise a molar excess of cholesterol is greatly increased by oxysterols. Oxidation products of cholesterol and phosphatidylcholine acting synergistically enhance enormously the solubility of cholesterol both in supersaturated solution in a triglyceride oil and in aqueous medium.

SUBCELLULAR LOCALIZATION AND QUANTIFICATION CHOLESTEROL IN CULTURED HUMAN FIBROBLASTS EXPOSED HUMAN LOW DENSITY LIPOPROTEIN. H.S. Kurth, J. Blanchette-Mackie, J. Avigan, W. Gamble, and M. Vaughan (Lab. of Cellular Metabolism, Nat'l. Heart, Lung, and Blood Inst., Nat'l. Inst. of Health, Bethesda, MD 20205) *J. Lipid Res. 23*:1128-1135 (1982). Subcellular localization of nonesterified cholesterol has been determined in normal human fibroblasts from cultures incubated with human low density lipoprotein (LDL). Nonesterified and esterified cholesterol content of fibroblasts, grown initially in the absence of cholesterol, increased significantly after a 1-hour incubation with LDL. Digitonin was used to localize nonesterified cholesterol that was accumulated within multivesicular and lamellar lysosomal inclusions. This was observed only in fibroblasts from cultures incubated with LDL. Accumulation of LDL-derived nonesterified cholesterol within lysosomes is consistent with the suggestion of other investigators that LDL is metabolized within lysosomes.

PLASMA LIPIDS AND LIPOPROTEINS IN JAPANESE MALE PATIENTS WITH CORONARY ARTERY DISEASE AND IN THEIR RELATIVES. H. Kukita, Y. Imamura, M. Hamada, T. Joh, and T. Kokubu (Second Department of Internal Medicine, Ehime University, School of Medicine, Onsen-gun, Ehime 791-02, Japan) *Atherosclerosis 42*(1):21-29 (1982). Plasma cholesterol (CH), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) were measured in 92 consecutive Japanese male subjects undergoing diagnostic coronary cineangiography. Sixty-nine of them were classified as having coronary heart disease (CAD), the remaining 23 subjects were classified as having normal coronary arteries (NCA). The CAD group had significantly lower HDL-C and higher TG levels than the NCA group. However, there was no significant difference in plasma CH between the two groups. First-degree relatives of the CAD patients were also investigated. The male blood relatives of the CAD patients also had significantly lower HDL-C and higher TG levels than the non-blood male relatives and healthy control males. The female blood relatives, however, showed no significant differences from the non-blood female relatives and the healthy control females in plasma CH, TG and HDL-C levels. These results suggest that low HDL-C and hypertriglyceridemia are the prevalent coronary risk factors, rather than hypercholesterolemia, in a population with a low fat intake such as the Japanese, and that these lipid abnormalities are related to sex and genetic factors.

EFFECTS OF ANTIMALARIAL DRUGS ON SEVERAL RAT-LIVER LYSOSOMAL ENZYMES INVOLVED IN PHOSPHATIDYLETHANOLAMINE CATABOLISM. H. Kunze, B. Hesse, and E. Bohn (Dept. of Biochemical Pharmacology, Max-Planck-Institute for Experimental Med., Gottingen, F.R.G.) *Biochim. Biophys. Acta 713* (1):112-117 (1982). The effects of three cationic amphiphilic antimalarial drugs (chloroquine, mepacrine and primaquine) on the intralysosomal catabolism of phosphatidylethanolamine and several of its metabolites were studied with rat-liver lysosomes which had been isolated from animals previously treated with Triton WR-1339. The activities of each of the various enzymes involved in the main pathways of intralysosomal phosphatidylethanolamine degradation (Kunze, H., Hesse, B., and Bohn, E. (1982) *Biochim. Biophys. Acta 711*:10-18) exhibited almost identical inhibitory sensitivities towards mepacrine and primaquine. In contrast, chloroquine inhibited the activities of the various enzymes to different extents, lysophospholipid acylhydrolase (EC 3.1.1.5) being the most sensitive enzyme, followed by phospholipase A_1 (EC 3.1.1.32) and monoacylglycerol lipase, and eventually lysophospholipid monoacylglycerol hydrolase as the least sensitive enzyme. The relative inhibitory potencies towards phospholipase A_1 activity of chloroquine were increased with increasing pH, and the mode of inhibition was competitive. In contrast, the inhibitory potencies towards monoacylglycerol lipase activity of chloroquine increased only up to pH 5 but decreased above this value, and the mode of inhibition was noncompetitive.

INTERACTION OF CHOLESTEROL AND LYSOPHOSPHATIDYLCHOLINE IN DETERMINING RED CELL SHAPE. Y. Lange and J.M. Slayton (Dept. of Pathology and Biochem., Rush Med. College, Chicago, IL 60612) *J. Lipid Res. 23*:1121-1127 (1982). The effect of lysolecithin on the shape of human erythrocytes of varied cholesterol content was examined by scanning electron microscopy. Under the conditions of these experiments, all of the (^{14}C)lysolecithin incubated with cells was shown to be located in the external membrane leaflet. The membrane lysolecithin required to induce echinocytosis (spiculation) in normal cells (0.8 mol cholesterol/mol phospholipid) was approximately 0.08-0.10 μmol/10^{10} cells, which contributed 1.6-2.0 μm^2 or 1% of the cell surface area. This value is consistent with the premise that echinocytosis was caused by a slight differential expansion of the outer surface of the bilayer. The lysolecithin required for echinocytosis decreased as the membrane cholesterol content increased; from 0.14 to 0.03 μmol/10^{10} cells at 0.5 to 1.4 mol cholesterol/ml phospholipid. Data were interpreted in terms of a bilayer couple mechanism. Assuming that the two amphipaths acted additively, the amount of lysolecithin required to induce echinocytosis was used to estimate the partition of cholesterol between the two leaflets of the red cell membrane. A value of about 51:49% in favor of the outer leaflet was found at all cholesterol levels.

INGUINAL FAT PAD WEIGHT VERSUS BODY WEIGHT AS A METHOD OF GENOTYPE IDENTIFICATION IN 16-DAY-OLD ZUCKER RATS. M. Lavam and R. Bazin (Unite de Recherches sur la Physiopathologie de la Nutr., Inserm U 177, Inst. Biomedical des Cordeliers, 15, rue de l"Ecole de Med., 750006 Paris, France) *J. Lipid Res. 23*(6):941-943 (1982). By plotting the weights of inguinal fat pad versus body weights in littermates from *fa/fa* × *Fa/fa* crosses, we observed that the data distributed along two widely separated regression lines as of 16 days of age. This procedure enabled us to determine unequivocally the genotype of every pup in seven litters. By its rapidity, its simplicity, and reliability, this method of genotype identification may be useful to many investigators.

LIPID ACCUMULATION IN ARTERIAL SMOOTH MUSCLE CELLS IN CULTURE. MORPHOLOGICAL AND BIOCHEMICAL CHANGES CAUSED BY LOW DENSITY LIPOPROTEINS AND CHLOROQUINE. D.S. Leake and T.J. Peters (Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, Great Britian) *Atherosclerosis 44*(3):275-291 (1982). Cultured smooth muscle cells from pig aortas were incubated with low density lipoproteins (LDL) and chloroquine for up to 5 days, as an in vitro model for lipid accumulation in atherosclerosis. Cells incubated with LDL alone had a normal morphology, except that some cells contained large lipid droplets. The activities of acid phosphatase, catalase and malate dehydrogenase were increased in homogenates prepared from these cells. Cells incubated with chloroquine alone developed large autophagic vacuoles. The activities of the three acid hydrolases, acid phosphatase, N-acetyl-β-glucosaminidase and β-glucuronidase, were decreased, as was the proteolytic activity of the cell homogenates at acid pH toward ^{125}I-labelled LDL. There was, however, a transient increase in the activity of malate dehydrogenase. Chloroquine by itself was toxic to the cells, but LDL protected against this toxic effect. Cells incubated with LDL and chloroquine together developed both autophagic vacuoles and large lipid droplets. The cholesteryl ester content of the cells was increased many-fold and the non-esterified cholesterol content was increased to a lesser extent. The above four acid hydrolase activities were decreased, as was the activity of catalase, whereas the activities of lactate dehydrogenase and malate dehydrogenase were increased.

INTERACTION OF FATTY ACIDS WITH THE CALCIUM-MAGNESIUM ION DEPENDENT ADENOSINETRIPHOSPHATASE FROM SARCOPLASMIC RETICULUM. A.G. Lee, J.M. East, O.T. Jones, J. McWhirter, E.K. Rooney, and A.C. Simmonds (Dept. of

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Biochem., Univ. of Southampton, Bassett Crescent East, Southampton, SO9 3TU, U.K.) *Biochem. 21*(25):6441-6446 (1982). The fluorescence emission spectrum of dansylundecanoic acid is sensitive to the environment and appears at a lower wavelength when the fatty acid is bound to protein than when it is bound to phospholipid. When bound to the $(Ca^{2+}$-$Mg^{2+})$-ATPase of sarcoplasmic reticulum, the emission spectrum can be resolved into separate components assigned to fatty acid bound to protein and to lipid. Efficiency of energy transfer from the tryptophan residues of the ATPase to dansylundecanoic is higher for protein-bound probe than for lipid-bound probe. Fluorescence titrations are consistent with three fatty acid binding sites per ATPase with a K_d of 7 μM, and these sites are postulated to occur at the protein-protein interface in ATPase oligomers. Fatty acid incorporated into the lipid component of the membrane appears to be bound outside the lipid annulus around the protein.

PROSTAGLANDINS MEDIATE INHIBITION OF GASTRIC ACID SECRETION BY SOMATOSTATIN IN THE RAT. M. Ligumsky, Y. Goto, H. Debas, and T. Yamada (Center for Ulcer Res. and Educ., Medical, Surgical and Res. Services, VA Wadsworth and Univ. of Calif. Med. Centers, Los Angeles, CA 90073) *Science 219*(4582): 301-303 (1982). Somatostatin, a tetradecapeptide with potent inhibitory actions on gastric acid secretion, potentiated carbamylcholine-induced synthesis and release of prostaglandin E_2 from isolated perfused rat stomachs. The ability of somatostatin to inhibit acid secretion was blocked by indomethacin, an inhibitor of postaglandin synthesis. These results suggest that postaglandins mediate gastric acid inhibition by somatostatin in the rat.

AGE-DEPENDENCY OF VASCULAR PHOSPHOLIPID DEACYLATION-REACYLATION IN SPONTANEOUSLY HYPERTENSIVE RATS. C. Limas, P. Goldman, and C.J. Limas (Dept. of Pathology, Veterans Administration Med. Center, and Lab. Med. Pathology and Med. (Cardiovascular Section), Univ. of Minnesota Schl. of Med., Minneapolis, MN 55455) *Biochim. Biophys. Acta 713*(2):446-455 (1982). We have studied the temporal relation to phospholipid turnover and prostaglandin synthesis to the evolution of hypertensive vascular disease in the spontaneously hypertensive rat. The incorporation of arachidonate into aortic phospholipids, its release by phospholipase A_2 and its utilization for prostaglandin synthesis were compared in spontaneously hypertensive and Wistar-kyoto rats aged 7, 20 and 42 weeks. When expressed per mg of protein in the assay medium, arachidonate incorporation into aortic phospholipids decreased, while prostaglandin synthesis increased, with age in both rat strains. No significant differences were noted between hypertensive and normotensive animals at 7 weeks of age whereas both enhanced phospholipid turnover and prostaglandin synthesis was demonstrated in hypertensive rats at 20 and 42 weeks of age. The higher phospholipase activity in hypertensive aortas was associated with a significant increase in the capacity for exogenous lysophosphatidehydrolysis. Transacylation and reacylation of lysolecithin, however, were not significantly enhanced in hypertensive aortas. These biochemical changes accompany, and may be related to, structural modifications of the aortic wall in the course of hypertension.

EFFECT OF DEXAMETHASONE ON 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE ACTIVITY AND CHOLESTEROL SYNTHESIS IN RAT LIVER. R.C. Lin and P.J. Snodgrass (Veterans Administration Med. Center, 1481 W. 10th St. and Dept. of Med., Indiana Univ. Schl. of Med., Indianapolis, IN 46202) *Biochim. Biophys. Acta 713*(2):240-250 (1982). Dexamethasone increases reductase activity in cultured liver cells after a lag period of 2 hr. The increases of activity are linear from 10^{-9} to 10^{-5} M dexamethasone, the maximum responses ranging from 2- to 4-fold. The increased reductase activity after dexamethasone treatment is not due to a change of the state of phosphorylation/dephosphorylation of the enzyme nor to an increase of cytosolic activating factor(s) for the reductase. Cholesterol synthesis, measured by incorporation of either [^{14}C] acetate of ^3H_2O, increases 3-fold after dexamethasone (10^{-6} M) treatment, as does the hydroxymethylglutaryl-CoA reductase activity, confirming that this enzyme is rate-controlling for cholesterol synthesis in cultured liver cells as it is in vivo. Dexamethasone (10 μg/100 g rat), given after onset of the light cycle, increases reductase activity over control rats at the nadir of the circadian cycle of this enzyme. When given after onset of the dark cycle, dexamethasone does not increase reductase activity over controls at the peak of their circadian cycle. Thus, physiologic doses of glucocorticoids partially reverse the decline in reductase activity due to the circadian rhythm.

CHANGES IN GLUCOSE TOLERANCE AND PLASMA INSULIN DURING LIPID-LOWERING TREATMENT WITH DIET, CLOFIBRATE AND NICERITROL. H. Lithell, B. Vessby and K. Hellsing (Dept. of Geriatrics and Dept. of Clinical Chem., Univ. of Uppsala, Uppsala, Sweden) *Atherosclerosis 43*(2,3):177-184 (1982). In an effort to reduce serum lipids in patients with atherosclerotic manifestations, a combined treatment with a conventional lipid-lowering diet, clofibrate and niceritrol was used. The effect on glucose metabolism of such treatment was studied. Among the 106 patients 66 took the full dose of both drugs and of these 51 were weight-stable and non-diabetic. The effects of the diet and the drugs were evaluated in this subsample. Diet had no effect on fasting blood glucose concentration, the K value of an intravenous glucose tolerance test (IVGTT) and concentrations of serum insulin. Niceritrol treatment was associated with increased blood glucose, decreased K value, elevated fasting serum insulin and serum insulin at 60 min during IVGTT. Clofibrate had the opposite effects to niceritrol and when both drugs were combined, carbohydrate metabolsim was unchanged compared with the pre-treatment state.

THE EFFECT OF TEMPERATURE ON GLYCERYL ETHERS IN *TETRAHYMENA PYRIFORMIS* W.S. Lund-Katz and R. Conner (Dept. of Biol. and Chem., Grad. Div., Bryn Mawr College, Bryn Mawr, PA 19010) *J. Lipid Res. 23*(9):1301-1307 (1982). The effect of temperature on the ether content of the glycerophospholipids of *Tetrahymena pyriformis* W was examined. The only ether detected was 1-*O*-hexadecyl glycerol (α-chimyl alcohol). The data provide evidence that the class 1-*O*-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphate (1-alkyl PsE), in addition to the previously reported 1-*O*-alkyl-2-acyl-*sn*-glycero-3-(2-aminoethyl)-phosphonate (1-alkyl PnE) and 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine (1 alkyl PC), exists in this ciliate species. A comparison was made of the ether content of the glycerophospholipids from cells grown at 15 and 28.5 C. An elevation in the amount of ether was noted in all glycerophospholipids at the lower temperature with the largest proportional change in 1-alkyl PsE. *Tetrahymena* species have a high γ-linolenic acid content in the *sn*-1 position of the glycerophospholipids in addition to the usual saturated acids and ether. The replacement at low temperature of γ-linolenic acid by a saturated hydrocarbon at the *sn*-1 position of the glycerophospholipids of *Tetrahymena pyriformis* W should increase the microviscosity of the membranes; thus, it is difficult to envision this alteration in the glycerophospholipids as an adaptive change beneficial for growth. These findings are in direct contrast to the situation in *Tetrahymena thermophila* where the percentage of ether glycerophospholipids increases at the expense of γ-linolenate as the temperature rises.

LIPID SYNTHESIS IN INOSITOL-STARVED *SACCHAROMYCES CEREVISIAE*. M.T. McCammon and L.W. Parks (Dept. of Microbiol., Oregon State Univ., Corvallis, OR 97331) *Biochim. Biophys. Acta 713*(1):86-93 (1982). Lipid synthesis was analyzed in an inositol-requiring mutant of *Saccharomyces cerevisiae* (MC13). Both rates and cellular amounts of [U-^{14}C] acetate incorporation into phospholipids, triacylglycerols, free sterols and steryl esters were elevated in an inositol-starved culture compared to the supplemented control at a time when the deprived culture was losing viability (inositol-less death). The rates at a later time were greatly reduced. During the period when de novo lipid synthesis was high in the starved culture, phospholipid turnover and presumed conversion to triacylglycerols was also accelerated; no differences were apparent in the turnover of the sterol fractions between the two cultures. No change in the fractional percent of ergosterol or of the sterol precursors could be attributed to inositol starvation. The synthesis and maintenance of membrane lipids (phospholipids and free sterols) and their coupling in cellular metabolism are discussed in light of these results.

THE ARACHIDONIC ACID METABOLIC CAPACITY OF CANINE MYOCARDIUM IS INCREASED DURING HEALING OF ACUTE MYOCARDIAL INFARCTION. E. McCluskey, P. Corr, B. Lee, J. Saffitz, P. Needleman (Depts. of Pharmac., Med. and Pathol., Washington Univ. Schl. of Med., St. Louis, MO) *Circ. Res. 51*:743-750 (1982). The relative capacity for metabolizing [^{14}C] arachodinic acid into biologically active products was studied in microsomes prepared from both normal and infarcted regions of myocardium at three different times after circumflex coronary artery occlusion in the dog. 3 days after infarction, when polymorphonuclear leukocytes were the predominant invading cell, the ability of infarcted left ventricle microsomes to produce arachidonic acid metabolites was greater than that of microsomes from normal areas of the same hearts. 3 weeks after infarction, when macrophages were the predominant infiltrating cell and there was a proliferation of blood vessels and fibroblasts, there continued to be significant increases in the production of both prostacyclin and thromboxane. This enhanced production was still seen 3 months after infarction at a time when histological examination of the tissue showed that it was still healing with both blood vessels and fibroblasts present. The production of 6-keto $PGF_{1\alpha}$ was 31.7 ± 4 picomoles per milligram protein

per hour in noninfarcted regions of left ventricle while production was significantly increased to 71.7 ± 15 at 3 days, 64.1 ± 10 at 3 weeks, and 67.2 ± 15 even 3 months after infarction. The thromboxane synthetase activity rose significantly from 30.1 ± 5 pmol mg per hr in noninfarcted regions to 73.7 ± 18 at 3 days, 71.2 ± 5 at 3 weeks, and 92.4 ± 40 at 3 months. The enhanced ability to metabolize arachidonic acid may result from the inflammatory cell invasion or fibroblast activation which accompany healing of acute infarcts.

TEMPORAL ASSOCIATION BETWEEN ARTERIAL CHOLESTEROL DEPOSITION, THYMIDINE INCORPORATION INTO DNA, AND ATHEROSCLEROSIS IN JAPANESE QUAIL FED AN ATHEROGENIC DIET. D.L. McCormick, J.D. Radcliffe, R.G. Mehta, C.A. Thompson, and R.C. Moon (Laboratory of Pathophysiology, Life Sciences Division, IIT Research Institute, Chicago, IL 60616) *Atherosclerosis 42*(1):1-13 (1982). Male Japanese quail (strain SEA) rapidly develop atherosclerotic lesions in the aorta and brachiocephalic arteries when fed an atherogenic diet containing 1.0% cholesterol and 0.5% cholic acid. The present study was conducted to determine time parameters of the atherosclerotic response. Groups of 20 quail fed the atherogenic diet were killed at 0 days, 1 day, 3 days, or weekly from 1 to 12 weeks. Quail fed the atherogenic diet for 1 day showed a significant increase in serum cholesterol; a plateau was reached by 2 weeks. A significant increase in arterial cholesterol was seen after 2 weeks on the atherogenic diet, and arterial cholesterol showed a linear increase with time from 2 to 12 weeks. Increased incorporation of tritiated thymidine into the DNA of arterial cells was first seen at 2 weeks; thymidine incorporation increased to a maximum value at 9 weeks, then declined to 50-60% of the 9-week value at weeks 11 and 12. Grossly visible atherosclerotic lesions were first seen at 3 weeks, and 90% of birds showed gross atherosclerotic lesions by 8 weeks. Atherosclerosis induced in Japanese quail by feeding cholesterol and cholic acid is characterized initially by lipid deposition in the arterial wall, followed by increased incorporation of tritiated thymidine and the appearance of gross lesions.

PHYSICAL FITNESS AND PLASMA HDL CHOLESTEROL CONCENTRATIONS IN MALE BUSINESS EXECUTIVES. J.R.L. Masarei, J.E. Pyke and F.S. Pyke (Depts. of Clinical Biochem. and Human Movement and Recreation Studies, Univ. of Western Australia, Perth, WA) *Atherosclerosis 42*(1):77-83 (1982). Endurance fitness has been measured objectively (physical work capacity at pulse rate of 170/min, W_{170}) in a group of middle-aged executives, and related to a number of other physical characteristics and aspects of coronary risk status: FEV_1, blood pressure, adiposity, smoking habit, alcohol consumption, plasma levels of total and non-high density lipoprotein cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C). The primary question was whether HDL-C levels could be shown to be related to endurance fitness levels over the range encountered in a fairly homogeneous population and hence whether there could be value in terms of lipid coronary risk status in encouraging a moderate increase in physical activity. HDL-C levels were significantly related to W_{170}. Fitness also separated the subjects in terms of adiposity, but not in terms of the other variables studied. Even though the trend was toward an index of physical activity being able to separate the subjects in terms of HDL-C this was not as clear-cut as the division in terms of endurance fitness. Alcohol and smoking were associated with higher triglyceride levels, but not with HDL-C. The variables mid-abdominal skinfold thickness, triglyceride, non-HDL-C and endurance fitness accounted for 53% of the variation in HDL-C levels in this population. Alterations in the levels of these probably related variables might be expected to have appreciable effects on levels of HDL-C.

STIMULATION OF SPECIFIC 1,25-DIHYDROXYVITAMIN D_3 BINDING PROTEIN IN CULTURED POSTNATAL RAT INTESTINE BY HYDROCORTISONE. E.R. Massaro, R.U. Simpson, and H.F. DeLuca (Dept. of Biochem., Univ. of Wisconsin, Madison, WI 53706) *J. Biol. Chem. 257*(22):13736-13739 (1982). During the third postnatal week, 1,25-dihydroxyvitamin D_3 specific binding activity begins to rise in intestine and increases to adult levels by the time of weaning. To determine factors directly involved in stimulating the appearance of the specific binding protein for 1,25-dihydroxyvitamin D_3, we have developed an organ culture system for postnatal rat intestine. Specific 1,25-dihydroxyvitamin D_3 binding activity was successfully maintained in explants cultured for 24 hr in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, insulin, and antibiotics. The influence of hydrocortisone on the appearance of 1,25-dihydroxyvitamin D_3 binding activity was examined in intestines from rats 14 and 16 days postpartum. Minimal binding activity was observed in intestines from rats 14 days postpartum and in explants thereof cultured in control medium for 24 hr. Explants from rats 14 days postpartum cultured in medium supplemented with 10^{-5} M hydrocortisone exhibited a 5-fold increase in 1,25-dihydroxyvitamin D_3 specific binding activity. Specific 1,25-dihydroxyvitamin D_3 binding activity significantly increased in intestines from rats 16 days postpartum when cultured in control medium for 24 hr. Binding activity was further elevated by exposure of explants to hydrocortisone-supplemented medium. These studies suggest that the appearance of 1,25-dihydroxyvitamin D_3 binding activity in postnatal rat intestine is initiated by glucocorticoids but also depends on intrinsic timing mechanisms.

DISTINCTIVE SELECTIVITY FOR DOCOSATETRAENOIC ACID INCORPORATION BY EHRLICH ASCITES TUMOR CELLS. Y. Masuzawa, Y. Nakagawa, K. Waku, and W. Lands (Faculty of Pharmaceutical Sciences, Teikyo Univ., Sagamiko, Tsukuigun Kanagawa 199-01 (Japan) and Dept. of Biol. Chem., Univ. of Illinois, Med. Center, Chicago, IL 60612 (U.S.A.)) *Biochim. Biophys. Acta 713*(2): 185-192 (1982). Three polyunsaturated fatty acids were incorporated into the lipids of Ehrlich ascites cells to examine the selectivity of the synthetic enzymes for chain length and the number of double bonds. Arachidonic acid (20:4*n*-6) and timnodonic acid (20:5*n*-6) showed approximately similar patterns of incorporation into neutral acylglycerols, choline glycerophospholipids and ethanolamine glycerophospholipids. Further division of the lipids into diacyl and alkylacyl categories established that 20:4*n*-6 was incorporated at relatively lower amounts in the triacylglycerols and in the alkylacyl and alkenylacyl forms of the glycerophospholipids. The pattern of incorporation of radioactive 20:5*n*-3 into the phospholipids was similar to that for 20-4*n*-6, but there was no evidence for a selectively restrained incorporation into the triacylglycerols. The 22-carbon acid (22:4*n*-6) differed greatly from its shorter homolog (20:4*n*-6) in being incorporated in much greater amounts into triacylglycerols than into phospholipids. It did not appear to be present at an appreciably higher relative amount in either the alkyl diacylglycerols or in the ether-containing choline glycerophospholipids. Nevertheless, it occurred with exceptionally high specific activities in the alkylacyl and alkenylacyl forms of ethanolamine glycerophospholipids in a manner that suggests marked discrimination by the cytidine-mediated ethanolamine phosphotransferase. This distinctive selectivity provides evidence for special role for a 22-carbon acid in lipid metabolism.

INCORPORATION OF EXOGENOUS FATTY ACIDS INTO PHOSPHOLIPIDS BY CULTURED HAMSTER FIBROBLASTS. EFFECT OF SV40 TRANSFORMATION. C. Maziere, J.-C. Maziere, L. Mora and J. Polonovski (Laboratoire de Chimie Biologique, ERA 481 du CNRS, CHU Saint-Antoine, 27 rue de Chaligny, 75012 Paris, France) *Biochim. Biophys. Acta 712*(3):712-715 (1982). In situ incorporation of two saturated (palmitic, 16:0; stearic, 18:0) and three unsaturated fatty acids (oleic, 18:1,; linoleic, 18:2,; arachidonic, 20:4) into the four major phospholipids, sphingomyelin, PC, PI and PE, was followed. Transformed cells incorporated unsaturated fatty acids more rapidly, whereas no significant differences were found concerning saturated fatty acids. In vitro determination of phospholipid acylation showed that incorporation of coenzyme A-activated forms of two saturated fatty acids (16:0 and 18:0) and one unsaturated fatty acid (18:1) into phospholipids was increased in transformed cells. Comparison of results obtained in situ and in vitro strongly suggests that incorporation of fatty acids into phospholipids in cultured cells is not limited by acyltransferase activities.

EFFECT OF VITAMIN A DEPLETION ON STRESS-INDUCED CHANGE IN URINARY OUTPUT OF CATECHOLAMINES. R. Mizutani and K. Nakano (Dept. of Nutr. Regulation, Res. Inst. for Biochem. Regulation, Nagoya Univ., Chikusa, Nagoya 464, Japan) *J. Nutr. 112*(12):2205-2211 (1982). The effect of vitamin A depletion on stress-induced change in sympathoadrenal medullary activity was studied in rats. Four consecutive hours daily of immobilization provoked a marked increase in urinary excretion of free norepinophrine (NE) and epinephrine (E), confirming previous findings. The stress caused a significant decrease in output of free dopamine (DA). In contrast, the vitamin A-depleted rats in the resting state excreted threefold more NE in urine as compared with the normal animals. The urinary NE response to the stress was markedly diminished in the depleted rats, although E and DA responses to the stress were similar in magnitude to those in the normal animals. These results suggest that vitamin A depletion causes derangement of the neurosympathetic system; hence, the animals cannot appropriately respond to the stress. Alternatively, the state of vitamin A depletion may be in fact a stress, and in consequence the animals have already been in a state of maximal response before immobilization.

Abstracts

EFFECT OF HIGH DOSE OF NICOTINIC ACID ON BILE ACID METABOLISM IN RATS FED CHOLESTEROL-FREE AND CHOLESTEROL-CONTAINING DIET. E.T. Varghese, A. Mathew and P.A. Kurup *Indian J. Biochem. Biophys. 19*(3):228-229 (1982). Effects of administration of high dose of nicotinic acid on the concentration of aortic cholesterol and fecal excretion of neutral sterols and bile acids have been studied in rats fed cholesterol-free and cholesterol-containing diets. High dose of nicotinic acid as compared to adequate dose caused significant decrease in aortic cholesterol and increase in the fecal excretion of neutral sterols and bile acids.

THE EFFECT OF POLYUNSATURATED FATTY ACIDS OF THE N-3 AND N-6 SERIES ON PLATELET AGGREGATION AND PLATELET AND AORTIC FATTY ACID COMPOSITION IN RABBITS. F.W. Vas Dias, M.J. Gibney and T.G. Taylor (Dept. of Nutr., Schl. of Biochem. and Physiological Sci., Univ. of Southampton, Southampton S09 5NH Great Britain) *Atherosclerosis 43* (2,3):245-257 (1982). Four groups of 6 New Zealand white rabbits were fed for 60 days on a commercial rabbit diet supplemented (60 g/kg) with 1 of 4 sources of fat: corn oil, linseed oil and fish oil, which respectively provided rich sources of linoleic acid (C18: 2, n-6), α-linolenic acid (C18:3, n-3) and eicosapentaenoic acid (C20:5, n-3), and coconut oil, low in all polyunsaturated fatty acids (PUFA). Platelet-rich plasma was prepared and aggregation induced by ADP (final concentration 0.29-74.3 μM), collagen (2.5-20 μg/400 μl, final concentration) and 2.5 U bovine thrombin, and recorded with a Peyton aggregometer. Platelet aggregation induced by both thrombin and collagen was significantly lower with either n-3 PUFA (fish or linseed oil) than with corn oil (n-6 PUFA) or the low PUFA coconut oil. ADP-induced platelet aggregation was significantly reduced only in animals fed fish-oil. Changes in platelet aggregation were accompanied by increased platelet lipid content of C20:5, n-3 and decreased content of C20:4, n-6, with little change in platelet total C20 fatty acids. Platelet levels of C20:5, n-3 were significantly increased with both the preformed C20:5, n-3 and its precursor C18:3, n-3 in the diet. However, aortic lipid accumulation of C20:5 only occurred with rabbits fed on fish oil. It was concluded that, for collagen and thrombin induced aggregation, C18:3, n-3 and C20:5, n-3 were equally antiaggregatory in rabbits. The implications of this in community nutrition programmes are discussed.

INFLUENCE OF POLYUNSATURATED FATS ON COMPOSITION OF PLASMA LIPOPROTEINS AND APOLIPOPROTEINS. G.L. Veta, E. Groszek, R. Wolf, and S.M. Grundy (Veterans Administration Med. Center and Univ. of California, San Diego, 3350 La Jolla Village Dr., San Diego, CA 92161) *J. Lipid Res. 23*(6):811-822 (1982). The mechanisms of the hypocholesterolemic effect of polyunsaturated fats (PUSF) are not well understood. One possibility is that these fats uniquely reduce the cholesterol content of lipoproteins. The present study was carried out to determine specifically whether the ratio of cholesterol-to-protein (or apoB) in LDL (or other lipoproteins) is reduced by PUSF; also, lipoprotein composition was examined for other possible changes. Eight men and two women with different levels of plasma cholesterol were studied on the metabolic ward for 8 weeks. They were given a diet high in saturated fats (SF) for 4 weeks and another rich in PUSF for 4 weeks. On PUSF diets, mean plasma cholesterol decreased by 25% (SF=296±27 (SEM0 vs. PUSF=223±21 mg/dl) as did total plasma apoB (155±8 vs. 116±13 mg/dl). LDL-Cholesterol decreased by 26%, and LDL-apoB fell by 29%. The mean ratio of cholesterol-to-apoB did not change significantly (SF=1.52±0.04 vs. PUSF=1.48± 0.07). Likewise, HDL-cholesterol decreased by 15% (SF=51±5 vs. PUSF=43±4 mg/dl), and total plasma apoA-I was reduced by 19% (95±15 vs. 77±6 mg/dl); also, no change in the cholesterol-to-apoA-I in HDL was noted. Finally, there were no changes in cholesterol/ apoB or triglyceride/apoB ratios in VLDL despite a 23% decrease in plasma triglycerides on PUSF. Thus, the hypocholesterol effect of PUSF was uniform for all lipoproteins and usually was accompanied by a corresponding decrease in concentrations of apoprotein constituents.

DISCREPANCIES BETWEEN DATA ON ATHEROSCLEROTIC INVOLVEMENT OF HUMAN CORONARY ARTERIES FURNISHED BY GROSS INSPECTION AND BY LIGHT MICROSCOPY. C. Velican and D. Velican (Inst. of Internal Med., Colentina Hospital, 72202, Bucharest 10, Rumania) *Atherosclerosis 43*(1):39-49 (1982). Intimal areas located at centimere intervals from the points of origin of the anterior descending, circumflex and right coronary arteries, as well as intimal areas located at the main branching points of the coronary tree, were compared on macro- and microscopic levels, irrespective of the presence or absence of atherosclerotic lesions in the regions selected. The study, carried out on 606 subjects aged 1-70 years, revealed important discrepancies between data furnished by gross inspection and by light microscopy. These discrepancies occurred in the recorded character of atherosclerotic lesions: the meaning of the terms "fibrous plaque", "fatty streak" and "normal intima"; the age period in which the first atherosclerotic lesions were detected; the features of these early lesions and their sequence of development; and the presence of atherosclerotic plaques in the main branch vessels. The fibromuscular and mucoid plaques, intimal necrotic areas and incorporated microthrombi, occurring as early stages of atherosclerotic involvement, were visualized only by light microscopy. In mature adults and elderly people the intimal surface of many coronary artery samples had a different appearance from the basal intimal regions.

INTESTINAL CHOLESTEROGENESIS. A. Venugopala Rao and S. Ramakrishnan (Dept. of Biochem., Jawaharial Inst. of Postgraduate Med. Education and Res., Pondicherry 605 006) *Indian Journal of Biochemistry and Biophysics 19*(3):195-200 (1982). Synthesis of cholesterol was greater in small intestines than in the liver of humans, rabbits, and rats. The second segment of rat small intestines showed higher synthesis than the first and third segments when equally divided into three portions lengthwise in the controls and experimental animals with either increased or decreased hepatic cholesterogenesis. The second segment also exhibited greater secretory capacity than the other two segments. The active nature of the second segment was confirmed by its increased hydroxymethyl glutaryl CoA (HMG-CoA) reductase activity. HMP-shunt pathway, and increased [^{14}C] leucine incorporation into protein. In the second segment of control rats, villi exhibited greater cholesterol synthesis than crypts and muscle wall. This was also supported by increased HMP-shunt pathway and [^{14}C] leucine incorporation into proteins.

DIVERGING EFFECTS OF CHOLESTYRAMINE ON APOLIPOPROTEIN B AND LIPOPROTEIN LP (A). A DOSE–RESPONSE STUDY OF THE EFFECTS OF CHOLESTYRAMINE IN HYPERCHOLESTEROLAEMIA. B. Vessby, G. Kostner, H. Lithell and J. Thomis (Department of Geriatrics, University of Uppsala, Uppsala, Sweden) *Atherosclerosis 44*(1):61-71 (1982). Nineteen hypercholesterolaemic patients were randomly treated with either 16 or 8 g cholestyramine with a changeover after 6 weeks for a second 6-week period. During a third consecutive 6-week period all patients received 4 g cholestyramine daily. The low density lipoprotein (LDL) cholesterol and triglyceride concentrations decreased significantly (-11%, -21%, and -26% for LDL cholesterol on 4, 8 and 16g, respectively) with a dose-response effect. However, the increase from 8 g to 16 g only caused a modest additional reduction of the lipid levels. The serum concentration of apolipoprotein (apo) B was correlated to the LDL cholesterol and decreased similarly in a dose-response fashion. However, the average reduction of apo B was less pronounced (-4%, -13% and -17% on 4, 8 and 16 g of cholestyramine, respectively) resulting in a significant change of the apo B/LDL cholesterol ratio during treatment. There was a significant increase of the high density lipoprotein (HDL) cholesterol concentration, which was similar at all dose levels. Also, the apo A-I concentration in serum increased significantly but the relative decrease was less pronounced than that of HDL cholesterol, causing a significant decrease of the apo A-I/HDL cholesterol ratio. The apo A-II concentration in serum was unchanged or slightly decreased and the apo A-I/apo A-II ratio increased significantly.

LACK OF EFFECT OF ASCORBIC ACID ON SERUM LIPOPROTEIN CONCENTRATIONS IN PATIENTS WITH HYPERTRIGLYCERIDAEMIA. G. Wahlberg and G. Walldius (King Gustaf V Res. Inst. and Dept. of Internal Med., Karolinska Hospital, S-104 01 Stockholm Sweden) *Atherosclerosis 43*(2,3):283-288 (1982). Previous studies on the possible effects of ascorbic acid on lowering serum triglycerides have given conflicting results. We have treated 9 patients with stable type IV hyperlipoproteinaemia despite adequate dietary treatment with placebo for 1 month, followed by ascorbic acid at 1 g twice daily for another month. Ascorbic acid did not change either triglyceride or cholesterol in whole serum or in any lipoprotein fraction. We concluded that treatment for 1 month with 2 g of ascorbic acid per day has no effects on lowering serum triglyceride concentrations.

SERUM LIPIDS IN LONG-LACTATING AFRICAN MOTHERS HABITUATED TO A LOW-FAT INTAKE. A.R.P. Walker, B.F. Walker, D. Bhamjee and Y. Ntola (Med. Res Council Human Biochem. Res. Unit, South African Inst. for Med. Res., Johannesburg (South Africa)) *Atherosclerosis 44*(2):157-179 (1982). African women, accustomed to a low fat intake, have relatively low blood lipid levels, and are virtually free from coronary heart disease. Among lactating mothers, the amount of fat voided daily in their breast milk is of the same order as that ingested in their habitual diet. However, investigations showed that long-lactating mothers,

compared with appropriate control groups, did not exhibit significant hypolipidaemia.

SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN AFRICAN SCHOOLCHILDREN LIVING NEAR OR VERY FAR FROM SCHOOL. A.R.P Walker, B.F. Walker and Q.N. Mngomezulu (Med. Res. Council Human Biochem. Res. Unit, South African Inst. for Med. Res., Johannesburg, South Africa) *Atherosclerosis 41*:35-40 (1982). In African populations, coronary heart disease (CHD) is rare. Serum high density lipoprotein (HDL) cholesterol levels, negatively associated with CHD, understandably are significantly higher in African children and adults, compared with their White counterparts. On enquiring into the role of physical activity, observations at 3 rural African schools showed that children of 10-12 years, who regularly walked long distances attending school (average about 10 km daily) had only slightly (although significantly) elevated mean HDL cholesterol levels, compared with groups who lived near by. It is considered that the diet of pupils (*inter alia*, having low fat and high fiber contents), associated with the high level of activity which prevails generally, share responsibility for their high HDL cholesterol levels.

GENETIC VARIATION IN HUMAN APOLIPOPROTEIN E. M.R. Wardell, P.A Suckling, and E.D. Janus (Dept. of Cardiology and Biochem., The Princess Margaret Hospital, Christchurch, New Zealand) *J. Lipid Res. 23*(8):1174-1182 (1982). Genetic variation in human apoprotein E was studied using the technique of isoelectric focusing applied to delipidated very low density lipoprotein from 426 Christchurch blood donors and 7 patients with type III hyperlipoproteinemia. Six phenotypes were distinguishable by the relative proportions of the apoprotein E isoforms. In the blood donors, observed frequencies for these were: E3/3 = 51.4%; E4/3 = 25.0%; E4/4 = 1.0%; E3/2 = 20.0%; E4/2 = 1.2%; and E2/2 = 1.4%. All seven patients with type III hyperlipoproteinemia exhibited phenotype E2/2. Family studies of apoprotein E variants support a mode of inheritance controlled by three alleles acting at one gene locus. On this basis, the alleles occurred in the blood donor population with frequencies of 0.72 for the $\epsilon 3$ allele, 0.12 for the $\epsilon 2$ allele, and 0.16 for the $\epsilon 4$ allele. The $\epsilon 2$ allele influenced plasma cholesterol. The mean plasma cholesterol in subjects heterozygous for the $\epsilon 2$ allele was 5.32 mmol/l, very significantly less ($P<0.01$) than the mean of 5.84 mmol/l in an age- and sex-matched group of subjects without this allele in their genotype. The mean plasma cholesterol value of 4.92 mmol/l for the five individuals homozygous for the $\epsilon 2$ allele was also significantly less ($P<0.05$) than for age- and sex-matched subjects without the $\epsilon 2$ allele, whose mean was 5.80 mmol/l.

FATTY ACID OXIDATION OF RAT BRAIN MICROVESSELS IN HYPERTENSION, AGING AND EXPERIMENTAL DIABETES. N. Morisaki, Y. Saito, and A. Kumagai (Second Dept. of Internal Med., Schl. of Med., Chiba Univ., Chiba, Japan) *Atherosclerosis 42*(2,3): 221-227 (1982). Microvessels were prepared from rat brain and their fatty acid oxidation was investigated. This activity was much higher in brain microvessels than in other vessels or organs, suggesting that brain microvessels have a high capacity for energy production. The activity was decreased in some pathological conditions, such as hypertension, aging and diabetes mellitus. The relationship between changes in fatty acid oxidation activities and injuries of brain microvessels is discussed.

LIPID METABOLISM IN ARTERIOSCLEROTIC ARTERIAL WALL OF RATS. N. Morisaki, Shun-ichi Murano, M. Shinomiya, N. Sasaki, K. Shirai, N. Matsuoka, M. Mizobuchi, B. Akikusa, Y. Saito, and A. Kumagai (Second Dept. of Internal Med. and Dept. of Pathology, Schl. of Med., Chiba Univ., Chiba, Japan) *Atherosclerosis 43*(1):51-57 (1982). Arteriosclerotic lesions were formed in rat aorta by the administration of vitamin D_2, a high-fat diet and a thyroid suppressing agent. This treatment increased the serum total cholesterol level to 12 times the control level. In the arteriosclerotic lesions that were induced the activities of lysosomal enzymes, such as acid phosphatase and acid lipase, were higher than in controls, that of acid cholesterol esterase was decreased, those of microsomal lipid-synthesizing enzymes – such as acyl-CoA synthetase and cholesterol ester synthesizing activity – were increased and that of neutral cholesterol esterase was decreased. These data suggest that lipid metabolism in arteriosclerotic lesions was changed, resulting in the accumulation of cholesterol esters in the aorta. Administration of high-fat diet and a thyroid suppressing agent also increased the serum cholesterol levels to 12-fold the control level, but did not induce arteriosclerotic lesions. After this treatment the activities of hydrolyzing enzymes, such as acid and neutral cholesterol esterase and lipase, in the aorta increased, but the activities of lipid synthesizing enzymes also increased. These data suggest that lipid metabolism in the aorta in this condition changed to compensate for the large influx of serum lipids and to prevent arteriosclerosis. The roles of the serum lipid level, cell injury and lipid metabolism in the aorta in forming arteriosclerotic lesions are discussed on the basis of these results.

CHANGES IN STEROL METABOLISM IN THE SKIN OF DEVELOPING CHICK EMBRYO AND ALTERATIONS IN THE PRESENCE OF AN ANTICHOLESTEROLEMIC AGENT AND A CHEMICAL CARCINOGEN. T. Moroyama, K. Yoshiga, K. Takada and K. Okuda (Dept. of Oral and Maxillofacial Surgery I and Dept. of Biochem., Hiroshima Univ. Schl. of Dentistry, Hiroshima 734, Japan) *Biochim. Biophys. Acta 712*(3):659-666 (1982). Changes in sterol metabolism in the skin of chick embryo during its development were studied with embryonal chick skin and with the cultured skin tissues. Changes in sterol metabolism of the skin of chick embryo began to appear at day 17, as observed by the accumulation of dihydrolanosterol, and the ratio of dihydrolanostrol:cholesterol increased thereafter until hatching. A similar change in sterol metabolism was also observed with the cultured skin tissue of chick embryo, although the stages of development seem to have been delayed by 3 days. The active sterol metabolism of the cultured skin tissue was also confirmed by studies of incorporation of [2-^{14}C] acetate into sterols. 20,25-Diazacholesterol almost completely inhibited the incorporation of [2-^{14}C] acetate into C_{27} sterols, whereas a chemical carcinogen, 4-hydroxyaminoquinoline 1-oxide, inhibited the incorporation of [2-^{14}C] acetate into lathosterol but not that into cholesterol.

STEREOSPECIFIC SYNTHESIS AND ENZYME STUDIES OF CDP-DIACYLGLYCEROLS. P.P.N. Murthy and B.W. Agranoff (Neurosci. Lab. Bldg., The Univ. of Michigan, 1103 E. Huron, Ann Arbor, MI 48109) *Biochim. Biophys. Acta 712*(3):473-483 (1982). Fatty acid specificity of two enzymes that metabolize CDPdiacylglycerol, CDPdiacylglycerol hydrolase and CDPdiacylglycerol:inositol phosphatidyltransferase has been examined in guinea pig brain. Mixed CDP diacylglycerols were stereospecifically synthesized by the following sequence: (i) hydrolysis of a homodiacyl lecithin to 1-acyl lysoPC by action of snake venom phospholipase A_2, (ii) reacylation with the anhydride of the desired second fatty acid and dimethylaminopyridine, (iii) hydrolysis of the resultant heterodiacyl lecithin to phosphatidate with cabbage phospholipase D, and (iv) reaction of phosphatidate with CMPmorpholidate to give CDPdiacylglycerol. CDPdiacylglycerol:inositol phosphatidyltransferase showed the following rates of conversion of 40-μM suspensions of CDPdiacylglycerol in 0.15% Triton X-100 to phosphatidylinositol relative to the 1-stearoyl-2-oleoyl derivative: dipalmitoyl, 70%; distearoyl, 38%; diarachidonoyl, 9%; 1-arachidonoyl-2-stearoyl, 6%; 1-stearoyl-2-arachidonoyl, 4%. The composition of isolated phosphatidylinositol and related lipids is not explained by the fatty acid specificity of the biosynthetic enzymes and supports the intervention of a deacylation-reacylation sequence. Rates of hydrolysis of the synthetic CDPdiacylglycerols at 75 μM, in 0.3% Triton X-100, by the CDPdiacylglycerol hydrolase relative to the 1-stearoyl-2-oleoyl derivative were: dipalmitoyl, 70%; distearoyl, 32%; 1-arachidonoyl-2-stearoyl, 30%; 1-stearoyl-2-arachidonoyl, 28%; diarachidonoyl, 22%. Inhibition of this enzyme by AMP was shown to be non-competitive, with a K_i of 40 μM. The lysosomal localization of the mammalian hydrolase was confirmed.

CHANGES IN THE COMPOSITION OF FATTY CHAINS OF DIACYL, ALKACYL AND ALKENYLACYL ETHANOLAMINE AND CHOLINE PHOSPHOGLYCERIDES DURING THE DEVELOPMENT OF CHICK HEART VENTRICULAR CELLS. HIGH ACCUMULATION OF 22-CARBON FATTY ACID IN ETHER PHOSPHOLIPIDS. Y. Nakagawa, K. Waku, and Y. Ishima (Faculty of Pharmaceutical Sci., Teikyo Univ., Sagamiko, Tsukuigun, Kanagawa 199-01, Japan) *Biochim. Biophys. Acta 712*(3):667-676 (1982). The phospholipids of embryonic chick ventricular cells were analysed at various developmental stages with respect to the composition of alkylacyl, alkenylacyl and diacyl ethanolamine and choline phosphoglycerides and for the fatty chain composition of these lipid classes. The percentage of alkylacyl and alkenylacyl choline phosphoglycerides increased in the course of the development. The fatty chain composition of ether-linked phosphoglycerides was significantly different from that of the diacyl compound. In general, both ether choline and ethanolamine phosphoglycerides consisted of a significantly higher percentage of 22-carbon fatty chains, such as 22:4, 22:5 and 22:6, compared to that of the diacyl compounds, throughout the earlier stage of development. During development, there was a dramatic increase of 20:4 and a decrease of 22:6, mainly in total ethanolamine phosphoglycerides but also in choline phosphoglycerides. A particularly significant decrease of 22:6 was found with diacyl ethanolamine phosphoglycerides. When ^{14}C-labeled 22:4 and ^{3}H-labeled 20:4 were incorporated into the ethanolamine and

choline phosphoglycerides of ventricles in vitro, it was observed that 22:4 was mainly associated with ether phospholipids, especially the ethanolamine alkyl ether phospholipids, suggesting that there is a high selectivity of 22-carbon fatty acid to ether phospholipids during the synthesis of these compounds.

PLATELETS STIMULATE AORTIC SMOOTH MUSCLE CELL MIGRATION IN VITRO – INVOLVEMENT OF 12-L-HYDROXY-5,8,10,14-EICOSATETRAENOIC ACID. J. Nakao, T. Ooyama, Wen-Chang Chang, Sei-itsu Murota, and H. Orimo (Dept. of Internal Med., Tokyo Metropolitan Geriatric Hospital, Itabashi-ku, Tokyo-173) *Atherosclerosis 43*(2,3):143-150 (1982). The migration of rat aortic smooth muscle cells was measured in modified Boyden chambers. Smooth muscle cells were motile in vitro and their migration was stimulated (time- and dose-dependently) by a platelet-derived factor. Treatment of platelets with indomethacin resulted in a significant increase in smooth muscle cell migration, whereas treatment with 5,8,11,14-eicosatetraenoic acid inhibited it. Purified 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid at a very low concentration (6×10^{-15}-6×10^{-13} g/ml) significantly stimulated smooth muscle cell migration. The locomotion induced by 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid was chemokinetic. These findings point to the physiological importance of a platelet 12-lipoxygenase product of arachidonic acid in the early phase of atherosclerosis.

COMPARATIVE EFFECT OF LIPOXYGENASE PRODUCTS OF ARACHIDONIC ACID ON RAT AORTIC SMOOTH MUSCLE CELL MIGRATION. J. Nakao, T. Ooyama, H. Ito, W-C. Chang, and S-I. Murota (Department of Internal Medicine, Tokyo Metropolitan Geriatric Hospital, and Department of Pharmacology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo-173, Japan) *Atherosclerosis 44*(3):339-342 (1982). We investigated the effects of mono-hydroxyeicosatetraenoic acids (HETEs) and *N*-formyl-methionyl-leucyl-phenylalanine (F-Met-Leu-Phe) on rat aortic smooth muscle cell migration in modified Boyden chambers. 12-HETE showed the most potent stimulatory effect on smooth muscle cell migration among the mono-HETEs tested. The optimal concentrations for cell migration were 3×10^{-15} and 3×10^{-13} g/ml for 12-HETE and 10^{-8} g/ml for 15-HETE. 5-HETE and F-Met-Leu-Phe were inactive with these cells. As 12-HETE is biosynthesized from arachidonic acid by the 12-lipoxygenase pathway in platelets and macrophages, and 15-HETE by the 15-lipoxygenase pathway in granulocytes, the present results indicate an important role for such cells in the early phase of atherosclerosis.

AN EVALUATION OF PHOSPHOLIPIDS AS REGULATORS OF MONOAMINE OXIDASE A AND MONOAMINE OXIDASE B ACTIVITIES. C. Navarro-Welch and R.B. McCauley (Dept. of Pharmacology, Wayne State Univ. Schl. of Med., Detroit, MI 48201) *J. Biol. Chem. 257*(22):13645-13649 (1982). When rat liver mitochondria are treated with the phospholipase A_2 isolated from *Naja naja* venom in amounts sufficient to cause about 70% loss of mitochondrial phospholipids, both of the monoamine oxidase isozymes monoamine oxidase A and monoamine oxidase B are reduced in activity. With both monoamine oxidases, phospholipase treatment affects the enzyme's affinity for the amine substrates rather than the maximum velocity, but monoamine oxidase A retains sensitivity to the irreversible monoamine oxidase A inhibitor, clorgyline, while monoamine oxidase B is still resistant. Reconstitution and analysis of the monoamine oxidases in the presence of high concentrations of phospholipids suggested that enzymatic activity could be restored by phosphatidylinositol, phosphatidylserine, or phosphatidylcholine but not by phosphatidylethanolamine. On the other hand, these same phospholipids also could activate the monoamine oxidase in mitochondria that had not been treated with the phospholipase. The restoration of activity by the phospholipids was abolished if the excess phospholipids which were not incorporated into the mitochondria were removed. Our data indicate that, while phospholipase A_2 treatment of mitochondria inactivates both monoamine oxidase A and monoamine oxidase B, there is no evidence that reconstitution of membrane phospholipids will selectively or collectively activate monoamine oxidase A or monoamine oxidase B. However, high concentrations of unreconstituted phospholipids in the incubation medium will apparently stimulate both monoamine oxidase A and monoamine oxidase B activities.

PHOSPHOLIPIDS AS ADJUNCTS FOR CALCIUM ION STIMULATED RELEASE OF CHROMAFFIN GRANULE CONTENTS: IMPLICATIONS FOR MECHANISMS OF EXOCYTOSIS. R. Nayar, M.J. Hope, and P.R. Cullis *Biochem. 21*(19):4583-4589 (1982). Structure-function relationships for the lipid component of chromaffin granules isolated from the bovine adrenal medulla have been investigated by employing ^{31}P nuclear magnetic resonance (NMR), freeze-fracture, and spectrophotometric techniques. Two aspects have been studied in detail: the structural preferences of lipids in the isolated granule membrane and derived liposomal model membrane systems as well as the influence of exogenous lipid on the Ca^{2+}-stimulated release of granule contents. At least 90% of endogenous granule membrane phospholipids assume a liquid-crystalline bilayer configuration at physiological temperatures. Liposomal dispersions of total granule lipid exhibit bilayer structure, consistent with a structural role of phospholipids in vivo. Incubation of intact isolated granules in the presence of up to 10 mM Ca^{2+} does not induce significant release of contents above background levels. However, incubation of granules in the presence of sonicated phospholipid systems undergoing structural transitions in the presence of Ca^{2+} cause immediate and total release of granule contents at Ca^{2+} levels of 2 mM or more. This is attributed to disruption of granule membrane integrity due to fusion of vesicle systems with chromaffin granules. Direct evidence for such fusion is obtained by freeze-fracture electron microscopy. With the assumption that the inner leaflet of the adrenal cell plasma is composed predominantly of phosphatidylethanolamine and phosphatidylserine, a mechanism of Ca^{2+}-stimulated exocytotic release of catecholamines in vivo is proposed.

THE OCCURRENCE OF PSYCHOSINE AND OTHER GLYCOLIPIDS IN SPLEEN AND LIVER FROM THE THREE MAJOR TYPES OF GAUCHER'S DISEASE. O. Nilsson, J. Mansson, G. Hakansson, L. Svennerholm (Dept. of Psychiatry and Neurochem., Univ. of Goteborg, St. Jorgen's Hospital, S-422 03 Hisings Backa, Sweden) *Biochim. Biophys. Acta 712*(3):453-463 (1982). Glycolipid changes in spleen autopsy specimens were determined in four cases of Gaucher's disease type I, three cases of type II, and twelve cases of type III. These changes were also determined in liver autopsy specimens from three cases of type II and in nine cases of type III. The concentration of glucosylceramide in spleen was of the same magnitude in all three types. In liver there were large differences in the glucosylceramide concentration between splenectomized and non-splenectomized cases. In the non-splenectomized type III cases it was 9.9 ± 3.0 mmol/kg; in the splenectomized type III cases it was 24.1 ± 6.1 mmol/kg. The accelerated deposition of glucosylceramide in liver after splenectomy was also demonstrated by analyses of liver biopsy specimens. A 2-6-fold increase of gangliosides was found in liver and spleen from the three types, with no significant differences between the types. The increase of gangliosides was limited to GM3. Glucosylsphingosine was demonstrated in all samples from Gaucher's livers and spleens. The concentration in spleen was in type II, 0.16 ± 0.05 mmol/kg, in type III, 0.19 ± 0.05 mmol/kg, while in type I it was significantly lower, 0.07 ± 0.03 mmol/kg. In liver, the highest concentrations occurred in the splenectomized type III subjects, 0.16 ± 0.08 mmol/kg, while in the non-splenectomized type III cases it was 0.06 ± 0.02 mmol/kg and in type II 0.09 ± 0.02 mmol/kg.

MICROPEROXISOMES AND MITOCHONDRIA OF BROWN ADIPOSE TISSUE. HYDRODYNAMIC PARAMETERS, ISOLATION AND CAPACITY OF LONG-CHAIN FATTY ACID OXIDATION. P.T. Normann and T. Flatmark (Dept. of Biochem., Univ. of Bergen, Arstadveien 19, N-5000 Bergen, Norway) *Biochim. Biophys. Acta 712*(3):621-627 (1982). 1. Analytical differential centrifugation of brown adipose tissue homogenates from cold-acclimated guinea pigs revealed a polydispersity of both mitochondria and peroxisomes, with at least two populations of each organelle. The estimated values were $\bar{s}_H$=16685 ± 4220S and $\bar{s}_L$=4792 ± 951S (mitochondria) and $\bar{s}_H$=3364 ± 1706S and $\bar{s}_L$=889 ± 177S (peroxisomes). Based on these s values, an optimal procedure is described for the isolation of subcellular fractions enriched in mitochondria and peroxisomes, respectively. 2. When the mitochondrial and peroxisomal fractions were subjected to isopycnic gradient centrifugation on a self-generating gradient of poly(vinylpyrrolidone)-coated colloidal silica particles (Percoll) in 0.25M sucrose medium, buoyant densities of about 1.11 g/cm^3 9main fraction of mitochondria) and 1.07 g/cm^3 (main fraction of peroxisomes) were obtained. A value of 1.06 g/cm^3 was found for the microsomal fraction. 3. The main peroxisomal fraction, isolated by gradient centrifugation, did not reveal any significant oxidation of palmitoyl-CoA as measured by conventional polarographic technique, whereas a small rate of oxidation (about 2.7 ± 0.2 nmol/min per mg peroxisomal protein) was observed when measured as NAD^+ reduction. This rate contributes no more than 1% of the mitochondrial oxidation of this fatty acid and it is, therefore, concluded that peroxisomal oxidation of the predominant long-chain fatty acids found in this tissue does not make a quantitatively significant contribution to fatty acid oxidation.

INCREASED PROPORTION OF ARACHIDONIC ACID IN PLASMA LIPIDS AFTER 2 WEEKS ON A DIET OF TROPICAL SEAFOOD. K. O'Dea and A.J. Sinclair (Baker Medical Research Institute, Commercial Road, Praham, Vic 3181, Australia) *Am. J. Clin. Nutr. 36*(5):868-872 (1982). Using capillary GLC we analyzed the plasma fatty acids in a group of full-blood Aborigines in north western Aus-

tralia before and after 2 wk on a diet in which over 90% of the energy was derived from tropical fish and shellfish. The proportion of saturated fatty acids did not change and all monoenoic and ω6 fatty acids, except arachidonic, fell significantly. The proportions of arachidonic and all ω3 PUFA rose significantly on the diet. This striking rise in arachidonic was evident in all lipid fractions (phospholipids, cholesterol esters, and triglycerides). Total triglycerides in fasting plasma fell from 1.32 to 0.61 mM after the diet while total cholesterol, which was low initially, did not fall significantly. Analysis of the fatty acids in lipid extracts from the tropical seafood eaten in the study revealed an arachidonic acid content ranging from 4.8 to 14.3% of the total fatty acids. The seafood contained almost no linoleic acid but was, as expected, a rich source of ω3 fatty acids (13.6 to 31.0% of the total fatty acids). From these data we are able to conclude that seafood from tropical waters, unlike seafood from colder waters, is a natural source of polyunsaturated fatty acids from both the ω6 and ω3 series.

EFFECTS OF NICERITROL (PENTAERYTHRITOL TETRANICOTINATE) ON PLASMA LIPOPROTEIN CONCENTRATION: INCREMENT OF HIGH DENSITY LIPOPROTEIN (HCL) CHOLESTEROL AND HDL-CHOLESTEROL/LOW DENSITY LIPOPROTEIN CHOLESTEROL RATIO IN HYPO-HIGH DENSITY LIPOPROTEINEMIA. K. Oida, T. Nakai, T. Tamai, Y. Kutsumi, T. Kobayashi, T. Hayashi, S. Yamada and R. Takeda (The 2nd Dept. Int. Med., Schl. Med., Kanazawa Univ. 13-1, Takaramachi, Kanazawa City, Japan 920). *Artery 10*(4):266-285 (1982). Effects of niceritrol were examined. (A) Niceritrol, 750 mg/day was given for first 12 weeks and 1,500 mg/day for an additional 12 weeks to 12 subjects. In six of them, the HDL cholesterol (Ch) levels were less than 45 mg/100 ml with normal plasma cholesterol levels and with plasma triglyceride levels of less than 250 mg/100 ml. In the other six, HDL-Ch, plasma cholesterol and triglyceride levels were all within normal limits except in one with a higher triglyceride level. Plasma lipoproteins were fractionated by sequential ultracentrifugation and analyzed for cholesterol, triglyceride (TG), phospholipid (PL) apolipoprotein (Apo) B and Apo A-I, every 4 weeks. Niceritrol decreased plasma-Ch, VLDL-Ch, LDL-Ch, plasma-TG, VLDL-TG, plasma-PL, VLDL-PL and LDL-PL. Niceritrol increased HDL-Ch and the HDL-Ch/LDL-Ch ratio. Effects were more significant with 1,500 mg/day than 750 mg/day and were more marked in patients with lower pretreatment HDL-Ch levels. Apo B level at 20 weeks was significantly lower than at pretreatment. Initial plasma apo A-I levels were about one-half of the control plasma. After treatment with niceritrol, apo A-I concentration tended to increase. (B) Changes of lipids concentration in HDL_2 and HDL_3 fraction were investigated in 5 patients during treatment with niceritrol. HDL_2-Ch levels tended to increase without significant changes of HDL_3-Ch levels. HDL_2-Ch/HDL_3-Ch ratio had a tendency to increase. Significant but weak inverse correlation between changes of VLDL-TG and HDL-Ch was observed, suggesting that the increment of HDL might be partly due to promoted lipolysis of TG-rich lipoproteins.

DOSE-RESPONSE STUDY OF THE EFFECT OF CIPROFIBRATE ON SERUM LIPOPROTEIN CONCENTRATIONS IN HYPERLIPOPROTEINAEMIA. A.G. Olsson and L. Orö (King Gustaf V Res. Inst. and Dept. of Med. at Karolinska Hospital and Ersta Hospital, Stockholm, Sweden) *Atherosclerosis 42*(2,3):229-243 (1982). The effect of ciprofibrate, 2[*p*-(2,2-dichlorocyclopropyl)-phenoxyl]-2-methyl propionic acid, in daily doses of 50, 100 and 200 mg was studied in 50 patients with hyperlipoproteinaemia (21 type IIA, 10 type IIb an 19 type IV). Ciprofibrate was convenient to take and was without subjective side effects. The greatest hypolipidaemic effects were reached for all lipoproteins with 200 mg daily. In type IIA and IIB, mean low density lipoprotein (LDL) cholesterol was normalized on the 200 mg dose. The effect was higly dependent on initial LDL cholesterol concentrations, decreases being observed above 4 mmol/l and increases below that concentration. Mean very low density lipoprotein (VLDL) triglyceride concentrations decreased on 200 mg per day by 48-59%. HDL cholesterol increased in all types of hyperlipoproteinaemia by 6-19%, the change being unrelated to changes in VLDL lipids. With a dosage of 200 mg daily the effects were maintained for the following period of 6 months. It is concluded from this study that it would be appropriate to start patients on 100 mg daily and then titrate their dose according to response. The optimal dosage for ciprofibrate seems to be 200 mg daily.

NUTRITIONAL STATUS IN A HEALTHY ELDERLY POPULATION: VITAMIN D. J.L. Omdahl, P.J. Garry, L.A. Hunsaker, W.C. Hunt, and J.S. Goodwin (Univ. of New Mexico Schl. of Med., Depts. of Biochem., Pathology (PJG, WCH), and Med. (JSG), Albuquerque, NX) *Am. J. Clin. Nutr. 36*(6):1225-1233 (1982). The vitamin D status in a group of healthy free-living elderly people was determined by measuring dietary and supplemental vitamin D intakes and the plasma concentration of 25-hydroxyvitamin D (25-OHD). Median dietary intake was 88 IU for vitamin D, with 26% of the population taking a median supplement of 400 IU. Plasma 25-OHD was significantly lower in the elderly (15.5 ng/ml) compared to a younger control (29.1 ng/ml) population. Within the elderly population, the plasma 25-OHD demonstrated a seasonal influence (nadir in January, zenith in September) and was consistently higher for men compared to women. People taking vitamin D supplements had higher plasma 25-OHD concentrations regardless of seasonal influence. Plasma alkaline phosphatase, an index for bone loss, was inversely related to the plasma 25-OHD concentration. Inadequate dietary vitamin D intake and inadequate sunlight exposure appeared to be contributory to the observed low vitamin D status. It is suggested that American elderly consider using a combination of moderate vitamin D supplementation and increased sunlight exposure in order to improve their vitamin D nutriture.

RAT PLASMA LIPOPROTEINS: RE-EVALUATION OF A LIPOPROTEIN SYSTEM IN AN ANIMAL DEVOID OF CHOLESTERYL ESTER TRANSFER ACTIVITY. Y. Oschry and S. Eisenberg (Lipid Res. Lab., Dept. Med. B, Hadassah Univ. Hospital, Jerusalem, Israel) *J. Lipid Res. 23*(8):1099-1106 (1982). Plasma lipoproteins from male rats were isolated by rate zonal centrifugation. Four lipoproteins were identified: VLDL, LDL, HDL_1, and HDL_2. LDL, HDL_1, and some HDL_2 distributed within the salt density interval of 1.006-1.085 g/ml, while HDL_2 was found in the 1.063-1.21 g/ml interval. HDL_3 was not identified in the rat. Rat VLDL is poor in cholesteryl esters (1.5-3.0% of total mass) and nearly lacks the smaller and denser particle subpopulation which is predominant in humans. Rat LDL, containing a relatively large amount of triglyceride (20.2% of total mass) and a small amount of cholesteryl ester (27.5%), could be isolated free of apoproteins other than apoB. HDL_1 is a cholesteryl ester-rich lipoprotein that occupies a density interval overlapping both LDL and HDL_2. ApoE is the major protein constituent of HDL_1; apoA-I, A-IV, and C are also present. ApoA-I-rich HDL_2 is the only human-like HDL subpopulation found in rats. Lipoproteins from fasted and non-fasted rats were essentially similar. Palmitic, palmitoleic, and oleic acids were the major cholesteryl ester fatty acids in VLDL and LDL. In vitro incubation of biosynthetically labeled HDL_2 cholesteryl ester with rat plasma demonstrated minimal transfer of the labeled cholesteryl ester to VLDL and LDL. These results indicate biological immiscibility of HDL cholesteryl esters with those of lower density lipoproteins. The finding of cholesteryl ester-poor VLDL and LDL and the presence of HDL as larger and less dense subpopulations is compatible with the absence of cholesteryl ester transfer activity in an animal with pronounced LCAT activity.

EFFECT OF VALIUM (DIAZEPAM) ON EXPERIMENTAL ATHEROSCLEROSIS IN ROOSTERS. D.J. Patel, H.Y.C. Wong, H.A.I. Newman, T.E. Nightingale, C. Frasinel, F.B. Johnson, S. Patel and B. Coleman (Dept. Phys. and Biophys., Coll. of Med., Howard Univ., 520 W. St., N.W., Washington, D.C. 20059) *Artery 10*(4): 237-249 (1982). Development of aortic and coronary atherosclerotic plaques were investigated in roosters fed an atherogenic diet with and without the addition of Valium (0.2 mg/kg twice daily) over a period of 5 months, as a step toward understanding the role of emotional factors in atherogenesis. Plasma levels of cholesterol and triglycerides, hemodynamic parameters, and body weight were measured. There was a progressive and quantitatively similar, body weight gain in all birds. In addition, there were no significant differences in values for blood pressure, cardiac output, and heart rate between the experimental groups. Conversely, birds receiving the atherogenic diet, as well as those receiving the atherogenic diet along with Valium, exhibited a marked hypercholesterolemia which reached a peak of 600 mg/dl in 4-6 weeks, before decreasing to between 200 and 300 mg/dl by the 10th week. Plasma triglyceride levels followed a qualitatively similar pattern as plasma cholesterol. Birds fed an atherogenic diet alone developed atherosclerotic lesions on the aortic surface which was more pronounced on the abdominal than the thoracic aorta. Aortas from birds given Valium along with the atherogenic diet were completely free of lesions. Histological sections of coronary arteries showed severe lesions in 4 out of 7 birds fed the atherogenic diet alone, whereas birds given Valium also had only an occasional slight lipid deposit. It was concluded that Valium provides some protection against the development of atherosclerosis in roosters fed an atherogenic diet.

CHARACTERISTIZATION OF LIPOPROTEIN IN A KINDRED WITH FAMILIAL HYPERCHOLESTEROLEMIA. W. Patsch, R. Ostlund, I. Kuisk, R. Levy, and G. Schonfeld (Lipid Res. Ce., Dept. of Pre. Med., Washington, Univ., St. Louis, MO) *J. Lipid Res. 23*(8): 1196-1205 (1982). To study possible consequences of decreased numbers of cellular LDL receptors on plasma lipoproteins, we char-

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acterized the low density and high density lipoproteins in fasting plasmas of a kindred with receptor-defective hypercholesterolemia. Both fast and slow floating LDL were found among affected members of the kindred. From molecular weights and chemical compositions, number of molecules of lipid components per LDL particle were calculated. Numbers of phospholipid, free cholesterol, and cholesteryl ester molecules were each strongly correlated with molecular weights of LDL particles. Thus, differences in mass of LDL resulted from alterations primarily of the phospholipid, free cholesterol, and cholesteryl ester contents per particle, whereas amounts of protein and triglyceride per particle were relatively constant. LDL of members of the kindred affected with familial hypercholesterolemia (FH) differed from LDL of unaffected members by containing more molecules of cholesteryl ester and less triglyceride, even when LDL were matched for molecular weight. Thus, FH affected the core lipid composition of LDL. "Hepatic" apoB (B-100, B-74, and B-26) comprised 96% of the protein moiety in all subjects, while "intestinal" apoB (B-48) was not found in any of the LDL preparations. Therefore, LDL of both normal and affected members probably is derived from hepatic lipoproteins. There was no correlation between the presence or absence of HDL_2 and FH status. There appeared to be a tendency toward lower LDL-cholesterol in those affected subjects whose plasma contained HDL_2. This suggestive inverse relationship between LDL and HDL_2 needs confirmation.

25-HYDROXYVITAMIN D_3-24-HYDROXYLASE IN RAT KIDNEY MITOCHONDRIA. J.I. Pedersen, H.H. Shobaki, I. Homberg, S. Bergseth, and I. Bjorkhem (Inst. for Nutr. Res., Schl. of Med., Univ. of Oslo 3, Norway) *J. Bio. Chem. 258*(2):742-746 (1983). Assay conditions for the measurement of 25-hydroxy-vitamin D_3-24-hydroxylase activity in rat kidney mitochondria have been worked out. The product, 24,25-dihydroxyvitamin D_3 was quantitated either by high pressure liquid chromatography or by isotope dilution mass spectrometry. By these procedures, the enzyme activity could be measured with saturating concentration ($> 2.5 \times 10^{-6}$ M) of substrate. Pretreatment of the animals by aminophylline (Kulkowski, J.A., Chow, T., Martinez, J., and Chazarian, J.G. (1979) Biochem. Biophys. Res. Commun. 90, 50-57) stimulated the 24-hydroxylase activity in vitro at least 2 to 3-fold. The identity of the product was verified by gas chromatography-mass spectrometry. The rates of the reaction varied between 1.5 and 5 pmol/mg of mitochondrial protein·min (at 25 C), and the K'_m. Malate, succinate, and isocitrate were all able to support the reaction. Low O_2 tension, CO, KCN, and the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone inhibited the reaction, while the respiratory inhibitor rotenone had no effect. Metyrapone inhibited the reaction with 50% inhibition at a concentration of 2.5 μmol/ml. The enzyme was found to be localized inside the inner mitochondrial membrane. The results indicate that in the rat the renal mitochondrial 25-hydroxyvitamin D_3-24-hydroxylase is a cytochrome P-450 and that the reducing equivalents are primarily supplied by NADPH via the energy-dependent transhydrogenase.

LONG-TERM EFFECT OF HYPERLIPIDEMIC SERUM ON THE SYNTHESIS OF GLYCOSAMINOGLYCANS AND ON THE RATE OF GROWTH OF RABBIT AORTIC SMOOTH MUSCLE CELLS IN CULTURE. K. Pietila (Department of Biomedical Sciences, University of Tampere, P.O. Box 607, SF-33101 Tampere 10, Finland) *Atherosclerosis 42*(1):67-75 (1982). Rabbit aortic smooth muscle cells (SMCs) were successfully subcultured in 10% hyperlipidemic rabbit serum (HLS) for at least 9 passages. SMCs grown in HLS grew into higher cell densities than SMCs cultured in normolipidemic rabbit serum (NLS) for at least 4-5 passages in NLS and HLS, respectively. However, cells cultured in NLS and HLS for up to 7 passages had similar growth characteristics when they were trypsinised and seeded to grow in 10% fetal calf serum (FCS). Incorporation of [^{3}H] glucosamine into GAGs was taken to represent their rate of synthesis. As compared with cultures incubated in 10% NLS, incubation of rabbit aortic SMCs in the presence of 10% HLS increased the synthesis of sulphated GAGs secreted into the pericellular space by 35% during the first 24 hr of contact with HLS. After preincubation for one week in the presence of HLS the synthesis of sulphated GAGs secreted into the incubation medium and into the pericellular space was stimulated by 95% and 34%, respectively. The stimulation of the synthesis of sulphated GAGs by HLS continued for up to 4 weeks at least if the contact of the cells with HLS was maintained. When the cells were subcultured in the presence of NLS and HLS and seeded to grow in FCS after the 1st, 3rd and 7th trypsinisations, the synthesis of sulphated GAGs in cultures of cells from both sources was similar.

EFFECT OF BEZAFIBRATE ON PLASMA LIPIDS, LIPOPROTEINS, APOLIPOPROTEINS AI, AII AND B AND LCAT ACTIVITY IN HYPERLIPIDEMIC, NON-INSULIN-DEPENDENT DIABETICS. R. Prager, G. Schernthaner, G.M. Kostner, I. Mühlhauser, R. Zechner and W. Dorda (Dept. of Med. II and Inst. for Med. Computer Science of the Univ. of Vienna, Ludwig Boltzmann-Inst. for Clinical Endocrinology, Vienna, Austria) *Atherosclerosis 43*(2,3): 321-327 (1982). The effect of bezafibrate on plasma lipids, lipoproteins, apolipoproteins AI, AII and B, and LCAT activity was investigated in 16 hyperlipidemic, non-insulin-dependent diabetics, who were treated for 8 weeks with either placebo or bezafibrate in a double-blind, cross-over design. Bezafibrate induced a significant decrease in plasma triglycerides ($P<0.01$), cholesterol ($P<0.05$), VLDL triglycerides ($P<0.05$) and VLDL cholesterol ($P<0.01$), and a significant increase in HDL cholesterol ($P<0.01$), whereas LDL cholesterol remained unchanged. The apolipoprotein AI/apolipoprotein B ratio increased significantly ($P<0.05$), although individual changes in these apolipoproteins were not significant. Apolipoprotein AII increased significantly ($P<0.0001$) and the apolipoprotein AI/apolipoprotein AII ratio decreased ($P<0.0001$), indicating an increase in the HDL_3 rather than the HDL_2 fraction. No significant change in LCAT activity was observed.

CHARACTERIZATION OF INTERMEDIATES UP TO LIPID-LINKED HEPTASACCHARIDE IMPLICATED IN THE BIOSYNTHESIS OF *SACCHARAOMYCES CEREVISIAE* MANNOPROTEINS. C. Prakash and I.K. Vijay (Dept. Dairy Sci., Univ. Maryland, College Park, MD 20742) *Biochem. 21*(19):4810-4818 (1982). Lipid-linked oligosaccharide Glc_3Man_9-(GlcNAc)2 serves as a precursor for the biosynthesis of the inner core portion of asparagine-linked polysaccharide of *S. cerevisiae* mannoproteins. As a prelude to establishing its detailed structure and assembly, lipid-linked oligosaccharides belonging to the general structure Man_n-$(GlcNAc)_2$, n=1-5, and presumably serving as intermediates in the assembly sequence were isolated from an in vitro incubation of *S. cerevisiae* microsomes with UDP-*N*-acetyl-glucosamine and GDP-[^{14}C]mannose. On the basis of size, elution characteristics on a column of concanavalin A-sepharose, exo- and endoglycosidase digestions, acetolysis, and methylation analysis, we found the structures for the major species within the tri- through heptasaccharides. These structures are identical with those of the major intermediates involved in biosynthesis of asparagine-linked glycoproteins in animal tissues. Minor isomers were observed in the tetra- through heptasaccharides and structurally characterized. The inner core of *S. cerevisiae* mannoproteins has some structural differences from the high mannose glycoproteins of animal origin. Our studies indicate that the lipid-linked assembly of the precursor unit for the inner core of *S. cerevisiae* mannoproteins might be similar to that in animal systems. The precise role of the minor isomers within the lipid-linked oligosaccharides in the assembly of the precursor oligosaccharide is unclear; possibly these arise due to a lack of specificity of the mannosyltransferases for acceptor substrates during the assembly process.

EFFECTS OF OLEATE AND COMPACTIN ON THE METABOLISM AND SECRETION OF CHOLESTEROL AND CHOLESTERYL ESTER BY RAT HEPATOCYTES. C.R. Pullinger and G.F. Gibbons (Med. Res. Council Lipid Metabolism Unit, Hammersmith Hospital, Ducane Road, London W12 0HA U.K.) *Biochim. Biophys. Acta 713* (2):323-332 (1982). Incubation of rat hepatocytes with oleate for a period of 1 hr gave rise to a decrease in the total (esterified plus unesterified) cholesterol associated with very-low-density lipoprotein (VLDL). This effect was no longer apparent after longer incubation periods. The rate of cholesterol biosynthesis decreased during the first hour of incubation in the presence of oleate. After longer incubation periods, however, more cholesterol was synthesized in the presence of oleate than in its absence. The extracellular presence of oleate gave rise to a 2-fold increase in the concentration of cellular cholesteryl ester. Under these conditions cholesteryl ester contributed a larger proportion of the total cholesterol secreted with the VLDL. The cholesteryl ester associated with VLDL was derived predominantly from cholesteryl ester synthesized intracellularly. Inhibition of cholesterol synthesis with compactin did not significantly alter the rate of secretion of VLDL-cholesterol. Newly synthesized non-esterified cholesterol equilibrated with the bulk of pre-existing cellular cholesterol before secretion with the VLDL. This was true irrespective of the rate of endogenous cholesterol synthesis.

ISOLATION AND CHARACTERIZATION OF AN ACYL CARRIER PROTEIN FROM PIGEON LIVER FATTY ACID SYNTHETASE BY CONTROLLED PROTEOLYSIS WITH ELASTASE. R.N. Puri and J.W. Porter (Lipid Metabolism Lab., William S. Middleton Memorial Veterans Hospital and the Dept. of Physiological Chem., Univ. of Wisconsin, Madison, WI 53706) *Biochim. Biophys. Acta 712*(3):576-589 (1982). Controlled proteolytic cleavage of 4'-phospho[^{14}C] pantetheine-labeled pigeon liver fatty acid synthetase generates two 4'-phospho[^{14}C] pantetheine-labeled peptides, E_c1

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and E_c2. These are separated from each other and the core enzyme by gel permeation chromatography on a Sephadex G-75 column. The two radioactively labeled peptides constitute 50% of the radioactivity initiatially present in the 4'-phospho[^{14}C] pantetheine-labeled fatty acid synthetase. The remaining label in the core enzyme is released quantitatively by proteolytic cleavage with trypsin. The molecular weights of E_c1 and E_c2 peptides are 12000 and 6000. E_c1 is characterized as an acyl carrier protein by the transacylation reaction between the unlabeled E_c1 peptide and radioactively labeled acetyl- and malonyl-CoA. Since E_c2 peptide also contains the prosthetic group present in the E_c1 peptide, the E_c2 peptide appears to result from the proteolytic cleavage of E_c1. Amino acid composition of the acyl carrier protein shows the presence of 1 mol of 4'-phosphopantetheine per mol of protein. 2 mol of acyl carrier protein are present per mol of the fatty acid synthetase. The amino acid analysis is in good agreement with the molecular weight of the E_c1 peptide, N-Terminal amino acid analysis of this peptide shows the presence of an arginine residue.

TRANSPORT OF IONS ACROSS AN ARTIFICIAL MEMBRANE INCORPORATED WITH SOME PHOSPHOLIPIDS. R.K. Ram, S.A. Rizvi and O.N. Tripathi (Dept. Chem., Lucknow Univ., Lucknow, India) *Indian J. Biochem. Biophys. 19*(3):213-216 (1982). EMF with transport (E_t) and current (I') across a model membrane consisting of Whatman filter paper No. 42 (WP) and phospholipids (phosphatidylcholine, phosphatidylserine, and phosphatidylinositol) have been measured for chloride salts of potassium, sodium and calcium at varying salt concentrations in two half diffusion cells. Transport number, permselectivity (P_S) and ionic flux (J) have been calculated. The transport numbers of K^+ and Na^+ increased on introducing the phospholipids into the filter paper; P_S for K^+ and Na^+ was found between 0.06 and 0.2 depending on the concentration ratios of the electrolytes used. There is a great increase in the flux ratios (J^+/J^-) when WP was coated with phospholipids than filter paper alone. This showed that such membranes behaved as cation elective membranes. Transport number and J^+/J^- also increased as the concentration ratio of the electrolytes in two half cells decreased. Only at a low concentration ratio, Ca^{2+} shows a behavior similar to that of K^+ and Na^+.

EFFECT OF PANTETHINE ON THE BIOSYNTHESIS OF CHOLESTEROL IN HUMAN SKIN FIBROBLASTS. S. Ranganathan, R.L. Jackson, and J.A.K. Harmony (Division of Lipoprotein Research, Departments of Biological Chemistry and Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267) *Atherosclerosis 44*(3):261-273 (1982). Panthethine [D-bis-(N-pantothenyl-β-aminoethyl)-disulfide] is a compound used clinically to decrease plasma triglycerides and to increase HDL cholesterol. To understand the mechanism of action of this drug, its effect on the synthesis of cholesterol in cultured skin fibroblasts was assessed. The addition of pantethine (100-200 μM) to cultured cells caused an 80% inhibition in cholesterol synthesis as measured by the incorporation of radiolabeled acetate or mevalonolactone. Inhibition occurred within 4 hr of adding the drug and was specific for pantethine; other sulfur-containing compounds such as dithiothreitol, glutathione, coenzyme A and cystine did not inhibit. The inhibition of cholesterol synthesis resulted in the accumulation of radiolabeled methyl sterols. The drug also inhibited total fatty acid synthesis. The amount of [^{14}C] pantethine detected in the cells is very low and represented less than 0.5% of the radiolabeled pantethine added in the medium. At low pantethine concentrations, the drug had negligible effects on the biosynthesis of DNA, protein and phospholipid.

FAILURE OF CHRONIC CIGARETTE SMOKE EXPOSURE TO ALTER PLASMA LIPOPROTEINS OF STUMPTAILED MACAQUES (*MACACA ARCTOIDES*). T.L. Raymond, A.J. Delucia, L.R. Bryant (Depts. of Internal Med. and Surgery, East Tennessee State Univ., Quillen-Dishner Coll. of Med., Johnson City, TN 37614) *Atherosclerosis 41*:27-33 (1982). Twenty-one 8-14 kg adult stumptailed macaques, *Macaca arctoides*, were fed a standard laboratory diet and divided into 3 groups. The high-dose group and low-dose group were exposed to cigarette smoke at the human equivalent of 3 packs and 1 pack per day, respectively, 7 days per week, for 3-5 years. Eight animals served as cage and sham controls. Peak blood carboxyhemoglobin (COHb) levels measured immediately after smoking showed levels of 0.5 ± 0.1%, 3.6 ± 1.0% and 5.7 ± 2.8% for sham controls, low, and high dose smokers, respectively. Hemoglobin and hematocrit values were 2-7% higher (N.S. to $P < 0.05$) for smoking groups, presumably as a consequence of chronically elevated COHb levels. No significant differences were seen in total plasma cholesterol and lipoprotein cholesterol concentration measured at four intervals over a period of one year. We conclude from these data that, while fed a low fat diet, chronic cigarette smoke inhalation fails to alter plasma lipoprotein levels in this animal model.

FURTHER EVIDENCE FOR THE ROLE OF HIGH DENSITY LIPOPROTEIN IN THE REMOVAL OF TISSUE CHOLESTEROL IN VIVO. D. Reichl, D.N. Rudra, N.B. Myant, and J.J. Pflug (MRC Lipid Metabolism Unit, Hammersmith Hospital, DuCane Road, London W12 0HS, Great Britain) *Atherosclerosis 44*(1):73-84 (1982). The lipoproteins of human peripheral lymph and plasma were separated according to particle size by polyacrylamide gradient gel electrophoresis. All samples of lymph contained lipoproteins that moved to the same positions on the gel as plasma LDL and plasma HDL. Some samples of lymph also contained lipoproteins with the mobility of VLDL and IDL. The lymph lipoproteins corresponding to plasma LDL reacted with anti-LDL serum and those corresponding to plasma HDL reacted with anti-HDL serum. In the lipoprotein fraction with the mobility of HDL, the proportion of particles larger than catalase was greater in lymph than in plasma. It is suggested that the shift in size distribution towards larger HDL particles in lymph compared with plasma is due to uptake of cholesterol from extravascular tissue by HDL particles after they have reached the interstitial fluid from the plasma, rather than to preferential movement of larger particles across the capillary walls.

YUCATAN MINIATURE SWINE AS A MODEL FOR DIET-INDUCED ATHEROSCLEROSIS. J.S. Reitman, R.W. Mahley, and D.L. Fry (Natl. Inst. of Arthritis, Metabolism, and Digestive Diseases, Natl. Inst. of Health, Phoenix, AZ 85016) *Atherosclerosis 43*(1):119-132 (1982). Nine female Yucatan miniature swine, a breed not previously evaluated for their potential usefulness as a model for experimental atherosclerosis studies, were fed a high-fat, high-cholesterol diet for 10-12 months. These swine and 4 control (low-fat, low-cholesterol-fed) swine underwent a complete necropsy at the end of this period to characterize the atherosclerosis both by gross and microscopic examinations. Cholesterol feeding led to elevated serum cholesterol levels and the development of accelerated atherosclerosis. Control animals on a low-cholesterol diet had little gross or microscopic atherosclerosis. All of the cholesterol-fed swine had more extensive atherosclerosis than any of the controls by gross inspection of the Sudan-stained arterial tissue. There was individual variation suggesting the interaction of factors in addition to the plasma cholesterol which determine the extent and severity of atherosclerosis. However, it was possible to show a positive correlation between hypercholesterolemia and (1) intimal thickening in the terminal abdominal aorta and mesenteric artery, and (2) increased fat deposition in the mesenteric artery. The cholesterol-induced atherosclerosis was characterized by the deposition of lipid in and around cells. Complicated atherosclerotic lesions similar to human atherosclerosis were characterized by marked intimal proliferation, necrosis, cholesterol crystal deposition, and calcification. It is concluded that the Yucatan miniature swine represent an important additional animal model in which to study certain aspects of atherosclerosis.

LASER-INDUCED EUROPIUM (III) LUMINESCENCE AS A PROBE OF THE METAL ION MEDIATED ASSOCIATION OF HUMAN PROTHROMBIN WITH PHOSPHOLIPID. M.-J. Rhee, W. DeW. Horrocks, Jr., and D.P. Kosow (Plasma Derivatives Lab., Bethesda, Maryland 20014) *Biochem. 21*(19):4524-4528 (1982). $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy of Eu(III) has been used to investigate the Eu(III) and phospholipid binding properties of human prothrombin. The results indicate that human prothrombin contains four high-affinity Eu(III) binding sites which are distributed into two classes of binding sites. When 4 equiv of Eu(III) is bound to prothrombin, the prothrombin is capable of binding to phospholipid vesicles. The deuterium isotope effect on the lifetime of the Eu(III)-prothrombin complex and the Eu(III)-prothrombin-phospholipid complex was used to determine the number of water molecules coordinated to the Eu(III). In both complexes, each of the Eu(III)'s coordinated to 2.5 ± 0.5 water molecules. These results indicate that the binding of the Eu(III)–prothrombin complex to the phospholipid does not require the formation of a prothrombin-Eu(III)–phospholipid bridge.

LIPOPROTEIN PRODUCTS OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE AND CHOLESTERYL ESTER TRANSFER. H.G. Rose and P. Ellerbe (Lipid Res. Lab., Veterans Admin. Med. Center, Bronx, NY 10468) *Biochim. Biophys. Acta 712*(3):547-556 (1982). High-density lipoprotein substrates and products of human plasma lecithin: cholesterol acyltransferase have been labelled with radioisotopic cholesteryl esters in order to facilitate identification. [^{3}H] Cholesteryl esters were formed by endogenous HDL_3/VHDL enzyme following incubation with mixed vesicles of phosphatidylcholine, unesterified cholesterol and ^{3}H-labelled unesterified cholesterol. Transfer of labelled esters to acceptor lipoproteins was employed to distinguish a hypothetical transfer complex. Separation of labelled HDL_3/VHDL was by gel-permeation chromatography. The results indicate that a subpopulation of labelled HDL_3/VHDL cholesteryl esters were removed by VLDL/LDL during a 3 hr transfer

period and these derive from the smaller lipoproteins of the spectrum. HDL carrying non-transferable [^{3}H] cholesteryl esters localize to the larger HDL_3. Transfer rates were proportional to ratios of acceptor to donor lipoproteins. Net transfer of cholesteryl esters from the smaller HDL_3 also occurred, but was smaller in magnitude. Acyltransferase assays indicated that enzyme distribution is skewed to larger-sized HDL_3, suggesting that the non-transferable components might be lecithin: cholesterol acyltransferase-containing parent complexes while the smaller transfer products contain little acyltransferase. The results fit the hypothesis that a parent HDL_3-lecithin: cholesterol acyltransferase complex generates a smaller-sized lipoprotein product which is active in cholesteryl ester transport.

FATTY ACID AND GLUCOSE INCORPORATION INTO HUMAN ADIPOSE TISSUE IN NON-INSULIN-DEPENDENT DIABETES AND IN INSULINOMA. INVERSE RELATIONS WITH PLASMA TRIGLYCERIDE AND GLUCOSE CONCENTRATIONS. P. Rubba, G. Pezzella, A. Rivellese and A. Postiglione (Center for Atherosclerosis and Metabolic Diseases, Institute of Semeiotica Medica, 2nd Faculty of Medicine, University of Naples, Naples, Italy) *Atherosclerosis 42*(1):31-40 (1982). Decreased fatty acid and glucose incorporation into human adipose tissue (FIAT and GLIAT) are frequently found in primary hypertriglyceridemia (HTG) and might also contribute to the defective removal of lipoprotein triglyceride (TG) in non-insulin-dependent diabetes mellitus (NIDDM). To study this possible mechanism, FIAT and GLIAT were determined in needle biopsy specimens from 14 patients with newly diagnosed NIDDM and in 14 age- and weight-matched controls. A patient with insulinoma and hyperinsulinism was also studied. FIAT and GLIAT processes were markedly reduced in patients with NIDDM that developed at the onset of maturity. Insulinoma patients, with normal plasma TG, showed FIAT-GLIAT values in the high to normal range before operation. A direct, highly significant correlation ($P<0.001$) was demonstrated between FIAT and GLIAT in diabetics, insulinoma and controls when considered together. Plasma TG and glucose concentrations were inversely related to FIAT and GLIAT. These relationships were independent of the degree of obesity. It is suggested that impaired FIAT and GLIAT might contribute to defective TG removal and HTG which are often demonstrated in NIDDM.

PLATELET LIPOXYGENASE-DEPENDENT OXYGEN BURST. EVIDENCE FOR DIFFERENTIAL ACTIVATION OF LIPOXYGENASE IN INTACT AND DISRUPTED HUMAN PLATELETS. A.I. Schafer, N.A. Turner and R.I. Handin (Hematology Div. and Hemostasis Unit, Dept. of Med., Brigham and Women's Hospital, and Harvard Med. Schl., 75 Francis St., Boston, MA 02115) *Biochim. Biophys. Acta 712*(3):535-541 (1982). The metabolism of arachidonic acid in platelets by both cyclooxygenase and lipooxygenase involves the rapid consumption of molecular oxygen. However, selective inhibition of cyclooxygenase completely abolishes the arachidonate-induced oxygen burst in intact platelets. This is in contrast to platelet lysates, in which approximately 50% of the arachidonate-induced oxygen burst remains detectable following inhibition of cyclooxygenase with acetylsalicylic acid. This lipoxygenase oxygen burst is blocked by preincubation of the platelets with ETYA, which inhibits both cyclooxygenase and lipoxygenase. In cell-free 100,000 X g supernatants of platelet lysates, which contain only lipoxygenase activity, archidonate induces an oxygen burst which is not blunted by preincubation with aspirin but is completely abolished by preincubation with ETYA. The finding of a lipoxygenase-dependent oxygen burst in platelet lysates but not in intact platelet suspensions suggests differential activation or differential availabilityof platelet lipoxygenase in intact and disrupted platelets. This was confirmed by a 5 min. lag in the generation of [^{14}C] HETE (the major lipoxygenase product) from [^{14}C] arachidonic acid in intact platelets, but an almost immediate initiation of [^{14}C] HETE production in platelet lysates. In contrast, the synthesis of [^{14}C] thromboxane B_2 (the major cyclooxygenase product) from [^{14}C] arachidonic acid began immediately in both intact and disrupted platelet preparations and peaked within 5 min. These observations provide new insight into factors controlling platelet hydroxy acid production and help to explain the nature of the platelet oxygen burst.

TRANSFER OF HUMAN LYMPH CHYLOMICRON CONSTITUENTS TO OTHER LIPOPROTEIN DENSITY FRACTIONS DURING IN VITRO LIPOLYSIS. E. Schaefer, M. Wetzel, G. Bengtsson, R. Scow, H. Brewer, Jr., T. Olivecrona (Molecular Disease Branch, Natl. Heart, Lung, and Blood Inst., Natl. Inst. of Health, Bethesda, MD) *J. Lipid Res. 23*(9):1259-1273 (1982). To ascertain whether chylomicron constituents would be transferred to low density lipoprotein (LDL, d 1.019-1.063 g/ml) and high density lipoprotein (HDL, d 1.063-1.21 g/ml) density fractions during lipolysis in the absence of other lipoproteins, the in vitro effect of bovine milk lipoprotein lipase on human thoracic duct lymph chylomicrons in the presence of albumin was examined. In incubations without lipase, over 90% of chylomicron constituents remained in the 1.006 g/ml supernate, and large particles ranging in diameter mainly from 750-6000 Å were observed by electron microscopy. After the addition of lipase, lipolysis ranged from 69.0-94.6% and numerous collapsed particles with redundant surface were seen, as well as smaller particles within the LDL and HDL density region. With lipolysis, the majority of chylomicron cholesterol and phospholipid mass was transferred to LDL and HDL, while chylomicron apolipoprotein (apo) A-I, A-II, and C-II mass was transferred mainly to HLD. Utilizing either radioiodinated apoA-I and apoA-II reassociated with chylomicrons or radiolabeled chylomicrons, a similar redistribution of apoA-I and apoA-II radioactivity was noted with lipolysis. In contrast, chylomicron apoB (mainly B-48) radioactivity was transferred predominantly to LDL with lipolysis.

HUMAN APOLIPOPROTEIN A-I AND A-II METABOLISM. E.J. Schaefer, L.A. Zech, L.L. Jenkins, T.J. Bronzert, E.A. Rubalcaba, F.T. Lindgren, R.L. Aamodt, and H.B. Brewer, Jr. (Building 10, Room 7N117, National Institutes of Health, Bethesda, MD 20205) *J. Lipid Res. 23*(6):850-862 (1982). The kinetics of the major apolipoproteins (apo) of plasma high density lipoproteins (HDL), apoA-I and apoA-II, were examined in a total of 44 individual tracer studies in 22 normal male and female subjects. Our data were consistent with the following concepts: 1) labeling of apoA-I and apoA-II as apolipoproteins or on HDL does not affect their specific radioactivity decay within HDL; 2) the mean residence time of apoA-I both in plasma and in HDL is significantly shorter than that of apoA-II; 3) the increased apoA-I levels seen in female subjects are due to increased apoA-I synthesis; and 4) the plasma apoA-I residence time, which is inversely correlated with plasma triglyceride levels, is an important determinant of apoA-I concentration in both males and females.

"LOW DOSE" COLESTIPOL IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS WITH FAMILIAL HYPERCHOLESTEROLEMIA. G. Schlierf, K. Mrozik, C.C. Heuck, G. Middelhoff, P. Oster, W. Riesen, B. Schellenberg (Medizinisch Universitätsklinik, Bergheimer Strasse 58, D-6900 Heidelberg, F.R.G.) *Atherosclerosis 41*:133-138 (1982). The effect of colestipol on plasma lipids and lipoproteins was studied in children, adolescents and young adults with familial hypercholesterolemia. 0.125 g or 0.25 g/kg body weight were given in randomized sequence for periods of 4 weeks. Total cholesterol was lowered by 13 and 18% with the smaller and larger dose, respectively, and LDl cholesterol lowered by 15% with the smaller and 12% with the larger dose. HDL cholesterol rose by 18 and 32%. LDL composition before and during the study was abnormal due to a markedly reduced triglyceride content. "Low-dose" colestipol is less effective in lowering total plasma and LDL cholesterol than conventional doses but may, due to very few side effects, be advantageously used in cases of familial hypercholesterolemia when plasma cholesterol levels after dietary management are only 15-20% above normal.

COMPARISON BETWEEN THE HYPERCHOLESTEROLAEMIA IN RABBITS INDUCED BY SEMIPURIFIED DIETS CONTAINING EITHER CHOLESTEROL OR CASEIN. K.E. Scholz, A.C. Beynen, and C.E. West (Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands) *Atherosclerosis 44*(1):85-97 (1982). Rabbits were fed a semipurified diet containing soy protein plus cholesterol (2 g/kg), or a semipurified diet containing casein as protein source. One group of rabbits was fed the soy protein diet throughout the entire experimental period. Blood samples from the animals were taken after an overnight fast. The rabbits transferred to the soy plus cholesterol diet and to the casein diet showed a significant increase in serum cholesterol concentration after 1 and 3 days, respectively. The cholesterol and protein content of the LDL_1 ($1.019 < \rho_{20} < 1.040$) fraction was markedly increased after 3 days on the casein and soy plus cholesterol diets. Thereafter the cholesterol, but not the protein concentration increased in the IDL_1 ($1.006 < \rho_{20} < 1.012$) and VLDL ($\rho_{20} < 1.006$) fractions, the effect being earlier and more pronounced in the soy plus cholesterol-fed animals. When compared to the soy-fed animals, the casein and soy plus cholesterol-fed animals showed a marked increase in the apoprotein E content of their VLDL and IDL fractions. It is concluded that cholesterol- and casein-induced hypercholesterolaemia in rabbits develop in a similar manner. In both hypercholesterolaemias the cholesterol concentration increases first in the LDL fraction and subsequently in the IDL and VLDL fractions.

HIGH DENSITY LIPOPROTEINS UNAFFECTED BY DIETARY FAT MODIFICATION. P. Schwandt, P. Janetschek, and P. Weis-

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weiler (Medical Department II, Klinikum Grosshadern, University of Munich, Marchioninistr. 15, D-8000 Munich 70) *Atherosclerosis 44*(1):9-17 (1982). Since diets containing a high P/S ratio have been reported to have detrimental effects on HDL, the effect of a moderately modified fat diet (P/S ratio 1.0, cholesterol content 250 mg/day) was investigated in 30 healthy male volunteers, divided into 2 groups. They were either given a modified fat diet or an isocaloric control diet (P/S ratio 0.3, cholesterol content 370 mg/day) for 3 months each in a cross-over design. After 3 months of the polyunsaturated fat diet LDL cholesterol was significantly lowered by 19 and 13%, respectively, in both groups. This effect was already apparent after 4 weeks. Apo A-I and cholesterol in serum and the subfractions HDL_2 and HDL_3 remained unchanged. Consequently, the ratio LDL/HDL cholesterol was decreased by this diet. Dietary adherence was good according to the typical changes of the linoleic acid content in serum cholesterol esters, to the dietary recalls and to the constant body weight. We conclude that a moderately modified fat diet supplied from mixed general good is acceptable for longer periods, effectively lowers LDL cholesterol, even in normolipoproteinemic subjects, and has no detrimental effects on HDL.

EFFECTS OF BEZAFIBRATE ON THE COMPOSITION OF VERY LOW DENSITY LIPOPROTEINS IN TYPE IV HYPERLIPOPROTEINEMIA. P. Schwandt, P. Weisweiler, M. Drosner and P. Janetschek (Med. Dept. II, Klinikum Grosshadern, Univ. of Munich, Marchioninistr. 15, D-8000 Munich 70, F.R.G.) *Atherosclerosis 42*(2,3): 245-249 (1982). As compared to normolipoproteinemic controls 10 male subjects with primary type IV hyperlipoproteinemia had higher apolipoprotein E and lower apolipoprotein C-II concentrations in the very low density lipoprotein (VLDL) fraction. After 8 weeks of treatment with 0.6 g/day bezafibrate, cholesterol and triglyceride in the serum and VLDL were significantly lower. The decrease of VLDL lipids was accompanied by a significant decrease of the apolipoproteins and of the lipid/protein ratio in VLDL. The analysis of the soluble VLDL apolipoproteins revealed a decrease of apo E and an increase of apo C1 and apo C2, resulting in a decrease of the apo E/apo C ratio.

SELECTIVE INHIBITION OF PLATELET LIPOXYGENASE BY ESCULETIN. K. Sekiya, H. Okuda, and S. Arichi (Dept. of Med. Biochem. Schl. of Med., Ehime Univ., Shigenobu, Onsen-gun, Ehime 791-02, Japan) *Biochim. Biophys. Acta 713*(1):68-72 (1982). The effects of coumarin and its derivatives on rat platelet lipoxygenase and cyclooxygenase activities were studied. Esculetin (6,7-dihydroxycoumarin) was found to inhibit the lipoxygenase more strongly than the cyclooxygenase; its concentration for 50% inhibition (IC_{50}) was 0.65 μM for platelet lipoxygenase and 0.45 mM for platelet cyclooxygenase. Esculin (the 6-glucoside of esculetin) and umbelliferone (7-hydroxycoumarin) also selectively inhibited the lipoxygenase, though less strongly (IC_{50} = 290 and 500 μM, respectively). 4-Hydroxycoumarin and coumarin had no inhibitory effect on either enzyme at concentrations up to 1 mM. The mechanism of the lipoxygenase inhibitin by esculetin was non-competitive. Other antioxidants (hydroquinone, gallic acid and ascorbic acid) were less inhibitory to both enzymes and showed little selectivity.

PROPERTIES OF ACID AND NEUTRAL CHOLESTEROL ESTER HYDROLASES IN RAT AND PIGEON AORTAS. D.L. Severson and T. Fletcher (Div. of Pharmacology and Therapeutics, Faculty of Med., Univ. of Calgary, Calgary T2N 1N4 Canada) *Atherosclerosis 41*(1):1-14 (1982). The activity of cholesterol ester hydrolase was measured in subcellular fractions from rat and pigeon aortas using a glycerol-dispersed cholesterol oleate substrate preparation. The specific activity of acid cholesterol ester hydrolase (assayed at pH 5) in adventitia tissue fractions was 40-50 fold greater than in media-intima fractions from rat aorta. Soluble and particulate subcellular fractions from rat aorta (media-intima) were observed to have cholesterol ester hydrolase activity with both an acid (pH 4.5-5) and a neutral (pH 7.5) pH optimum. A comparison of the subcellular distribution of acid cholesterol ester hydrolase with the lysosomal marker enzyme, N-acetylglucosaminidase, suggests that the acid hydrolase activity originated in aortic lysosomes; the neutral cholesterol ester hydrolase was predominantly soluble. Acid and neutral cholesterol ester hydrolases could also be distinguished on the basis of the effects of $MgCl_2$ and NaCl on hydrolase activity and on rates of thermal denaturation. Both acid and neutral hydrolases from rat aorta (media-intima) were inhibited by chloroquine (half-maximal at 2-4 mM), and both hydrolases were characterized as having the same apparent affinity for the glycerol-dispersed cholesterol oleate substrate. Acid and neutral cholesterol ester hydrolases were also observed in preparations from pigeon aortas. The specific activity for both acid and neutral hydrolases was higher in atherosclerosis-susceptible White Carneau pigeon aortas in comparison to Show Racer pigeon aortas.

SPECIFIC BINDING OF PROSTAGLANDIN D_2 TO RAT BRAIN SYNAPTIC MEMBRANE. T. Shimizu, A. Yamashita, and O. Hayaishi (Dept. of Med. Chem., Kyoto Univ. Faculty of Med., Sakyo-ku, Kyoto 606, Japan) *J. Biol. Chem. 257* (22): 13570-13575 (1982). Prostaglandin (PG) D_2 bound specifically to a particulate fraction rich in the synaptic membrane of rat brain. The binding was dependent on time and temperature, equilibrium being reached after 5 min at 37 C. The specific binding constituted about 70% of the total binding at 37 C, and 55% at 0 C. The maximal binding was obtained in the presence of 100 mM sodium ion and at pH 8. The equilibrium dissociation constant and the maximal concentration of binding sites as determined by Scatchard analysis were 28 ± 7 nM and 0.45 pmol/mg of protein(n=3), respectively. Hill coefficient was 1.15, indicating a single entity of binding sites and no cooperativity. The binding sites were highly specific for PGD_2; the K_i values for PGD_1 and $PGF_{2\alpha}$ were 523 and 693 nM, respectively. Other PGs including 13,14-dihydro-15-keto-PGD_2, an inactive metabolite of PGD_2, had 150- to 1000-fold lower affinites than PGD_2. The binding was inhibited by boiling or treatment with proteases, phospholipases, or β-galactosidase. The specific activity of PGD_2 binding was highest in the pituitary gland, followed by the hypothalamus and the olfactory bulb of the rat brain, this pattern being almost parallel to that of the cytosolic NADP-linked PGD_2 dehydrogenase activity. The results suggest that PGD_2 plays a significant role in these regions of the rar brain.

ABSENCE OF EFFECT OF PROSTAGLANDINS ON CHOLESTERYL ESTER METABOLISM OF 3T3 MOUSE FIBROBLASTS GROWN IN TISSUE CULTURE. S.P. Singh, F.A. Shamgar and A.J. Day (Dept. of Physiology, Univ. of Melbourne, Parkville, Vic. 3052 (Australia)) *Atherosclerosis 42*(1):109-119 (1982). This study examines the effect of prostaglandin E_2 and 6-keto $F_{1\alpha}$ on the cholesteryl ester metabolism of cells grown in tissue culture. When 3T3 mouse fibroblasts were incubated with cationized low density lipoprotein (LDL) and ^{3}H-labelled oleic and ^{14}C-labelled linoleic acids a marked increase in cholesteryl ester content of cells was observed. Oleic acid was the preferred substrate for cholesterol esterification. However, the presence of prostaglandin E_2 or 6-keto $F_{1\alpha}$ (up to 10 μg/ml) did not affect the cholesteryl ester content or the uptake of labelled fatty acids into cellular lipids. Following preincubation with cationized LDL and labelled fatty acids the cells were reincubated in normal medium with or without prostaglandins. The presence of PGE_2 or 6KPGF$_{1\alpha}$ (up to 10 μg/ml) did not appreciably change the rate of removal of cholesteryl ester or labelled lipids. This indicates that these prostaglandins even when present in relatively large doses in the incubation medium do not affect lipid metabolism of cells grown in tissue culture.

INCREASED LIVER OLEIC ACID SYNTHESIS IN CHOLESTEROL-FED RABBITS. M.R. Sivaramakrishnan and T.I. Pynadath (Chem. Dept., Kent State Univ., Kent, OH 44242) *Atherosclerosis 41*(1):21-25 (1982). Several investigators have observed increased levels of esterified oleic acid in tissue cholesterol esters and phospholipids of atherosclerotic humans and cholesterol-fed animals. However, the cause of this is still unknown. Increased synthesis, increased esterification, or both of oleic acid can account for this. In the present investigation, hepatic synthesis of oleic acid is studied in cholesterol-fed rabbits. A nearly 3-fold increase in oleic acid synthesis was observed after 3 weeks of cholesterol feeding. This increase continued for at least 6 weeks. Since acyl acceptors like glycerol-3-phosphate are known to increase liver oleic acid synthesis, it is possible that the observed increase in oleic acid formation was partially due to an increased availability of acyl acceptors in the system.

INTERACTIONS BETWEEN PHOSPHOLIPID HEAD GROUPS AT MEMBRANE INTERFACES: A DEUTERIUM AND PHOSPHOROUS NUCLEAR MAGNETIC RESONANCE AND SPIN-LABEL ELECTRON SPIN RESONANCE STUDY. F. Sixl & A. Watts (Biochem. Dept., Univ. of Oxford, Oxford, OX1 3QU United Kingdom) *Biochemistry 21*(25):6446-6452 (1982). The head group interactions in fully hydrated, mixed bilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol, specifically deuterated in the head groups at the α- and β-methylene and $N(CD_3)_3$ positions, have been investigated by deuterium and phosphorus-31 nuclear magnetic resonance (NMR) and 2,2,6,6-tetramethylpiperidinyl-1-oxy (Tempo) spin-label electron spin resonance (ESR) studies at pH 7.5. The results indicate that some reorientations in the lipid

head groups and changes in their amplitudes of motion are induced in the two-component bilayers by the presence of one lipid on the other but their rates of motion remain rather similar.

REGULATION OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN MAMMALIAN CELLS. R. Sleight and C. Kent (Dept. of Biochem., Purdue Univ., West Lafayette, IN 47907) *J. Bio. Chem. 258*(2):824-830 (1983). Addition of phospholipase C from *Clostridium perfringens* to cultures of Chinese hamster ovary (CHO) cells resulted in rapid degradation of cellular phosphatidylcholine with concomitant release of phosphocholine. The rate of incorporation of radiolabeled choline into lipids was increased 2-fold in phospholipase C-treated CHO cells compared to untreated controls. The only enzyme in the pathway of phosphatidylcholine biosynthesis with increased activity in phospholipase C-treated cells was CTP:phosphocholine cytidylyltransferase, indicating that the cytidylyltransferase plays an important role in the stimulation of phosphatidylcholine biosynthesis. The phospholipase treatment was toxic to a CHO mutant cell line with abnormally low cytidylyltransferase activity. Mouse LM fibroblasts were resistant to enzymatic attack by phospholipase C, and cytidylyltransferase activity in LM cells did not change upon phospholipase C treatment.

REGULATION OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN MAMMALIAN CELLS. R.Sleight and C. Kent (Dept. of Biochem., Purdue University, West Lafayette, IN 47907) *J. Bio. Chem. 258*(2):836-839 (1983). The activity and subcellular distribution of CTP:phosphocholine cytidylyltransferase in LM and Chinese hamster ovary cells in which the phospholipid composition had been altered by supplementary feeding with choline analogues were examined. Decreased levels of cellular phosphatidylcholine with corresponding increased levels of either phosphatidylethanolamine, phosphatidylmonomethylethanolamine, or phosphatidyldimethylethanolamine resulted in increased CTP: phosphocholine cytidylyltransferase activity in cell homogenates. In addition, a significantly larger fraction of the total cytidylyltransferase activity was membrane-bound in these cells. The activity of the cytidylyltransferase from cytosolic extracts of both cell types was found to be greatly increased when assayed in the presence of either phosphatidyldimethylethanolamine. The lysophosphatide forms of these lipids were found to be poor activators of the cytidylyltransferase. These findings suggest that the regulation of phosphatidylcholine biosynthesis is at least partially dependent on information transfer from membranes to CTP:phosphocholine cytidylyltransferase. That is, in the presence of phosphatidylcholine-deficient membranes of cytidylyltransferase becomes activated and associated with the membranes.

REGULATION OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN MAMMALIAN CELLS. R. Sleight and C. Kent (Dept. of Biochem., Purdue Univ., West Lafayette, IN 47907) *J. Bio. Chem. 258*(2):831-835 (1983). CTP:phosphocholine cytidylyltransferase was located in both the cytosolic and particulate fractions from Chinese hamster ovary cells. The activity of the cytosolic from of the enzyme was greatly enhanced by incubation with sonicated preparations of several different lipids, although incubations with either phosphatidylcholine or 1,2-*sn*diolein did not increase activity. The activation of the cytidylyltransferase in Chinese hamster ovary cells treated with phospholipase C from *Clostridium perfringens* occurred with a concomitant shift in the subcellular distribution of the enzyme from cytosolic to particulate fractions. This shift was rapid and did not require protein synthesis. Removal of phosphatidylcholine, a decrease in the activity of cytidylyltransferase, and a loss of the membrane-bound form of the enzyme. Similar experiments with LM cells, which are resistant to exogenous phospholipase C, showed no change in subcellular distribution of cytidylyltransferase, suggesting that the activation of CTP:phosphocholine cytidylyltransferase required a change in membrane phospholipid composition. The results presented are discussed in terms of a mechanism of regulation of phosphatidylcholine production involving monitoring of membrane phospholipid composition.

TOXICITY OF VITAMIN D STEROIDS TO LAYING HENS. J. Soares, Jr., D. Kaetzel, J. Allen, and M. Swerdel (Dept. Poultry Sci., Univ. Maryland, College Park, MD 20742) *Poultry Sci. 62*(1):24-29 (1982). Two experiments were conducted with 56-week-old or 104-week-old Leghorn hens to determine if feeding vitamin D steroids in excess of requirement levels caused any marked affects on eggshell quality. In the first experiment caged hens had reduced feed consumption, egg shell quality, and egg production as early as 6 weeks after initially consuming a basal diet supplemented with 6.8 μg 1α-hydroxycholecalciferol (1α-OH-D_3)/kg. The second experiment confirmed the previous results and showed that extensive weight loss occurred with continued feeding of 10 or 15 μg 1α-OH-D_3/kg diet. No adverse affects were observed in either experiment when the level of 1α-OH-D_3 supplementation was 5.0 μg/kg diet or less. No toxic effects were observed when the hormone precurser 25-OH-D_3 was supplemented to diets at 6 or 12 μg/kg. It is suggested that the pathological effects observed are related to the potent calcium homeostatic properties of 1α-OH-D_3 that at elevated levels may cause aberrations in circulating calcium.

ApoLDL: EVIDENCE FOR AN AGGREGATING SYSTEM OF HETEROGENEOUS SUBUNITS. L. Socorro, F. López, A. López, G. Camejo (Lab. de Lipoproteinas, Centro de Biofisica y Bioquimica, Inst. Venezolano de Investigaciones Cientificas (IVIC), Caracas, Venezuela) *J. Lipid Res. 23*(9):1283-1291 (1982). The protein moieties of low density lipoprotein and subfractions I, II, III and IV were obtained by a mild delipidation procedure involving attachment of LDL to an ionic-exchange column and a gradient of the nonionic detergent Brij-36T. The apoLDL appeared to be made up of several polypeptide components with molecular weights between 250,000 and 14,000. The complex pattern was observed in the apoprotein of LDL subfractions I to IV; the distribution of the most prominent components was different for each density range. These results discounted the possibility that the presence of bands with molecular weights below 250,000 could be caused by contamination with VLDL or HDL or nonspecific proteolysis. Delipidation of subfractions I to IV with organic solvents produced simple patterns of highly aggregated apoLDL subfractions, where most of the protein had molecular weights above 250,000. The more disaggregated apoLDL preparations were those obtained from LDL rapidly isolated by single-spin centrifugation in KBr gradients, from fresh plasma immediately mixed with EDTA, phenylmethyl sulfonylfluoride, and chloramphenicol. ApoLDL fractionated by single-pore polyacrylamide gel electrophersis appeared to be a system of heterogeneous antigens unevenly distributed on those obtained by peptide components.

BINDING PROPERTIES OF HIGH-DENSITY LIPOPROTEIN SUBFRACTIONS AND LOW-DENSITY LIPOPROTEINS TO RABBIT HEPATOCYTES. P. Soltys, O. Portman, and J. O'Malley (Oregon Reg. Primate Res. Center, Beaverton, OR 97006) *Biochimica et Biophysica Acta 713*:300-314 (1982). Primary cultures of rabbit hepatocytes which were preincubated for 20 hr in medium containing lipoprotein-deficient serum subsequently bound, internalized and degraded ^{125}I-labeled high-density lipoproteins$_2$ (HDL_2). As the concentration of HDL_2 increased, binding reached saturation. Unlabeled low-density lipoproteins (LCL) inhibitited only at low concentrations of ^{125}I-labeled HDL_2. Quantification of ^{125}I-labeled HDL_2 binding to a specific receptor yielded a dissociation constant of $1.45 \cdot 10^{-7}$ M. Preincubation of hepatocytes in the presence of HDL resulted in only a 40% reduction in specific HDL_2 receptors, whereas preincubation with LDL, largely suppressed LDL receptors. HDL_2 and LDL from control and hypercholesterolemic rabbits inhibited the degradation of ^{125}I-labeled HDL_2, but HDL_3 did not. Treatment of HDL_2 and LDL with cyclohexanedione eliminated their capacity to inhibit ^{125}I-labeled HDL_2 degradation which suggested that apolipoprotein E plays a critical role in triggering the degradative process. The effect of incubation with HDL on subsequent ^{125}I-labeled LDL binding was time-dependent. The binding of ^{125}I-labeled LDL to isolated liver cellular membranes demonstrated saturation kinetics at 4 C and was inhibited by EDTA or excess LDL. EDTA did not affect the binding of either HDL_2 or HDL_3 to isolated liver membranes. Hepatocytes incubated with [2-^{14}C] acetate in the absence of lipoproteins incorporated more label into cellular cholesterol, nonsaponifiable lipids and total cellular lipid than hepatocytes incubated with [2-^{14}C] acetate in the presence of any lipoprotein fraction. However, the level of ^{14}C-labeled lipids released into the medium was higher in the presence of medium lipoproteins, indicating that the effect of those lipoproteins was on the rate of release of cellular lipids rather than on the rate of synthesis.

ACYL-CoA SYNTHASE AND ACYLTRANSFERASE ACTIVITY IN DEVELOPING SKELETAL MUSCLE MEMBRANES. P.B. Smith, R.C. Reitz, and D. Kelley (Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103) *Biochim. Biophys. Acta 713*(1): 128-135 (1982). Enzymes participating in the activation and esterification of fatty acids for complex lipid biosynthesis were characterized in neonatal and adult rabbit skeletal muscle membranes. The activity of acyl-CoA synthase was 1.4-1.6-fold greater

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in neonatal vs. adult sarcoplasmic reticulum for the fatty acid substrates linoleic (2.6 vs. 1.61 nmol 18:2-CoA/min per mg), stearic (0.94 vs. 0.66 nmol 18:0-CoA/min per mg) and palmitic (2.43 vs. 1.51 nmol 16:0-CoA/min per mg) acids. Enzyme activity was identical between neonate and adult for coenzyme A, ATP and linoleic acid concentration dependence. Glycerol-3-phosphate acyltransferase activity was 6-fold greater in neonatal than adult sarcoplasmic reticulum for both linoleoyl-CoA (1.4 vs. 0.22 nmol 18:2/min per mg) and stearoyl-CoA (1.1 vs. 0.13 nmol 18:0/min per mig) donor substrates, whereas lysophosphatidylcholine acyltransferase activity was similar. Enriched fractions of sarcolemmal membranes possessed the highest activity for lysophosphatidylcholine acyltransferase activity, being 2-4-fold greater than sarcoplasmic reticulum. In contrast to sarcoplasmic reticulum, lysophosphatidylcholine acyltransferase activity was 2-3-fold greater in neonatal compared to adult sarcolemma for lineoleic (18.9 vs. 8.5 nmol 18:2/min per mg) and the stearic (2.8 vs. 0.68 nmol 18:0/min per mg) acid incorporation. The greater capacity of neonatal membranes for acylation by the de novo pathway is in accord with the requirements for neonatal muscle to effect high rates of triacylglycerol and phospholipid synthesis essential for oxidative metabolism and membrane synthesis during postnatal development and growth.

THE METABOLISM OF VERY LOW DENSITY LIPOPROTEINS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA. A.K. Soutar, N.B. Myant, and G.R. Thompson (Med. Res. Council Lipid Metabolism Unit, Hammersmith Hospital, London W12 0HS, Great Britain) *Atherosclerosis 43*(2,3):217-231 (1982). The metabolism of apolipoprotein B (apoB) in very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) was studied in normal subjects and in patients with familial hypercholesterolaemia (FH) after an intravenous injection of autologous VLDL labelled with ^{125}I. There were no significant differences in half life, pool size and turnover rate (mg/kg/h) of VLDL-apoB between the normal subjects, the FH heterozygotes and the FH homozygotes. IDL-apoB metabolism in the FH patients differed significantly from that in the normal subjects. In the FH patients, the rise to the maximum of the specific activity curve was slower, the half life of the descending limb of the specific-activity curve was longer, the fractional rate of turnover was lower and the plasma concentration was higher than in the normals. The effect of cholestyramine on IDL-apoB metabolism in the normal subjects did not differ from that in the FH heterozygotes and homozygotes, though cholestyramine is known to stimulate hepatic uptake of low density lipoprotein (LDL) by the LDL receptor. It is suggested that in normal human subjects the LDL receptor makes some contribution to the hepatic uptake of IDL-apoB derived from VLDL, but that IDL uptake is mediated partly by a separate receptor that recognizes apolipoprotein E but not apoB.

INTERACTION BETWEEN MACROPHAGES AND MESENCHYMAL CELLS. EFFECT OF LDL- OR HDL-CONTAINING MEDIA, ADDED TO CHOLESTERYL ESTER-LOADED MACROPHAGES, ON CHOLESTEROL ESTERIFICATION IN MESENCHYMAL CELLS. O. Stein, Y. Stein, and G. Halperin (Dept. of Exper. Med. & Cancer Res., Hebrew University-Hadassah Med. School and Lipid Res. Lab., Dept. of Med. B, Hadassah Univ. Hospital, Jerusalem, Israel) *Biochim. Biophys. Acta 712*(3): 597-604 (1982). Mouse peritoneal macrophages were loaded with cholesteryl ester by incubation with acetylated LDL for 72 hr. Next, the loaded cells were incubated for 48 hr in Dulbecco-Vogt medium containing 1% bovine serum albumin alone or together with low or high density lipoproteins or acetylated LDL. Bovine aortic smooth muscle cells and human skin fibroblasts were labeled for 72 hr with medium containing free [^3H]cholesterol. The labeled cells were then incubated for an additional 48 hr with media conditioned in the presence of macrophages. The cholesteryl ester content of the labeled smooth muscle cells incubated with macrophage-conditioned media containing LDL or acetylated LDL increased 5-fold and there was also a 10-fold increase in the amount of labeled cholesteryl ester over that recorded prior to exposure to the macrophage-conditioned medium. No enhancement of cholesterol esterification was seen with macrophage-conditioned media containing albumin only. Increase in cellular cholesteryl ester content and in labeled cholesteryl ester after incubation with macrophage-conditioned media containing LDL or acetylated LDL was obtained also in human skin fibroblasts. Cells derived from control donors and from a patient with familial homozygous hypercholesterolemia responded in a similar manner. The enhanced cholesterol esterification in human skin fibroblasts, incubated with macrophage-conditioned medium containing acetylated LDL, was counteracted by addition of HDL to the human skin fibroblast medium.

EFFECTS OF BEZAFIBRATE ON RECEPTOR-MEDIATED AND RECEPTOR-INDEPENDENT LOW DENSITY LIPOPROTEIN CATABOLISM IN TYPE II HYPERLIPOPROTEINAEMIC SUBJECTS. J.M. Stewart, C.J. Packard, A.R. Lorimer, D.E Boag, and J. Shepherd (University Departments of Medical Cardiology and Biochemistry, Royal Infirmary, Glasgow G4 OSF, Great Britain) *Atherosclerosis 44*(3):355-365 (1982). This study examines the effects of bezafibrate (200 mg t.i.d.) on LDL metabolism in 7 type II hyperlipoproteinaemic subjects. Eight weeks of treatment lowered plasma cholesterol and triglyceride by 10% and 30%, respectively ($P < 0.02$). These reductions were associated with a fall in circulating VLDL (31%, $P < 0.02$) and LDL (11%, $P < 0.05$), while HDL cholesterol stayed the same. LDL metabolism changed during therapy. The plasma fractional clearance rate (FCR) of autologous [^{125}I]LDL normalized from a low value of 0.256 ± 0.048 (mean ± SD) to 0.298 ± 0.040 pools/day ($P < 0.001$). This was attributable to a 65% increase ($P < 0.01$) in receptor-mediated LDL catabolism since the clearance of simultaneously injected 1,2-cyclohexanedione-modified [^{131}I] LDL, which measures the receptor-independent pathway, was unaltered (FCR of [^{131}I]cyclohexanedione/LDL in control phase = 0.194 ± 0.030 pools/day; during drug treatment = 0.194 ± 0.024 pools/day). We conclude that bezafibrate lowers plasma LDL in type II hyperlipoproteinaemia by promoting its degradation via high affinity receptors.

LIPID ANALYSIS IN NON-FASTING DIABETICS. A. Stott, S.B. Cohen, R.Dale, I.J.L. Goldberg, and M.B. Macauley (Depts. of Chemical Pathology and Medicine, Fazakerley Hospital, Liverpool, Great Britain) *Atherosclerosis 44*(2):137-140 (1982). Serum cholesterol and triglyceride concentrations were measured in diabetic and non-diabetic inpatients in the fasting state and at 1, 2, 2½ and 3 hours after breakfast. No significant changes in these parameters were observed during this period. It is suggested that the use of non-fasting samples for lipid analysis in cardiovascular disease studies is feasible.

ETHER PHOSPHOLIPIDS IN GUINEA PIG POLYMORPHONUCLEAR LEUKOCYTES AND MACROPHAGES. T. Sugiura, Y. Onuma, N. Sekiguchi and K. Waku (Faculty of Pharmaceutical Sci., Teikyo Univ., Sagamiko, Kanagawa, 199-01 Japan) *Biochim. Biophys. Acta 712*(3):515-522 (1982). Significant proportions of the choline phosphoglycerides (CPG) were found to contain alkyl ether-type moieties (e.g., 1-*O*-alkyl-2-acyl-giycero-3-phosphocholine) in both guinea pig peritoneal exudate polymorphonuclear leukocytes (16.4%) and macrophages (13.5%). High proportions of the ethanolamine phosphoglycerides (EPG) contained alkenyl ether moieties in both cells (37.2 and 41.2%), while the proportions of the CPG containing alkenyl moieties and of the EPG containing alkyl moieties were shown to be small. The ether phospholipid composition as well as the fatty chain profiles of these two types of cells had relatively similar patterns. However, the fatty chains at the 1- and 2-positions for alkenyl ether, alkyl ether and diacyl phosphoglycerides showed considerable differences. The amount of 16:0 at the 1-position was higher in alkyl compounds than that in diacyl compounds of the CPG. This was also the case in ether-containing and diacyl EPG. The most predominant fatty acids at the 2-position was 18:2, in each lipid class, except for the alkenyl CPG. The amounts of 20:4 and other polyunsaturated fatty acids were low in every lipid class, though ether compounds contained higher amounts of 20:4 than diacyl compounds, particularly for EPG.

EFFECT OF CARBON MONOXIDE ON ATHEROGENESIS IN FORMAL PIGS AND PIGS WITH VON WILLEBRAND'S DISEASE. D.L. Sultzer, K.M. Brinkhous, R.L Reddick and T.R. Griggs (Depts. of Pathology and Med. and the Center for Thrombosis and Hemostasis Res., Univ. of North Carolina Schl. of Med., Chapel Hill, NC) *Atherosclerosis 43*(2,3):303-319 (1982). The extent of coronary and aortic atherosclerosis was examined in pigs following balloon-catheter injury of coronary arteries and subsequent feeding of an atherogenic diet for 4 months. The pigs were either exposed intermittently to 100 ppm carbon monoxide or to ambient air alone. Three types of pigs were used: normals, homozygotes for von Willebrand's disease (bleeders), and heterozygotes (carriers). The 3 types of pigs developed coronary artery intimal lesions of similar thickness. Aortic lesions, quantified as percent of aortic surface involved with sudanophilia and raised fibrous plaques, were slightly less extensive in bleeder pigs than in normals. Carbon monoxide exposure did not increase the thickness of coronary artery intimal lesions, nor did it increase the percent of aortic surface involved with sudanophilia or raised fibrous lesions. These results suggest that exposure to low levels of carbon monoxide does not perceptibly enhance atherogenesis induced by hypercholesterolemia. None of 14 bleeder pigs showed

evidence of myocardial infarction, despite significant coronary artery narrowing. Of the 24 normal and carrier pigs, 5 showed myocardial infarction. Four of these 5 pigs were exposed to carbon monoxide, while 1 was not exposed. These findings suggest that exposure to low levels of carbon monoxide may increase the incidence of myocardial infarction and that the absence of von Willebrand factor may be protective.

EFFECT OF CLINOFIBRATE, A NEW HYPOLIPIDEMIC AGENT, ON BILIARY AND SERUM LIPIDS IN PATIENTS WITH HYPERLIPIDEMIA. N. Takeuchi, H. Kukita, G. Kajiyama, M. Fujiyama, K. Ishikawa, H. Miki, T. Mishima, K. Murata, and T. Asano (Central Laboratory, Ehime University Hospital, Shigenobucho, Ehime 791-02, Japan) *Atherosclerosis 42*(2,3): 129-139 (1982). Clinofibrate was given to 15 patients with hyperlipidemia, for 6-8 weeks at the daily dose of 600 mg, and its effect on 3 biliary lipid components (cholesterol, bile acids and phospholipids) and on the lithogenic index was investigated. After clinofibrate treatment, 6 of the patients were given 1.5 g/day clofibrate with that of clinofibrate. The molar percentages of biliary cholesterol and phospholipids to the total mol number of the 3 biliary lipid components decreased, and that of bile acids increased during clinofibrate administration. In this way, the molar ratio of bile acids to cholesterol increased during the treatment. Neither the lithogenic index calculated by the formula of Admirand and Small nor that of Hegardt, Dam and Holzbach was altered significantly by the treatment. There was no apparent relationship between the effect of the drug on the lithogenic index and any of the factors initial lithogenic index, rate of decrease of serum lipids, or type of hyperlipidemia. Although clofibrate had no significant effect on the maximum solubility of cholesterol in the bile, the molar percentage of biliary cholesterol was elevated and the lithogenic index increased as compared with the control and clinofibrate period. No significant influence on bile acid composition in the bile was observed, with either clinofibrate or clofibrate.

THE EFFECT OF SEMIPURIFIED DIETS CONTAINING DIFFERENT PROPORTIONS OF EITHER CASEIN OR SOYBEAN PROTEIN ON THE CONCENTRATION OF CHOLESTEROL IN WHOLE SERUM, SERUM LIPOPROTEINS AND LIVER IN MALE AND FEMALE RATS. A.H.M. Terpstra, G. Van Tintelen and C.E. West (Dept. of Human Nutr., Agri. Univ., De Dreijen 12, 6703 BC Wageningen (The Netherlands) *Atherosclerosis 42* (1):85-95 (1982). Male and female lean Zucker strain rats were fed cholesterol-enriched semipurified diets containing 2 levels (20% and 50%, w/w) of either casein or soybean protein for a period of 14 weeks. In the female rats, the feeding of casein diets resulted in significantly higher levels of serum cholesterol than when diets containing soybean protein were fed. In addition, the hypercholesterolemic effect of dietary casein could be enhanced by increasing the proportion of this protein in the diet. Modulations in the proportion of dietary soybean protein did not significantly affect the serum cholesterol levels. In the male rats, however, no such differential effects were observed, indicating a difference between male and female rats in susceptibility to the induction of changes in serum cholesterol levels by dietary means. Upon feeding casein diets, both the male and female rats exhibited a shift of cholesterol from the high density lipoproteins to the lipoproteins with a lower density. This effect was more pronounced in the female than in the male rats. Liver cholesterol concentrations were markedly affected by modulations both in the type and proportion of dietary protein in both sexes. The concentration of cholesterol in the liver of the rats was highest in those fed the 50% casein diet and progressively lower in the animals on diets containing 20% casein, 20% soybean protein and 50% soybean protein.

THE EFFECT OF FERMENTED AND UNFERMENTED MILKS ON SERUM CHOLESTEROL. L.U. Thompson, D.J.A. Jenkins, V. Amer, R. Reichert, A. Jenkins, and J. Kamulsky (Dept. of Nutr. Sci., Faculty of Med., Univ. of Toronto, Toronto, Ontario and Gay Lea Foods Co., Weston, Ontario, Canada) *Am. J. Clin. Nutr. 36*(6):1106-1111 (1982). Groups of 10 to 13 healthy volunteers were provided with 11 supplements of 25% butterfat milk (2% milk), whole milk, skim milk, yogurt, buttermilk, and sweet acidophilus milk daily for a 3-wk period. Despite increases in calorie intakes on all supplements, no significant increases were found in total, low-density, and high-density lipoprotein cholesterol. A significant weight gain was seen in subjects taking yogurt, acidophilus, buttermilk, and skim milk. Weight gain was, however, most marked in the yogurt and acidophilus groups; these were the only two groups showing significant rises in triglyceride levels. These results in normal volunteers from attention on the current practice of recommending only skim or 2% milk for hyperlipidemic individuals.

INFLUENCE OF CHOLESTEROL ON THE STRUCTURAL PREFERENCES OF DIOLEOYLPHOSPHATIDYLETHANOLAMINE-DIOLEOYLPHOSPHATIDYLCHOLINE SYSTEMS: A PHOSPHORUS-31 AND DEUTERIUM NUCLEAR MAGNETIC RESONANCE STUDY. C.P.S. Tilcock, M.B. Bally, S.B. Farren, and P.R. Cullis (Biochem. Dept., Univ. of British Columbia, Vancouver, V6T 1W5, Canada) *Biochem. 21*(19):4596-4601 (1982). The polymorphic phase behavior of mixtures of synthetic dioleoylphosphatidylethanolamine (DOPE) and dioleoylphosphatidylcholine (DOPC) and the influence of cholesterol on these phase preferences have been investigated by employing nuclear magnetic resonance (NMR) techniques. In particular, ^{31}P NMR procedures are utilized to study the overall phase preferences of these mixed systems, whereas ^{2}H NMR is employed to monitor the structural preferences of individual components of these systems by using versions of DOPE and DOPC which are deuterium (^{2}H) labeled at the C_{11} position of the acyl chains. The results obtained show that DOPE-DOPC systems containing as little as 20 mol % DOPC initially assume lamellar structure at 40 C, even though DOPE in isolation prefers the hexagonal (H_{II}) organization at this temperature. However, this lamellar organization appears to represent a metastable state, as incubation for extended periods at 40 C results in formation of a structure, possibly the cubic phase, in which the phospholipids experience isotropic motional averaging. The addition of cholesterol induces hexagonal (H_{II}) phase organization. ^{2}H NMR studies of appropriately labeled versions of these systems indicate that cholesterol does not produce such effects by associating preferentially with either DOPE or DOPC. Further, in situations where bilayer, hexagonal, or "isotropic" phases coexist in the same sample, the phospholipids exhibit apparently ideal mixing behavior.

PURIFICATION AND PROPERTIES OF AN ACID LIPASE FROM HUMAN GASTRIC JUICE. C. Tiruppathi and K.A. Balasubramanian (Wellcome Res. Unit, Christian Med. College Hospital, Vellore 632004, India) *Biochim. Biophys. Acta 712*(3): 692-697 (1982). An acid lipase (EC 3.1.1.3) from human gastric juice was purified by using poly(ethylene glycol)-6000 precipitation, ethanol fractionation and Sephadex G-75 gel filtration. A molecular weight of 44000 was obtained by SDS-polyacrylamide gel electrophoresis. pH-dependent aggregation was observed and by using Sephadex G-200 gel filtration, a molecular weight of 90000 was obtained at pH 6.0 and 45000 at pH 3.0, for the purified enzyme. A pH optimum of 5.3 was obtained using triolein as substrate. The apparent K_m for tributyrin and triolein was found to be 21 and 73 μmol, respectively. Diacylglycerol and free fatty acids were the major hydrolytic end products of this enzyme. Studies on the positional specificity of the enzyme showed that the preferred site of hydrolysis was *sn*-3 and *sn*-1, although a good percentage of the *sn*-2 position was also hydrolysed. Conjugated bile salts inhibited the enzyme when triolein was used as substrate, whereas they activated it when tributyrin was used. Some of the properties of the purified human gastric juice acid lipase resembles those of rat and human lingual lipase.

INHIBITION OF SMALL INTESTINAL BRUSH BORDER MEMBRANE Mg^{2+}-STIMULATED ADENOSINE TRIPHOSPHATASE BY LONG-CHAIN FATTY ACIDS. C. Tiruppathi, P.G. Hill and K.A. Balasubramanian (Wellcome Res. Unit, Christian Med. College and Hospital, Vellore 632004) *Indian Journal of Biochemistry and Biophysics 19*(3):186-190 (1982). Long-chain fatty acids were found to inhibit small intestinal brush border membrane Mg^{2+}-ATPase in vitro. Unsaturated fatty acids showed more inhibition compared to saturated and hydroxy fatty acids. Methylester of oleic acid did not inhibit the enzyme and oleic acid inhibition was of uncompetitive type. The inhibition of Mg^{2+}-ATPase by oleic acid was independent of the period of incubation and pH. The oleic acid inhibition was completely reversible by wash out. The brush border membrane Mg^{2+}-ATPase had a temperature optimum above 30 C and yielded an Arrhenius' plot with a break-point at 28 C. In the presence of oleic acid, however, the optimum temperature of the enzyme decreased to below 30 C, the activation energy of the reaction at temperature below 28 C was lowered from 7.058 kcal/mole to 6.157 kcal/mole and the enzyme had a linear Arrhenius plot. Oleic acid had no influence on solubilized and relipidated enzyme.

RELATIVE RATES OF STEROL SYNTHESIS IN THE LIVER AND VARIOUS EXTRAHEPATIC TISSUES OF NORMAL AND CHOLESTEROL-FED RABBITS. J.M. Andersen, S.D. Turley, J.M. Dietschy (Depts. of Internal Med. and Pediatrics, Univ. of Texas

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Health Sci. Center, Dallas, TX 75235) *Biochim. Biophys. Acta 711* (3): 421-430 (1982). The relative rates of sterol synthesis in the liver and ten extrahepatic tissues of normal and cholesterol-fed rabbits were determined by measuring the rates of incorporation of [1-^{14}C] octanoate into digitonin-precipitable sterols by tissue slices. In normal rabbits the rate of sterol synthesis in the liver was low compared to that in several extrahepatic tissues, particularly the small intestine. The rate of synthesis in the small intestine showed regional variation, with the highest rate occurring in the section proximal to the entry of the common bile duct and the lowest rate in the mid-sections of the intestine. The regional differences in intestinal sterol synthesis correlated inversely with the cholesteryl ester content of the tissue. Rabbits fed the cholesterol diet developed hypercholesterolemia, with much of the additional cholesterol appearing in the VLDL and LDL fractions. The cholesteryl ester content of the liver, small intestine and other extrahepatic tissues increased significantly. There was a marked suppression of sterol synthesis in the liver, small intestine, adrenal gland, kidney, lung, spleen and ovary. The rabbit, like the guinea pig, normally exhibits a low rate of hepatic sterol synthesis compared to that found in other species and manifests feedback inhibition of both hepatic and extrahepatic sterol synthesis when dietary cholesterol intake is increased. This general suppression of synthesis correlates with an accumulation of cholesteryl ester in the tissues, related to the uptake of lipoprotein cholesterol from the hypercholesterolemic plasma that develops under such dietary conditions.

24-HYDROXYLATION OF 25-HYDROXYVITAMIN D_3: IS IT REQUIRED FOR EMBRYONIC DEVELOPMENT IN CHICKS? S. Ameenuddin, M. Sunde, H.F. DeLuca, N. Ikekawa, and Y. Kobayashi (Dept. of Poultry Sci. Coll. of Agric. and Life Sci., Univ. of Wisconsin, Madison, WI 53706) *Science 217*(4558):451-452 (1982). As shown previously, laying hens given 1,25-dihydroxyvitamin D_3 as their source of vitamin D produce fertile eggs having normal shells, but only 35-55 percent of the embryos are normal. Giving these hens additional 25-hydroxyvitamin D_3, 24,25-dihydroxyvitamin D_3, or 24,24-difluoro-25-hydroxyvitamin D_3 at 1.25 nanomoles per day resulted in 90 to 100 percent normal embryos, and hence, hatchability. Since 24,24-difluoro-25-hydroxyvitamin D_3 cannot be 24-hydroxylated, 24-hydroxylation is not required for this function of 25-hydroxyvitamin D_3.

LIPID HAPTEN CONTAINING MEMBRANE TARGETS CAN TRIGGER SPECIFIC IMMUNOGLOBULIN E-DEPENDENT DEGRANULATION OF RAT BASOPHIL LEUKEMIA CELLS. K. Balakrishnan, F.J. Hsu, A.D. Cooper, H.M. McConnell (Depts. of Chem. and Med., Stanford Univ., Stanford, CA 94305) *J. Biol. Chem. 257*(11):6427-6433 (1982). We have studied the binding of liposomes containing dinitrophenylated lipid to rat basophil leukemia cells armed with monoclonal anti-dinitrophenyl IgE. The liposomes were either "fluid" at 37 C (dimyristoylphosphatidylcholine or an equimolar binary mixture dipalmitoylphosphatidylcholine and cholesterol) or "solid" (dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, or dibehanoylphosphatidylcholine). We have also studied the immune mediated degranulation of these cells induced by the above lipid membrane targets. In some cases both studies were carried out with liposomes containing various surface densities of lipid haptens. From these studies we conclude that freely mobile nonaggregated lipid haptens in bilayer membrane targets can trigger efficient serotonin release from rat basophil leukemia cells in the presence of specific antihapten IgE. Solid target membranes are also effective as stimulators of serotonin release. The release of serotonin depends strongly on the surface density of lipid haptens over a narrow range of surface densities. These studies with lipid membrane targets having well-defined physical properties indicate the need for generalized molecular models of receptor-mediated cell triggering.

AVAILABILITY OF DINITROPHENYLATED LIPID HAPTENS FOR SPECIFIC ANTIBODY BINDING DEPENDS ON THE PHYSICAL PROPERTIES OF HOST BILAYER MEMBRANES. K. Balakrishnan, S.Q. Mehdi, and H.M. McConnell (Stauffer Lab. for Phys. Chem., Stanford Univ., Stanford, CA 94305) *J. Biol. Chem. 257* (11):6434-6439 (1982). We have measured the binding of two radioiodinated monoclonal anti-dinitrophenyl antibodies (IgE and IgG_{2a}) to two dinitrophenylated lipid haptens in lipid bilayer membranes having various compositions and physical properties. These antibodies bind strongly to the lipophilic dinitrophenyl group in some membranes. Dimyristoylphosphatidylcholine and dipentadecanoylphosphatidylcholine containing 2 mol % dinitrophenyl lipid hapten bind anti-dinitrophenyl antibodies below the chain-melting transition temperatures of these lipids (22 and 35 C, respectively) but not above these temperatures. Evidently, the lipophilic dinitrophenyl group is partially or completely buried in the hydrophobic region of these bilayers at temperatures above the chain-melting transition temperatures. The inclusion of increasing concentrations of cholesterol in such membranes (e.g. in dimyristoylphosphatidylcholine at 37 C) results in a marked enhancement of antibody binding. It was found that a third lipid hapten containing the dinitrophenyl group does not show this strong dependence of antibody binding on the physical state of the lipid membrane. The weak immunologic degranulation of rat basophil leukemia cells by dimyristoylphosphatidylcholine membrane targets at 37 C can be attributed to a weak binding of anti-dinitrophenyl IgE to these membranes. However, if the antibody is first allowed to bind to this membrane below the lipid chain-melting transition temperature, these IgE-coated membrane targets are very effective in releasing serotonin from the rat basophil leukemia cells when the temperature is raised to 37 C.

LIPID DOMAINS IN THE CRYSTALLINE LIPOVITELLIN/PHOSVITIN COMPLEX: A PHOSPHORUS-31 AND DEUTERIUM NUCLEAR MAGNETIC RESONANCE STUDY. L.J. Banaszak, J. Seelig (Dept. of Biophys. Chem., Biozentrum, Univ. of Basel, Basel, Switzerland CH 4056) *Biochemistry 21*(10):2436-2443 (1982). The crystalline lipovitellin/phosvitin complex has a molecular weight of 456,000 and contains nearly 100 molecules of bound phospholipid. Earlier work using electron microscopy and three-dimensional image reconstruction methods established the symmetrical dimeric nature of this lipoprotein, but the organization of the lipid was unknown. Under conditions where the lipoprotein is in solution, the high-resolution ^{31}P nuclear magnetic resonance (NMR) spectra contain two well-resolved peaks which can be assigned to phosphoserine moieties in both lipovitellin and phosvitin and to the phospholipid microdomains. The spinlattice relaxation times, T_1, for the phosphoserines and the phospholipid headgroups are distinctly different, with the serine phosphates having faster reorientation rates. ^{31}P NMR spectra of crystalline lipoprotein contain a broad symmetric component with a chemical shielding anisotropy of about -50 ppm. By obtaining ^{31}P NMR spectra from several modified forms of the lipoprotein as well as from the extracted lipids, it is shown that the chemical shielding anisotropy is characteristic of phospholipid in a bilayer arrangement. As a further check on ^{31}P NMR results, samples of the lipovitellin/phosvitin complex containing deuterium-labeled 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine were studied by ^{2}H NMR methods. The resulting spectrum has characteristics similar to those obtained from model lipid systems in a lamellar state. The results of the ^{31}P and ^{2}H NMR experiments correlate with the low-resolution model of the crystalline lipovitellin complex obtained by diffraction studies. It is proposed that each subunit of lipovitellin contains a microdomain of phospholipid in a bi-layer like arrangement.

UPTAKE AND OXIDATION OF MALONALDEHYDE BY CULTURED MAMMALIAN CELLS. R.P. Bird and H.H. Draper (Dept. of Nutr., Coll. of Biol. Sci., Univ. 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 oxidized 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 cultture to relatively high MA concentrations.

LIPID ENVIRONMENTS IN THE YOLK LIPOPROTEIN SYSTEM. A SPIN-LABELING STUDY OF THE LIPOVITELLIN/PHOSVITIN COMPLEX FROM *XENOPUS LAEVIS*. G.B. Birrell, P.B. Anderson, P.C. Jost, O.H. Griffith, L.J. Banaszak, J. Seelig (Inst. of Molecular Biol. and Dept. of Chem., Univ. of Oregon, Eugene, OR 97403) *Biochemistry 21*(10):2444-2452 (1982). Lipid/protein and lipid/lipid interactions in the yolk lipoprotein complex from *Xenopus laevis* were examined by introducing a series of lipid spin-labels into the complex and observing the electron spin resonance spectra as a function of the position of the label along the lipid chains, temperature, pH, and charge on the lipid polar head group. Analyses of the spectra show a component with increased segmental flexibility and the greater temperature dependence characteristic of lipid/lipid interactions. These spin-labeling data and supporting compositional data indicate that much of the lipid is organized into a lipid-rich pool consistent with the earlier model derived from electron microscopy and diffraction data and with companion ^{31}P and ^{2}H nuclear magnetic resonance data. The bilayer-like component exhibits a greater restriction of motion compared to vesicles of the isolated lipids at

the same temperature. Phospholipids exchange between the two motionally distinguishable environments. The equilibrium binding undergoes a shift between these two environments as a function both of pH and of the charge on the phospholipid polar head group. This is opposite in direction to that reported for membrane proteins and implicates negatively charged groups on the protein that repel negatively charges phospholipids. This effect is greatly reduced by alkaline phosphatase treatment, suggesting that some of the lipid binding sites are in close proximity to phosphorylated residues on the protein.

EFFECT OF DIETARY FAT AND CHOLESTEROL ON UPTAKE OF OLEIC ACID AND TRIOLEIN BY EVERTED SACS OF BOVINE SMALL INTESTINE. J. Bitman, T.R. Wrenn, J.R. Weyant, D.L. Wood (Milk Secretion and Mastitis Lab., SEA-AR, US Dept. of Agric., Beltsville, MD 20705) *J. Dairy Sci. 65*(7):1148-1154 (1982). The influence of dietary fats on in vitro lipid absorption by bovine intestine was studied in 14 calves. Holstein bull calves were fed for 16 wk five liquid diets containing skim milk plus either 3.5% milk fat, 3.5% tallow, 3.5% tallow and .2% cholesterol, 7.0% tallow, or 7.0% tallow and .2% cholesterol. Uptake of oleic acid or triolein by everted jejunal or ileal sacs was measured after incubation for 30 min at 37 C in pH 7.4 micellar solutions containing tritium-labeled oleic acid or tritium-labeled triolein. Lipids were extracted from homogenates of sacs and separated into lipid classes by thin layer chromatography. Equal amounts of oleic acid were taken up by jejunal or ileal sacs. Triolein uptake was less than oleic acid uptake, but uptakes by jejunal or ileal sacs did not differ. Oleic acid incorporation into triglyceride was three to four times greater in intestinal sacs from milk fat-fed calves than in sacs from calves fed either 3.5 or 7% tallow. Oleic acid incorporation in intestinal sacs from calves fed cholesterol as well as tallow was equal to that in milk fat-fed calves. Intestinal uptake and metabolism of oleic acid proceeded faster when calves were fed milk fat than when fed tallow. The lower intestinal incorporation with tallow increased if cholesterol was fed, suggesting that cholesterol either stimulated absorption or increased esterification.

HETEROGENEITY OF RABBIT INTESTINE BRUSH BORDER PLASMA MEMBRANE CHOLESTEROL. B. Bloj, D. Zilversmit (Div. of Nutr. Sciences and Section of Biochem., Molecular and Cell Biol., Div. of Biol. Sciences, Cornell Univ., Ithaca, NY 14853) *J. Biol. Chem. 257*(13):7608-7614 (1982). Nonspecific lipid transfer protein accelerated cholesterol exchange from brush border vesicles according to a biphasic time course, but sonicated vesicles made from brush border phospholipids and glycosphingolipids showed a single phase exchange. Removal of surface protein with papain or opening brush border vesicles with deoxycholate did not abolish the biphasic exchange pattern. In brush border vesicles treated with cholesterol oxidase, 31 ± 10% of the free cholesterol was oxidized rapidly, and the remaining cholesterol was oxidized at a slower rate. Opening vesicles with sodium doexycholate or treatment with phospholipase C, which degraded 55% of the phospholipids, did not increase the size of the rapidly oxidizable cholesterol pool. The rapidly oxidizable cholesterol pools appear to represent the same fraction. In doubly-labeled brush border vesicles 27 ± 9% of the cholesterol is present in a readily accessible pool, which slowly equilibrates with the remaining membrane cholesterol. The fractional turnover rate of cholesterol in the readily accessible pool equals 0.07 ± 0.04 h^{-1} and is increased to 3.35 h^{-1} by 12 μg/ml of nonspecific lipid transfer protein. The heterogeneous distribution of cholesterol in the intact brush border vesicles may not reflect an inside-outside distribution or interaction of cholesterol with membrane lipids but rather an association of more than two-thirds of the membrane cholesterol with a membrane protein fraction.

PREVENTION OF LIPID ACCUMULATION IN EXPERIMENTAL VEIN BYPASS GRAFTS BY ANTIPLATELET THERAPY. L.I. Boncheck, L.E. Boerboom, G.N. Olinger, J.R. Pepper, J. Munns, L. Hutchinson, A.H. Kissebah (Depts. of Cardiothoracic Surgery and Med., Med. Coll. of Wisconsin, Milwaukee, WI) *Circulation 66*(2): 338-341 (1982). The ameliorative effect of antiplatelet therapy on atherogenesis of vein grafts was assessed in autologous cephalic veins grafted into femoral arteries of 16 normolipemic and 11 hyperlipemic stumptailed macaque monkeys. Before grafting, one half of each vein was distended at high pressure (700 mm Hg) and the other half at low pressure (350 mm Hg). Eight normolipemic monkeys were treated with aspirin, 80 mg/day, and dipyridamole, 50 mg/day, and eight were controls. When grafts were harvested at 12 weeks, tissue cholesterol and β-apoprotein content in grafts from untreated monkeys were significantly higher than in ungrafted, uninjured veins. Antiplatelet therapy eliminated the increase in lipid content of vein segments distended at low pressure, and significantly lowered lipid content of segments distended at high pressure, though not to control levels of ungrafted veins. Seven of the 11 hyperlipemic monkeys received antiplatelet drugs and four did not. The lipid content of all graft segments was significantly higher than ingrafted or ungrafted veins from normolipemic monkeys. Antiplatelet therapy again significantly reduced the lipid content in vein segments distended at both levels of pressure, and also reduced the elevated cholesterol content in ungrafted veins. Although this animal prepartion differs in many ways from human coronary bypass operations, these observations may be pertinent to the prevention of atherosclerosis in human vein bypass grafts.

CHOLESTEROL BIOSYNTHESIS AND MODULATION OF MEMBRANE CHOLESTEROL AND LIPID DYNAMICS IN RAT INTESTINAL MICROVILLUS MEMBRANES. T.A. Brasitus, and D. Schachter (Dept. of Physiology and Med., Columbia Univ. College of Physicians and Surgeons, New York, NY 10032) *J. Biochem. 21*(9): 2241-2246 (1982). Experiments were performed to test the hypothesis that cholesterol biosynthesis in the rat ileal enterocyte, the major absorptive cell lining the distal epithelium of the small intestine, can modulate the cholesterol content and the motional freedom of the plasma membrane lipids. Decreased sterol biosynthesis in vivo was elicited by feeding sodium taurocholate or by fasting the rats, whereas increased synthesis was induced by biliary ligation or feeding cholestyramine, a bile salt binding resin; these effects were monitored by assay of mucosal 3-hydroxy-3-methyl-glutaryl coenzyme A reductase. After each procedure, isolated microvillus membranes were examined to determine the lipid composition and the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene. The results demonstrate that variations in cholesterol biosynthesis in vivo can modulate the cholesterol content and the motional freedom of the lipids of the microvillus membrane; similar effects were not observed on the basolateral membrane. The observations suggest that the normal pattern of decreased lipid motional freedom in microvillus membranes of the distal as compared to the proximal small intestine of the rat results from higher rates of cholesterol biosynthesis in the distal mucosa.

POSITIONAL SPECIFICITY OF A RETICULOCYTE LIPOXYGENASE. CONVERSION OF ARACHIDONIC ACID TO 15S-HYDROPEROXY-EICOSATETRAENOIC ACID. R.W. Bryant, J.M. Bailey, T. Schewe, and S.M. Rapoport (George Washington Univ., Med. Schl., Washington, D.C. 20037) *J. Biol. Chem. 257*(11):6050-6055 (1982). The metabolism of arachidonic and other polyunsaturated fatty acids by various lipoxygenases is an important event in certain cell types, particularly cells of the reticuloendothelial system such as platelets and leucocytes. A particularly abundant lipoxygenase has also been isolated and purified by ammonium sulfate precipitation, DEAE-Sephadex A-50 chromatography, and isoelectric focusing from rabbit reticulocytes. We now report the positional specificity of this enzyme as determined by structural analysis of the products formed by the purified reticulocyte lipoxygenase acting on selected polyunsaturated fatty acids. The hydroperoxy fatty acid products were isolated from reaction mixtures by solvent extraction and further purified by high performance liquid chromatogarphy. The purified hydroperoxy compounds were reduced to the corresponding hydroxy acids with triphenylphosphine. These were converted to the trimethylsilyl ether methyl ester derivatives and their structures determined by gas chromatography/mass spectrometry. We found that reticulocytes contain 15-lipoxygenase in contrast to the 12- and 5-lipoxygenases which predominate in platelets and neutrophils, respectively. In common with platelets and neutrophils, reticulocytes have a peroxidase system which converts the lipoxygenase-generated hydroperoxy fatty acid to the corresponding hydroxy fatty acid.

REVERSIBLE MODIFICATION OF HUMAN PLASMA LOW DENSITY LIPOPROTEINS TOWARD TRIGLYCERIDE-RICH PRECURSORS. A MECHANISM FOR LOSING EXCESS CHOLESTEROL ESTERS. R.J. Deckelbaum, S. Eisenberg, Y. Oschry, E. Butbul, I. Sharon, and T. Olivecrona (Depts. of Gastroenterology and Pediatrics, Med. B and Lipid Res. Lab., Hadassah Univ. Hosp., Hebrew Univ.-Hadassah Med. Schl., Jerusalem, Israel) *J. Biol. Chem. 257*(11): 6509-6517 (1982). Since human very low density lipoprotein (VLDL) particles contain more cholesterol ester molecules than low density lipoprotein (LDL), we studied how excess cholesterol ester can be removed as VLDL is catabolized to LDL. The exchanges of core lipids and apoproteins between LDL and VLDL are dependent on factors present in human but not rat lipoprotein-poor plasma, independent of lecithin cholesterol acyltransferase activity, and dependent on time, concentrations, and temperature of incubations. The changes in M-LDL are reversible as most acquired triglyceride can be hydrolyzed by incubation with purified bovine milk lipoprotein lipase. A new smaller LDL particle results, again cholesterol ester-rich, but with less ester than original plasma LDL. Thus, a mechanism is proposed whereby in vivo excess VLDL cholesterol ester is

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lost from LDL in exchange for triglyceride. After lipolysis, a smaller LDL particle with less cholesterol ester is formed. Since similar changes are observed if intermediate density lipoproteins are incubated with VLDL, we suggest these mechanisms for removal of excess cholesterol ester operate all along the lipolytic pathway as VLDL is catabolized to LDL.

A TIME STUDY COMPARING THE APPEARANCE OF CATABOLITES OF 9α,11α,15(*S*)-TRIHYDROXYPROSTA-5,13-DIENOIC ACID IN BLOOD AND URINE DURING CONSTANT INTRAVENOUS INFUSION OF TRITIATED 9α,11α,15(*S*)-TRIHYDROXYPROSTA-5,13-DIENOIC ACID IN THE RAT. ISOLATION OF A NEW CIRCULATING CATABOLITE. N.S. Edwards, C.R. Pace-Asciak (Research Inst., The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8) *J. Biol. Chem. 257*(11):6339-6342 (1982). Profiles of 9α,11α,15(*S*)-trihydroxyprosta-5,13-dienoic acid ($PGF_{2\alpha}$) and its catabolites in blood and urine of male adult Wistar rats were determined by high pressure liquid chromatography during and after slow intravenous infusion of tritium-labeled $PGF_{2\alpha}$. In addition to $PGF_{2\alpha}$ and 15-keto-13,14-dihydro-$PGF_{2\alpha}$, a major catabolite in blood during the infusion was identified as *tetranor*-15-keto-13,14-dihydro-$PGF_{2\alpha}$ (VII). This product was absent in the corresponding urine samples. VII was still detected in blood 2 hr after the infusion was terminated. VII was previously shown by us to be an intermediate in the formation of certain urinary products of $PGF_{2\alpha}$. These other catabolites of $PGF_{2\alpha}$ were also observed in both blood and urine although their levels were greater in urine. Our observations indicate that a long lasting catabolite of $PGF_{2\alpha}$, i.e. VII, as well as all the urinary catabolites of $PGF_{2\alpha}$ appear in the circulation. These results suggest that these catabolites are mostly (although not exclusively) extrarenal in origin (probably all hepatic) entering the circulation prior to their excretion by the kidney.

PLASMA AND URINARY LIPIDS AND LIPOPROTEINS DURING THE DEVELOPMENT OF NEPHROTIC SYNDROME INDUCED IN THE RAT BY PUROMYCIN AMINONUCLEOSIDE. E. Gherardi and S. Calandra (instituto di Patologia Generale, Universitá di Modena, Via Campi 287, 41100 Modena (Italy)) *Biochim. et Biophys. Acta 710*(2):188-196 (1982). This study was undertaken to ascertain whether the alterations of plasma lipoproteins found in nephrotic syndrome induced by puromycin aminonucleoside were due to nephrotic syndrome per se, or, at least in part, to the aminonucleoside. The purpose was to investigate the changes in plasma and urinary lipoproteins during the administration of puromycin aminonucleoside and the subsequent development of nephrotic syndrome. Since massive albuminuria occurred after 6 days of treatment, the time-course study was divided into two stages: pre-nephrotic stage (day 1-5) and nephrotic stage (day 6-11). In pre-nephrotic stage the plasma level of fatty acids, triacylglycerol and VLDL decreased while that of phospholipid, cholesteryl esters and HDL remained constant. Plasma apolipoprotein A-I tended to increase. At the beginning of nephrotic stage, the concentration of plasma albumin dropped to a very low level, while that of apolipoprotein A-I increased abruptly and continued to rise in the following days. The plasma concentration of HDL followed the same pattern. Plasma VLDL and LDL increased at a later stage. Plasma apolipoprotein A-I was found not only in HDL, but also in the LDL density class. In the pre-nephrotic stage lipoproteinuria was negligible, while in the early nephrotic stage the urinary loss of plasma lipoproteins consisted mainly of HDL. These observations indicate that puromycin aminonucleoside alters plasma lipoproteins by lowering VLDL and increasing HDL. It is likely that the early and striking increase of plasma HDL found in nephrotic rats is related to a direct effect of the drug on HDL metabolism.

CRADLE-TO-GRAVE ATHEROSCLEROSIS: HIGH DENSITY LIPOPROTEIN CHOLESTEROL. C.J. Glueck (LIpid Res. Clinic, Gen. Clinical Res. Center, and CLINFO Center, Lipid Res. Div., Coll. of Med., Univ. of Cincinnati) *J. Amer. Coll. Nutr. 1*(1):41-48 (1982). This presentation reviews environmental and genetic factors that relate to high density lipoprotein cholesterol, the most potent independent lipoprotein risk factor for coronary heart disease. Although at least three decades of work have focused upon the primary atherogenic lipoprotein, low density lipoprotein cholesterol (C-LDL), which has a strong positive association with coronary heart disease (CHD), it has only been in the past decade that detailed epidemiologic and biochemical studies have revealed that high density lipoprotein cholesterol (C-HDL) is the most potent lipoprotein cholesterol related to coronary heart disease; this relationship is, however, inverse.

INTESTINAL AND HEPATIC CHOLESTEROL SYNTHESIS IN THE ALLOXAN DIABETIC RAT. M.W. Goodman, L.D. Michels, and W.F. Keane (Dept. of Med., Hennepin County Med. Center, and Univ. of Minnesota Hosp., Minneapolis, MN 55415) *Proc. Soc. Exp. Biol. and Med. 170*(3):286-290 (1982). The effects of alloxan diabetes and insulin treatment upon rat 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase was studied. In diabetes, hepatic HMG-CoA reductase specific and whole organ activities were reduced 85 and 89%, respectively, during the diurnal high period of enzyme activity. In contrast, whole small intestinal HMG-CoA reductase activity was increased twofold in diabetic rats during this period. The diabetic rats' whole gut HMG-CoA reductase activity approximated the entire liver's. Chronic insulin therapy markedly stimulated hepatic HMG-CoA reductase activity in diabetes rats but did not increase gut enzyme activity. Thus in alloxan diabetes, the intestine may be as important a source of endogenous cholesterol as the liver.

INCORPORATION OF RADIOLABELED LYSOPHOSPHATIDYLCHOLINE INTO CANINE PURKINJE FIBERS AND VENTRICULAR MUSCLE. R.W. Gross, P.B. Corr, B.I. Lee, J.E. Saffitz, W.A. Cra^{f}ford, Jr., B.E. Sobel (Cardiovascular Div., Washington Univ. Schl. of Med., St. Louis, MO) *Circ. Res. 51*(1):27-36 (1982). Lysophosphoglycerides including lysophosphatidylcholine (LPC), accumulate in ischemic myocardium, and in comparable concentrations induce electrophysiological alterations in vitro analogous to those seen in ischemic myocardium in vivo. The present study was performed to assess the amount of ^{14}C-LPC incorporated into isolated tissue required to induce electrophysiological effects, to localize the sites of incorporation by electron microscopic autoradiography, and to assess the association between electrophysiological recovery and metabolism of incorporated LPC. ^{14}C-LPC (200 μM) induced marked electrophysiological effects in Purkinje fibers when only 2.3% of cellular phospholipid was supplanted by exogenous LPC. Electrophysiological depression correlated with incorporation of LPC, and electrophysiological recovery correlated with metabolism of LPC to free fatty acid and phosphatidylcholine. Incubation of ventricular muscle strips with ^{14}C-LPC (100 μM) resulted in incorporation of 0.42 nmol-mg protein of exogenous LPC at pH 7.4. Incorporation was similar at pH = 6.7 (0.36 nmol/mg protein), although electrophysiological derangements were markedly enhanced. Electron microscopic autoradiography showed that incorporated LPC was localized to the sarcolemmal membrane. These findings indicate that incorporation of as little LPC as 1% of cellular phospholipid induces marked electrophysiological changes, that LPC rather than its major metabolites, fatty acid and phosphatidylcholine, are responsible for the electrophysiological alterations, and that reduction in pH enhances the membrane effects of LPC without increasing incorporation.

CHARACTERIZATION AND QUANTITATION OF APOLIPOPROTEINS A-I AND E- OF NORMAL AND CHOLESTEROL-FED GUINEA PIGS. L.S.S. Guo, R.L. Hamilton, J.P. Kane, C.J. Fielding, and G.C. Chen (Cardiovascular Res. Inst. and the Depts. of Anatomy, Physiology, and Med., Univ. of California, San Francisco, CA 94143) *J. Lipid Res. 23*(4):531-542 (1982). We have characterized and quantified the two major plasma apoproteins of high density lipoproteins (HDL), apolipoproteins A-I (apoA-I) and E (apoE), of guinea pigs fed standard chow (normal) or chow supplemented with 1% cholesterol (cholesterol-fed). ApoA-I isolated from plasma HDL of the normal guinea pig exists in six polymorphic forms (pI 5.75-5.40). A similar isoform pattern of this apoprotein was present in nascent HDL isolated from perfused livers of normal and cholesterol-fed animals. This apoprotein contains cysteine and isoleucine and is slightly different in overall amino acid composition from apoA-I of human and rat, but activates lecithin:cholesterol acyltransferase from human plasma with an activation curve almost identical to that obtained with human apoA-I. ApoE present in nascent VLDL and HDL from perfused liver of normal animals contains three isoforms (pI 5.42-5.34). Following cholesterol feeding, the numbers of apoE isoforms from perfused livers were increased from three to five or more by shifting the major component (pI 5.42) to more acidic isoforms (pI 5.28-5.17). This shifting was mostly reversible when apoE was treated with neuraminidase, suggesting that cholesterol feeding leads to a modification of apoE by increasing its content of sialic acid. Similar changes of apoE isoforms were also observed in plasma lipoproteins as early as 10 days after cholesterol feeding. The amino acid compositions of four apoE isoform fractions isolated from plasma HDL of cholesterol-fed guinea pigs were similar to that of parent apoE.

CHANGES IN LIVER LIPIDS AFTER ADMINISTRATION OF 2-DECANOYLAMINO-3-MORPHOLINOPROPIOPHENONE AND CHLORPROMAZINE. A.V. Hospattankar, R.R. Vunnam, N.S. Radin (Mental Health Res. Inst., Dept. of Psychiatry and Dept. of Biol. Chem., Univ. of Michigan, Ann Arbor, MI 48109) *Lipids 17*(8):538-543, (1982). The enzyme which forms glucocerebroside, ceramide:UDP-glucose glucosyltransferase, is inactivated in vitro by a cationic analog of cerebroside, 2-decanoylamino-3-morpholinopropiophenone. A study of the inhibitor using intraperitoneal injection

into young mice showed that the level of the enzyme activity in liver was appreciably lowered between 3 and 6 hr after injection. The activity increased subsequently, overshooting the normal level within 24 hr by about 20%, then returning to normal within the next 24 hr. Additional effects observed in liver were an increase in lipid content (primarily in the triglyceride fraction and ceramides) and a decrease in the glucocerebroside level. Body temperature dropped rapidly. Markedly similar effects were produced by injecting chlorpromazine, which was tried in order to reduce the hyperirritability and inhibitory effects on monoamine oxidase previously demonstrated by the glucosyltransferase inhibitor. Chlorpromazine did indeed block the hyperirritability and resulted in enhancement of the keto amine's effects on the enzyme and lipids. It is possible that the two drugs in combination would be helpful in ameliorating the symptoms due to the cerebroside accumulation that occurs in Gaucher disease. Diazepam also produced a reduced level of glucosyltransferase. A color reaction for chlorpromazine, possibly suitable for quantitative determination in tissues, was accidentally discovered.

EARLY CHOLESTEROL FEEDING: ARE THERE LONG-TERM EFFECTS IN THE RAT? G. Hulbron, R. Aubert, F. Bourgeois, and D. Lemonnier (Unite de Recherches sur la Nutrition et l' Alimentation, U.1. INSERM, Hopital Bichat, 170 Bd. Ney, 75877 Paris Cedex 18-France) *J. Nutr. 112*(7):1296-1305 (1982). The effects of exposure to cholesterol in early life on diet-induced hypercholesterolemia in adult rats were investigated. Experiment 1; dams and their offspring received either a control or a cholesterol-enriched diet during gestation and lactation; at 7 weeks of age half of the rats fed each diet were switched to the other diet for 50 weeks. Experiment 2: adult males 6 months old were fed one of the 2 experimental diets for 10 weeks, at which time half of the rats in each group were switched to the other diet for 50 weeks. Cholesterol, triglycerides and phospholipids were determined in liver, serum, heart and aorta at different ages. In both experiments feeding a cholesterol-enriched diet induced an increase in serum and liver cholesterol and triglyceride levels, but serum phospholipid were not influenced by diet. The cholesterol-enriched diet induced a decrease of liver phospholipid levels. Old rats (experiment 2) fed the cholesterol-enriched diet exhibited higher heart cholesterol level than controls. In experiment 1 rats fed the cholesterol-enriched diet in early life and thereafter had lower heart triglyceride levels than the 3 other groups and lower liver triglyceride levels than rats fed control diet in early life and cholesterol diet at 7 weeks. In both experiments, cholesterolemia was not influenced by the diet fed at the beginning of the test. The results indicate that cholesterol given in early life does not protect against diet-induced hypercholesterolemia in adult rats.

DEGRADED AND STABLE PHOSPHATIDYLGLYCEROL IN *ESCHERICHIA COLI* INNER AND OUTER MEMBRANES, AND RECYCLING OF FATTY ACYL RESIDUES. D. Joseleau-Petit, and A. Kepes (Univ. Paris VII, Centre Natl. de la Recherche Scientifique, Inst. de Recherche en Biol. Moleculaire, 2, place Jussieu, 75251 Paris Cedex 05 (France)) *Biochim. et Biophys. Acta 711*(1):1-9 (1982). The metabolic fate of membrane phospholipids in exponentially growing *Escherichia coli* was reexamined by incorporation and chase of labeled precursors: [^{32}P] phosphate, [2-^{3}H] glycerol and ^{3}H-labeled fatty acids. It was found that the well-known turnover of phosphatidylglycerol lasted only about two generation times; the remaining labeled phosphatidylglycerol was stable for the subsequent two generation times. The location of the stable phosphatidylglycerol pool remaining after the turnover in the outer and inner membrane was investigated. Both were found to contain stable phosphatidylglycerol so that the existence of a stable portion cannot be ascribed to its exclusive location in one leaflet. A small loss of labeled phosphatidylethanolamine was also observed, and upon fractionation this was found to occur exclusively in the outer membrane. [^{32}P] Phosphate and [2-^{3}H] glycerol labels of the degraded phospholipids were lose from lipid-soluble material, whereas labeled fatty acid, palmitate or oleate was reincorporated into newly synthesized phosphatidylethanolamine and phosphatidylglycerol, so that total fatty acid label remained constant in (membrane) phospholipid during chase. The recycling of the fatty acids under the form of diacylglycerols to phosphatidic acid does not appear to be the predominant pathway of reincorporation. After double labeling with [^{32}P] phosphate and [^{3}H] palmitate, a complete balance sheet of loss and reincorporation of fatty acid of the two envelopes could be established. Results indicate that fatty acid was reincorporated essentially in the inner membrane phospholipids. Movements of phospholipids and of fatty acids from one membrane to another and in the planc of each layer are discussed.

HYDROLYTIC DEGRADATION OF PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE BY ISOLATED RAT-LIVER LYSOSOMES. H. Kunze, B. Hesse, and E. Bohn (Dept. of Biochem. Pharmacol., Max-Planck-Institute for Experimental Med., Gottingen, F.R.G.) *Biochim. et Biophys. Acta 711*(1):10-18 (1982). Lysosomal catabolism of radioactively labelled phosphatidylethanolamine, phosphatidylcholine and several potential metabolites of these diacylphospholipids was studied using rat-liver lysosomes which had been isolated from Triton WR-1339-treated animals. Hydrolysis of these lipids seems to be restricted to the soluble lysosomal compartment. The initial intralysosomal degradation is predominantly catalyzed by phospholipase A_1 (EC 3.1.1.32) followed by lysophospholipase (EC 3.1.1.5). The end products of this pathway are free fatty acids and glycerophosphorylethanolamine or glycerophosphorylcholine. These phosphodiesters are not hydrolyzed further in lysosomes, as has been shown previously. The intermediary lysophospholipids, however, are also hydrolyzed by an alternative pathway, i.e. by a lysophospholipase which catalyzes the hydrolysis of the glycerophosphate ester bond, followed by a monoacylglycerol lipase and a phosphomonoesterase (EC 3.1.3.2), respectively. Besides these two catabolic routes of intralysosomal hydrolysis of phosphatidylethanolamine and phosphatidylcholine, additional pathways are possible, which seem, however, to be of minor importance, at least in the substrate concentration ranges employed in these studies. These additional reactions include attack by a phospholipase A_2 (EC 3.1.1.4) and – as discovered recently – by a phospholipase C (EC 3.1.4.3). Cations such as Mg^{2+}, Ca^{2+}, K^+ and Na^+ inhibit preferentially deacylation reactions.

ANTIOXIDANT ROLE OF RETINYL-PALMITATE ON CARBON-TETRACHLORIDE TOXICITY. P.S. Kumar, D.M. Vasudevan, N.J. Bai (Basic Res. Div., Regional Cancer Centre, Med. Coll. Campus, TRIVANDRUM-11, India) *Acta Vitaminol. Enzymol. 3*(4):214-218 (1982). Hepatic microsomal lipid peroxidation as measured by diene conjugation was markedly reduced by feeding 400,000 IU of retinyl palmitate to rats. A single oral dose of 0.3 ml of CCl_4 per 100 g body weight elevated microsomal lipid peroxidation by 1.5 fold, but the increase could be prevented by prior feeding of retinyl palmitate. Aminopyrene and p-nitroanisole demethylase were inhibited by retinyl ester and CCl_4 either alone or in combination with the vitamins. Serum transaminases and alkaline phosphatase were elevated by CCl_4 and the elevation was not affected by prior feeding of retinyl ester. It is concluded that retinyl palmitate acts as an antioxidant in vivo.

HUMORAL IMMUNE RESPONSE IN VITAMIN A DEFICIENT CHILDREN. P.M. Kutty, M. Mohanram, R. Vinodini (Natl. Inst. of Nutr. Indian Council of Med. Res. Jamai Osmania (P.O.) HYDERABAD-500 007, A.P., India) *Acta Vitaminol. Enzymol. 3*(4):231-235 (1982). Humoral immune response was evaluated in children with vitamin A deficiency. The percentage of B lymphocytes and the initial levels of plasma IgA, IgG and IgM were normal. Two weeks after the immunization with diptheria and tetanus toxoids, there was a marked increase in the antibody titres. There were no significant differences between the deficient and the normal children. The results indicate that the antibody production is not altered in children with vitamin A deficiency.

SELECTIVE DEPOSITION OF *TRANS*-8- AND *CIS*-9-OCTADECENOATES IN EGG AND TISSUE LIPIDS OF THE LAYING HEN. A.C. Lanser (Northern Regional Res. Center, Agric. Res. Service, U.S. Dept. of Agric., Peoria, IL 61604) *Lipids 17*(8):524-528 (1982). The deposition of *trans*-8-octadecenoate-8(9)-^{3}H was compared to *cis*-9-octadecenoate-10-^{14}C (9*c*-18:1-^{14}C) in the major egg yolk neutral lipids and phospholipids and in organ lipids from the laying hen, *trans*-8-Octadecenoate was preferentially incorporated into only the phosphatidylethanolamines (PE), whereas discrimination against 8*t*-18:1-^{3}H occurred in the phosphatidylcholines (PC), triglycerides (TG) and cholesteryl esters (CE). The 1-acyl position of both PE and PC contained three times more 8*t*-18:1-^{3}H than 9*c*-18:1-^{14}C. Almost total exclusion of the 8*t*-18:1-^{3}H from the 2-acyl position of these phospholipids was found. Preferential incorporation of 9*c*-18:1-^{14}C occurred at the combined 1- and 3-acyl positions and at the 2-acyl position of yolk TG. Tissue lipid analyses indicated that there was preferential deposition of 9*c*-18:1-^{14}C into all organs. Individual liver lipid classes displayed the same relative order of discrimination against 8*t*-18:1-^{3}H as did egg yolk lipids (CE>TG>PC>PE).

REACTION OF HUMAN LECITHIN CHOLESTEROL ACYLTRANSFERASE WITH SYNTHETIC MICELLAR COMPLEXES O APOLIPIPROTEIN A-I, PHOSPHATIDYLCHOLINE, AND CHOLESTEROL. C.E. Matz and A. Jonas (Dept. of Biochem. Schl. of Basic Med. Sci. and Schl. of Chem. Sci., Univ. of Illinois, Urbana, IL 61801) *J. Biol. Chem. 257*(8):4541-4546 (1982). Micellar, discoidal complexes of human apolipoprotein A-I (apo A-I) with phospha-

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tidylcholines and cholesterol, prepared by the method described in the preceding paper were used as substrates for human lecithin cholesterol acyltransferase, purified 10,000-fold. The micellar complexes of apo A-I·egg yolk-phosphatidylcholine·cholesterol were compared to commonly used substrates of lecithin cholesterol acyltransferase, consisting of small unilamellar vesicles of egg yolk-phosphatidylcholine and cholesterol in the presence of apo A-I. Under identical reaction conditions, the micellar complexes has 4- to 5-fold higher initial velocities and 3-fold greater capacities for cholesterol esters than did the corresponding vesicular substrates. Micellar complexes, labeled with 5-dimethylaminonaphthalene-1-sulfonyl fluorescent groups in the apolipoprotein, were isolated by density gradient centrifugation. After reaction with lecithin cholesterol acyltransferase, they had a shorter rotational relaxation time (290 ns) and smaller Stokes radius (47Å) than the unreacted complexes (530 ns and 57 Å, respectively). The characteristic stacked, discoidal particles observed on electron micrographs of negatively staind micellar, unreacted complexes disappeared after enzymatic reaction and were replaced by structures with spheroidal shapes.

SOME EFFECTS OF PHENOBARBITAL DOSING OF DAIRY CATTLE ON AFLATOXIN M_1 AND FAT IN MILK. P.B. McGrew, H.M. Barnhart, D.R. Mertens, R.D. Wyatt (Animal and Dairy Sci. Dept., Univ. of Georgia, Athens, GA) *J. Dairy Sci. 65*(7):1277-1233 (1982). Eight Holstein cows were selected randomly to determine effects of phenobarbital on the fate of orally administered aflatoxin B_1, milk fatty acid profiles, total milk fat, and milk production. Animals were grouped in pairs for one of four treatments: 1) control, 2) oral dosing with sodium phenobarbital for 5 days, 3) dosing with sodium phenobarbital followed by oral dosing with aflatoxin B_1, and 4) oral dosing with aflatoxin B_1 for 5 days. Daily composite raw milk samples were taken and assayed for aflatoxin M_1 by thin-layer chromatography, and milk fatty acid profiles were measured by gas-liquid chromatography. Milk production for all treatments was less than that of controls. Total milk fat was not affected. Both aflatoxin B_1 and phenobarbital affected fatty acid distribution, suppressing the amount of fatty acids carbons 8 through 14 while increasing the amount of 16-carbon fatty acids. No treatment effect was significant for the short chain fatty acids of 4 and 6 carbons and the 18-carbon acids oleic, stearic, and linoleic. Pre-treatment with phenobarbital resulted in a significant difference between treated and untreated animals. A greater than 50% reduction in the amount of aflatoxin B_1 excreted as M_1 in the milk was realized.

ACETYL GLYCERYLPHOSPHORYLCHOLINE INHIBITION OF PROSTAGLANDIN I_2-STIMULATED ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE LEVELS IN HUMAN PLATELETS. EVIDENCE FOR THROMBAXANE A_2 DEPENDENCE. O.V. Miller, D.E. Ayer, R.R. Gorman (Depts. of Exper. Biol. and Chem., The Upjohn Co., Kalamazoo, MI 49001) *Biochim. Biophys. Acta 711*(3): 445-451 (1982). Previous studies with AGEPC (1-*O*-hexadecyl/octadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) stress the independence of the proaggregatory activity of AGEPC from the platelet cyclooxygenase. However, our dose response analyses in human platelet-rich plasma show distinct primary and secondary waves of aggregation in response to AGEPC. Second wave aggregation is inhibited completely by either 10 μM indomethacin, a cyclooxygenase inhibitor, or 5.6 μM 9,11-azoprosta-5,13-dienoic acid, a thromboxane A_2 synthetase inhibitor. Simultaneous addition of AGEPC and prostaglandin I_2 to platelet-rich plasma results in a marked increase in platelet cyclic AMP, which is not different from the prostaglandin I_2 response alone. However, if prostaglandin I_2 is added to AGEPC-stimulated platelets at a point where secondary aggregation is just beginning, AGEPC can attenuate prostaglandin I_2-stimulated cyclic AMP accumulation. The inhibition by AGEPC is blocked by either cyclooxygenase or thromboxane A_2 synthetase inhibitors, and radioimmunoassay of thromboxane B_2 confirmed that the inhibition of prostaglandin I_2-stimulated cyclic AMP accumulation is due to thromboxane A_2 synthesis, and that AGEPC-stimulated secondary aggregation does not start until thromboxane A_2 is synthesized. These data suggest that much of the bioactivity of AGEPC is attributable to thromboxane A_2.

LIPID TRANSLOCATION ACROSS THE HUMAN ERYTHROCYTE MEMBRANE. N. Mohandas, J. Wyatt, S.F. Mel, M.E. Rossi, and S.B. Shohet (Depts. of Lab. Med. and Med., Cancer Res. Inst., Univ. of California, San Francisco, CA 94143) *J. Biol. Chem. 257* (11):6537-6543 (1982). A simple method based on the differential extraction of lysophosphatidylcholine (LPC) by saline and albumin solutions has been developed to study the factors that influence lipid translocation across intact human erythrocyte membrane. With this assay, the rate of LPC translocation across the bilayer at 37 C was found to be 1.87%/hr (0.0187 hr^{-1}). Identical translocation rates were derived for normal cells and cells in which the ATP was totally depleted, implying that the metabolic state of the cell had no influence. In contrast, the translocation rate was strongly influenced by temperature. Above 21 C, the rate doubled for every 51 C increase in temperature, suggesting an important role for diffusion through the lipid phase. Denaturation of a single major skeletal protein, spectrin, by heating cells to 5 C did not alter the translocation rate. However, oxidative cross-linking of a complex of membrane proteins by treatment with diamide significantly increased the rate of translocation at 37 C. Cholesterol enrichment of the cells decreased the apparent rate of translocation but not the total quantity of LPC translocated. Taken together, these data suggest that lipid translocation across the intact human erythrocyte membrane is not energy dependent, and that it is influenced by the organizational state of both the lipid and protein moieties of the membrane.

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 Biochem., Bowman Gray Schl. of Med., 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 comparable 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.

FATTY ACID BINDING PROTEIN. ISOLATION FROM RAT LIVER, CHARACTERIZATION, AND IMMUNOCHEMICAL QUANTIFICATION. R.K. Ockner, J.A. Manning, and J.P. Kane (Dept. of Med. and Liver Center, Univ. of California, San Francisco, CA 94143) *J. Biol. Chem. 257*(13):7872-7878 (1982). Fatty acid-binding protein (FABP) was identified and isolated from rat liver cytosol by gel filtration, thin layer isoelectric focusing, and affinity chromatography. FABP (M_r 12,080 ± 80) exists in several immunochemically identical forms differing in isoelectric pH, which may in part reflect differences in their respective complements of bound endogenous ligand. FABP-bound fatty acids accounted for 60% of total cytosolic long chain fatty acids but contained no detectable phospholipid; the substantial enrichment of FABP in 18:2 and 20:4 as compared with whole liver homogenate was no influenced by homogenization of tissue in EDTA. The amino acid composition of FABP suggest that it is closely related or identical with certain similar neutral and acidic cytosolic proteins reported from other laboratories. By quantitative radial immunodiffusion, FABp concentration in cytosol from livers of sexually mature female rats exceeded that from mature males (51.7 ± 3.0 versus 39.8 ± 4.0 μg/mg of protein, $p < 0.05$), confirming earlier studies in which sex steroid effects on rates of fatty acid utilization were correlated with FABP concentration as determined by means of a binding assay. The abundance of FABP, its importance in the cytosolic binding of endogenous as well as exogenous fatty acids, and its demonstrated correlation with rates of hepatocyte fatty acid utilization provide additional evidence for its relationship to the cellular metabolism of long chain fatty acids.

FATTY ACID SYNTHETASE SYSTEM IN THE REGULATION OF MEMBRANE LIPID SYNTHESIS IN *ESCHERICHIA COLI* AFTER SHIFTS IN TEMPERATURE. H. Okuyama, M. Saitoh, R. Hiramatsu (Dept. of Biol. Chem., Faculty of Pharmaceutical Sciences, Nagoya City Univ., 3-1 Tanabedori, Mizuhoku, Nagoya, Japan 467) *J. Biol. Chem. 257*(9):4812-4817 (1982). Fatty acid synthetase systems were prepared from *Escherichia coli* grown at 40 and 10 C. Both enzyme preparations synthesized fatty acid mixtures containing higher proportions of unsaturated then saturated fatty acids and having shorter average chain lengths when assayed at 10 C than when assayed at 40 C. The 40 C fatty acid synthetase synthesized a mixture of fatty acids with a higher U/S ratio and a longer average chain length than those fatty acids synthesized by the 10 C fatty acid synthetase at assay temperatures of 40 and 10 C, indicating that the two fatty acid synthetase systems are physically different.

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Differences were observed between the 40 C fatty acid synthetase and 10 C fatty acid synthetase prepared from *E. coli* B and *E. coli* K12 strains. The chain-shortening effect appears to be suppressed in vivo. When the effect of malonyl-CoA concentrations was examined as a possible suppressive factor, the 40 C fatty acid synthetase and 10 C fatty acid synthetase showed different responses; lowering resulted in decreases in both the U/S ratio and the average chain lengths of saturated and unsaturated fatty acids when 40 C fatty acid synthetase was used, while only the average chain length of saturated fatty acids decreased when 10 C synthetase was examined. These properties seem partially responsible for the temperature-controlled mechanism of fatty acid synthesis.

INHIBITION OF PHOSPHATIDYLETHANOLAMINE *N*-METHYLATION BY 3-DEAZAADENOSINE STIMULATES THE SYNTHESIS OF PHOSPHATIDYLCHOLINE VIA THE CDP-CHOLINE PATHWAY. P.H. Pritchard, P.K. Chiang, G.L. Cantoni, and D.E. Vance (Dept. of Biochem., Univ. of British Columbia, Vancouver, British Columbia V6T 1W5) *J. Biol. Chem.* 257(11):6361-6367 (1982). The effect of 3-deazaadenosine on phosphatidylcholine biosynthesis has been studied in rat liver in vivo and in adult rat hepatocytes maintained in monolayer culture. The drug had a marked inhibitory effect on phosphatidylcholine biosynthesis via the *N*-methylation of phosphatidylethanolamine. Treatment of rats or hepatocytes with the drug caused a 2- to 3-fold increase in phosphatidylcholine biosynthesis via CDP-choline. The effect was much less marked on phosphatidylcholine biosynthesis in rat spleen, and no significant effect was observed in rat brain. The reaction catalyzed by CTP:phosphocholine cyticylyltransferase was stimulated approximately 3-fold by treatment of the hepatocytes with the drug and this accounted for the increased rate of phosphatidylcholine biosynthesis. This appeared to be associated with a redistribution of enzyme protein from the cytosol to the microsomes. No effect was observed on the activity of microsomal phosphatidylethanolamine-*N*-methyltransferase. However, a metabolite of 3-deazaadenosine, *S*-3-deazaadenosyl-L-homocysteine, was shown to be a competitive inhibitor of the *N*-methyltransferase with respect to *S*-adenosyl-L-methionine. The results suggest that treatment with 3-deazaadenosine causes an inhibition of phosphatidylethanolamine-*N*-methylation by the accumulation of two competitive inhibitors, *S*-adenosyl-L-homocysteine and *S*-3-deazaadenosyl-L-homocysteine. There appears to be coordinate regulation of phosphatidylcholine biosynthesis via CDP-choline and *N*-methylation of phosphatidylethanolamine. However, the mechanism by which 3-deazaadenosine causes a stimulation and redistribution of the cytidylyltransferase reaction still remains to be elucidated.

WHOLE SUNFLOWER SEED AS A FAT SUPPLEMENT FOR LACTATING COWS. W. Rafalowski and C.S. Park (Department of Animal Science, North Dakota State University, Fargo, ND 58105) *J. Dairy Sci.* 65:1484-1492 (1982). Complete rations containing whole sunflower seed at 0, 10, 20, and 30% of concentrates were fed to 16 Holstein cows during early lactation. All rations consisted of corn silage, alfalfa hay, and grain mixtures and were formulated to be isonitrogenous at 16% protein and nearly isocaloric at 1.50 Mcal net energy lactation per kilogram of dry matter. Intakes of total dry matter were not different among ration groups. Cows fed 10% sunflower seed produced more milk and were more efficient energetically. Treatment did not affect milk composition. Average secretion of shorter chain fatty acids in milk from caproate to palmitate was depressed, whereas oleate was increased by sunflower seed. Molar percentage of acetate was increased, and remaining variable measured in rumen fluid were not altered by dietary treatment. No health or feeding problems associated with whole sunflower seed supplement were observed during the entire trial. Graded increase of sunflower seed in the diet elevated cholesterol in blood serum with no effect on cholesterol in milk. Blood urea nitrogen remained unchanged, and total serum protein decreased with increasing sunflower seed in the diet.

MECHANISM OF CHOLESTEROL EFFLUX FROM CELLS. EFFECTS OF ACCEPTOR STRUCTURE AND CONCENTRATION. G.H. Rothblat, M.C. Phillips (Dept. of Physiol. and Biochem., Med. Coll. of Pennsylvania, Philadelphia, PA 19129) *J. Biol. Chem.* 257(9):4775-4782 (1982). The kinetics of removal of [^{3}H]cholesterol from Fu5AH rat hepatoma, WIRL-3C rat liver cells, and human skin fibroblasts growing in culture to phospholipid-containing acceptor particles in the extracellular medium (0.05-1.2 mg of phosphatidylcholine (PC)/ml) has been measured. The rate of release of 1/3 of the cholesterol in either monolayer or suspension culture is first order. The rate constants ($t_{1/2}$) are a function of the cell type and the concentration and structure of the extracellular acceptor particles. At high acceptor concentrations, the $t_{1/2}$ is independent, and the rate-limiting step is the desorption of cholesterol molecules from the cell plasma membrane into a layer of unstirred water surrounding the cell. The $t_{1/2}$ values are Fu5AH<WIRL-3C<fibroblasts for all acceptors used and reflect differences in plasma membrane structures. At lower concentrations of egg PC vesicles, apo-high density lipoprotein/egg PC complexes efflux decreases with decreasing PC concentration in the extracellular medium, because the lower frequency of collisions between desorbed cholesterol molecules and acceptor particles causes the diffusion barrier of the unstirred water layer to reduce the cholesterol flux. The $t_{1/2}$ is a function of acceptor composition so that $t_{1/2}$ values are in the order: PC vesicles>apo-high density lipoprotein/PC complexes>sodium taurocholate/PC micelles. Particle size is important because comparison of different acceptors on the basis of the total surface area presented to the cells normalizes their performances to a large extent.

BILIARY METABOLITES OF *ALL-TRANS*-RETINOIC ACID IN THE RAT: ISOLATION AND IDENTIFICATION OF A NOVEL POLAR METABOLITE. K.L. Skare, H.K. Schnoes, and H.F. DeLuca (Dept. of Biochem., Col. of Agric. and Life Sci., Univ. of Wisconsin-Madison, WI 53706) *Biochemistry* 21(14):3308-3317 (1982). The biliary metabolites from normal rats dosed with either pharmacological or physiological doses of *all-trans*-[11,12-$^3H_2$]retinoic acid were investigated. Biliary metabolites excreted during the first 24 hr account for approximately 60-65% of the radiolabeled dose. A major polar metabolite was purified to homogeneity by using Sephadex LH-20 chromatography and several high-performance liquid chromatographic procedures. This metabolite was negatively charged as revealed by high-performance liquid chromatography on ion-exchange columns and accounts for 10% of the total biliary radioactivity (6% of the dose). The polar compound was positively identified by using Fourier transform proton nuclear magnetic resonance spectroscopy, high- and low-resolution mass spectrometry, fast atom bombardment mass spectrometry, ultraviolet absorption spectrophotometry, Fourier transform infrared spectroscopy, amino acid analysis, and chemical derivatization as 2-[8-[6-(hydroxymethyl)-2,6-dimethyl-3-oxo-1-cyclohexen-1-yl]-2,6-dimethyl-5,7-octadienamido]ethanesulfonic acid. The metabolic transformations required for the generation of this metabolite from *all-trans*-retinoic acid are the following: (1) allylic oxidation at carbon 4 of the cyclohexene ring to produce a 4-keto group, (2) hydroxylation of one of the methyl groups at carbon 1 of the cyclohexene ring, (3) saturation of the two terminal double bonds in the side chain, (4) loss of the terminal carboxyl group of the side chain via decarboxylation, and (5) conjugation of the resulting retinoid with taurine. This may represent the first taurine conjugate of a fat-soluble vitamin to be identified.

TOCOPHEROL ABSORPTION AND METABOLISM IN THE CHICK AND TURKEY. D. Sklan, I. Bartov, and S. Hurwitz (Faculty of Agr., Hebrew Univ., Rehovot 76100, Israel and Agr. Res. Organization, The Volcani Center, P.O. Box 6, Bet Dugan 50250, Israel) *J. Nutr.* 112(7):1394-1400 (1982). Chickens and turkeys were fed from hatching basal diets to which tocopherol was added at levels of 10,50 and 250 mg/kg for 28 days. During the last 4 days [^{3}H]tocopherol and ^{141}Ce were included in the diets. Plasma and liver tocophenrol levels were correlated with dietary tocopherol in both chickens and turkeys, but concentrations were 1.5- to 4.5-fold lower in turkeys. Disappearance (absorption + catabolism) of tocopherol between feed and lower ileum was 78-90% of the ingested vitamin, and no significant differences were found with dietary intake or between chickens and turkeys. Of the ^{3}H-labeled material found in the duodenum, 24-40% was not extractable by organic solvents and comprised mainly tocopheryl glucuronides. The duodenal secretion of glucuronides increased with dietary tocopherol intake, and less than 30% of the secreted glucuronides were reabsorbed by the small intestine. The duodenal organic solvent-extractable ^{3}H contained 30-40% material that appeared to be tocopheryl quinone. This proportion increased with distance from the pylorus. Turkeys excreted 2.5- to 7-fold more glucuronides than chickens. This explains in part the lower plasma and tissue concentrations of tocopherol observed in turkeys.

EFFECT OF HIGH FAT WEANLING DIETS CONTAINING EITHER MEDIUM-CHAIN TRIGLYCERIDES OR LONG-CHAIN TRIGLYCERIDES ON THE DEVELOPMENT OF OBESITY IN THE ZUCKER RAT. I.J. Turkenkopf, C.A. Maggio, M.R.C. Greenwood (Dept. of Biol., Vassar Coll., Poughkeepsie, NY 12601) *J. Nutr.* 112(7):1254-1263 (1982). Zucker rats were early weaned onto either medium-chain (MCT) or long-chain triglycerides (LCT) to examine the effect on the development of obesity. Preobese and lean pups were weaned at 16 days to isocaloric, isonitrogenous liquid diets containing either 65% MCT or LCT (by calories) or to a "stocklike" (5.5% fat, 72.6% carbohydrate) control diet or were pair-fed stocklike diet to MCT-fed rats until day 45. MCT-feeding lowered

body weight gain and fat pad weight in obese and lean rats compared to stocklike-fed controls. Additionally, fat cell size and lipoprotein lipase (LPL) activity and hepatic acetyl CoA carboxylase activity were reduced in obese MCT-fed rats compared to obese controls fed stocklike diet. Except for altered LPL activity the effects produced by MCT-feeding were attributable to its anorectic effect. All obese rats, including the MCT group, developed an obese body composition and were hyperinsulinemic. The developmental sequence leading to obesity may be derived from a fundamental cellular defect that results in metabolic alterations in different tissues at critical periods of development. Thus, effective treatment of this genetic obesity requires a better understanding of *fa* gene action.

ROLE OF EXOGENOUS CHOLESTEROL IN REGULATION OF ADRENAL STEROIDOGENESIS IN THE RAT. A.H. Verschoor-Klootwyk, L. Verschoor, S. Azhar, and G.M. Reaven (Dept. of Med., Stanford Univ. Schl. of Med. and Veterans Admin. Med. Center, Palo Alto, CA 94304) *J. Biol. Chem. 257*(13):7666-7671 (1982). Rat steroidgenic tissues take up cholesterol, and it has been suggested that this process plays a regulatory role in steroid hormone synthesis. To provide evidence for this hypothesis, we carried out studies in lipoprotein-deficient rats. Lipoprotein deficiency, achieved by treating male rats with pharmacological amounts of estradiol, led to profound lowering of plasma cholesterol (8 ± 2 versus 54 ± 4 mg/dl) and adrenal cholesteryl ester content (113 ± 57 versus 747 ± 108 μg/organ). Basal serum corticosterone levels were decreased by 50%, and the response to adrenocorticotropic hormone (ACTH) was totally abolished. Injection of high density lipoprotein (HDL) to estradiol-treated animals restored the response of coricosterone to ACTH. Comparable in vitro studies with adrenal cell suspensions obtained from lipoprotein-deficient rats confirmed the in vivo data. Measurement of [^{14}C]-acetate incorporation and uptake of both HDL- and low density lipoprotein (LDL)-cholesterol in these adrenal cell showed a progressive increase with the duration of estradiol treatment, and neither of these two phenomena was altered by ACTH. These results provide in vitro and in vivo evidence for the hypothesis that normal adrenal steroidogenesis depends upon cholesterol delivery from plasma. Furthermore, under the conditions studied, ACTH does not stimulate adrenal de novo cholesterol biosynthesis nor the uptake of either HDL- or LDL-cholesterol.

1,25-DIHYDROXYVITAMIN D RECEPTORS IN AN ESTABLISHED BONE CELL LINE. CORRELATION WITH BIOCHEMICAL RESPONSES. M.R. Walters, D.M. Rosen, A.W. Norman, and R.A. Luben (Dept. of Biochem. and Div. of Biomed. Sci., Univ. of California, Riverside, CA 92521) *J. Biol. Chem. 257*(13):7481-7484 (1982). A stable cell line derived from mouse bone (cell line MMB-1) has been used for studies of the cellular receptor for 1,25-dihydroxyvitamin D_3 in osteoblasts. Previous studies have demonstrated that collagen synthesis in the MMB-1 cell line is specifically inhibited by 1,25-dihydroxyvitamin D_3 as well as by other bone-regulating hormones. Incubation of cell homogenates with [^{3}H] 1,25-dihydroxyvitamin D_3 indicated the presence of a specific receptor which was located primarily in the chromatin fraction. Optimum conditions for the receptor assay required the inclusion of 500 kallikrein-inactivating units of Trasylol/ml and 10 mM $NaMoO_4$. Under these conditions the receptors were stable for 2 hr at 23 C and for 24 hr at 4 C. Cellular content of receptors was dependent upon the state of confluency of the cells: fully confluent cells contained minimal concentrations of receptors. In cultures of 70-80% confluency, the 1,25-dihydroxyvitamin D_3 receptors demonstrated linear Scatchard plots with K_d = 0.4 nM. Peak receptor activity was found at 3.7 S in linear sucrose gradient fractions of cell homogenates. The synthesis of collagen by MMB-1 cells was inhibited by 1,25-dihydroxyvitamin D_3 indirect proportion to the concentration of cellular receptors at varying levels of culture confluence. The data indicate that MMB-1 cells contain cytoplasmic/nuclear receptors for 1,25-dihydroxyvitamin D_3 which are similar to the receptors found in other target tissues for this hormone and suggest that these receptors are mediators of the effects of 1,25-dihydroxyvitamin D_3 on collagen synthesis.

PLASMA LIPOPROTEIN COMPOSITION IN ALCOHOLIC HEPATITIS: ACCUMULATION OF APOLIPOPROTEIN E-RICH HIGH DENSITY LIPOPROTEIN AND PREFERENTIAL REAPPEARANCE OF "LIGHT" – HDL DURING PARTIAL RECOVERY. S.W. Weidman, J.B. Ragland, and S.M. Sabesin (Div. of Gastroenterology, Dept. of Med., Univ. of Tennessee Center for the Health Sci., Memphis TN 38163) *J. Lipid Res. 23*(4):556-569 (1982). Abnormal lipoproteins accumulate in the plasma of alcoholic hepatitis patients in association with a deficiency of the cholesterol esterifying enzyme, lecithin:cholesterol acyltransferase. Most of these abnormal lipoproteins are found in the d > 1.006 g/ml density fraction. To investigate the composition and morphology of the lipoproteins at various times during the illness in four patients, we have employed density gradient ultracentrifugation combined with analyses of gradient fractions by polyacrylamide gel electrophoresis, electroimmunoassay, and electron microscopy. The results illustrated the diversity of abnormal lipoproteins in alcoholic hepatitis and the ability of density gradient ultracentrifugation combined with lipid and apolipoprotein quantitation, electron microscopy, and polyacrylamide gel electrophoresis to partially resolve those lipoproteins in the d > 1.006 g/ml plasma fraction.

THE EFFECT OF AGE ON THE DEVELOPMENT OF HYPERCHOLESTEROLEMIA IN RABBITS FED SEMIPURIFIED DIETS CONTAINING CASEIN. C.E. West, K. Deuring, J.B. Schutte, A.H.M. Terpstra (Dept. of Human Nutr., Agric. Univ., De Dreijen 12, 6703 BC Wageningen, The Netherlands) *J. Nutr. 112*(7):1287-1295 (1982). Young and adult male rabbits were alternately fed semipurified diets and a commercial diet over a period of 57 weeks. The semipurified diets, containing either casein or soy protein, and the commercial diet were fed either ad libitum or on a restricted basis. When a restricted feeding regime was applied, both in the young and adult rabbits, significantly higher levels of serum cholesterol were observed in the animals fed casein compared with their counterparts fed soy protein. However, during the first period of feeding the semipurified diets, the hypercholesterolemic response of the casein diet was significantly greater in the young than in the adult rabbits. During the second and third period, no significant differences in cholesterolemic response were observed between young and adult rabbits fed casein diets. Further, the cholesterolemic response to semipurified diets containing casein was progressively lower during the second and third period. Similar results were found when the rabbits were fed ad litibum. However, in the adult rabbits no significant differences were observed between the rabbits fed casein and soy protein. Thus, the results of this study show that adult rabbits are less susceptible to the induction of hypercholesterolemia by feeding casein diets than are young ones.

LIPID CHARACTERIZATION OF LONGISSIMUS AND BICEPS FEMORIS MUSCLES FROM BEEF ANIMALS EXSANGUINATED AT VARIOUS TIMES AFTER STUNNING. J.C. Williams, R.A. Field, G.J. Miller, J.E. Kunsman Jr., M.L. Riley, and R.J. Vimini (Food Sci. Sec., Div. of Animal Sci., Univ. of Wyoming, Laramie, WY 82071) *J. Food Sci. 47*(4):1384-1385 (1982). Lipid characteristics of bovine longissimus and biceps femoris muscle from 30 heifer carcasses which were exsanguinated 0 (control), 3 or 6 min after stunning were studied. Blood loss was greater when control animals were compared to animals stunned 3 to 6 min prior to exsanguination. However, total lipid, lipid phosphorus, cholesterol values and TBA numbers were similar among treatments. As time between stunning and exsanguination increased, monounsaturated and polyunsaturated fatty acids tended to increase. Delaying time between stunning and exsanguination had little influence on lipid characteristics of muscle even though large differences between lipid characteristics of muscle and blood exist.

PROTECTIVE ROLE OF VITAMIN E ON ESSENTIAL FATTY ACIDS. E. Turchetto and C. Pignatti (Centro Ricerche sulla Nutrizione–c/o Istituto di Chimica Biologica, Via Irnerio, 48 - 40126 Bologna, Italy) *Acta Vitaminol. Enzymol. 4*(3):267-277 (1982). The protective role of vitamin E against free-radical-mediated oxidations is discussed. In spite of the presence of vitamin E in cell membranes, as structural complex with polyunsaturated fatty acids (PUFA) of phospholipids, the question arises whether high PUFA containing diets, producing high deposition of PUFA in the tissues, can nevertheless lead to peroxidations in the body. It has been suggested that large amounts of dietary PUFA increase the requirement for vitamin E and deplete its tissue stores, particularly when PUFA are discontinued in the diet, also because of their longer half-life time than tocopherols. However, in physiological conditions, linoleic acid up to 10% of caloric intake seems to have no effects on vitamin E requirement. In contrast, in essential fatty acid (EFA) deficient animals, also the addition of small amounts of dietary EFA, by resulting in a proportional increase in PUFA content of membrane structural lipids, is associated with an increased need for vitamin E. This becomes particularly important in the case of dietary fish oils or other poorly protected fats.

EFFECT OF CAFETERIA FEEDING ON BROWN AND WHITE ADIPOSE TISSUE CELLULARITY, THERMOGENESIS, AND BODY COMPOSITION IN RATS. O.L. Tulp, R. Frink and E. Danforth, Jr. (Metabolic Unit, Dept. of Med. and Dept. of Anatomy and Neurobiology, Univ. of Vermont College of Med., Burlington, VT 05405) *J. Nutr. 112*(12):2250-2260 (1982). To determine the effects of cafeteria feeding on brown (BAT) and white (WAT) adipose tissue cellularity, thermogenesis and body

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composition, male Sprague-Dawley rats were fed a cafeteria or a Purina chow diet for 52 days postweaning. Interscapular BAT (IBAT) was removed from subgroups of rats on each diet, and the animals continued on the same regimens. The IBAT weight of rats fed cafeteria diets was 160% of controls after 3 days and 220% after 52 days of the dietary regimens, and brown adipocyte numbers were 130 and 300% those of stock diet-fed rats, respectively, during the same period. Brown adipocyte diameters were initially greater in rats fed cafeteria diet than in rats fed stock diet but were similar after 52 days. Norepinephrine-stimulated thermogenesis was greater in rats fed cafeteria diets than in rats fed stock diet. Surgical reduction of IBAT resulted in hypertrophy of WAT and improved efficiency of weight gain of similarly operated rats fed stock diet were unaltered from those of unoperated animals fed stock diet. These results are consistent with the development of a nutritionally induced hyperplasma and/or differentiation of BAT similar to that which follows cold acclimatization. BAT may play an active role in the expenditure of excess energy during periods of overnutrition, and thereby influence an animal's propensity for fatness.

COMPARTMENTALIZATION OF PHOSPHATIDYLETHANOLAMINE IN MICROSOMAL MEMBRANES FROM RAT LIVER. C. Valtersson and G. Dallner (Dept. of Biochem., Arrhenius Lab., Univ. of Stockholm, and Dept. of Pathology at Huddinge Hospital, Karolinska Inst., Stockholm, Sweden) *J. Lipid Res. 23* (6):868-876 (1982). Microsomal membranes from rat liver were treated with the cross-linking reagent 1,5-difluoro-2,4-dinitrobenzene (DFDNB). Experimental work showed that at a probe concentration of 0.75 mM all free phosphatidylethanolamine (PE) and phosphatidylserine (PS) were found as dinitrophenyl derivatives: 29% of PE was in monomeric form, 9% dimeric, 2% interacted with PS, and 63% cross-linked to protein. PS showed a greater percent in monomeric and dimeric form and only 31% was cross-linked to protein. The cross-linking pattern of PE was clearly different from that pattern which is present in the inner mitochondrial and erythrocyte membranes. In vivo labeling of PE with [^{3}H]glycerol and [^{3}H]ethanolamine followed by phospholipase A_2 treatment of isolated microsomes established a heterogeneous labeling pattern during the first 2 hours. During this period, the specific activity of the phospholipase A_2 sensitive compartment was considerably higher. The differential distribution of radioactivity after in vivo labeling in the part of the PE which reacted with increasing concentrations of DFDNB also indicated compartmentalization. After in vivo labeling with the precursors, the time course of the specific radioactivity demonstrated an initial high labeling, almost exclusively in the monomeric form, followed by a later appearance of the label in the protein-bound PE. The experiments indicate that the biosynthesis of PE takes place in a compartment that is more accessible to surface probes and that the labeled molecules are transferred in a time-dependent process to a second compartment where the lipid is not available for phospholipase A_2 action but is available for cross-linking to protein.

CHANGES IN THE CONCENTRATIONS AND DISTRIBUTIONS OF APOLIPOPROTEINS OF THE AGING RAT. B.J. Van Lenten and P.S. Roheim (Dept. of Physiology, Louisiana State Univ. Med. Center, New Orleans, LA 70119) *J. Lipid Res. 23* (8):1187-1195 (1982). The hyperlipidemia associated with aging was characterized in the rat by comparing the plasma lipid, lipoprotein, and apolipoprotein profiles of adult (12 weeks old) male rats. Compared with those of the adult rats, the VLDL concentrations of the old rats were reduced, but IDL, LDL, and HDL concentrations were elevated. Despite a reduced VLDL concentration, concentrations of triglycerides in the plasma of the old rats were elevated. This phenomenon was attributed to an enrichment of triglyceride in the other lipoprotein fractions. In the old rats, hypercholesterolemia was the result of elevated IDL- and HDL-cholesterol whereas elevated plasma concentrations of apolipoproteins B and E were attributed to elevated LDL and HDL concentrations, respectively. Although concentrations of apolipoproteins A-I and A-IV did not change significantly in the plasma of the old rats, the distribution pattern of the apoA-IV was altered dramatically. Compared with the adult rats, a shift of apoA-IV in the HDL to the "lipoprotein-free" fraction was observed in the old rats, as measured by agarose gel chromatography. The data demonstrate that the hyperlipidemia in the old rats is associated with selective changes in the apolipoprotein profile.

BIOORGANIC CHARACTERIZATION AND MECHANISM OF THE 2,3-OXIDOSQUALENE→LANOSTEROL CONVERSION. E.E. van Tamelen (Dept. of Chem., Stanford Univ., Stanford, CA 94305) *J. Am. Chem. Soc. 104* (23):6480-6481 (1982). In regard to the biological conversion of 2,3-oxidosqualene to lanosterol, previous studies of various enzymic and nonenzymic reactions of squalene oxide and its variants have led to inter alia the following observations and inferences regarding the cyclization process: (a) polycyclization involves A-ring formation with a high degree of S_N2-like participation of the neighboring, Δ^6 πbond and an ensuing series of conformationally rigid, partially cyclized carbocationic intermediates; (b) the oxide-tetra-π-bond sequence constitutes the essential substrate requirement for tetracyclization, the nonoxidic C-5 terminus and the methyls at C-6, -10, and -15 not being individually necessary; (c) the chiral, trisubstituted oxide, Δ^6, Δ^{10} array (a) currently represents the minimum requirement for significant cyclase action; (d) distances between and required conformational orientations of C-2 and C-7, C-6 and Δ^{10} as well as C-10 and Δ^{14} must be optimized; (e) except for the terminating C-9 proton loss and for behavior in the Δ^{14} area, all chemical (including conformational) behavior can be qualitatively simulated in nonenzymic, related systems. By contrast, illuminating biochemical information regarding relationships between the Δ^{10} and Δ^{18} sites and that at Δ^{14} (β) has been lacking, a shortcoming alleviated by the recent finding that 15'-nor-18,19-dihydrosqualene 2,3 oxide is transformed enzymically to the tricycle. Indications of the transient involvement of a five-membered C ring in lanosterol biosynthesis have been presented. The entirety of our results permits for the first time a comprehensive view of substrate behavior during the bioconversion of oxidoqualene to lanosterol and presumably other polycyclic triterpenes.

IN VITRO RECIPROCAL EXCHANGE OF APOPROTEINS AND NONPOLAR LIPIDS BETWEEN HUMAN HIGH DENSITY LIPOPROTEINS AND AN ARTIFICIAL TRIGLYCERIDE-PHOSPHOLIPID EMULSION (INTRALIPID). R.B. Weinberg and A.M. Scanu (Depts. of Med. and Biochem., The Univ. of Chicago, 950 E. 59th Street, Chicago, IL 60637) *Atherosclerosis 44* (2):141-152 (1982). To determine the nature of lipid and apoprotein exchange between human high density lipoprotein (HDL) and Intralipid particles of $S_f > 400$ (ILIP) we have studied their in vitro interaction during incubation in aqueous buffer and in lipoprotein-deficient serum (LPDS). We found that ILIP acquires apo A-I, apo A-IV and apo E from LPDS, and that this uptake is inhibited by the presence of HDL, which readily donate C-apoproteins to the ILIP surface. In the absence of LPDS exchange of only polar lipids occurred between ILIP and HDL, with HDL gaining phospholipid from, and donating free cholesterol to this fat emulsion. In the presence of LPDS the exchange of nonpolar lipids occurred between the two particles: in the case of HDL, cholesteryl ester content decreased, accompanied by an increase in triglyceride, causing a decrease in the hydrated density of the lipoprotein and an increase in its molecular weight; in the case of ILIP, reciprocal changes in lipid content were seen as a loss of triglyceride and the appearance of cholesteryl esters. When compared to literature data, out findings indicate that intralipid $S_f > 400$ particles exhibit an in vitro behavior which is remarkably similar to that of nascent chylomicrons with respect to the exchange of A- and C-apoproteins and surface polar lipids with HDL. We postulate that since ILIP and HDL can participate in a LPDS-dependent exchange of non-polar core lipids, that this process may occur when this fat emulsion is administered in vivo.

EFFECTS OF TRIIODOTHYRONINE AND PROPYLTHIOURACIL ON PLASMA LIPOPROTEINS IN MALE RATS. H.G. Wilcox, W.G. Keyes, T.A. Hale, R. Frank, D.W. Morgan and M. Heimberg (Dept. of Pharmacology, Univ. of Tennessee Center for the Health Sci., Memphis, TN 38163) *J. Lipid Res. 23* (8):1159-1166 (1982). Hyperalphalipoproteinemia, characterized by increased plasma concentrations of apoA-I and of HDL lipid and protein, was observed in rats treated with triiodothyronine (T_3) for 7 days. The increase in the plasma HDL apoproteins was general for apoC, apoE plus A-IV, and apoA-I, as determined by isoelectric focusing. Hypotriglyceridemia, characterized by decreased concentrations of VLDL and apoB, was also observed in the hyperthyroid state. Our observations suggest a regulatory role for thyroid hormones that determine concentration and composition of plasma HDL and other lipoproteins.

LIPID-INDUCED ORDERED CONFORMATION OF SOME PEPTIDE HORMONES AND BIOACTIVE OLIGOPEPTIDES: PREDOMINANCE OF HELIX OVER β FORM. C.-S. C. Wu, A. Hachimori and J.T. Yang (Cardiovascular Res. Inst., Univ. of California, San Francisco, CA 94143) *Biochem. 21* (19):4556-4562 (1982). The conformation of several naturally occurring peptide hormones and bioactive oligopeptides in phospholipid solutions was studied by circular dichroism. Phosphatidylcholine induced a partial helix in human gastrin I at neutral pH, but phosphatidylserine did not induce a partial helix unless the five consecutive glutamic acid residues in gastrin were protonated. Reduced somatostatin with two

lysines and substance P with one arginine and one lysine were partially helical in phosphatidylserine, but not phosphatidylcholine, solution. Both lipids induced a helical conformation in glucagon and its COOH-terminal fragment (19-29) probably because the helical segment is primarily located at the uncharged COOH terminus. Thus, polypeptides with a helix-forming potential can have the helical conformation only when the peptides carry no charge or charges opposite to those on the polar head of the lipid. Renin substrate, which has potentials for the β form and β turn, seemed to form a mixture of the two conformations in phosphatidylserine solution. Angiotensin I with a strong probability for the β form adopted the β form in phosphatidylserine solution and sleep peptide with no structure-forming potential remained unordered in lipid solutions. The helix usually predominated over the β form in lipid solutions if the peptide has potentials for both conformations. This could account for the preponderance of helices in bacteriorhodopsin of the purple membrane, which according to its amino acid sequence would have favored the β form.

EFFECT OF CHOLESTEROL FEEDING ON SERUM LIPOPROTEINS AND ATHEROSCLEROSIS IN ATHEROSCLEROSIS-SUSCEPTIBLE AND ATHEROSCLEROSIS-RESISTANT JAPANESE QUAIL. T.-C. Wu and W.E. Donaldson (Dept. of Poultry Sci., North Carolina State Univ., Raleigh, NC 27650) *Poultry Sci. 61* (12):2407-2414 (1982). Male and female Japanese quail from two strains bred for differing susceptibility to induction of atherosclerosis by dietary cholesterol (RES = resistant, SUS = susceptible) were fed a control diet or control diet + .5% USP cholesterol. The diets were fed for 20 weeks beginning when the quail were 7 weeks old. Only quail fed cholesterol developed atherosclerosis. Atherosclerosis (incidence and severity) was higher in males than females and higher in SUS than RES. Cholesterol feeding increased aortic, liver, and serum cholesterol concentrations and liver total fat content. There were high positive correlations between serum cholesterol and liver cholesterol in all cholesterol-fed groups but not in control groups. Diet, sex, and strain differences in cholesterol distribution among the various serum lipoproteins and ratios of β/α lipoproteins were observed. These differences are discussed in terms of their usefulness in predicting atherosclerosis in quail as compared with humans.

SEPARATION OF SERUM LIPOPROTEINS OF JAPANESE QUAIL BY DISC POLYACRYLAMIDE GEL ELECTROPHORESIS AND SINGLE DISCONTINUOUS DENSITY GRADIENT ULTRACENTRIFUGATION. T.-C. Wu and W.E. Donaldson (Dept. of Poultry Sci., North Carolina State Univ., Raleigh, NC 27650) *Poultry Sci. 61* (12):2398-2406 (1982). A method based on disc polyacrylamide gel electrophoresis (disc PAGE) for the separation of serum lipoproteins of Japanese quail *(Coturnix coturnix japonica)* prestrained with Sudan Black B is described and evaluated. Good separation was obtained by using 3% separating gel and decreasing the concentration of buffer solution A used for preparation of separating gel solution to one-half that recommended by Narayan (1975). Best resolution of the high density lipoproteins was achieved by using 7 to 10% of separating gel, which separated the high density lipoproteins into three bands. Clear lipoprotein bands were obtained after ultracentrifugation of prestained serum. For corresponding positions, the bands of unstained serum after staining had the same mobility in the disc (PAGE) as prestained lipoprotein bands. In quail, density smaller than 1.006 g/ml contains chylomicrons and very low density lipoproteins (VLDL); density from 1.018 to 1.05 g/ml contains the low density lipoproteins (LDL); and density more than 1.05 g/ml and less than 1.16 g/ml contains the high density lipoproteins (HDL). In the profile of serum lipoproteins after ultracentrifugation, two peaks were observed in the density range of HDL. There was one small peak with density 1.05 to 1.09 g/ml and one large peak with density 1.09 to 1.16 g/ml. Based on the lipoprotein profiles from both disc PAGE and ultracentrifugation, HDL is the predominant form, LDL is intermediate, and VLDL and chylomicrons are smallest in amount.

ISOLATION AND IDENTIFICATION OF 1α- AND 23- HYDROXYLATED METABOLITES OF 25-HYDROXY-24-OXOVITAMIN D_3 FROM *IN VITRO* INCUBATES OF CHICK KIDNEY HOMOGENATES. S. Yamada, M. Ohmori and H. Takayama (Dept. of Biochem., Schl. of Dentistry, Showa Univ., 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan) *J. Biol. Chem. 258* (1):457-463 (1982). Five major metabolites (peaks I-V) of 25-hydroxy-24-osovitamin D_3 (25(OH)24-oxo-D_3) have been isolated in pure form from *in vitro* incubates containing kidney homogenates of vitamin D-deficient chicks and chicks given 65 nmol of vitamin D_3; peaks II, III, and V are from vitamin D-deficient chicks and peaks I, II, and IV are from vitamin D-supplemented birds. The structures of the metabolites were unequivocally identified as 23,25-dihydroxy-24-oxo-vitamin D_3 (peak I), 24,25-dihydroxyvitamin D_3 (24,25$(OH)_2D_3$) (peak II), α,25-dihydroxy-24-oxovitamin D_3 (peak III), 24,25,25-trihydroxyvitamin D_3 (peak IV), and 1α,24,25-trihydroxyvitamin D_3 (peak V) by means of ultraviolet absorption spectrometry, mass spectrometry, and specific chemical reactions. It is concluded that 25(OH)24-oxo-D_3 is further hydroxylated at the 1α-position in the kidney of vitamin D-deficient chicks and at the 23-position in that of vitamin D-supplemented animals. Formation of 24,25$(OH)_2D_3$ from 25(OH)24-oxo-D_3 in both vitamin D-deficient and vitamin D-supplemented animals provides evidence for the presence of an enzyme to reduce the 24-oxo group irrespective of the vitamin D status.

MYOCARDIAL-ISCHEMIC RATS (MIR). CORONARY VASCULAR ALTERATION INDUCED BY A LIPID-RICH DIET. Y. Yamori, M. Kihara, Y. Nara and R. Horie (Department of Pathology, Shimane Medical University, Izumo 693, Japan) *Atherosclerosis 42* (1):15-20 (1982). A new strain of rats in which there is a high incidence of heart failure (myocardial-ischemic rats: MIR) has been inbred in our laboratory from a substrain of stroke-prone spontaneously hypertensive rats (SPSHR). The clinicopathological manifestations of MIR on a high-fat-cholesterol diet (HFCD) were found to be similar to those seen in ischemic heart disease (IHD) in man, in terms of vectorcardiographical (VCGcal) alterations, coronary fat deposition, thrombosis and resultant myocardial fibrosis. MIR should serve as a good experimental model of IHD.

INCREASED POLYUNSATURATED FATTY ACIDS IN DEVELOPING AND REGENERATING PERIPHERAL NERVE. J.K. Yao (Lipid Biochem. Lab., Peripheral Nerve Res. Center, Dept. of Neurology, Mayo Clinic and Foundation, Rochester, MN 55905) *Biochim. Biophys. Acta 712* (3):542-546 (1982). Characteristic fatty acids of peripheral nerve myelin are mainly saturated and monosaturated. A marked increase of polyunsaturated fatty acids, particularly arachidonic acid, was found in endoneurial phosphatidylethanolamine of both developing and regenerating rat sciatic nerve, suggesting a close association between polyunsaturated fatty acids and peripheral nerve myelination.

ALPHA-TOCOPHEROL LEVEL IN LIVER DISEASES. T. Yoshikawa, S. Takemure and M. Kondo (First Dept. of Med., Kyoto Prefectural Univ. of Med., Kamikyo-ku, Kyoto 602, Japan) *Acta Vitaminol. Enzymol. 4* (4):311-318 (1982). Serum levels of α-tocopherol (vitamin E) were determined in various types of liver diseases, and as a result, it was revealed that serum α-tocopherol was significantly depressed in acute hepatitis ($p < 0.01$, $n = 22$), alcoholic hepatitis ($p < 0.001$, $n = 9$) and fulminant hepatitis ($p < 0.001$, $n = 6$). There was a significant correlation between serum levels of α-tocopherol and β-lipoprotein ($r = 0.92$, $p < 0.001$, $n = 17$). Though there was no correlation between serum levels of α-tocopherol and triglyceride, there was a significant correlation between α-tocopherol and cholesterol ($r = 0.57$, $p < 0.01$, $n = 21$), and phospholipid ($r = 0.49$, $p < 0.05$, $n = 18$). There was no correlation between serum levels of α-tocopherol and other liver function tests. These facts suggested that the diminished serum vitamin E in patients with liver diseases is ascribable to the depression in blood level of β-lipoprotein that results from liver disorders, because the liver is the major supply source of β-lipoprotein.

PREVENTING HYPERPHAGIA NORMALIZES 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE ACTIVITY IN SMALL INTESTINE AND LIVER OF DIABETIC RATS. N.L. Young, C.D. Saudek, L. Walters, J. Lapeyrolerie and V. Chang (Dept. of Med., Cornell Univ. Med. College, 515 E. 71st St., New York, NY 10021) *J. Lipid Res. 23* (6):831-838 (1982). Rats with streptozotocin-induced diabetes stop growing, develop high cholesterol and triacylglycerol levels in plasma, and have decreased activity of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl CoA reductase (EC 1.1.1.34), in liver and increased activity in small intestine. They also eat more than normal. To determine the contribution of hyperphagia to these changes in lipid metabolism, we restricted intake of chow to the amount eaten ad lib by normal rats. Rats were meal-fed for 8 or 22 days from the time diabetes was induced. This regimen normalized reductase activity in both liver and intestine at mid-dark and mid-light, and all but eliminated high plasma cholesterol and triacylglycerol levels, although plasma insulin remained low and glucose remained high. Activation of hepatic reductase by endogenous phosphatase in vitro was reduced in hyperphagic diabetic rats but was normal in diabetic rats eating a normal amount of food. We conclude that hyperphagia, rather than direct effects of insulin deficiency as is usually assumed, is responsible for perturbations of lipid metabolism in chronically diabetic rats. These results support the proposal that hyperphagia increases the input of dietary and newly synthesized cholesterol

from the small intestine, and that this increased input raises plasma cholesterol level and inhibits reductase activity in liver.

PROTEIN-INDUCED AGGREGATION OF LIPID VESICLES. MECHANISMS OF THE MYELIN BASIC PROTEIN-MYELIN INTERACTION. P.R. Young, D.A. Vacante and W.R. Snyder (Department of Chemistry, University of Illinois at Chicago, Chicago, IL 60680) *J. Am. Chem. Soc. 104* (25):7287-7291 (1982). When the light scattered by suspensions of lipid vesicles during protein-induced aggregation is monitored, changes in the physical size of the vesicle aggregates can be obtained along with kinetic constants for the reaction. The aggregation of phosphatidylserine vesicles, induced by polylysine and by bovine myelin basic protein (MBP), is monitored by this technique. Polylysine rapidly induces high-order aggregation to produce relatively stable vesicle trimers and tetramers. Myelin basic protein is much less efficient at inducing the aggregation reaction, producing dimers that rapidly coalesce to form units of more compact structure. With vesicles prepared from whole myelin suspensions, however, dimers are initially induced by both proteins, and myelin basic protein is much more efficient than polylysine at inducing this dimer formation. The induction of myelin dimers is a rapid equilibrium process displaying simple saturation kinetics with respect to [MBP]. The MBP-induced dimerization is markedly pH dependent, becoming unfavorable in acid, and dissociation constant follows a simple titration curve with an apparent pK_a of 5.9. It is concluded that the interaction between MBP and the myelin membrane if largely electrostatic. The significance of the pH instability to myelin phagocytosis in demyelinating diseases is discussed.

ADIPOSE TISSUE CELLULARITY IN WOODCHUCKS: EFFECTS OF SEASON AND CAPTIVITY AT AN EARLY AGE. R.A. Young, L.B. Salans and E.A.H. Sims (Metabolic Unit, Dept. of Med., Univ. of Vermont, College of Med., Burlington, VT 05405) *J. Lipid Res. 23* (6):887-892 (1982). The objectives of this study were to determine the roles of adipocyte hypertrophy and hyperplasia in the prehibernatory weight gain of adult woodchucks and in the increased body weight of woodchucks born in captivity. The seasonal increase in weight in wild adult woodchucks was associated with an increase approaching tenfold in both body fat and in subcutaneous and retroperitoneal adipocyte size. There was no increase in total adipocyte number. Four groups of woodchucks were used in the study of the effect of captivity: I) animals born to females bred in the laboratory; II) those born to females captured just before parturition; III) those captured at weaning; and IV) animals captured at 12 months of age. At 14 months non-fat body weight and subcutaneous adipocyte size were equal in the four groups. The males but not the females in Groups I, II, and III had both an increased body fat content and a significantly increased total adipocyte number in comparison to the males in Group IV and the adults in the seasonal study. This study demonstrates that captivity at an early age, unlike prehibernatory weight gain, is associated with an increased adipocyte number in male woodchucks, and this increase can occur after weaning.

BIOSYNTHESIS OF S-METHYL-N-OLEOYLMERCAPTOETHYLAMIDE FROM OLEOYL COENZYME A AND S-ADENOSYLMETHIONINE. M. Zatz, S.J. Engelsen and S.P. Markay (Lab. of Clinical Sci., Natl. Inst. of Mental Health, Bethesda, MD 20205) *J. Biol. Chem. 257* (22):13673-13678 (1982). Addition of oleoyl-CoA to incubations containing rat lung membranes and S-adenosyl [methyl-^{3}H] methionine resulted in the formation of a previously unidentified nonpolar methylated lipid. The product was formed enzymatically, with an apparent K_m for S-adenosylmethionine (AdoMet) of about 0.3 μM and half-maximal activity using about 0.1 mM oleoyl-CoA. Activity was highest in microsomes but present in other membranous fractions, including plasma membranes from mature human erythrocytes. Intact red blood cells formed the nonpolar methylated lipid intracellularity upon incubation with [*methyl*-^{3}H] methionine and oleoyl-CoA. Product formation differed among membranes from various tissues. The nonpolar methylated lipid was analyzed by TLC, high performance liquid chromatography, and gas chromatography with radiodetection. It was identified as S-methyl-N-oleoylmercaptoethylamide by gas chromatography-mass spectrometry. Products obtained from oleoyl-CoA or palmitoyl-CoA, incubated with nonradioactive or [*methyl*-^{3}H] AdoMet, were compared using electron impact and/or chemical ionization mass spectrometry. Inferred structures were confirmed using authentic standards. The methyliated product was apparently formed by tissue as follows: a) cleavage of oleoyl-CoA by an amidase to form S-oleoylmercaptoethylamine; b) spontaneous rearrangement to form N-oleoylmercaptoethylamide; and c) enzymatic methylation of the free thiol by AdoMet. Participation of the amidase was suggested by the biosynthesis of the amide (free thiol) using [1-^{14}C] oleoyl-CoA.

INTESTINAL ABSORPTION OF POLYENEPHOSPHATIDYLCHOLINE IN MAN. O. Zierenberg and S.M. Grundy (Veterans Administration Med. Center and Univ. of California, San Diego, La Jolla, CA) *J. Lipid Res. 23* (8):1136-1142 (1982). The metabolic fate of 1g of ^{3}H/^{14}C-labeled dilinoleoglycerophosphocholine was studied in five patients after oral administration. The ^{3}H label was in choline and ^{14}C was in the two linoleic acid residues. More than 90% of both isotopes was absorbed from the intestine. Seventy to 90% of the ^{3}H radioactivity in blood was linked to phosphatidylcholine (PC) whereas ^{14}C was associated with both PC and nonpolar lipids. At peak activity, the ^{3}H/^{14}C ratio of plasma PC was twice that of oral PC; this suggests that most oral PC was hydrolized to lysolecithin before absorption. The mean maximum concentration in total blood volume was 20% of the administered dose for ^{3}H and 28% for ^{14}C. Examination of lipoproteins revealed that the specific activity of PC in high density lipoprotein (HDL) was 2 to 6 times higher than in apoB-containing lipoproteins, and 2 to 20 times than that of red blood cells or total blood. Thus, absorbed PC seemingly was incorporated preferentially into the HDL fraction of plasma.

BILIARY LIPIDS IN NEW WORLD MONKEYS: DIETARY CHOLESTEROL, FAT, AND SPECIES INTERACTIONS. M.J. Armstrong, Z. Stephan, and K.C. Hayes (Department of Nutrition, Harvard School of Public Health, Boston, MA) *Am. J. Clin. Nutr. 36* (4): 592-601 (1982). The separate effects contributed by dietary cholesterol and dietary fat on several parameters of biliary lipid metabolism thought to be important in the genesis of cholesterol gallstones were examined in squirrel and cebus monkeys fed diets containing either corn or coconut oil from birth. Half the monkeys were also fed cholesterol. In gallstone-susceptible squirrel monkeys, corn oil tended to decrease the bile acid pool size and decrease the percentage of taurochenodeoxycholic acid. Dietary cholesterol effected major changes in gallbladder bile molar percent lipid composition with significantly increased cholesterol saturation indices that exceeded the metastable-labile limits. The supersaturated biles notwithstanding, none of the monkeys developed gallstones and only one had cholesterol crystals in its bile. By contrast, the gallstone-resistant cebus monkeys experienced less remarkable shifts in biliary lipid composition during dietary challenges of cholesterol and fat. The data are consistent with the hypothesis that neither a diminished bile acid pool size nor bile supersaturated with cholesterol are sufficient in themselves to result in gallstone formation in immature monkeys.

THE BIOLOGICAL ACTIVITY OF 25-HYDROXYCHOLECALCIFEROL AND 1,25-DIHYDROXYCHOLECALCIFEROL FOR RAINBOW TROUT *(SALMO GAIRDNERI)*. B.J. Barnett, G. Jones, C.Y. Cho and S.J. Slinger (Dept. of Nutr., College of Biol. Science, Univ. of Guelph, Guelph, Ontario N1G 2W1, Canada) *J. Nutr. 112* (11):2020-2026 (1982). Dietary 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol showed vitamin D activity in rainbow trout. However, inclusion of dietary cholecalciferol (vitamin D_3), ergocalciferol (vitamin D_2), 25-OH-D_3 or 1,25-$(OH)_2D_3$ did not result in the presence of detectable levels of vitamin D or 25-OH-D in the blood plasma of the fish. Fish fed the diet devoid of vitamin D over an extended period of time showed symptoms of a droopy-tail or "lordosis-like" syndrome that appeared to be related to muscle weakness since x-ray examination indicated no abnormality in vertebral development. The requirement for vitamin D as cholecalciferol was in excess of 1600 IU/kg diet and may be as high as, or higher than, 2400 IU/kg diet.

RELATIVE BIOPOTENCY OF DIETARY ERGOCALCIFEROL AND CHOLECALCIFEROL AND THE ROLE OF AND REQUIREMENT FOR VITAMIN D IN RAINBOW TROUT *(SALMO GAIRDNERI)*. B.J. Barnett, C. Young Cho and S.J. Slinger (Dept. of Nutr., College of Biol. Science, Univ. of Guelph, Guelph, Ontario N1G 2W1, Canada) *J. Nutri. 112* (11):2011-2019 (1982). A growth assay was conducted for six consecutive 28-day periods by using triplicate groups of 110 rainbow trout with an average initial body weight of 3.0 g. Ergocalciferol and cholecalciferol were included to provide levels of 200, 400 and 800 IU/kg in a semipurified casein, gelatin diet. Further treatments with 0 vitamin D and 1600 IU/kg of D_3 were also included. The resulting growth curves were significant for parallelism. Statistical analysis showed that D_3 was 3.27 times as potent as D_2. The dietary requirement for D_3 was found to be in excess of 800 IU/kg of diet. Vitamin D-deficient fish showed no change in bone ash but exhibited clinical manifestations of tetany with no hypocalcemia. A complete absence of tetany was

seen only in the groups fed 800 and 1600 IU of D_3 per kilogram. None of the levels of D_2 used were sufficient to completely alleviate symptoms of this disorder. These studies of rainbow trout provide evidence that vitamin D is required for the normal functioning of white muscle without altering the calcium content of the plasma or epaxial musculature.

CHOLESTEROL METABOLISM IN GNOTOBIOTIC GERBILS. K.F. Bartizal, Jr., M.H. Beaver and B.S. Wostmann (Dept. of Microbiology, Univ. of Notre Dame, Notre Dame, IN 46556) *Lipids 17* (11):791–797 (1982). Germfree gerbils were associated with a murine-derived hexaflora which produced only minor changes in the primary bile acid pattern of rats. These hexaflora-associated gerbils had relatively small ceca (4% of body weight) and reproduced well. Although serum cholesterol levels of both conventional and hexaflora-associated gerbils increased in response to dietary cholesterol, the hexaflora-associated gerbil showed a greater elevation in serum cholesterol than the conventional gerbil maintained on a diet containing 0.1% cholesterol. This increase in serum cholesterol manifested itself almost totally in the very low density lipoprotein and low density lipoprotein fractions. The fecal bile acids of the hexaflora-associated gerbil were largely deconjugated, but very little further modification of either cholic or chenodeoxycholic acid had taken place. The data suggest that in the absence of elements of the intestinal microflora that can express a bile acid-modifying potential, and particularly a 7-α-dehydroxylating capacity, catabolism of cholesterol to bile acids is reduced, and cholesterol accumulates in the very low density and low density serum lipoprotein fractions.

IMMUNOBLOT ANALYSIS OF LOW DENSITY LIPOPROTEIN RECEPTORS IN FIBROBLASTS FROM SUBJECTS WITH FAMILIAL HYPERCHOLESTEROLEMIA. U. Beisiegel, W.J. Schneider, M.S. Brown and J.L. Goldstein (Depts. of Molecular Genetics and Internal Med., Univ. of Texas Health Science Center at Dallas, Dallas, TX 75235) *J. Biol. Chem. 257* (21):13150–13156 (1982). This paper describes a sensitive method for study of the isoelectric point and molecular weight of immunoreactive low density lipoprotein (LDL) receptors of cultured human fibroblasts. The fibroblast receptors are solubilized with Triton X-100, partially purified by batch elution from DEAE-cellulose, and subjected to two-dimensional isoelectric focusing/sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins are transferred electrophoretically to nitrocellulose paper which is then incubated with a mouse monoclonal antibody (IgG-C7) directed against the LDL receptor, followed by an ^{125}I-labeled antibody against mouse IgG. The receptor-bound monoclonal antibody is localized by autoradiography. By this technique, the immunodetectable LDL receptors from normal human fibroblasts migrate as a single spot with an isoelectric point of 4.3 and a M_r of ~160,000. In one patient with homozygous familial hypercholesterolemia whose cells fail to bind ^{125}I-labeled IgG-C7, no immunoreactive LDL receptor spot was detected after electrophoresis. We also studied LDL receptors from three homozygotes whose cells bind ^{125}I-IgG-C7, i.e., cross-reacting material-positive mutants. Their immunodetectable receptors were indistinguishable from normal receptors in terms of isoelectric point and molecular weight. Similarly, the receptors from one patient with the internalization-defective form of familial hypercholesterolemia showed normal electrophoretic migration. The immunoblotting technique should prove useful in analyzing structural alterations, if they exist, in LDL receptors from other subjects with cross-reacting material-positive forms of familial hypercholesterolemia.

BIOCHEMICAL AND ANTHROPOMETIC DETERMINANTS OF SERUM β- AND PRE-β-LIPOPROTEINS IN CHILDREN. G.S. Berenson, L.S. Webber, S.R. Srinivasan, A.W. Voors, D.W. Harsha and E.R. Dalferes, Jr. (Depts. of Medicine, Biometry, Biochemistry and Preventive Medicine and the Specialized Center of Research-Arteriosclerosis of Louisiana State University Medical Center, New Orleans, Louisiana) *Arteriosclerosis 2* (4):325–334 (1982). A special in-depth substudy was conducted on 388 children from a total biracial (black-white) population, who were stratified on levels of serum β- and pre-β-lipoprotein cholesterol to explore factors associated with lipoprotein levels in childhood. Biochemical parameters on venous blood samples were obtained both on fasting subjects and after an abbreviated glucose tolerance test, along with selected anthropometric measures like height, weight, and skinfolds. Biochemical and anthropometric relationships were minimal for children with elevated β-lipoprotein cholesterol and low pre-β-lipoprotein cholesterol. On the other hand, children with higher levels of pre-β-lipoprotein cholesterol, with or without elevated β-lipoprotein cholesterol, showed associations with fatness and slightly higher levels of glucose and insulin, with other biochemical parameters considered within normal levels. These differences noted among free-living children with different levels of serum lipoproteins provide clues to mechanisms involved in the early natural history of coronary artery disease.

RAPID DECREASE OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE IN THROMBIN-STIMULATED PLATELETS. M.M. Billah and E.G. Lapetina (Department of Molecular Biology, Wellcome Research Laboratories, Research Triangle Park, NC 27709) *J. Biol. Chem. 257* (21):12705–12708 (1982). The addition of thrombin to horse platelets prelabeled with ^{32}P induces a rapid decrease of the radioactivity from phosphatidylinositol 4,5-bisphosphate. Maximum loss of the radioactivity from phosphatidylinositol 4,5-bisphosphate occurs within 10 s of stimulation and is followed by an increased incorporation of ^{32}P into this lipid. The stimulation of phosphatidylinositol 4,5-bisphosphate loss by thrombin is concentration-dependent. The ionophore A23187, which mobilizes Ca^{2+}, is ineffective in inducing the degradation of phosphatidylinositol 4,5-bisphosphate. Measurements of polyphosphoinositides by phosphorus estimation show that, 10 s after thrombin stimulation, there is a decrease of 15–20% of the total phosphatidylinositol 4,5-bisphosphate without any significant change in phosphatidylinositol 4-monophosphate. It appears that thrombin causes a rapid and transient degradation of phosphatidylinositol 4,5-bisphosphate and that this effect might be related to the initiation of platelet activation.

SYNTHESIS OF APOLIPOPROTEIN AI BY PERIPHERAL TISSUES OF THE ROOSTER. M. Blue, P. Ostapchuk, J. Gordon, and D. Williams (Depts. of Pharmac. Sci. and Anatom. Sci., Health Sciences Center. State Univ. of New York at Stony Brook, Stony Brook, NY 11794) *J. Biol. Chem. 257* (18):11151–11159 (1982). After *in vitro* incubation with radiolabeled amino acids, extracts of chicken tissues were reacted with antiserum against apolipoprotein AI of plasma high density lipoprotein. Radiolabeled apo-AI was found in liver, intestine, kidney, and a variety of peripheral tissues. The immunoreactive apo-AI synthesized by peripheral tissues had the same mobility as plasma apo-AI and newly synthesized apo-AI exists in at least four isoforms in each of the tissues examined. Comparisons of isoform patterns suggest that newly synthesized apo-AI isoforms have identical charge properties in each tissue. Plasma apo-AI shows four isoforms with the same isoelectric points, but quantitative distribution among isoforms is different. The two basic isoforms predominate in newly synthesized apo-AI, while plasma apo-AI consists of the two acidic isoforms. Messenger RNA directed the synthesis of apo-AI in a wheat germ extract. The apo-AI cell-free product directed by each tissue RNA had the same molecular weight on sodium dodecyl sulfate-polyacrylamide gels. These products are 2000 daltons larger than mature apo-AI synthesized by liver tissue. All results are discussed with respect to the potential role of peripheral apo-AI in cellular cholesterol efflux and/or the movement of cholesterol from peripheral tissues to the liver.

HYDROXYLATIONS IN BIOSYNTHESIS OF BILE ACIDS. ISOLATION OF SUBFRACTIONS WITH DIFFERENT SUBSTRATE SPECIFICITY FROM CYTOCHROME P-450LM_4. H. Boström and K. Wikvall (Department of Pharmaceutical Biochemistry, University of Uppsala, S-751 23 Uppsala, Sweden) *J. Biol. Chem. 257* (19):11755–11759 (1982). Chromatography of electrophoretically homogeneous cytochrome P-450LM_4 from cholestyramine-treated rabbits on octylamine-Sepharose resulted in the isolation of two subfractions, cytochrome P-450LM_4 I and cytochrome P-450LM_4 II, with different catalytic properties. The original cytochrome P-450LM_4 fraction catalyzed 7α-hydroxylation of cholesterol, 12α-hydroxylation of 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α, 12α-triol, 6β-hydroxylations. Cytochrome P-450LM_4 I was inactive in cholesterol 7α-hydroxylation, but catalyzed the other hydroxylations. Cytochrome P-450LM_4 II catalyzed efficient cholesterol 7α-hydroxlyation. It also catalyzed the other hydroxylations, although at lower rates than cytochrome P-450LM_4 I. Emulgen inhibited all steroid hydroxylase activities in cytochrome P-450LM_4 II except the cholesterol 7α-hydroxylase activity. Cytochrome P-450LM_4 I and cytochrome P-450LM_4 II showed the same apparent molecular weight and spectral properties as the original cytochrome P-450LM_4 fraction. The two subfractions differed in amino acid composition. They produced similar but not identical one-dimensional peptide maps upon limited proteolysis with papain, chymotrypsin, and trysin. The results show that cytochrome P-450LM_4 from cholestyramine-treated rabbits contains at least two species with different amino acid compositions and different substrate specificities towards C_{27}-steroids involved in biosynthesis of bile acids.

Abstracts

VITAMINS E AND A IN VASCULAR DISEASES. U. Butturini (Instituto di Clinica Medica Generale e Terapia medica dell'Universita degli Studi di Parma- 43100 Parma, Italy) *Acta. Vitaminol. Enzymol.* 4(1-2):15-19 (1982). The major pathogenetic factors of the atherosclerotic diseases are: a) vasal endothelium distress; b) rheological disturbs; c) alterations in plasma lipid pattern; d) dietary intake of saturated and polyunsaturated fatty acids; e) alteration of mithocondrial and microsomal membranes; f) vascular injury induced by immune complexes; g) increased lipid peroxidations. Many well documented reports state a positive effect of vitamins A and E on some of the factors previously considered. Vitamins A and E have an endothelium-protective activity and an antiperoxidative effect; they act as antiaggregant factors, affect O_2 transport and utilization processes, increase HDL-cholesterol, potentiate the hypolipemic action of the nicotinic acid.

STEROL CARRIER PROTEIN$_2$. DELIVERY OF CHOLESTEROL FROM ADRENAL LIPID DROPLETS TO MITOCHONDRIA FOR PREGNENOLONE SYNTHESIS. R. Chanderbhan, B.J. Noland, T.J. Scallen and G.V. Vahouny (Department of Biochemistry, George Washington University, School of Medicine and Health Services, Washington, D.C. 20037) *J. Biol. Chem.* 257(15):8928-8934 (1982). The ability of sterol carrier protein$_2$ (SCP$_2$) to mediate transfer of unesterified cholesterol from adrenal lipid inclusion droplets to mitochondria has been tested in an *in vitro* model system. Unlike mitochondrial utilization of cholesterol added in acetone or dimethyl sulfoxide, the unesterified cholesterol of lipid droplets did not provide a readily available source of substrate for mitochondrial pregnenolone production, without the addition of a transport mediator. Addition of SCP$_2$, but not albumin, stimulated mitochondrial utilization of droplet cholesterol in a concentration-dependent manner. In the absence of mitochondria, SCP$_2$ sequestered lipid droplet cholesterol, and in the presence of mitochondria, which were unable to convert cholesterol to pregnenolone, this cholesterol was quantitatively accumulated by mitochondria. Both processes were concentration-dependent and demonstrated a molar ratio of SCP$_2$ and cholesterol for both binding and transport of 1. SCP$_2$ also enhanced pregnenolone formation by mitochondria which were incubated in the absence of an extramitochondrial source of cholesterol. However, SCP$_2$ had no effect on steroid release from a crude particulate fraction. These studies suggest that the effects of SCP$_2$ are related to delivery of cholesterol from preformed stores to and into mitochondria for initiation of steroid hormone synthesis, and may represent an important modulator of sterol metabolism in adrenal cortical cells.

DECREASED RENAL PROSTAGLANDIN METABOLISM IN URETERAL OBSTRUCTION. A. Chaudhari and M.A. Kirschenbaum (Division of Nephrology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024) *Biochim. Biophys. Acta* 713(1):10-15 (1982). Studies were performed in rabbits to assess whether alterations in renal prostaglandin metabolism occur with ureteral obstruction. After 3 days, the cortical metabolism of prostaglandin E$_2$ (as measured by prostaglandin E$_2$-9-ketoreductase activity) decreased by almost 40% in unilateral and 50% in bilateral ureteral obstruction when compared to appropriate control levels. Medullary prostaglandin E$_2$ metabolism decreased by 70% after unilateral ureteral obstruction but was unchanged in response to bilateral ureteral obstruction. Enzyme kinetics data from bilateral ureteral obstructed and sham-operated kidneys revealed noncompetitive inhibition. In contrast to metabolism, cortical prostaglandin E$_2$ biosynthesis increased by 122% over the control levels in unilateral, and 205% in bilateral ureteral obstruction. However, medullary prostaglandin E$_2$ synthesis fell by almost 40% after bilateral obstruction. These data suggest that decreased renal prostaglandin metabolism, together with increased biosynthesis, could have significant effects on local prostaglandin tissue concentrations and thus explain the enhanced activity of these lipids previously noted in this model.

LOW DENSITY LIPOPROTEIN METABOLISM IN CULTURED FIBROBLASTS FROM A NEW GROUP OF PATIENTS PRESENTING CLINICALLY WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA. G.A. Coetzee, D.R. van der Westhuyzen, G. Berger, H. Henderson and W. Gevers (MRC/UCT Muscle Research Unit, Dept. of Med. Biochem., Univ. of Cape Town Med. Schl., Observatory 7925, Cape Town, South Africa) *Arteriosclerosis* 2(4):303-311 (1982). The metabolism of low density lipoproteins (LDL) was studied in cultured fibroblasts obtained from five local patients diagnosed as having the homozygous form of familial hypercholesterolemia. LDL receptor function was assessed by measuring the binding, internalization, and degradation of ^{125}I-labeled LDL, and by measuring the stimulation of cellular acyl-CoA cholesterol acyltransferase (ACAT) activity which followed exposure to LDL. Fibroblasts from two cases showed receptor activities which were approximately 10% of the values obtained with normal cells, while ACAT stimulation by LDL was very low. These two patients were classified as homozygous for a receptor-defective abnormality. Fibroblasts from the other three patients showed greater than 25% of normal receptor activity. Receptor activities of the cells from the available parents, assessed on the basis of LDL binding and degradation or of ACAT stimulation, were not clearly distinguishable from those of normal cells. These results add to the growing evidence of genetic heterogeneity underlying the clinical picture associated with familial hypercholesterolemia in different geographical distributions.

FEEDBACK REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE IN VASCULAR ENDOTHELIAL CELLS. D. Cohen, S. Massoglia and D. Gospodarowicz (Cancer Res. Inst. and the Dept. of Med., Univ. of California, Med. Center, San Francisco, CA 94143) *J. Biol. Chem.* 257(18):11106-11112 (1982). The "multivalent-regulation" of 3-hydroxy-3-methylglutaryl coenzyme A reductase has been investigated using confluent cultures of adult bovine aortic endothelial cells. At confluence, these form a monolayer composed of nondividing cells, which in serum-free medium have a minimal need either for *de novo* cholesterol synthesis or for exogenous cholesterol made available by low density lipoprotein. The HMG-DoA reductase activity of such cultures can be increased 5- to 10-fold by the presence of high density lipoproteins or compactin. Observations suggest that the induction by HDLs results from a deficiency in sterols, while that of compactin results from a deficiency in mevalonate or non-sterol isoprenes. 1) Mevalonate prevents the induction of HMG-CoA reductase by compactin at concentrations 30-fold lower than those at which it prevents the HMG-CoA reductase induction by HDLs. 2) LDL can completely counteract the inductions of HMG-CoA reductase by compactin. 3) HDLs and compactin interact synergistically in inducing HMG-CoA reductase activity, indicating that at some level their inductive mechanisms are separable. 4) Compacting causes morphological changes which can be prevented and reversed by mevalonate, but not by LDL. HDLs also prevent and reverse the morphological changes caused by exposure to compactin, correlating well with their induction of HMG-CoA reductase activity. This indicates that one of the primary roles of mevalonate in quiescent cells may be related to the maintenance of cell shape.

TRANSFER OF [^{14}C]PHOSPHATIDYLCHOLINE BETWEEN LIPOSOMES AND HUMAN PLASMA HIGH DENSITY LIPOPROTEIN. PARTIAL PURIFICATION OF A TRANSFER-STIMULATING PLASMA FACTOR USING A RAPID TRANSFER ASSAY. J. Damen, J. Regts and G. Scherphof (Laboratory of Physiological Chemistry, State University, Bloemsingel 10, 9712 KZ Groningen, The Netherlands) *Biochim. Biophys. Acta* 712(3):444-452 (1982). A simple method was developed for the rapid determination of [^{14}C]phosphatidylcholine transfer from small unilamellar liposomes to human plasma HDL, based on the selective precipitation of liposomes by heparin and $MnCl_2$. The assay was utilized to monitor the progress in the partial purification of a phospholipid transfer factor from human plasma. The purification procedure included ultracentrifugation at d = 1.25 g/ml, hydrophobic chromatography on phenyl-Sepharose, affinity chromatography on heparin- Sepharose and gel filtration. The partially purified protein(s) catalyzed the net transfer of phospholipid from small unilamellar phosphatidylcholine liposomes to isolated HDL. The transfer of (^{14}C)phosphatidylcholine from liposomes consisting of phosphatidylcholine/phosphatidylserine/cholesterol (molar ratio, 4:1:5) to HDL was stimulated without affecting the permeability barrier of the liposomal membranes and is, therefore, taken to represent exchange with HDL phospholipid rather than net transfer.

INTRAHEPATIC ASSEMBLY OF VERY LOW DENSITY LIPOPROTEINS. EFFECT OF FATTY ACIDS ON TRIACYLGLYCEROL AND APOLIPOPROTEIN SYNTHESIS. R.A. Davis and J.R. Boogaerts (Cell Biol. Unit, Dept. of Physiology, Louisiana State Univ. Med. Schl., New Orleans, LA 70112) *J. Biol. Chem.* 257(18):10908-10913 (1982). Cultured rat hepatocytes were used to examine the rapidly invoked mechanisms through which fatty acids stimulate very low density lipoprotein (VLDL) secretion. Fatty acids bound to albumin were added to the serum-free culture medium of hapatocytes. Within 15 min, there was a significant 2-fold increase in the secretion of [^{3}H]triacylglycerol. The stimulation lasted for at least 4 h. For three 18 carbon fatty acids studied, there was an inverse relationship between ability to stimulate triacylglycerol secretion and the number of carbon-carbon double bonds. Since oleic acid caused the greatest stimulation of triacyl-

glycerol secretion, its effects were studied further. Isolation of VLDL from the culture medium showed that oleic acid increased the content of both triacylglycerol and phospholipid. Isolation of a total lipoprotein fraction by both ultracentrifugation and antibody affinity chromatography showed that oleic acid did not affect total apolipoprotein synthesis. When added to hepatocytes obtained from rats having different lipogenic states, oleic acid stimulated [^{3}H] triacylglycerol secretion in the same order as the rate of apolipoprotein synthesis. Although oleic acid stimulated [^{3}H] triacylglycerol secretion in all hepatocyte preparations, apolipoprotein synthesis was unaffected. Inhibition of protein synthesis by cyclohexamide totally prevented the stimulation of triacylglycerol secretion by oleic acid. Although protein synthesis is required for VLDL triacylglycerol secretion, rapid stimulation of triacylglycerol secretion is not accompanied by similar changes in apolipoprotein synthesis. However, rates of apolipoprotein synthesis do play a role in determining the capacity of the hepatocyte to augment triacylglycerol secretion in response to oleic acid.

ATHEROSCLEROSIS IN CHOLESTEROL-FED JAPANESE QUAIL: EVIDENCE FOR AMELIORATION BY DIETARY VITAMIN E. W.E. Donaldson (Nutrition Program, North Carolina State University, Raleigh, NC 27650) *Poult. Sci. 61* (10):2097–2102 (1982). Three experiments were conducted with adult male Japanese quail *(Coturnix coturnix japonica)* from 5 through 14 weeks of age. In Experiment 1, quail fed a cholesterol-free diet were compared with quail fed .5% of United States Pharmacopia (USP), recrystallized (RCR), or oxidized (OXI) cholesterol preparations. In Experiment 2, .5% OXI cholesterol was fed alone and with .1% butylated hydroxytoluene (BHT) or 100 mg *d*-alpha-tocopherol acetate/kg of diet and compared with .5% RCR cholesterol. Experiment 3 was the same as Experiment 2 except the BHT treatment was deleted. In comparison to RCR-treated quail, OXI-treated quail exhibited significantly increased serum ($P < .05$) and liver ($P < .01$) cholesterol concentrations and increased severity of atherosclerotic lesions ($P < .05$). Addition of vitamin E to the OXI cholesterol diet appeared to reduce severity of atherosclerotic lesions. Vitamin E did not completely prevent atherosclerosis nor did it change the proportion of the quail population that exhibited lesions.

PHYSICAL PROPERTIES OF ESTROGEN RECEPTOR COMPLEXES IN MCF-7 HUMAN BREAST CANCER CELLS. R.L. Eckert and B.S. Katzenellenbogen (Dept. of Physiol. and Biophys., Univ. of Illinois, Urbana, IL 61801) *J. Biol. Chem. 257* (15):8840–8846 (1982). We examined the binding of two high affinity radiolabeled anti-estrogens, [^{3}H] CI628M and [^{3}H] *trans*-hydroxytamoxifen to the estrogen receptor from MCF-7 human breast cancer cells and used hydrodynamic methods to determine the molecular properties of estrogen and anti-estrogen receptor complexes from these cells. Each compound binds predominantly to a single class of high affinity binding sites with K_d of 1.3×10^{-10} M for estradiol (E_2), 1.4×10^{-10} M for [^{3}H] *trans*-hydroxytamoxifen, and 2.2×10^{-10} M for [^{3}H] CI628M. Differences are seen in the sedimentation rate and chromatographic properties of the nuclear estrogen receptor when complexed with antiestrogen as opposed to the estrogen, estradiol (E_2). The nuclear E_2 receptor sediments at 4.1 ± 0.03 S on high salt sucrose gradients; receptor complexed with CI628M or *trans*-hydroxytamoxifen sediments as a 5.5 ± 0.06 S peak. These correspond to calculated molecular weights of 83,000 for the nuclear E_2 receptor complex and 137,000 for the nuclear CI628M and *trans*-hydroxytamoxifen, or E_2 have similar sedimentation coefficients of 4.1 ± 0.03 S and Stokes radii of 4.39 ± 0.30 nm, corresponding to a molecular weight of 76,000. Anti-estrogen-promoted change may be an important aspect of the estrogen-antagonist and growth-inhibiting properties of these compounds.

DECOMPOSING POTASSIUM PEROXYCHROMATE PRODUCES HYDROCYL RADICAL ('OH) THAT CAN PEROXIDIZE THE UNSATURATED FATTY ACIDS OF PHOSPHOLIPID DISPERSIONS. J. Colin Edwards and P.J. Quinn (Department of Biochem., Chelsea College, University of London, Manresa Road, London 3W3 6LX, United Kingdom) *J. of Lipid Res. 23* (7):994–1000 (1982). The unsaturated fatty acyl residues of egg yolk lecithin are selectively removed when bilayer dispersions of the lipid are exposed to decomposing peroxychromate at pH 7.6 or pH 9.0. Mannitol (50 mM or 100 mM) partially prevents the oxidation of the phospholipid due to decomposing peroxychromate at pH 7.6 and the amount of lipid lost is inversely proportional to the concentration of mannitol. N, N-Dimethyl-p-nitrosoaniline, mixed with the lipid in a molar ratio of 1.3:1, completely prevents the oxidation of lipid due to decomposing peroxychromate at pH 9.0, but some linoleic acid is lost if the incubation is done at pH 7.6. If the concentration of this quench reagent is reduced tenfold, oxidation of linoleic acid by decomposing peroxychromate at pH 9.0 is observed. Hydrogen peroxide is capable of oxidizing the unsaturated fatty acids of lecithin dispersions. Catalase or boiled catalase (2 mg/ml) protects the lipid from oxidation due to decomposing peroxychromate at pH 7.6 to approximately the same extent, but their protective effect is believed to be due to the non-specific removal of 'OH. It is concluded that 'OH is the species responsible for the lipid oxidation caused by decomposing peroxychromate. This is consistent with the observed bleaching of N, N-dimethyl-p-nitrosoaniline and the formation of a characteristic paramagnetic 'OH adduct of the spin trap, 5,5-dimethylpyrroline-1-oxide.

EFFECT OF HIGH-CHROMIUM BREWER'S YEAST ON HUMAN SERUM LIPIDS. J.C. Elwood, D.T. Nash, and D.H.P. Streeten (Department of Biochemistry and Department of Medicine, State University of New York, Upstate Medical Center at Syracuse) *J. Am. College Nutr. 1* (3):263–274 (1982). A group of 11 normolipidemic and a group of 16 hyperlipidemic adult subjects were given orally 20 gm daily of a high-chromium brewer's yeast (2,4 μg Cr^{+++}/gm, i.e., 48 μg Cr^{+++} daily) for 8 weeks. A significant decrease in total cholesterol in both groups of subjects was observed (24–26 mg/dl). High density lipoprotein cholesterol (HDL-C) was significantly increased (5–6 mg/dl) in normo- and hyperlipidemic subjects by brewer's yeast supplementation. However, following supplementation, the triglyceride blood levels were not changed in either the normo- or hyperlipidemic group. When the multiple complex risk factor (total cholesterol/HDL-C) was calculated, 84% of all subjects receiving brewer's yeast showed a decrease in this ratio, and the mean decrease in this ratio in all subjects was significant at $P < 0.01$. A second group of 19 normolipidemic, predominantly male, adult subjects was given orally 10 gm of a high-chromium brewer's yeast (2.4 μg/gm, i.e., 24 μg Cr^{+++} daily) for 8 weeks. The total circulating serum cholesteral was significantly increased (4 mg/dl). The total cholesterol/HDL ratio was decreased in 79% of the subjects, and the mean TC/HDL-C decrease of the entire group was significant at $P < 0.01$.

FRACTIONATION OF PLASMA TRIGLYCERIDES LIPOPROTEINS OF THE DAIRY COW: EVIDENCE OF CHYLOMICRON-SIZE PARTICLES. L.F. Ferreri and R.C. Elbein (Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, VA) *J. Dairy Sci. 65* (10):1912–1920 (1982). Electron micrographs of bovine triglyceride-rich lipoproteins demonstrated spherical particles with diameters up to 3000 Å. Chylomicron-size particles (larger than 750 Å) were isolated specifically by rate zonal centrifugation. Subfractionation by rate zonal centrifugation, isopycnic centrifugation, and gel filtration chromatography all indicated a wide range of bovine triglyceride-rich lipoproteins from chylomicrons to very low density lipoproteins. Chemical analysis of subfractions from gel filtration showed changes in the ratio of triglyceride/protein characteristic of chylomicrons versus very low density lipoproteins. Electrophoretic analysis on agarose gels of bovine chylomicrons obtained by centrifugation and gel filtration showed migrating bands similar to very low density lipoproteins, unlike human chylomicrons. Evidence of bovine chylomicrons provides support for the concept of a significant contribution to milk fat from intestinally absorbed lipid in the dairy cow.

VITAMINS AND LIPID METABOLISM. A. Fidanza and M. Audisio (Institute of General Physiology, Faculty of Pharmacy, University of Rome, 00100 Rome, Italy) *Acta Vitaminol. Enzymol. 4* (1–2): 105–114 (1982). Vitamins play an essential role in lipid metabolism reactions and their presence is therefore absolutely necessary for these reactions to occur. The effect of pantothenic acid, niacin and riboflavin is here described. By transformation into coenzymes these vitamins are involved in fatty acid synthesis and oxidation reactions. Other vitamins, like vitamin B_{12}, folic acid, vitamin C, and essential fatty acids influence lipid metabolism by different mechanisms. Coenzyme B_{12} and folate coenzyme provide to balance, by methionine synthesis, the pool of methyl radicals necessary for phospholipid biosynthesis. By its involvement in the microsomal respiratory chain, vitamin C promotes cholesterol transformation into bile acids. The essential fatty acids, mainly linoleic acid, are directly connected with cholesterol transport and plasma cholesterol decrease. It is suggested that many lipid metabolism disorders may be due to primary and secondary hypovitaminosis. Nicotinic acid and its derivatives have a particular pharmacological effect since they cause a HDL increase with LDL decrease and improve cholesterol transfer from LDL to HDL. Results of several experiments on the influence of pantothenic acid on polyunsaturated fatty acid metabolism are eventually reported, and these data are related to the effect of the administration of vitamin C at high

doses on total cholesterol, triglyceride, lipoprotein, vitamin C and fatty acids of the different plasma lipid fractions.

EVIDENCE FOR THE SEPARATION OF ALBUMIN- AND APO A-I-DEPENDENT MECHANISMS OF CHOLESTEROL EFFLUX FROM CULTURED FIBROBLASTS INTO HUMAN PLASMA. C.J. Fielding and K. Moser (Cardiovascular Res. Inst. and Dept. of Physiology, Univ. of California Med. Center, San Francisco, CA 94143) *J. Biol. Chem. 257* (18):10955-10960 (1982). The role of albumin has been studied in the plasma-mediated efflux of cholesterol from cultured fibroblasts. Immunoaffinity chromatography of plasma on immobilized anti-albumin antibody decreased by 25-50% total efflux catalyzed by plasma. The remainder of the efflux-promoting capacity of plasma was deleted by immunoaffinity chromatography on antibody to apolipoprotein A-I, the major apoprotein of high density lipoprotein. Both components of efflux were saturable with half-saturation at 0.5-1.0% (v/v) plasma. However, the net transport of sterol from cells to medium, catalyzed by lecithin:cholesterol acyltransferase, was not reduced by the deletion of the albumin-catalyzed component of efflux. This finding was confirmed with congenitally analbuminemic plasma. These results indicate that efflux to albumin and to high density lipoprotein in plasma represent independent mechanisms; only the latter is coupled to net transport.

THE SYNTHESIS IN VIVO OF CHOLINE AND ETHANOLAMINE PHOSPHOGLYCERIDES IN DIFFERENT BRAIN AREAS DURING AGING. A. Gaiti, M. Brunetti, G.L. Piccinin, H. Woelk and G. Porcellati (Instituto di Chimica Biologica, Universita di Perugia, 06100 Perugia, Italy) *Lipids 17* (4):291-296 (1982). The biosynthesis of choline and ethanolamine phosphoglycerides was tested in vivo in different brain areas of the rat during aging. Mixtures of [2-^{3}H] glycerol and [*Me*-^{14}C] choline or [2-^{3}H] glycerol and [2-^{14}C] ethanolamine were injected into lateral ventricle of the brain as lipid precursors and their incorporation into corresponding phospholipid was examined. A significant decrease of synthesis of both phosphoglycerides takes place in cerebral cortex and in the striatum, and is already apparent at 9 months of age with no further decrease or change thereafter. No significant change takes place in the cerebellum. The unchanged absorption of injected water-soluble precursors, together with the lack of any significant change of phospholipid/protein ratio in all examined brain areas, suggests that the incorporation of both glycerol and nitrogen bases are affected by aging.

ALTERATIONS OF HDL CHOLESTEROL DISTRIBUTION INDUCED BY INCUBATION OF HUMAN SERUM. P. Gambert, C. Lallemant, A. Athias and P. Padieu (Laboratoire de Biochimie Medicale, Faculte de Medecine, 7bd. Jeanne d'Arc, 21033 Dijon, France) *Biochim. Biophys. Acta 713* (1):1-9 (1982). The study of high density lipoprotein (HDL) alterations induced by serum incubation was undertaken by a new approach. Subfractions of HDL were separated by gradient gel electrophoresis, without preliminary ultracentrifugation, and were characterized by their size range. After dissolution of the polyacrylamide gel, each subfraction was analyzed for its total- and unesterified-cholesterol content by gas chromatography. We have observed that a general displacement of HDL cholesterol towards the subspecies of high size range occurred during serum incubation at 37°C, contemporaneously with cholesterol esterification. This displacement could not be identified with a HDL_3 to HDL_2 conversion since it occurred with HDL_3 and HDL_2. It is probably the indication of complex HDL conversion leading to particles of increased sizes. HDL alterations occurring upon serum incubation appear to be the consequence of the activity of an HDL conversion factor, which is thermolabile, non-dialysable, present in the $d > 1.25$ serum fraction and differs from lecithin: cholesterol acyltransferase and cholesteryl ester transfer protein. They could be considered as preliminary enzymatic transformation, necessary for the action of lecithin:cholesterol acyltransferase and as the first step of a metabolic sequence including, successively, HDL conversion, cholesterol esterification by lecithin:cholesterol acyltransferase and cholesteryl ester transfer.

RECEPTOR-MEDIATED UPTAKE OF HYPERTRIGLYCERIDEMIC VERY LOW DENSITY LIPOPROTEINS BY NORMAL HUMAN FIBROBLASTS. S.H. Gianturco, F.B. Brown, A.M. Gotto, Jr. and W.A. Bradley (Dept. of Med., Baylor College of Med., and The Methodist Hospital, Houston, TX) *J. Lipid Res. 23* (7):984-993 (1982). To determine if functional abnormality of hypertriglyceridemic VLDL resulted from differences in uptake of the VLDL by the low density lipoprotein (LDL) receptor pathway, we isolated VLDL subclasses from the d < 1.006 g/ml fraction of normal and hypertriglyceridemic plasma by flotation through a discontinuous salt gradient for direct and competitive binding studies in cultured human fibroblasts. In direct binding studies, radiolabeled VLDL from hypertriglyceridemic but not normolipemic subjects were bound, internalized, and degraded with high affinity and specificity by normal fibroblasts. We conclude that 1) hypertriglyceridemic VLDL S_f 60-400 are bound, internalized, and degraded by normal fibroblasts primarily by the high affinity LDL receptor-mediated pathway; 2) by contrast, normal VLDL S_f 60-400 are bound, internalized, and degraded by normal fibroblasts primarily by nonspecific, nonsaturable routes; and 3) of the normal VLDL subclasses, only the smallest S_f 20-60 fraction is bound and internalized via the LDL pathway.

CRADLE-TO-GRAVE ATHEROSCLEROSIS: HIGH DENSITY LIPOPROTEIN CHOLESTEROL. C.J. Glueck (Lipid Research Clinic, General Clinical Research Center, and CLINFO Center, Lipid Research Division, College of Medicine, University of Cincinnati, Cincinnati, OH) *J. Am. College Nutr. 1* (1):41-48 (1982). This presentation reviews environmental and genetic factors that relate to high density lipoprotein cholesterol, the most potent independent lipoprotein risk factor for coronary heart disease. Although at least three decades of work have focused upon the primary atherogenic lipoprotein, low density lipoprotein cholesterol (C-LDL), which has a strong positive association with coronary heart disease (CHD), it has only been in the past decade that detailed epidemiologic and biochemical studies have revealed that high density lipoprotein cholesterol (C-HDL) is the most potent lipoprotein cholesterol related to coronary heart disease; this relationship is, however, inverse.

FATTY ACID SYNTHESIS IN ISOLATED SPERMATOCYTES AND SPERMATIDS OF MOUSE TESTIS. W.M. Grogan and J.W. Lam (Department of Biochemistry, Box 614 MCV Station, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298) *Lipids 17* (9):605-611 (1982). In vitro incorporation of [1-^{14}C] acetate into fatty acids and lipid classes of spermatocytes, round spermatids and condensing spermatids enriched by Staput 1 × g sedimentation was measured by thin layer and gas radiochromatography. All three cell fractions showed the full range of de novo synthetase, elongation and desaturase activities necessary for biosynthesis of fatty acids characteristic of mouse testis, but synthesis of fatty acids of > 16 carbons declined with progressive stages of differentiation. The magnitudes and patterns of distribution of fatty acid synthesis in the germinal cells were similar to those of whole testis incubated in vitro or injected in vivo with [^{14}C] acetate. On the other hand, complex lipid synthesis was much more variable and incorporation into triacylglycerol was generally much lower in dispersed germinal cells than in whole testis in vitro or in vivo. Cells remained viable throughout the 15-hr incubation. Thus, isolated germinal cells are fully capable of synthesizing their constituent fatty acids, including the long-chain polyenoic acids which they accumulate, but the intratubular environment or association with Sertoli cells may be necessary for maintenance of adequate complex lipid synthesis.

REGULATION OF TRIACYLGLYCEROL SYNTHESIS IN THE LIVER. H.P. Haagsman, C.G.M. de Haas, M.J.H. Geelen and L.M.G. van Golde (Lab. of Vet. Biochem., State Univ. of Utrecht, Biltstraat 172, 3572 BP Utrecht, The Netherlands) *J. Biol. Chem. 257* (18):10593-10598 (1982). Acyl-CoA:1,2-diacylglycerol *O*-acyltransferase of rat liver microsomes could be inactivated *in vitro* by incubation with ATP, Mg^{2+} and a 105,000 × *g* rat liver supernatant. ATP was the most effective nucleotide in inactivating diacylglycerol acyltransferase. The rate of inactivation was not influenced by the addition of cAMP. Treatment of rat liver microsomes with high concentrations of the catalytic subunit of cAMP-dependent protein kinase did not result in the inactivation of diacylglycerol acyltransferase. The activity of diacylglycerol acyltransferase was lower in microsomes isolated in the presence of fluoride than in control microsomes isolated from homogenates containing chloride instead of fluoride. The activity of diacylglycerol acyltransferase in microsomes isolated in the presence of fluoride and that in control microsomes were both reduced to the same level upon treatment of the microsomes with Mg^{2+}, ATP and 105,000 × *g* supernatant. To find if inactivated diacylglycerol acyltransferase could be reactivated, microsomes were reisolated, washed and incubated with 105,000 × *g* supernatant of rat liver. Microsomal diacylglycerol acyltransferase could be reactivated by a factor present in the 105,000 × *g* supernatant. The activating factor appeared to be heat-labile, nondialyzable, and trypsin-sensitive. The reactivation of microsomal diacylglycerol acyltransferase was inhibited in the presence of fluoride. Inactivation and reactivation of diacylglycerol acyltransferase appeared to be reversible processes. These findings are consistent with a model in which microsomal diacylglycerol acyltransferase is interconvertible between catalytically inactive

and active states, possibly via a phosphorylation-dephosphorylation mechanism.

LIPID: THE UNIDENTIFIED FACTOR FOR ALLEVIATING FATTY LIVER SYNDROME. F. Haghighi-Rad and D. Polin (Department of Animal Science, Michigan State University, East Lansing, Michigan 48824) *Poult. Sci. 61* (10):2075-2082 (1982). Laying hens were fed diets based on either corn-soybean meal (CS) or wheat-soybean meal (WS). The WS diets were formulated to be isocaloric to the CS diet by supplementing with either corn oil, corn starch, or wheat starch. Hens fed the WS diets with either of the starches had significantly ($P \leq .01$) higher percentages of hepatic lipid than those fed WS diets with corn oil. Values for hepatic lipid of hens fed the CS diets were intermediate to those of the wheat-based diets. The supplementation of fish meal or a selenium salt to supply .4 mg Se per kilogram of diet did not prevent or alleviate the problem of fatty livers caused by feeding diets composed mostly of wheat-soy and starch. The data revealed that wheat does not have an unidentified factor preventing FLS. The indication is that lipid at proper amounts in the diet acts through feed-back mechanisms to prevent excessive hepatic lipid accumulation that starches enhance.

ESSENTIAL FATTY ACID-SUPPLEMENTED DIET DECREASES RENAL EXCRETION OF IMMUNOREACTIVE ARGININE-VASOPRESSIN IN ESSENTIAL FATTY ACID-DEFICIENT RATS. H.S. Hansen (Biochemical Laboratory, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark) *Lipids 17* (4):321-322 (1982). Essential fatty acid (EFA)-deficient rats have been reported to have very concentrated urine and low urinary prostaglandin E_2 (PGE_2) excretion. Both parameters were normalized by feeding an EFA-supplemented diet. The urinary excretion rate of immuno-reactive arginine-vasopressin (iAVP) has been determined in these rats. The iAVP excretion rate was high: ca. 4.8 mU/24 hr, during the EFA-deficient period compared to the controls, 0.7-1.3 mU/24 hr. One day after the dietary change, iAVP excretion rate was still high, but it decreased significantly ($p < 0.05$) at the second measurement 7 days later. It is suggested that the water-conserving effect of vasopressin 1 day after the dietary change was suppressed by the very high PGE_2 production, resulting in normal renal water excretion. PGE_2 and water excretion data were published in the paper just cited.

INFLUENCE OF PARTIALLY HYDROGENATED VEGETABLE AND MARINE OILS ON LIPID METABOLISM IN RAT LIVER AND HEART. G. Hølmer, C.-E. Høy, and D. Kirstein (Department of Biochemistry and Nutrition, The Technical University of Denmark, Bldg. 224, DK-2800 Lyngby, Denmark) *Lipids 17* (9):585-593 (1982). Partially hydrogenated marine oils containing 18:1-, 20:1-, and 22:1-isomers and partially hydrogenated peanut oil containing 18:1-isomers were fed as 24-28 wt % of the diet with or without supplement of linoleic acid. Reference groups were fed peanut, soybean, or rapeseed oils with low or high erucic acid content. Dietary monoene isomers reduced the conversion of linoleic acid into arachidonic acid and the deposition of the latter in liver and heart phosphatidylcholine. This effect was more pronounced for the partially hydrogenated marine oils than for the partially hydrogenated peanut oil. The content of *trans* fatty acids in liver phospholipids was similar in groups fed partially hydrogenated fats. The distribution of various phospholipids in heart and liver was unaffected by the dietary fat. The decrease in deposition of arachidonic acid in rats fed partially hydrogenated fats. The distribution of various phospholipids in heart and liver was unaffected by the dietary fat. The decrease in deposition of arachidonic acid in rats fed partially hydrogenated marine oils was shown in vitro to be a consequence of lower Δ 6-desaturase activity rather than an increase in the peroxisomal β-oxidation of arachidonic acid. The lower amounts of arachidonic acid deposited may be a result of competition in the Δ 6-desaturation not only from the C22- and C20-monoenoic fatty acids originally present in the partially hydrogenated marine oil, but also from C18- and C16-monoenes produced by peroxisomal β-oxidation of the long-chain fatty acids.

METABOLISM OF APOLIPOPROTEIN E IN PLASMA HIGH DENSITY LIPOPROTEINS FROM NORMAL AND CHOLESTEROL-FED RATS. F.V. Hooft and R.J. Havel (Cardiovascular Research Institute, University of California, San Francisco, CA 94143) *J. Biol. Chem. 257* (18):10996-11001 (1982). High density lipoproteins of rat blood plasma were labeled *in vitro* with radioiodinated apolipoprotein E and biologically with [^{3}H] cholesteryl esters. These two components were removed slowly from perfused livers and the labeled apolipoprotein E was also removed slowly from the blood of intact rats. When labeled serum was subjected to ultracentrifugation at a density of 1.21 g/ml before the floating apolipoprotein E-labeled high density lipoproteins were separated by chromatography, the labeled protein was rapidly removed from the blood of intact rats by uptake into the liver. About one-half of the labeled apolipoprotein E associated with high density lipoproteins was dissociated during ultracentrifugation, but most of it reassociated with these lipoproteins when the floating lipoproteins were remixed with the sedimented serum proteins. The apolipoprotein E in such reassociated high density lipoproteins was removed from the blood of intact rats at the slow rate observed when the high density lipoproteins were separated chromatographically from whole serum. About 90% of the labeled apolipoprotein E in uncentrifuged or centrifuged high density lipoproteins was shown by affinity chromatography to be associated with particles containing apolipoprotein A-I. Rapid hepatic uptake of apolipoprotein E in centrifuged high density lipoproteins may result from an altered conformation of the apolipoprotein E on the particle surface.

CHANGES IN LIVER LIPIDS AFTER ADMINISTRATION OF 2-DECANOYLAMINO-3-MORPHOLINOPROPIOPHENONE AND CHLORPROMAZINE. A.V. Hospattankar, R.R. Vunnam and N.S. Radin (Mental Health Research Institute, Department of Psychiatry and Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor, MI 48109) *Lipids 17* (8):538-543 (1982). The enzyme which forms glucocerebroside, ceramide:UDP glucose glucosyltransferase, is inactivated in vitro by a cationic analog of cerebroside, 2-decanoylamino-3-morpholinopropiophenone. A study of the inhibitor using intraperitoneal injection into young mice showed that the level of the enzyme activity in liver was appreciably lowered between 3 and 6 hr after injection. The activity increased subsequently, overshooting the normal level within 24 hr by about 20%, then returning to normal within the next 24 hr. Additional effects observed in liver were an increase in lipid content (primarily in the triglyceride fraction and ceramides) and a decrease in the glucocerebroside level. Body temperature dropped rapidly. Markedly similar effects were produced by injecting chlorpromazine which was tried in order to reduce the hyperirritability and inhibitory effects on monoamine oxidase previously demonstrated by the glucosyltransferase inhibitor. Chlorpromazine did indeed block the hyperirritability and resulted in enhancement of the keto amine's effects on the enzyme and lipids. It is possible that the two drugs in combination would be helpful in ameliorating the symptoms due to the cerebroside accumulation that occurs in Gaucher disease. Diazepam also produced a reduced level of glucosyltransferase. A color reaction for chlorpromazine, possibly suitable for quantitative determination in tissues, was accidentally discovered.

PREVENTION OF PARTURIENT HYPOCALCEMIA: EFFECT OF A SINGLE ORAL DOSE OF 1,25-DIHYDROXYVITAMIN D_3. K. Hove and T. Kristiansen (Department of Animal Nutrition, Agricultural University of Norway, 1432 Ås-NLH, Norway) *J. Dairy Sci. 65* (10):1934-1940 (1982). Norwegian Red cows 4 yr or older were fed a high calcium diet the last 2 to 4 wk before calving to increase the severity of hypocalcemia at parturition. An oral dose of pellets of fat-encapsulated 1,25-dihydroxyvitamin D_3 (500 μg) was given to 15 cows, and placebo pellets to 12 cows. Treated cows were grouped according to time of treatment, 1) 4 cows treated within 24 hr of calving, 2) 8 cows treated 1 to 3 days before, and 3) 3 cows treated 4 to 5 days before calving. Minerals in blood plasma were measured from day −10 to day +10 (calving: day 0). Average calcium concentration of cows treated 1 to 3 days before calving decreased from 2.6 to 2.4 and of placebo treated from 2.5 to 1.8 mmol/liter from day −1 to day +1. Treatment with 1,25-dihydroxyvitamin D_3 in plasma were 200 to 250 pg/ml on days −2 and −1 in 5 cows treated on days −3 and −2. Similar concentrations were reached by placebo cows 1 to 2 days after parturition. A single oral dose of 500 μg 1,25-dihydroxyvitamin D_3 given 1 to 3 days before parturition can prevent hypocalcemia at calving. Difficulties in judging actual time for parturition led to optimal treatment of only 8 to 15 cows.

FECAL STEROIDS IN DIARRHEA: IV. CHOLERA. C.T.L. Huang, M.M. Levine, G.S. Daoud, D.R. Nalin and B.L. Nichols (Section of Nutrition and Gastroenterology, Dept. of Pediatrics, Baylor College of Medicine, Houston, TX 77030) *Lipids 17* (9):612-616 (1982). Fecal bile acid and neutral sterol patterns were studied in eight healthy adult volunteers who were challenged with *Vibrio cholerae* classical Ogawa 395 strain in the course of vaccine development studies. Bacterial 7α-dehydroxylation of cholic and chenodeoxycholic acids was not altered during experimentally induced cholera diarrhea, despite the fact that fecal weight in g/day (wet wt) was increased greatly during diarrhea (1913 ± 390 vs 161 ± 11 in controls, $p < 0.005$). Consistent with the findings on bile acids, no

significant changes in the production of coprostanol, epicoprostanol, or coprostanone were observed although the percentage of unmodified cholesterol was increased during the diarrheal episode (20.7 ± 3.3% vs 11.9 ± 2.3, $p < 0.02$). Total concentrations of both bile acids and cholesterol in mg/g of feces (wet wt) were decreased considerably as a result of diarrhea. However, total bile acid and neutral steroid excretions in mg/kg/day in subjects with and without diarrhea do not appear to be different. Intentinal transit times, measured in eight subjects by the use of carmine red dye, were found to be shortened in diarrhea (5.8 ± 1.1 hr vs 23.4 ± 4.1 hr in controls, $p < 0.001$). The results from this study are similar to those observed in experimentally induced traveller's diarrhea associated with toxigenic *Escherichia coli*, but they are in striking contrast to the changes in gastrointestinal steroid metabolism observed in acute shigellosis, an invasive intestinal infection.

EFFECTS OF DIETARY 9-*TRANS*,12-*TRANS* LINOLEATE ON ARACHIDONIC ACID METABOLISM IN RAT PLATELETS. D.H. Hwang, P. Chanmugam and R. Anding (Louisiana Agricultural Experiment Station, Human Nutrition, Home Economics Building, Louisiana State University, Baton Rouge, LA 70803) *Lipids 17* (4):307–313 (1982). In order to determine the minimal amount of dietary 9-*trans*,12-*trans*-linoleate which can decrease endoperoxide metabolites synthesized and their precursor in rat platelets, graded amounts (0, 0.1, 0.5, 1.0, 2.5%) of the *trans*-linoleate were fed to rats with a constant amount of all-*cis*-linoleate (2.5%) for 12 weeks. Arachidonic acid levels in platelet phospholipids of groups receiving the *trans*-linoleate at 2.5 and 1.0% were significantly ($p < 0.01$) lower than that of the control receiving no *trans*-linoleate. Concentrations of TXB_2 and $PGF_{2\alpha}$ in sera of the group receiving 2.5% *trans*-linoleate were significantly ($p < 0.05$) lower than those of the control; however, there was no difference between the group receiving 1.0% *trans*-linoleate and the control. To determine whether the difference in serum concentrations of endoperoxide metabolites could be manifested if rats were fed for longer period of time, 2 groups of rats were again fed diets containing 0 and 1.0% *trans*-linoleate, respectively, for 16 weeks. Arachidonic acid in platelet phospholipids of the group receiving the *trans*-linoleate was again significantly ($p < 0.01$) lower than that of the control group. Concentrations of TXB_2 and $PGF_{2\alpha}$, and 12-hydroxyeicosatetraenoic acid formed in platelets, were smaller in the group receiving *trans*-linoleate than the control group; however, the difference was not statistically significant. These results indicated that all-*trans*-linoleate can reduce arachidonic acid metabolites formed in rat platelets when its dietary level is equal to or exceeds the level of all-*cis*-linoleate.

ENDOCYTOSIS OF VERY LOW DENSITY LIPOPROTEIN REMNANTS BY LIVER OF FASTED RATS. M.M. Ittmann and C. Cooper (Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106) *J. Biol. Chem. 257*(20):11953–11959 (1982). Radioactive lipoproteins in the very low density lipoprotein (VLDL) density range were taken up by rat liver *in vivo*. The radioactivity became associated with an intracellular particle of $d = 1.11$ that did not correspond to lysosomes, endoplasmic reticulum, or plasma membrane as determined by marker enzyme distribution. Radioactive VLDL remnants could be released from these particles by passage through a hydraulic press, hypotonic shock, or sonication. The release of radioactivity from the particles by one of these methods became more complete with increasing time after injection. The injection of colchicine inhibited the breakdown of the VLDL triglyceride and cholesterol ester and caused an accumulation of radioactive material in the $d = 1.11$ particles. In contrast, injected chloroquine inhibited breakdown of VLDL triglyceride and cholesterol ester and caused an accumulation in lysosomes. We have concluded VLDL remnants are metabolized in liver by an endocytosis-lysosomal digestion pathway and that the $d = 1.11$ particles are endocytic vesicles. The existence of a releasable pool of VLDL within endocytic vesicles makes it possible to examine the internalized remnant.

VARIATION OF ELASTASE-TYPE PROTEASE ACTIVITY AND ELASTIN BIOSYNTHESIS IN RABBIT AORTA INDUCED BY CHOLESTEROL DIET AND IMMUNIZATION WITH ELASTIN PEPTIDES. M.P. Jacob, D. Bréchemier, L. Robert and W. Hornebeck (Laboratoire de Biochimie du Tissu Conjonctif, Faculté de Médecine, Université Paris-Val de Marne, 8 rue du Général Sarrail, 94010 Créteil Cédex, France) *Artery 10*(5):310–316 (1982). Elastase-type proteases are shown to be produced by arterial smooth muscle cells and fibroblasts in culture and are probably involved in the development of the arterio-atherosclerotic process. The present investigation was aimed at the quantitative determination of the elastase-type enzyme activity in the aortas of rabbits submitted to two different ather-arteriosclerosis inducing treatments: high cholesterol diet and immunization with κ-elastin peptides. Simultaneously we determined the incorporation of ^{14}C-lysine in cross-linked elastin peptides by the surviving (organ culture) aorta preparations. Elastase-type activity of aorta extracts, determined with a synthetic substrate (Suc-$(Ala)_3$-pNA) increased two fold after 1.5 month cholesterol diet, and three fold after 8 weeks of immunization with κ-elastin in complete Freund's adjuvant. The incorporation of ^{14}C-lysine in cross-linked elastin slightly increased (+20%) in cholesterol-fed aorta-explants and strongly decreased (−65%) in the immunized aorta-explants, on a DNA basis. These results confirm our contention that atherogenic stimuli produce an increase of elastase-type enzyme activity in the arterial wall. This increase appears to be correlated with elastic fibers degradation. It may also be accompanied by a decrease of elastin biosynthesis as in the κ-elastin immuno-arteriosclerosis model.

LINOLEIC ACID METABOLISM AND PROSTAGLANDIN PRODUCTION BY CULTURED BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS. T.L. Kaduce, A.A. Spector and R.S. Bar (Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242) *Arteriosclerosis 2*(5):380–389 (1982). When bovine pulmonary artery endothelial cells are cultured in a medium supplemented with linoleic acid, their capacity to produce prostacyclin (PGI_2) is reduced by about 60%. This reduction occurs when PGI_2 formation is stimulated by the addition of either the calcium ionophore A23187 or arachidonic acid. In addition, supplementation with linoleic acid reduced the proportion of prostaglandin E_2 and $F_{2\alpha}$ from 1-^{14}C-arachidonic acid by more than 50%. The capacity of cultured bovine pulmonary vein and aortic endothelial cells to convert extracellular arachidonic acid into PGI_2 also was reduced by about 50% when the growth medium was supplemented with linoleic acid. Although bovine pulmonary artery endothelial cells incorporated large amounts of 1-^{14}C-linoleic acid into cellular phospholipids and triglycerides, a maximum of only 2.3% of the radioactivity was converted to arachidonic acid in 24 hours. The most prevalent radioactive metabolite was eicosadienoic acid, the elongation product of linoleic acid. As compared with linoleic acid, the bovine endothelial cells incorporated 30% more 1-^{14}C-arachidonic acid into phospholipids and 60% more into triglycerides. When the growth medium was supplemented with linoleic acid, the percentage of this fatty acid in cellular lipids increased 3- to 4.5-fold and eicosadienoic acid accumulated, accounting for up to 9% of the cellular fatty acids. This increase was accompanied by a 30% to 45% reduction in arachidonic acid. These findings suggest that an inability to convert large amounts of linoleic to arachidonic acid and a suppressive effect of linoleic acid enrichment on prostaglandin production may be general properties of endothelial cells.

HORMONAL REGULATION OF STEAROYL COENZYME A DESATURASE ACTIVITY AND LIPOGENESIS DURING ADIPOSE CONVERSION OF 3T3-L1 CELLS. R. Kasturi and V.C. Joshi (Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030) *J. Biol. Chem. 257*(20):12224–12230 (1982). During differentiation of 3T3-L1 cells to adipocytes the desaturation of exogenous [^{14}C]palmitate and of *de novo* synthesized fatty acids from exogenous [^{14}C]acetate to monounsaturated fatty acids increased by 5,9- and 255-fold, respectively. Under similar conditions, fully differentiated 3T3-L1 adipocytes showed a maximal induction of 100-, 63-, and 50-fold, respectively, in the activities of stearoyl-CoA desaturase, fatty acid synthetase, and malic enzyme. The extent of differentiation into adipocytes and the increase in enzyme activities occurred more slowly when the differentiation was induced by insulin alone than when differentiation was induced by dexamethasone, 1-methyl-3-isobutylxanthine, and insulin. These results indicate that insulin supports phenotypic expression as well as the induction of lipogenesis and the activities of stearoyl-CoA desaturase and fatty acid synthetase during adipose conversion of 3T3-L1 cells.

INCREASED THROMBOXANE B_2 BIOSYNTHESIS IN PLATELETS. H. Kawaguchi, T. Ishibashi and Y. Imai (Dept. of Biochem., Schl. of Med., Hokkaido Univ., Sapporo 060, Japan) *Lipids 17*(9):577–584 (1982). The synthesis of thromboxane B_2 is increased in platelets from rabbits with experimental hypercholesterolemia, but the increase is not due to increased phospholipids hydrolysis. We have clarified the mechanism for the increased thromboxane synthesis. The biosyntheses of prostaglandin H_2 and thromboxane B_2 were unaffected by superoxide dismutase, xanthine oxidase, mannitol, or benzoate in experiments studying the possible involvement of reactive oxygen species. $O_2^{\cdot-}$ and $OH^{\cdot}$ were not likely to be involved as intermediates in the synthesis of prostaglandin H_2 and thromboxane B_2 in platelets. The rate of prostaglandin H_2 biosynthesis was promoted in deuterium oxide, and this was reversed by 2,5-dephenylfuran, suggesting that singlet oxygen may

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be involved in prostaglandin H_2 biosynthesis. The biosynthesis of prostaglandin H_2 was promoted by ADP-Fe^{3+} but inhibited by EDTA and EDTA-Fe^{3+}. The effect of ADP-Fe^{3+} could not be replaced by EDTA-Fe^{3+}. The effects of glutathione, glutathione peroxidase and H_2O_2 on cyclooxygenase and thromboxane synthetase were studied. Glutathione and glutathione peroxidase inhibited the activity of cyclooxygenase but not that of thromboxane synthetase. H_2O_2 caused the inactivation of cyclooxygenase, but the addition of H_2O_2 did not inhibit the formation of thromboxane B_2 from prostaglandin H_2. The observed alterations in glutathione levels and glutathione peroxidase activity are large enough to cause increased thromboxane B_2 synthesis in platelets but the possibility that other unidentified factors may also contribute cannot be excluded.

CHOLESTEROL HOMEOSTASIS OF SKIN FIBROBLASTS AFTER INCUBATION WITH POSTABSORPTIVE AND POSTPRANDIAL LIPOPROTEINS. THE EFFECT OF A FATTY MEAL. R.D. Kenagy, C.-H. Floren, E.L. Bierman, B. Kudchodkar and J.J. Albers (Division of Metabolism and Endocrinology, Dept. of Med., RG-20, Univ. of Washington, Seattle, WA 98195) *Arteriosclerosis 2* (4):290–295 (1982). To determine if lipoproteins formed after a fatty meal deliver more cholesterol to cultured skin fibroblasts than do lipoproteins in the basal state, very low density lipoproteins and remnants (d < 1.019), low density lipoproteins (LDL), and high density lipoproteins (HDL) were isolated from plasma obtained before, and 3 and 6 hours after, consumption of a high fat-cholesterol formula by seven normal males. Binding of ^{125}I-LDL to cells and cell cholesterol content were determined after incubation of normal human skin fibroblasts for 48 hours with the lipoprotein fractions at 5% or 15% of plasma concentration. Activity of 3-hydroxy-3-methyglutaryl coenzyme A reductase was also measured after preincubation of cells with HDL for 48 hours. Despite a 40% increase in unesterified cholesterol in the d < 1.019 fraction at 3 hours compared to the 0-hour fraction, the 3-hour d < 1.019 fraction did not decrease LDL binding or increase cell cholesterol more than did the 0-hour fraction. Preincubation of cells with LDL, concentrations of which were unchanged by feeding, decreased LDL binding and increased cellular cholesterol. These effects also were not altered by the meal. HDL lipids and apo A-I were decreased at 3 hours, but not at 6 hours. Effects of HDL and LDL binding and cellular cholesterol were not altered by feeding, but the 3-hour and 6-hour fractions increased 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, while the 0-hour fraction had little effect. These data indicate that consumption of a high fat-cholesterol meal as a bolus does not acutely alter the cholesterol delivery capacity of serum lipoproteins of normal male subjects.

DECREASED PLASMA PHOSPHATIDYLCHOLINE/FREE CHOLESTEROL RATIO AS AN INDICATOR OF RISK FOR ISCHEMIC VASCULAR DISEASE. A. Kuksis, J.J. Myher, K. Geher, G.J.L. Jones, W.C. Breckenridge, T. Feather, D. Hewitt and J.A. Little (Department of Medical Research, University of Toronto, 112 College St., Toronto, Ontario, Canada M5G 1L6) *Arteriosclerosis 2* (4):296–302 (1982). As part of a population survey and a follow-up study of plasma lipid profiles by high temperature gas-liquid chromatography, we have determined the quantities and relative proportions of all major chemical classes and molecular species of lipids of plasma from 1200 subjects at Visit 2 of the Toronto-McMaster Lipid Research Clinic Prevalence Study. We have compared these values between our 24 subjects with ischemic vascular disease and 73 control subjects matched for age, sex, and plasma total cholesterol and triacylglycerols. The phosphatidylcholine/free cholesterol ratio showed the highest association with ischemic vascular disease of any of over 10 other lipid parameters and all the common risk indicators except high density lipoprotein cholesterol. The phosphatidylcholine/free cholesterol ratio had a relative risk ratio of 20/4 (95% confidence limits, 15/9, 23/1) and high density lipoprotein cholesterol, a risk ratio of 23/1 (95% confidence limits, 24/0, 19/5) for ischemic vascular disease. The average ratio of phosphatidylcholine/free cholesterol for the ischemic vascular disease group was 1.36 and for the controls 1.51, the population average being 1.50. Plasma high density lipoprotein cholesterol had a significant correlation (R = 0.15) with the phosphatidylcholine/free cholesterol ratio in the total population sample. The increased risk for ischemic vascular disease from a lower phosphatidylcholine/free cholesterol ratio may possibly be explained on the basis of decreased fluidity and stability of the lipoproteins due to a relatively oversaturation with free cholesterol.

THE EFFECTS OF ENDOGENOUS ENERGY, TYPE OF DIET, AND ADDITION OF BILE SALTS ON TRUE METABOLIZABLE ENERGY VALUES IN YOUNG CHICKS. R. Kussaibati, J. Guillaume and B. Leclercq (Station de Recherches Avicoles, Institut National de la Recherche Agronomique, Nouzilly – 37380 Monnaie, France) *Poultry Sci. 61* (11):2218–2223 (1982). A trial was carried out using 3-week-old chickens of a commercial breed to study the effects of either a fat-free diet or a diet containing 150 g/kg of animal fat on the endogenous energy losses measured with starved birds. The effects of the addition of different levels of bile salts to such diets and the accuracy of true metabolizable energy (TME) with respect to the other modes of expression of metabolizable energy were also examined. The excreted endogenous energy values were shown to vary not only according to the type of diet (P < .01) but also in relation to the dietary intake level (P < .01). Because it is directly related to endogenous energy, TME proved to be an inaccurate parameter in young chicks unless values were corrected for N-balance, they are normally comparable and independent of the dietary intake level if the diet contains virtually no added fat. These findings indicate that most of the endogenous excreta are composed of nitrogenous metabolites. However, neither AME nor TME values of fat-rich diets are independent of dietary intake. The addition of bile salts had no effect on the metabolizable energy values of the fat-free diet. However, in the case of the diet rich in saturated fats, they compensated either for insufficient bile secretion or for endogenous bile salts degraded by the intestinal microflora. Thus, the digestive utilization of dietary fat, especially that of the saturated fatty acids, palmitic and stearic acids, was increased. Metabolizable energy was significantly improved (P < .01) by the addition of bile salts when the dietary intake level increased to the *ad libitum* level.

SELECTIVE DEPOSITION OF *TRANS*-8- AND *CIS*-9-OCTADECENOATES IN EGG AND TISSUE LIPIDS OF THE LAYING HEN. A.C. Lanser (Northern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, Peoria, IL 61604) *Lipids 17* (8):524–528 (1982). The deposition of *trans*-8-octadecenoate-8(9)-^{3}H (8*t*-18:1-^{3}H) was compared to *cis*-9-octadecenoate-10-^{14}C (9*c*-18:1-^{14}C) in the major egg yolk neutral lipids and phospholipids and in organ lipids from the laying hen. *Trans*-8-octadecenoate was preferentially incorporated into only the phosphatidylethanolamines (PE), whereas discrimination against 8*t*-18:1-^{3}H occurred in the phosphatidylcholines (PC), triglycerides (TG) and cholesteryl esters (CE). The 1-acyl position of both PE and PC contained three times more 8*t*-18:1-^{3}H than 9*c*-18:1^{14}C. Almost total exclusion of the 8*t*-18:1-^{3}H from the 2-acyl position of these phospholipids was found. Preferential incorporation of 9*c*-18:1^{14}C occurred at the combined 1- and 3-acyl positions and at the 2-acyl position of yolk TG. Tissue lipid analyses indicated that there was preferential deposition of 9*c*-18:1-^{14}C into all organs. Individual liver lipid classes displayed the same relative order of discrimination against 8*t*-18:1-^{3}H as did egg yolk lipids (CE > TG > PC > PE).

LOW DENSITY LIPOPROTEIN RECEPTOR BINDING IN AGING HUMAN DIPLOID FIBROBLASTS IN CULTURE. H-C. Lee, M.A. Paz and P.M. Gallop (Departments of Biological Chemistry and Oral Biology, Harvard Schools of Medicine, Boston, MA 02115) *J. Biol. Chem. 257* (15):8912–8918 (1982). High affinity cell surface receptors for low density lipoproteins (LDL) are inducible in cultured human lung fibroblasts by the removal of lipoproteins from the cell culture medium. The binding, uptake, and degradation of ^{125}I-LDL by fibroblasts decrease with increasing number of population doublings. The affinity of LDL receptor binding, however, remain unchanged at different population doubling levels. Hence, the difference in LDL binding activity in the aging fibroblasts can be attributed to a reduction in the number of receptor sites on the cell membrane. Cellular uptake of [4-^{14}C] cholesterol and 2-deoxy-D-[1-^{14}C]glucose mediated through mechanisms independent of the LDL receptor pathway revealed no significant difference in early and late passage fibroblasts. This suggests that the alteration in the LDL receptor binding in serially passaged fibroblasts is an "age-related" phenomenon. The late population doubling fibroblasts require more LDL in the culture medium for feedback inhibition of LDL receptor synthesis. Thus, aging fibroblasts are both progressively less inducible and less suppressible in the regulation of their cell surface LDL receptors. Similar results were also obtained with respect to the regulation of DL-3-hydroxy-3-methyl-glutaryl coenzyme A reductase in the aging fibroblasts in culture; the enzyme has become less inducible and less suppressible as the fibroblasts approach the limit of their *in vitro* lifespan. These age-related alterations in the cellular metabolism of LDL and cholesterol might contribute to our understanding of the increased risk of atherosclerosis in our aging population.

REVERSIBLE EXCHANGE OF GLYCOSPHINGLOLIPIDS BETWEEN HUMAN HIGH AND LOW DENSITY LIPOPROTEINS. J.A. Loeb and G. Dawson (Departments of Pediatrics and Biochemistry, Joseph P. Kennedy, Jr. Mental Retardation Research

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Center, Pritzker School of Medicine, University of Chicago, Chicago, IL 60637) *J. Biol. Chem. 257* (20):11982-11987 (1982). Both human serum low density lipoprotein (LDL) and high density lipoprotein (HDL) can acquire [^{3}H]glycosphingolipids from glycosphingolipid-coated hydrophobic glass beads, but the process produces variable denaturation of LDL. However, endogenous LDL glycosphingolipid can be ^{3}H-labeled by the galactose oxidase/NaB[^{3}H]$_4$ technique without structural modification. We have now demonstrated that ^{3}H-labeled neutral glycosphingolipids can be reversibly exchanged under physiological conditions between HDL and LDL. Maximal exchange was achieved following 4 to 8 h of incubation at 37°C when the ratio of HDL to LDL concentration was 1:1 by weight. Only a small fraction, 10-15%, of the total glycosphingolipid contents of both HDL and LDL was available for exchange, indicating that at least two separate pools of glycosphingolipid exist on the surface of lipoprotein particles. When lipoprotein-deficient serum was added, the amount of glycolipid exchanged was not stimulated significantly. The level of phosphoglyceride exchange was 2-fold greater, and that of neutral lipids 4-fold greater, than neutral glycosphingolipids. Based on this and on our previous observations, we propose that high density lipoprotein can be used to modify the glycosphingolipid content and thus the biological properties of membranes.

FACTOR Va-FACTOR Xa INTERACTION. EFFECTS OF PHOSPHOLIPID VESICLES OF VARYING COMPOSITION. T. Lindhout, J.W.P. Govers-Riemslag, P. van de Waart, H.C. Hemker and J. Rosing (Department of Biochemistry, Biomedical Center, Rijksuniversiteit Limburg, Maastricht, The Netherlands) *Biochemistry 21* (22):5494-5502 (1982). The interaction between factor Xa and factor Va was investigated both in solution and in the presence of phospholipid vesicles with varying contents of phosphatidylserine. The binding parameters were inferred from the kinetics of prothombin activation. Factor Xa and factor Va form in solution an equimolar complex with a dissociation constant of 3.3×10^{-9} M. Phospholipid vesicles promote the formation of the factor Xs-Va complex. The K_d of complex formation is dependent on both the phospholipid concentration and the composition of the phospholipid vesicle. For the interaction between factor Xa and factor Va in the presence of phospholipid vesicles containing 40 mol % dioleoylphosphatidylserine (DOPS) and 60 mol % dioleoylphosphatidylcholine (DOPC), the K_d increases linearly with increasing phospholipid concentration. In the presence of 10 μM phospholipid (DOPS/DOPC, 40/60 mol/mol) $K_d = 3 \times 10^{-11}$ M. When the mole percentage of DOPS in the phospholipid vesicles is lowered from 20 to 5 mol %, there is a gradual increase of the K_d. In the presence of 10 μM phospholipid vesicles containing 5 mol % DOPS and 95 mol % DOPC $K_d = 2.8 \times 10^{-10}$ M. The K_d measured in the presence of phospholipid vesicles containing 5 mol % DOPS and 95 mol % DOPC is independent of the phospholipid concentration. Two models are discussed that can quantitatively explain the effect of the polypeptides with M_r 80000 and M_r 94000 of which factor Va is composed on the K_d of the factor Xa-Va complex suggest that factor Xa binding to factor Va requires a Ca^{2+}-mediated interaction between the two polypeptides.

RAT ADIPOCYTE UTILIZATION OF DIFFERENT SUBSTRATES: EFFECTS OF CELL SIZE AND THE CONTROL OF LIPOGENESIS. J.M. May (Dept. of Med., Med. College of Virginia, Richmond, VA 23298) *Lipids 17* (9):626-633 (1982). The metabolism of labeled glucose, pyruvate and acetate was compared in adipocytes isolated from old, obese rats (> 500 g) and young, lean rats (130-150 g). The larger cells from old, obese rats had markedly reduced rates of glucose, pyruvate and acetate conversion to glyceride-fatty acids, indicating that large cell fatty acid formation is reduced at some point beyond the entry of pyruvate and acetate into glucose metabolism. No evidence of a primary block in the pentose phosphate cycle of cells from old, obese rats was found. In spite of diminished glucose metabolism to several products in the large cells, both basal and insulin-stimulated rates of glyceride-glycerol synthesis from glucose and pyruvate were similar in each cell type. This indicates a relative diversion of carbon flow to α-glycerophosphate and reesterification in the large cells. Addition of low concentrations of glucose increased glyceride-fatty acid synthesis from acetate (both cell types) or pyruvate carbon (small cells), but decreased glyceride-glycerol synthesis from pyruvate carbon (both cell types). The acceleration of small fatty acid synthesis from pyruvate carbon by glucose and insulin was shown to be related to provision of NADPH from glucose metabolism in the pentose cycle. These studies indicate that, although the block in lipogenesis in adipocytes from old, obese rats appears to reside in the pathway of fatty acid synthesis itself, provision of additional α-glycerophosphate or NADPH from glucose metabolism may, under certain conditions, increase lipogenesis in cells from old, obese and young, lean rats.

EFFECT OF ALTERED STEROL COMPOSITION ON THE OSMOTIC BEHAVIOR OF SPHAEROPLASTS AND MITOCHONDRIA OF *SACCHAROMYCES CEREVISIAE*. C.A. McLean-Bowen and L.W. Parks (Department of Microbiology, Oregon State University, Corvallis, OR 97331) *Lipids 17* (9):662-665 (1982). The effect of sterols on the osmotic stability of mitochondrial and plasma membranes of yeast wild-types and mutants that are defective in ergosterol biosynthesis has been studied. Incorporation of the nonfungal sterol, cholesterol, into yeast membranes reduces membrane elasticity which is observed as an increased susceptibility to osmotic lysis. However, the wild-type and nystatin-resistant strains which were examined indicate that qualitative alterations in endogenously generated sterols do not affect resistance to swelling. Although these strains exhibit differences in membrane fluidity, which is influenced by the sterol accumulated by the organisms, the membrane stretching capacity shows no distinct dependence on sterol structure or bilayer fluidity.

GENETIC SUSCEPTIBILITY AND RESISTANCE TO DIET-INDUCED ATHEROSCLEROSIS AND HYPERLIPOPROTEINEMIA. J.D. Morrisett, H-S. Kim, J.R. Patsch, S.K. Datta and J.J. Trentin (Division of Experimental Biology, Baylor College of Medicine, Houston, TX 77030) *Arteriosclerosis 2* (4):312-324 (1982). Male mice of a genetically susceptible and a genetically resistant strain were fed either a normal or an atherogenic diet. After 20 weeks on a normal diet, neither the resistant nor the susceptible strain mice had atherosclerosis; resistant strain mice had serum cholesterol of 66 ± 11 while the susceptible strain mice had 90 ± 1 mg/dl serum cholesterol, and lipoproteins were dominated by a single α-migrating HDL. After 20 weeks on an atherogenic diet, resistant strain mice had 185 ± 55 mg/dl cholesterol, their lipoproteins remained dominated by α-migrating HDL, and two of eight mice had mild atherosclerosis lesions; susceptible strain mice had 510 ± 94 mg/dl cholesterol, multiple α- and pre-β-migrating lipoprotein species, and all 13 had advanced aortic atherosclerosis. The resistant strain mice had an apolipoprotein E/total lipoprotein ratio of 0.42 on the normal diet and 0.53 on the atherogenic diet, which the susceptible strain mice had the significantly lower ratios of 0.07 and 0.31, respectively. These data indicate that genetic resistance to diet-induced aortic atherosclerosis in mice is correlated with capacity to prevent large increases in serum cholesterol, to suppress abnormal α- and pre-β-migrating lipoproteins, and to maintain elevated serum apolipoprotein E/total lipoprotein ratios. Our data do not preclude the possibility of additional gene control at the level of arterial end organ response.

PURIFICATION AND CHARACTERIZATION OF LIPID TRANSFER PROTEIN(S) FROM HUMAN LIPOPROTEIN-DEFICIENT PLASMA. R.E. Morton and D.B. Zilversmit (Division of Nutritional Science and Section of Biochem., Molecular and Cell Biol., Division of Biol. Sciences, Cornell University, Ithaca, NY 14853) *J. Lipid Res. 23* (7):1058-1067 (1982). Lipid transfer activities from human plasma have been characterized to determine whether triglyceride and cholesteryl ester transfer proteins are identical. After sequential purification by phenyl-Sepharose, CM-cellulose, chromatofocusing, and gel filtration, both triglyceride and cholesteryl ester transfer activities were purified ~15,000-fold compared to lipoprotein-deficient plasma, with a 14% recovery of both transfer activities. The gel filtration fraction showed two bands, M_r 58,300 and 66,400, as determined by electrophoresis in sodium dodecyl sulfate. Two samples, each containing predominantly one of the two bands, were obtained by selectively combining the eluates from the gel filtration column. The specific activities of triglyceride and cholesteryl ester transfer promoted by the larger protein were within 10% of those for the smaller protein. The relative rates of transfer for cholesteryl ester, triglyceride, retinyl ester, and cholesteryl ether for each fraction were the same. The transfer of triglyceride by either the large or small molecular weight component was almost completely inhibited by mercurial compounds, whereas cholesteryl ester transfer was relatively unaffected. We conclude that triglyceride and cholesteryl ester are transferred by the same plasma protein(s).

THE EFFECTS OF FASTING AND STREPTOZOTOCIN DIABETES ON THE TRIGLYCERIDE LIPASE ACTIVITY OF RAT LIVER PLASMA MEMBRANES. T. Nomura, A. Iguchi, H. Matsunaga and N. Sakamoto (The Third Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, 466, Japan) *Lipids 17* (8):573-575 (1982). The activity of hepatic triglyceride lipase (H-TGL) of plasma membranes isolated from rat liver is shown to be reduced by fasting. Refeeding restores the enzyme activity. The suppressed activity of H-TGL in streptozotocin diabetic rats is restored by insulin treatment. The behavior of the enzyme activities in both situations coincides with that of plasma insulin levels. The results suggest that the H-TGL of rat liver plasma membranes is uncer hormonal regulation by insulin.

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OCCURRENCE OF OCTADECENOIC FATTY ACID ISOMERS FROM HYDROGENATED FATS IN HUMAN TISSUE LIPID CLASSES. J.B. Ohlrogge, R.M. Gulley and E.A. Emken (Northern Regional Res. Center, Agr. Res. Service, U.S. Dept. of Agr., Peoria, IL 61604) *Lipids 17* (8):551-557 (1982). The level of trans-18:1 isomers in several isolated lipid classes of human liver, heart, red blood cells and plasma was determined. Phospholipids contained substantially fewer trans-18:1 isomers than triglycerides. The double bond distribution of the cis and trans octadecenoate fraction of triglycerides and phosphatidylcholines from human liver and heart was determined. Whereas the double bond distribution of the triglycerides correlated closely with the pattern found in dietary hydrogenated vegetable oils, the phosphatidylcholine fraction showed evidence of selective incorporation or metabolism of specific *trans* positional isomers. In general, isomers with double bonds near the methyl terminus were present at levels higher than expected from their relative abundance in the diet. Refinements in methodology needed to analyze octadecenoate double bond configurations and location in human tissues are presented.

EXERCISE AND ESTRUS CYCLE INFLUENCES ON THE PLASMA TRIGLYCERIDES OF FEMALE RATS (41487). W.K. Palmer and J.R. Davis (Dept. of Phys. Educ., Univ. of Illinois at Chicago, Box 4348, Chicago, IL 60680) *Proc. Soc. Exper. Biol. Med. 171* (1): 120-125 (1982). Since exercise and estrogens have a significant influence on plasma triglyceride (TG) concentration, this study was performed to determine the effects of exercise on the TG titers of female rats in the four stages of the estrus cycle. Normal female rats in the various phases of the estrus cycle, ovariectomized females, ovariectomized rats receiving estradiol, and normal male rats, all of comparable age, were run to exhaustion. At the time of exhaustion, the runner and a weight-matched control were anesthetized and exsanguinated. Ovariectomized animals receiving estrogen replacement ran 61% longer than the male rats. However, this difference probably resulted from body weight differences, because when positive work was calculated, all group means were equivalent. Resting plasma TG levels were higher in normal male rats than in any other group. Ovariectomy had no effect on plasma TG levels but estrogen administration increased the concentration by 35%. Phase of the estrus cycle had no effect on resting TG levels. Exercise reduced plasma TG levels in all groups. The exercise-induced plasma TG response was not influenced by the phase of the estrus cycle. The concentration of TG at exhaustion was equivalent for all groups regardless of the preexercise TG level. These findings suggest that, during exercise, animals with high resting TG titers divert a greater portion of this fuel to oxidation than to tissue TG synthesis.

PHOSPHATIDYLINOSITOL BREAKDOWN INDUCED BY VASOPRESSIN AND EPINEPHRINE IN HEPATOCYTES IS CALCIUM-DEPENDENT. V. Prpić, P.F. Blackmore and J.H. Exton (Laboratories for the Studies of Metabolic Disorders, Howard Hughes Medical Institute and the Department of Psychology, Vanderbilt University School of Medicine, Nashville, TN 37232) *J. Biol. Chem. 257* (19):11323-11331 (1982). The hormonal regulation of phosphatidylinositol breakdown in isolated rat hepatocytes was studied using isotopic and chemical means. Phospholipids extracted from hepatocytes prepared from rats injected 18-20 h previously with [^{3}H]*myo*-inositol showed radioactivity almost exclusively confined to phosphatidylinositol. Subsequent incubation of these hepatocytes resulted in the release of trichloroacetic acid-soluble radioactivity, 95% of which was shown to be *myo*-inositol. Two major conclusions are drawn from these data: a) the breakdown of phosphatidylinositol induced by vasopressin and epinephrine in hepatocytes is too slow to be responsible for the changes in cell Ca^{2+} which underlie their metabolic actions, and b) the breakdown of phosphatidylinositol caused by these agents as well as by angiotensin II and ATP is Ca^{2+}-dependent and may well result from the changes in cell Ca^{2+}.

LIBERATION, PURIFICATION, AND PROPERTIES OF THIOESTERASE COMPONENT OF PIGEON FATTY LIVER ACID SYNTHETASE COMPLEX. R.N. Puri, J.W. Porter and S.S. Katiyar (Department of Chemistry, Indian Institute of Technology, Kanpur 208016, India) *Biochim. Biophys. Acta 713* (1):29-38 (1982). Proteolysis of pigeon liver fatty acid synthetase with elastase results in the quantitative cleavage of the thioesterase component from the enzyme complex. This thioesterase component is two or three times more active catalytically in the isolated state than in the native fatty acid synthetase, and its activity is not affected by the presence or absence of reducing thiols. The proteolytically cleaved thioesterase is separated from the core enzyme in one step by size-exclusion chromatography on a Sephadex G-75 column. The peptide obtained by gel permeation is homogeneous with respect to size and charge, as shown by polyacrylamide gel electrophoresis in the presence and absence of SDS. Size-exclusion chromatography on Bio-Gel A 0.5 m and Sephadex G-75 columns, sucrose density gradient ultracentrifugation, and the *N*-terminal amino acid analysis also indicate that the proteolytically cleaved thioesterase is homogeneous. The sedimentation coefficient of the thioesterase is approximately 2.9S. Proteolytic cleavage with elastase also quantitatively releases the [1,3-^{14}C]- or [1,3-^{3}H] diisopropylphosphofluoridate-labeled thioesterase component from the correspondingly labeled fatty acid synthetase. Binding studies with ^{14}C- or ^{3}H-labeled diisopropylphosphofluoridate and fatty acid synthetase show that 2 mol of the label are bound per mol of the enzyme when complete loss of fatty acid-synthetizing activity occurs. The molecular weight of the thioesterase component is estimated to be 36000 by size-exclusion chromatography, SDS-polyacrylamide gel electrophoresis and amino acid analysis.

ROLES OF LIPOPROTEIN LIPASE AND HEPATIC TRIGLYCERIDE LIPASE IN THE CATABOLISM *IN VIVO* OF TRIGLYCERIDE-RICH LIPOPROTEINS. M.F. Reardon, H. Sakai and G. Steiner (Room 7302, Medical Sciences Bldg., Univ. of Toronto, Toronto, Ontario, Canada M55 1A8) *Arteriosclerosis 2* (5):396-402 (1982). To define the roles *in vivo*, of hepatic triglyceride lipase and lipoprotein lipoase in the catabolism of triglyceride-rich lipoproteins, we investigated the relationship between the activities of the above enzymes in postheparin plasma and the fractional removal rates of very low density lipoproteins (VLDL) and VLDL-remnant particles. In 22 patients, the fractional removal rates of VLDL and VLDL-remnant particles were determined from analyses of the disappearance of radioiodinated Sf 60-400 and Sf 12-60 lipoprotein B apoprotein. The maximal activities of hepatic triglyceride lipase and lipoprotein lipase were determined in plasma samples drawn 2-60 minutes after heparin injection (60 U/kg). A positive correlation was observed between the fractional removal rate of VLDL and postheparin plasma lipoprotein lipase activity ($r = 0.65$). Our data is consistent with the following: 1) lipoprotein lipase plays a key regulatory role in the catabolism of triglyceride-rich lipoproteins; 2) this role applies only to those catabolic processes involving the formation of particles of higher density VLDL remnants and low density lipoprotein; and 3) hepatic triglyceride lipase plays no rate-limiting role in the catabolism of VLDL or VLDL-remnant particles.

EFFECTS OF TAUROCHOLATE ON THE SIZE OF MIXED LIPID MICELLES AND THEIR ASSOCIATIONS WITH PIGMENT AND PROTEINS IN RAT BILE. A. Reuben, K.E. Howell and J.L. Boyer (Liver Study Unit, Department of Medicine, Yale University School of Medicine, New Haven, CT) *J. Lipid Res. 23* (7):1039-1052 (1982). Mixed lipid micelles were isolated from rat bile on taurocholate-equilibrated Sephadex G100 and G200 columns (5-60 mM) to study relationships between lipids and other constituents of bile. Phospholipid, cholesterol, and a bile salt peak co-eluted as mixed micelles at all taurocholate concentrations. The micellar radius, derived from the elution profile, increased progressively from ~1.6 nm to ~3.5 nm when the column taurocholate concentration was reduced from 40-60 mM to 5 mM (the physiological range for rat bile). Biliary bile pigment and bromsulphthalein, added in vivo, eluted as self-aggregates that were smaller than the lipid micelles. In contrast, on bromsulphthalein-equilibrated columns, unconjugated bromsulphthalein associated weakly with lipid micelles but this association accounted for less than 10% of the unconjugated dye in bile. No associations were found between lipid and proteins when SDS-polyacrylamide gel electrophoretic polypeptide patterns of column fractions were compared with the lipid elution profiles at different taurocholate concentrations. Two high molecular weight protein aggregates were demonstrated in bile ($> 222{,}000$ M_r) by Sephadex G200 chromatography. These studies provide a reliable estimate of rat bile lipid micelle size and suggest that bile pigment, bromsulphthalein and proteins do not form strong associations with biliary mixed micelles but exist in bile predominantly as self-aggregate.

RECONSTITUTION OF THE RECEPTOR FOR IMMUNOGLOBULIN E INTO LIPOSOMES. CONDITIONS FOR INCORPORATION OF THE RECEPTOR INTO VESICLES. B. Rivnay and H. Metzger (Section on Chemical Immunology, Arthritis and Rheumatism Branch, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205) *J. Biol. Chem. 257* (21):12800-12808 (1982). The receptor with high affinity for monomeric IgE on mast cells and basophils is a complex of at least two polypeptides and mediates the initial phase of antigen-triggered, IgE-mediated degranulation. This study defines the conditions needed for solubilization and reincorporation of ^{125}I-IgE-receptor complexes in crude extracts into lipid mem-

branes (vesicles). Reincorporation was assessed by using sucrose density gradients. More than half of the receptors in cell extracts prepared with octylglucoside at a high detergent to phospholipid ratio were found to be impared in their capacity to reincorporate into the lipids. Even with mild conditions successful reincorporation of the receptor for IgE depended on the combination of detergent and lipid used. The IgE continued to be bound with high affinity to the reincorporated receptors and was largely oriented such that it could bind ligands on Sepharose beads and be digested by added trypsin.

REGULATION OF PHOSPHOLIPID SYNTHESIS IN *ESCHERICHIA COLI*. COMPOSITION OF THE ACYL-ACYL CARRIER PROTEIN POOL *IN VIVO*. C.O. Rock and S. Jackowski (Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101) *J. Biol. Chem. 257* (18):10759-10765 (1982). The regulation of membrane lipid biogenesis was investigated by measuring the levels of the acyl-acyl carrier protein (acyl-ACP) intermediates in the biosynthetic pathway. In particular, the role of the *sn*-glycerol-3-phosphate acyltransferase was assessed by focusing on the size and composition of the long chain acyl-ACP pool. The ACP pool was specifically labeled *in vivo* with β-[3-^{3}H]-alanine and the ACP subspecies analyzed by reversed phase liquid chromatography and conformationally sensitive gel electrophoresis. The acyl-ACP pool was found to be a small fraction of the total ACP in normally growing cells and was particularly devoid of chain lengths that could serve as acyl-transferase substrates. Inhibition of phospholipid synthesis at the acyltransferase step resulted in a rapid increase in the content of acyl-ACP, and analysis showed the presence of chain lengths that are acyl-transferase substrates. Acyl-CoAs were not detected during interruption of acyl transfer activity. These results show that 1) acyl-ACPs are the acyl donors for phospholipid synthesis *in vivo*, 2) the acyltransferase does not play a role in the regulation of the lipid biosynthetic rate or the composition of phospholipid acyl moieties, 3) the primary regulatory site in phospholipid biosynthesis is at an early step in fatty acid biosynthesis, 4) feedback regulation by long chain acyl-ACPs is not a controlling mechanism for fatty acid synthesis under normal physiological circumstances, and 5) enzymes that utilize acyl-ACPs are involved in kinetic competition for the scarce acyl-ACP substrates.

CIRCADIAN RHYTHMS IN *NEUROSPORA CRASSA*: OSCILLATIONS IN FATTY ACIDS. P.E. Roeder, M.L. Sargent and S. Brody (Dept. of Biol., Univ. of California, San Diego, La Jolla, CA 92093) *Biochemistry 21* (20):4909-4916 (1982). In the band strain of *Neurospora crassa*, a circadian rhythm of spore formation is expressed at the growing front of the mycelia by sequential periods of conidiating and nonconidiating growth. A region, ca. 7 mm wide, of the growth front of the mycelium of the band (*bd csp-1*) strain was sampled at different times over a 40-h interval, and the amount and composition of the fatty acids in the total lipids and in the phospholipid subfractions were determined. In the growing front, the mole percentages of linoleic acid (18:2) and linolenic acid (18:3) oscillated out of phase with each other in the total lipids and in the phospholipids. The oscillations in 18:2 and 18:3 content nearly compensated for each other; i.e., when the 18:2 content in the total lipid from *bd csp-1* increased from 34 to 38 mol %, the 18:3 content decreased from 41 to 36 mol %. No oscillations could be detected in the mole percentages of the other fatty acids or in the total lipid content. The oscillations in the content of 18:2 and 18:3 were shown to have two properties of a circadian rhythm: their periods were about 20-h long and they were phase set by an initial exposure to light. While firm conclusions as to whether or not these oscillations have a role in the mechanism of the circadian cloak cannot be drawn from the available data, several criteria and experimental approaches to his problem are discussed. The possibility that the oscillations were merely part of the developmental conidiation rhythm was eliminated by demonstrating similar oscillations in the 18:2 and 18:3 content in the total lipid and in the phospholipid subfractions of samples isolated from a strain (*bd+ csp-1*) that does not express the spore-forming rhythm under the growth conditions used.

RIBOFLAVIN DEFICIENCY AND β-OXIDATION SYSTEMS IN RAT LIVER. T. Sakurai, S. Miyazawa, S. Furuta and T. Hashimoto (Department of Biochemistry, Shinshu University School of Medicine, Matsumoto, Nagano, 390, Japan) *Lipids 17* (9):598-604 (1982). Weanling rats were fed a riboflavin-deficient diet. The mitochondrial fatty acid oxidation in liver was depressed in riboflavin deficiency but restored after supplementation of riboflavin. Among the enzymes involved in this system, only the acyl-CoA dehydrogenase (EC 1.3.99.2 and 1.3.99.3) activities varied with the change in fatty acid oxidation. An accumulation of the apoforms of acyl-CoA dehydrogenases was found in riboflavin deficiency. The levels of electron transfer flavoprotein and other enzymes involved in the β-oxidation system remained unchanged. The peroxisomal fatty acid oxidation and levels of individual enzymes of this system remained constant. No accumulation of apoform of acyl-CoA oxidase was observed under simple, riboflavin-deficient conditions. However, accumulation of a large amount of apo-acyl-CoA oxidase was observed when the peroxisomal system was induced by administration of a peroxisome proliferator, di(2-ethylhexyl)phthalate, under riboflavin-deficient conditions.

EFFECT OF DETERGENTS ON IN VITRO 7α-HYDROXYCHOLESTEROL FORMATION BY RAT LIVER MICROSOMES. A. Sanghvi, E. Grassi, C. Bartman and W.F. Diven (Dept. of Pathology, Univ. of Pittsburgh Schl. of Med., Pittsburgh, PA 15261) *Lipids 17* (9):644-649 (1982). Formation of 7-α-hydroxycholesterol by rat liver microsomes was quantitated using a gas chromatograph-mass spectometer (GC/MS) operated in selected ion monitoring (SIM) mode. Microsomes from normal rat livers incubated for different periods were found to yield increased 7α-hydroxycholesterol with time. This was also true when incubations contained Tween-80, but in this instance, the rate of 7α-hydroxycholesterol production was lower and dependent on the concentration of Tween used. Similarly, Triton X-100, Renex-30, Kyro EOB, Cutscum, and Emulgen 911 all lowered the formation of 7α-hydroxycholesterol by rat liver microsomes, whereas Triton WR-1339 stimulated its production. Analysis of data obtained from following the enzyme reaction over an extended period using an integrated Michaelis-Menten equation indicated that enzyme possesses a very significant affinity for the product ($K_S > K_P$). Similar analysis shows that Tween-80 is a noncompetitive inhibitor of the enzyme.

PROTEOLIPID OF ADENOSINETRIPHOSPHATASE FROM YEAST MITOCHONDRIA FORMS PROTON-SELECTIVE CHANNELS IN PLANAR LIPID BILAYERS. H. Schindler and N. Nelson (Biocenter of the Univ. of Basel, CH-4056 Basel, Switzerland) *Biochemistry 21* (23):5787-5794 (1982). Proteolipid isolated from yeast mitochondrial adenosinetriphosphatase by butanol extraction is reincorporated into lipid vesicles from which planar membranes are formed. The proteolipid permits electric conductance through the membrane. This conductance occurs through membrane channels which are highly selective for protons. Proton channels in the membrane are directly observed at high proton concentrations in the aqueous phases. Channels open and close independently from each other; their open-state conductances and lifetimes are monodisperse but influenced by the applied voltage (12 pS and 3 s, respectively, at pH 2.2 and 100 mV). Proton channels do not occur in single proteolipid molecules; the conducting structure consists of at least two polypeptide chains since channels form in a (reversible) bimolecular reaction of nonconducting forms of proteolipid. The number of proton channels at a constant proteolipid concentration changes in sharp transitions and by orders of magnitudes upon critical changes of membrane composition and pH. These transitions are caused by transitions of proteolipid organization in the membrane from a dispersed state (equilibrium between channel-forming "dimers" and a large pool of "monomers") to a state of almost complete aggregation of proteolipid which stabilizes large proton-conducting structures (probably associates of channel-forming dimers). This self-association of isolated proteolipid into structures containing proton-selective channels suggests that the six proteolipids in the adenosine-triphosphatase complex exist as a self-associating entity containing most likely three proton channels.

LOW DENSITY LIPOPROTEIN RECEPTOR ACTIVITY IN HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA FIBROBLASTS. C.F. Semenkovich, R.E. Ostlund, Jr., R.A. Levy and S.R. Osa (Metabolism Div., Dept. of Med., Washington Schl. of Med., St. Louis, MO 63110) *J. Biol. Chem. 257* (21):12857-12865 (1982). We identified specific low affinity low density lipoprotein receptors in skin fibroblasts from two patients classified as having LDL receptor-negative homozygous familial hypercholesterolemia (FHC). K_m and maximum capacity for cell-associated and degraded ^{125}I-LDL were determined by i) adding increasing amounts of ^{125}I-LDL until receptor saturation was achieved and ii) a new technique in which the displacement of a small amount of ^{125}I-LDL tracer was observed during the addition of variable amounts of unlabeled ^{125}I-LDL. The K_m for specific cell-associated ^{125}I-LDL in FHC cells was 3.5-7.3 times that of normal cells and the maximum specific capacity was reduced to 11% of normal. Thus, some FHC cells have reduced affinity and reduced capacity for LDL. The FHC cell receptors share many properties of the normal skin fibroblasts LDL receptor. Specific degradation of bound ^{125}I-LDL occurred concomitantly with LDL binding and was greatly reduced by adding chloroquine. Preincubation of FHC cells with choles-

terol or LDL resulted in significant suppression of receptor function. Modification of lysine residues of LDL abolished receptor activity in both normal and FHC cells. Treatment of FHC cells with compactin resulted in increases in specific ^{125}I-LDL binding and degradation compared to FHC cells without compactin treatment. Normal cells also showed increases in ^{125}I-LDL binding and degradation, but the mean percentage increase in specific ^{125}I-LDL degradation was greater in FHC cells than in normal cells.

INFLUENCE OF VITAMIN E AND NITROGEN DIOXIDE ON LIPID PEROXIDATION IN RAT LUNG AND LIVER MICROSOMES. A. Sevanian, A.D. Hacker and N. Elsayed (Univ. of California–Los Angeles, Schools of Med. and Public Health, Los Angeles, CA 90024) *Lipids* 17(4):269–277 (1982). Rat lung and liver microsomes were used to examine the effects of dietary vitamin E deficiency on membrane lipid peroxidation. Microsomes from vitamin-E-deficient rats displayed increased lipid peroxidation in comparison to microsomes from vitamin-E-supplemented controls. The extent of lipid peroxidation, as determined by measurement of thiobarbituric acid reacting materials, was enhanced by addition of reduced iron and ascorbate (or NADPH). Rats fed a vitamin-E-supplemented diet and exposed to 3 ppm NO_2 for 7 days did not exhibit increases in microsomal lipid peroxidation compared to air-breathing controls. However, increases were found in microsomes prepared from rats fed a vitamin-E-deficient diet and exposed to NO_2. Lung microsomes from vitamin-E-fed rats contained almost 10 times as much vitamin E as liver microsomes when expressed in terms of polyunsaturated fatty acid content. The extent of lipid peroxidation was, in turn, considerably less in lung than in liver microsomes. Lipid peroxidation in lung microsomes from vitamin-E-deficient rats was comparable to liver microsomes from vitamin-E-supplemented rats as was the content of vitamin E in these respective microsomal samples. A combination of vitamin E deficiency and NO_2 exposure resulted in the greatest increases in lung and liver microsomal lipid peroxidation with the largest relative increases occurring in lung microsomes. An inverse relationship was found between the extent of lipid peroxidation and vitamin E content. Most of the peroxidation in lung microsomes appeared to proceed nonenzymatically whereas peroxidation in liver was largely enzymatic. Vitamin E appears to be assimilated by the lung during oxidant-inhalation, but with dietary vitamin E deprivation, the margin for protection in lung may be less than in liver.

PROSTAGLANDIN AND ACYL CHAIN EFFECTS ON GLUTAMATE DEHYDROGENASE ACTIVITY. P.T. Shafer and A.M. Fiskin (Med. Res. Service, Veterans Admin. Med. Center, 4801 Linwood Boulevard, Kansas City, MO 64128) *Lipids* 17(4):297–306 (1982). Prostaglandins A_1 (PGA_1), A_2, B_1, B_2, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, and 19 esterified natural fatty acids were tested as effectors of beef liver glutamate dehydrogenase (L-glutamate: NAD(P)$^+$ oxidoreductase [deaminating], EC 1.4.1.3). All prostaglandins tested are found to activate the enzyme initially, but only $PGA_2 > PGB_2 \geqslant PGA_1$ cause a subsequent time-dependent loss (not inhibition) of NADH oxidation activity. Both PGA_1 and PGA_2 desensitize glutamate dehydrogenase to allosteric activation by ADP, whereas PGA_2 and PGB_2 desensitize to allosteric inactivation by GTP. We conclude from the action of the PG and structural analogs that the initial activation of glutamate dehydrogenase is caused by α,β-unsaturated monoketo cyclopental structures. GTP inhibition is blocked primarily by diketo structures which eventually inactivate the enzyme. ADP activation is blocked by sulfhydryl binding of the unsaturated cyclopental keto structure of the PG. Appearance of a 270 nm absorbance simultaneous to the acyl effects on the enzyme suggests that conjugated unsaturations are responsible for the precursor's qualitatively similar action to that of the PG.

HYPOLIPIDEMIC EFFECTS OF GOSSYPOL IN CYNOMOLGUS MONKEYS *(MACACA FASCICULARIS)*. L.N. Shandilya and T.B. Clarkson (Arteriosclerosis Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103) *Lipids* 17(4):285–290 (1982). The effect of gossypol acetic acid (gossypol) on plasma lipid concentrations was studied in adult male cynomolgus monkeys consuming a diet containing 0.19 mg cholesterol/Kcal. Gossypol was administered orally at 5 (n = 4) or 10 (n = 3) mg/kg/day for 6 months. A significant decrease in total plasma cholesterol (TPC) and low density lipoproteins and very low density lipoprotein-cholesterol (LDL + VLDL-chol) concentrations was observed without any significant decrease in plasma high density lipoprotein-cholesterol (HDL-chol) levels among 10 mg/kg/day gossypol-treated animals. This is a new therapeutic property of gossypol that has not been previously reported. No appreciable differences were observed in plasma levels of TPC and LDL + VLDL-chol among 5 mg/kg/day gossypol-treated animals when compared to controls until the gossypol dosage was increased to 10 mg/kg/day, thus suggesting that hypolipidemic effect of gossypol is dose-dependent. In general, no adverse clinicopathological findings were noted except a temporary diarrhea and loss of appetite among 10 mg/kg/day gossypol-treated animals during the initial stages of treatment. In conclusion, it is tempting to speculate that gossypol might possibly reduce the intestinal absorption of dietary cholesterol or it may reduce the hepatic synthesis of low density lipoproteins. These results also suggest that gossypol may be a particularly useful drug in lowering plasma cholesterol concentrations in addition to its previously demonstrated antifertility properties in males.

FATTY ACID PATTERNS IN IRON-DEFICIENT MATERNAL AND NEONATAL RATS. A.R. Sherman, S.J. Bartholmey and E.G. Perkins (Dept. of Foods and Nutr. and Dept. of Food Sci., Univ. of Illinois, Urbana, IL 61801) *Lipids* 17(9):639–643 (1982). To determine the effects of maternal iron deficiency on lipid composition and fatty acid patterns in offspring, rats were fed ad libitum diets containing 5 ppm iron (deficient) (n = 8) or 320 ppm iron (control) (n = 7) and deionized water from day-1 of gestation through day-18 of lactation. On day-2 of lactation, litters were standardized to three male and three female pups. On day-18, pups were fasted for 4 hr before tissue and blood collection. Significant changes in serum and liver lipid concentrations and fatty acid patterns were observed in deficient pups. Serum triglycerides, cholesterol and phospholipids and liver triglycerides, cholesterol, and cholesteryl esters were increased. In deficient pups, percentage total fatty acids of 14:0, 16:1, 18:1, 18:2 from serum lipids were increased; in liver, 14:0, 18:2, 18:3 were increased; 18:0 and 20:4 were decreased in both serum and liver. Dam serum lipids did not differ between groups. Lipid changes observed in iron-deficient pups did not consistently reflect the milk, serum or liver lipid patterns observed in dams. Altered lipid composition and fatty acid patterns of iron-deficient pups thus appear to be of endogenous origin.

ASSOCIATION AND ASSEMBLY OF TRIGLYCERIDE AND PHOSPHOLIPID WITH GLYCOSYLATED AND UNGLYCOSYLATED APOPROTEINS OF VERY LOW DENSITY LIPOPROTEIN IN THE INTACT LIVER CELL. P. Siuta-Mangano, D.R. Janero and M.D. Lane (Dept. of Physiological Chem., The Johns Hopkins Univ. Schl. of Med., Baltimore, MD 21205) *J. Biol. Chem.* 257(19):11463–11467 (1982). Using estrogen-induced chick liver cells which synthesize and secrete large amounts of very low density lipoprotein (VLDL), we have previously shown (P. Siuta-Mangano, S. Howard, W.J. Lennarz and M.D. Lane (1982) *J. Biol. Chem.* 257, 4292–4300) that the major protein constituent of VLDL, the 350,000 molecular weight apoprotein (apoprotein B), is synthesized as a single polypeptide to which core oligosaccharides are added co-translationally. This system has now been employed to study the assembly of the apoproteins of VLDL with their glycerolipid (triglyceride and phospholipid) components and the secretion of the VLDL glycerolipids. In the presence of cycloheximide such that VLDL apoprotein synthesis is inhibited 98%, the secretion of lipids labeled from a [^{3}H] palmitate pulse by hepatocyte monolayers was halted only after completed apoprotein chains had cleared the cell. Under conditions whereby tunicamycin inhibited [^{3}H]-glucosamine incorporation into apoprotein B by 98% and [^{3}H]-leucine incorporation into the VLDL apoproteins minimally, the unglycosylated form of apoprotein B assembled with the usual complement of triglyceride and phospholipid as did glycosylated apoprotein B to form a VLDL which was readily secreted by the hepatocyte. Taken together, these findings demonstrate that whereas apoprotein synthesis is necessary for the secretion of the major lipid components of VLDL, glycosylation of apoprotein B is not required for either the assembly of VLDL glycerolipids or for the secretion of the VLDL particle.

LIPID COMPOSITION AND INTERRELATIONSHIPS OF MAJOR SERUM LIPOPROTEINS. OBSERVATIONS IN CHILDREN WITH DIFFERENT LIPOPROTEIN PROFILES. BOGALUSA HEART STUDY. S.R. Srinivasan, L.S. Webber and G.S. Berenson (Department of Medicine, Louisiana State University Medical Center, 1542 Tulane Ave., New Orleans, LA 70112) *Arteriosclerosis* 2(4): 335–345 (1982). Cholesterol (C) and triglyceride (T) contents and their relationship within serum lipoproteins, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL), were determined in subgroups of children (n = 360) from a total biracial community whose earlier β- and/or pre-β-lipoprotein cholesterol levels were in the extreme percentiles of the distribution. The lipid content of lipoproteins was analyzed following fractionation by ultracentrifugation. In addition to previously known race- and sex-related differences for lipoprotein cholesterol levels, results indicated that white children had significantly higher VLDL-T and LDL-T and lower VLDL-C/T

and LDL-C/T ratios than black children. Although HDL-T levels were the same in both races, girls had significantly higher levels than boys. Children with low pre-β-lipoprotein cholesterol levels earlier had consistently higher VLDL-C/T, LDL-C/T, and HDL-C/T ratios than children with high pre-β-lipoprotein cholesterol. Children with high β- and low pre-β-lipoprotein cholesterol and low pre-β-lipoprotein cholesterol earlier showed the highest LDL-C/T ratio, suggesting that LDL particles in this group may have less fluid lipid core. These data provide baseline values for children with different lipoprotein profiles and may help identify at an early stage children with lipoprotein patterns and characteristics that are potentially atherogenic.

USE OF ^{3}H-CHOLESTERYL LINOLEYL ETHER FOR THE QUANTITATION OF PLASMA CHOLESTERYL ESTER INFLUX INTO THE AORTIC WALL IN HYPERCHOLESTEROLEMIC RABBITS. Y. Stein, O. Stein and G. Halperin (Lipid Res. Lab., Dept. of Med. B, Hadassah University Hospital, Jerusalem, Israel) *Arteriosclerosis 2* (4):281–289 (1982). In this study use was made of ^{3}H-cholesteryl linoleyl ether (CLE), a nondegradable analogue of cholesteryl ester (CE) to measure plasma lipoprotein CE influx into rabbit aorta. Autologous serum labeled with ^{3}H-CLE was injected into seven hypercholesterolemic rabbits, and more than 90% of the label was recovered in the plasma compartment 10 minutes after injection. Between 4 hours and 3 days the label was cleared from the circulation with a $t_{1/2}$ of about 24 hours. Between 4 and 24 hours the lipoproteins isolated at $d < 1.006$, $d < 1.019$, and $d < 1.063$ approached similar specific activity, assuming that ^{3}H-CLE had mixed with the lipoprotein CE pool. The rabbits were killed 7 to 14 days after injection when plasma radioactivity decreased to $< 0.03\%$ of injected dose/ml. Total recovery of CLE ranged from 70% to 95% and 48% to 72% were found in the liver. The minimum influx of plasma CE into the aortic intima was determined by dividing the label found in the artery by the mean specific activity of the labeled compound in the plasma. The minimum influx into regions with atheromatous involvement ranged from 0.8 to 3.4 μg CE/cm^2 hr. The rate of influx was highly correlated with the amount of CE mass in the intima and media indicating that the bulk of aortic CE is derived from plasma lipoprotein CE.

REDUCED PYRIDINE NUCLEOTIDES AND CYTOCHROME b_5 AS ELECTRON DONORS FOR PROSTAGLANDIN SYNTHETASE RECONSTITUTED IN DIMYRISTYL PHOSPHATIDYLCHOLINE VESICLES. P. Strittmatter, E.T. Machuga and G.J. Roth (Department of Biochemistry and the Division of Hematology/Oncology, Department of Medicine, University of Connecticut School of Medicine, Farmington, CT 06032) *J. Biol. Chem.* 257 (20):11883–11886 (1982). Prostaglandin synthetase has been reconstituted in dimyristyl phosphatidylcholine vesicles. These vesicles in either 80 or 2 μM flufenamate utilized NADH or NADPH as electron donor in the reductive peroxidative step of prostaglandin H_2 formation. Vesicles containing bound cytochrome b_5 reductase and cytochrome b_5 in order to complete an NADH cytochrome b_5 reductase system also utilized reduced cytochrome b_5 as the electron donor for this peroxidative step. In systems containing cytochrome and reductase, the rate of NADH oxidation exceeded that of NADPH oxidation, indicating that reduced cytochrome b_5 is an effective electron donor for prostaglandin H_2 formation, enhancing both the initial rate and the extent of the reaction. The concentrations of reduced pyridine nucleotides and cytochrome b_5 employed in these experiments to provide reductants for prostaglandin synthetase are within the concentration ranges that obtain under physiological conditions.

VITAMIN K DEPENDENT IN VITRO PRODUCTION OF PROTHROMBIN. J.C. Swanson and J.W. Suttie (Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, WI 53706) *Biochemistry 21* (23):6011–6018 (1982). During prothrombin biosynthesis, glutamyl residues in prothrombin precursor proteins are carboxylated to γ-carboxyglutamyl residues by a vitamin K dependent carboxylase. Calcium-dependent and calcium-independent rat prothrombin antibody subpopulations have been produced and utilized to study the liver microsomal precursors of prothrombin that accumulate when vitamin K action is blocked. A substantial portion of the precursor pool accumulating in the vitamin K deficient or warfarin-treated rat will react with a Ca^{2+}-dependent antibody at high calcium concentration and appears to be partially carboxylated. During in vitro incubation in the presence of vitamin K, the fraction of the precursor pool which is tightly bound to the microsomal membrane appears to be the preferred substrate for the vitamin K dependent carboxylation. A small amount of completely carboxylated rather than a large amount of partially carboxylated products are produced during these incubations. Treatment with a Sepharose-bound prothrombin antibody demonstrated that about 20–25% of the total carboxylated microsomal protein precursor pool consists of prothrombin precursors. This treatment removes an equal amount of total carboxylase activity, and the enzyme is active in this carboxylase precursor-antibody complex.

HEPATIC GOLGI LIPOPROTEINS: PRECURSORS TO PLASMA LIPOPROTEINS IN HYPERCHOLESTEROLEMIC RATS. L.L. Swift, P.D. Soule and V.S. LeQuire (Dept. of Pathology, Vanderbilt Univ. Schl. of Med., Nashville, TN 37232) *J. Lipid Res. 23* (7): 962–971 (1982). Two classes of nascent lipoproteins can be isolated from Golgi apparatus-rich fractions of liver from hypercholesterolemic rats. Golgi very low density lipoproteins (VLDL, $d < 1.006$ g/ml) are enriched in cholesteryl esters and are similar in many respects to hypercholesterolemic plasma B-VLDL. Golgi low density lipoproteins (LDL, d 1.006–1.04 g/ml) are cholesteryl ester-rich beta-migrating lipoproteins similar to hypercholesterolemic plasma LDL. To determine if this latter lipoprotein is a precursor to plasma LDL, control and hypercholesterolemic rats were injected with Triton WR 1339 (400 mg/kg) to block intraveascular lipoprotein catabolism followed in 30 min with 100 μCi[^{3}H]-leucine. At time intervals up to 3 hours after [^{3}H] leucine injection, rats were killed, and plasma lipoproteins and, in some experiments, Golgi lipoproteins were isolated. Three hours after radioisotope injection, 52% of the total lipoprotein radioactivity was found in the plasma VLDL of hypercholesterolemic rats compared to 82% in chow-fed control rats. Twenty-four percent of the total lipoprotein radioactivity appeared in the plasma IDL fraction in hypercholesterolemic rats, while only 3% was found in the same fraction in control rats. After Triton, the time course of specific activities of the Golgi and plasma lipoproteins was consistent with Golgi VLDL and LDL being precursors to plasma VLDL and IDL, respectively. The time course of specific activities of the tetramethylurea-insoluble proteins of plasma and Golgi lipoproteins provided additional evidence in support of this relationship. Furthermore the composition of plasma VLDL and IDL after Triton injection resembled their hepatic Golgi counterparts.

VITAMIN D AND ITS METABOLITES. ADVANCES IN THE DIAGNOSIS AND TREATMENT OF RICKETS. R. Tojo, P. Pavon, J. Antelo and A. Mourino (Department of Pediatrics and Organic Chemistry, University of Santiago De Compostela, Santiago De Compostela, Spain) *Acta Vitaminol. Enzymol. 4* (1-2):1–11 (1982). Diagnostic and therapeutical uses of vitamin D_3 and its metabolites are reviewed. Special emphasis is dedicated to the fetomaternal relationships of 1,25 $(OH)_2D_3$ and 25-OH-D_3 at term. The serum levels of 1,25 $(OH)_2D_3$ have been found to be higher in the maternal serum than in the corresponding fetus (85.3 pg/ml and 50.9 pg/ml, respectively). The highest serum levels of 1,25 $(OH)_2D_3$ were found in October and the lowest ones in January showing that there is a dependence on the ultraviolet light. It has been found that there is a correlation between the fetomaternal serum levels of 1,25 $(OH)_2$-D_3 and 25-OHD. However, there is no correlation between the serum levels of 1,25 $(OH)_2D_3$ and 25-OHD_3, neither in the fetus nor in the mother.

LIPOPROTEIN LIPASE IN CULTURED PIG AORTIC SMOOTH MUSCLE CELLS. J.E. Vance, J.C. Khoo and D. Steinberg (Department of Medicine, Division of Metabolic Disease, University of California, San Diego, La Jolla, CA 92093) *Arteriosclerosis 2* (5):390–395 (1982). Acetone powder extracts prepared from cultured pig aortic smooth muscle cells and the culture medium from these cells (particularly when incubated with heparin) were shown to contain a lipolytic enzyme which was identified as lipoprotein lipase by the following criteria: 1) stimulation by apolipoprotein C-II; 2) an optimal activity at approximately pH 8.0; 3) inhibition by NaCl; and 4) binding to a heparin-Sepharose affinity column. In addition, we found that cultured arterial smooth muscle cells from guinea pig and rabbit secreted a similar lipase into the culture medium. In contrast, studies using cultured bovine aortic endothelial cells yielded no evidence for either the synthesis or secretion of lipoprotein lipase by these cells. The production of lipoprotein lipase by the smooth muscle cells of the artery may play a role in the process of atherogenesis.

NEW ROLE FOR 15-HYDROXYEICOSATETRAENOIC ACID. ACTIVATOR OF LEUKOTRIENE BIOSYNTHESIS IN PT-18 MAST/BASOPHIL CELLS. J.Y. Vanderhoek, N.S. Tare, J.M. Bailey, A.L. Goldstein and D.H. Pluznik (Dept. of Biochemistry, The George Washington University School of Medicine and Health Sciences, Washington, DC 20037) *J. Biol. Chem.* 257 (20):12191–12195 (1982). Leukotrienes are vasoactive arachidonic acid metabolites which are released by mast cells during hypersensitivity reac-

tions. The mechanisms for regulating leukotriene biosynthesis are not well understood. A murine mast/basophil cell line (PT-18) was used to investigate this problem. Exogenously supplied [^{14}C] arachidonic acid is not appreciably converted to leukotrienes by untreated PT-18 cells. However, when the cells were preincubated with the lymphocyte product 15-hydroxyeicosatetraenoic acid (15-HETE), addition of [^{14}C]arachidonic acid consistently resulted in a dose-dependent synthesis of large amounts of both [^{14}C]leukotriene B_4 and [^{14}C]5-HETE. These metabolites were isolated by high pressure liquid chromatography, converted to the methyl ester trimethylsilyl ether derivatives, and the structures confirmed by gas chromatography-mass spectrometry. These findings indicate that 15-HETE induces a direct activation of a cryptic 5-lipoxygenase in these cells. The closely related 12-HETE was ineffective. The activation phenomenon occurs rapidly and is reversible. Furthermore, the activation appears to be highly cell- and enzyme-specific, since lipoxygenase in three primary cell types including one that contains a 5-lipoxygenase and six other cell lines did not show this specific induction of leukotriene biosynthesis by 15-HETE. This report is the first evidence that 15-HETE, a major arachidonate metabolite in lymphocytes, can act as a signal to activate leukotriene production by susceptible mast cells.

STUDY OF LIPOPROTEIN LIPASE CONTENT IN Ob17 PREADIPOCYTES DURING ADIPOSE CONVERSION. IMMUNOFLUORESCENT LOCALIZATION OF THE ENZYME. C. Vannier, H. Jansen, R. Négrel and G. Ailhaud (Centre de Biochimie, CNRS-LP 7300, Universite de Nice, Parc Valrose, 06034 Nice, Cedex, France) *J. Biol. Chem. 257* (20):12387–12393 (1982). The development of lipoprotein lipase has been examined during adipose conversion of preadipocyte Ob17 cells. These cells have been previously shown to differentiate in clusters of fat cells. The lipoprotein lipase activity, which increases 20–50-fold during adipose conversion, is fully inhibited by anti-lipoprotein lipase γ-globulins. Monoacylglycerol lipase activity is not inhibited by anti-lipoprotein lipase antibodies and thus represents a different molecular entity. Identical curves for immunotitration are obtained for the lipoprotein lipase activity present in cell homogenates and for the heparin-releasable activity, indicating no significant difference in antigenicity between the different activities. Studies by indirect immunofluorescence reveal the absence of lipoprotein lipase in early confluent cells. Immediately thereafter adipose conversion is accompanied by the appearance of the enzyme in cells present in developing fat clusturs. A double labeling procedure using fluorescein and rhodamine immunoglobulin conjugates allows a topographical distinction between cell surface and intracellular lipoprotein lipase. Lipoprotein lipase activities are enhanced in confluent cells after chronic exposure to physiological concentrations of insulin and triiodothyronine. Immunotitration experiments lead to the conclusion that changes in activity correspond to parallel changes in enzyme levels. The results show a direct modulation by both hormones of the enzyme cell content of adipose cells in culture.

HYDROXYLATION OF PROSTAGLANDINS BY INDUCIBLE ISOZYMES OF RABBIT LIVER MICROSOMAL CYTOCHROME P-450. K.P. Vatsis, A.D. Theoharides, D. Kupfer and M.J. Coon (Department of Pharmacology, Northwestern University Medical and Dental Schools, Chicago, IL 60611) *J. Biol. Chem. 257* (19): 11221–11229 (1982). The hydroxylation of prostaglandin (PG) E_1, PGE_2, and PGA_1 was investigated in a reconstituted rabbit liver microsomal enzyme system containing phenobarbital-inducible isozyme 2 or 5,6-benzoflavone-inducible isozyme 4 of P-450, NADPH-cytochrome P-450 reductase, phosphatidylcholine, and NADPH. Significant metabolism of prostaglandins by isozyme 2 occurred only in the presence of cytochrome b_5. Isozyme 4 of P-450 differed markedly from isozyme 2 in that it catalyzed prostaglandin hydroxylation at substantial rates in the absence of cytochrome b_5, was regiospecific for position 19 of all three prostaglandins, and had an order of activity $PGA_1 > PGE_1 > PGE_2$. Cytochrome b_5 was required for maximal metabolism of all three prostaglandins, but did not alter the regionspecificity or the order of activity of P-450 isozyme 4 with the individual substrates. In the presence of cytochrome b_5, the prostaglandin hydroxylase activities of isozyme 4 were two to six times higher than those of isozyme 2.

STUDIES ON THE SUBSTRATE SPECIFICITY OF PURIFIED HUMAN MILK LIPOPROTEIN LIPASE. C.W. Wang, A. Kuksis and F. Mangaro (Lab. of Lipid and Lipoprotein Studies, Oklahoma Medical Res. Foundation, Oklahoma City, OK) *Lipids 17* (4):278–284 (1982). The fatty acid specificity of purified human milk lipoprotein lipase was studied using the C_{18} to C_{54} (total acyl carbon number) saturated and the C_{54} mono-, di- and triunsaturated monacoid triacylglycerols. Kinetic determinations indicated that the medium-chain triacylglycerols were better substrates than long- or very short-chain saturated triacylglycerols. The unsaturated triacylglycerols were hydrolyzed at rates comparable to that of tricaprylin with triolein having the highest rate of hydrolysis of the unsaturated more readily than the secondary ester bond. The purified humanmilk lipoprotein lipase showed a preferential stereospecific lipolysis of the *sn*-1-position of the triacylglycerol molecule.

CATALYTIC ACTIVITY OF PARTIALLY PURIFIED RENAL 25-HYDROXYVITAMIN D HYDROXYLASES FROM VITAMIN D-DEFICIENT AND VITAMIN D-REPLETE RATS. M. Warner (Dept. of Pharmac. and Therapeutics, McGill Univ., Montreal, Quebec, Canada U3G 1Y6) *J. Biol. Chem. 257* (21):12995–13000 (1982). A method based on affinity and hydrophobic chromatography has been developed for the partial purification of renal mitochondrial cytochrome P-450. 2,4-Dichloro-6-phenylphenoxyethylamine coupled to Sepharose 4B provides the chromatographic medium which in the presence of emulgen 911 and cholate retained P-450 but not most other mitochondrial proteins. P-450 was eluted from the column by increasing the detergent concentrations. The method allows for the spectral quantitation of the mitochondrial P-450 and provides starting material for further purification. The specific content of the P-450 eluted varied between 0.5 and 2 nmol/mg of protein. The concentration of P-450 in the renal mitochondria of control rats was 0.26 ± 0.2 nmol/g of kidney or 0.016 nmol/mg of mitochondrial protein; renal mitochondria from vitamin D-deficient rats contained similar amounts of P-450. The solubilized partially purified P-450 fraction catalyzed both the 1α- and 24-hydroxylations of 25-hydroxyvitamin D_3. In vitamin D-replete animals, the turnover numbers of the 1α- and 24-hydroxylation reactions were 0.38 and 0.18 pmol/pmol of P-450/30 min. In vitamin D deficiency, there was an increase in the turnover number of both the 1α- and 24-hydroxylations. The apparent K_m values of the 1α- and 24-hydroxylations were 50 and 25 nM, not different for control or vitamin-D deficient animals. SKF 525A inhibited these reactions by 70%. Metyrapone enhanced the 24-hydroxylase from vitamin D-replete animals by 100% but had no effect on the other three activities. Calcium causes a 2-fold stimulation of both hydroxylations while phosphate ions inhibited both reactions.

THE LIPID COMPOSITION OF HUMAN LIVER MICROSOMES. L. Waskell, D. Koblin and E. Canova-Davis (Department of Anesthesia, Veterans Administration Medical Center, and University of California, San Francisco, CA 94143) *Lipids 17* (4):317–320 (1982). The lipid composition of human liver microsomes isolated from liver biopsy samples obtained at abdominal surgery has been determined. Human liver microsomal phospholipid is composed of 49% phosphatidylcholine, 31% phosphatidylethanolamine, 14% phosphatidylserine + phosphatidylinositol and 6% sphingomyelin, very similar to the phospholipid composition of rat liver microsomes. The fatty acid composition of human liver microsomes is remarkable only for its content of polyunsaturated fatty acids, with 20% of the fatty acids consisting of arachidonic, docosatetraenoic, docosapentaenoic and docosahexaenoic acids. This value contrasts with 33% in rats and 9% in rabbits. The molar cholesterol/phospholipid ratio in human liver microsomes is 0.069, similar to the ratio in rat and rabbit microsomes.

EFFECT OF DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLE CURVATURE ON THE REACTION WITH HUMAN APOLIPOPROTEIN A-I. J.R. Wetterau and A. Jonas (Department of Biochemistry, School of Basic Medical Sciences and School of Chemical Sciences, University of Illinois, Urbana, IL 61801) *J. Biol. Chem. 257* (18):10961–10966 (1982). Large unilamellar vesicles of dipalmitoylphosphatidylcholine (DPPC) were prepared by sonication and were fractionated by gel filtration on Sepharose C1-2B in the size range from 180- to 380Å Stokes radii. Negatively stained electron micrographs of these preparations indicated the presence of unilamellar, spheroidal structures of the expected size. Fluorescence polarization of diphenylhexatriene, dissolved in the vesicles, revealed progressively broader phase transitions, shifted to lower temperatures for vesicles of decreasing sizes. The fractionated unilamellar vesicles and multilamellar vesicles of DPPC were reacted with human apolipoprotein A-I at 41°C for periods from 1 to 120 h. The reaction mixtures were then passed through a Bio-Gel A-5m column to separate unreacted lipid vesicles and protein from micellar complexes of DPPC with apolipoprotein A-I. Smaller vesicles were much more reactive than larger vesicles or multilamellar vesicles with the apolipoprotein. This difference in reactivity was explained by the increasing bilayer curvature of smaller vesicles which changes the packing of DPPC molecules in the bilayer and facilitates its penetration by the apolipoprotein.

PRODUCT ACTIVATION OF PANCREATIC LIPASE. LIPOLYTIC ENZYMES AS PROBES FOR LIPID/WATER INTERFACES.

Abstracts

T. Wieloch, B. Borgstrom, G. Pieroni, F. Pattus and R. Verger (Department of Physiological Chemistry, University of Lund, P.O. Box 750, S-220 07 Lund 7, Sweden and the Centre de Biochimie et de Biologie, Moléculaire, Centre National de la Recherche Scientifique, 31 Chemin Joseph Aiguier, 13274, Marseille Cedex 2, France) *J. Biol. Chem. 257*(19):11523-11528 (1982). During the action of pancreatic lipase and colipase on racemic 1,2-didodecanoylglycerol monolayers in the absence of bile salts, biphasic kinetics was observed under conditions of high lipid packing. Similar kinetics has earlier been reported using phospholipid-emulsified triolein droplets. These kinetics are characterized by a lag time τ_d, dependent on products accumulation at the substrate/water interface. This lag time is differentiated from the previously described enzyme concentration independent lag time τ_i in systems of low lipid packing. Both τ_i and τ_d reflect a rate-limiting step due to the slow enzyme penetration into the substrate interface. The variation of τ_d under different conditions (change in pH and concentration of Ca^{2+}, enzyme, bovine serum albumin, and lipolytic products) lead us to propose a model for the product activation during lipolysis. We will discuss the use of the pancreatic lipase-colipase system to probe the lipid packing of emulsified triglyceride particles and lipoproteins using τ_d as a reference value.

CLUSTERING OF ANTHROPOMETRIC PARAMETERS, GLUCOSE TOLERANCE, AND SERUM LIPIDS IN CHILDREN WITH HIGH AND LOW β- AND PRE-β-LIPOPROTEINS. BOGALUSA HEART STUDY. A.W. Voors, D.W. Harsha, L.S. Webber, B. Radhakrishnamurthy, S.R. Srinivasan and G.S. Berenson (Louisiana State Univ. Med. Ctr., 1542 Tulane Avenue, New Orelans, LA 70112) *Arteriosclerosis 2*(4):346-355 (1982). Children initially aged 2½ to 14 years living in Bogalusa, Louisiana (n = 2350) were examined twice, 3 years apart, for fasting serum pre-β- and β-lipoprotein cholesterol (β-LPC) levels. Based on averages of these levels, the children were ranked for pre-β- and β-LPC in combinations of extreme quintiles (low-low, high-high) or quartiles (low-high, high-low), n = 388, and were reexamined for serum lipids, lipoprotein cholesterol, glucose tolerance and anthropometry. Skinfolds were thicker in whites than in blacks except for subscapular skinfold. Children in the high-high stratum were heavier and more obese. The postglucose insulin level was positively correlated with fasting serum triglycerides and pre-β-LPC. Compared with other strata, high-high strata showed more clustering among half-hour and 1-hour plasma insulin, serum triglycerides and pre-β-LPC, and trunk skinfolds. We conclude that the racial differences in lipid and carbohydrate metabolism occur in all four strata, and that a strong clustering occurs more in the high-high strata, which may, in part, explain the coincidence of several high cardiovascular risk factor levels observed in the same children. These observations document in free-living children changes of obesity, plasma glucose, and insulin metabolism related to serum lipoproteins that are involved in the early natural history of atherosclerosis.

EFFECT OF EICOSATETRAYNOIC ACID ON LIVER AND PLASMA LIPIDS. R. Wood (Dept. of Biochem. and Biophys., Texas Agricultural Exper. Station, Texas A&M Univ. System, College Station, TX 77843) *Lipids 17*(11):763-770 (1982). Groups of rats were fed a fat-free diet supplemented with 0.5% safflower oil (control) or the control diet containing 0.5% of 5,8,11,14-eicosatetraynoic acid (TYA). Blood was collected weekly and plasma lipids analyzed. After 4 weeks, the animals were killed and the liver lipids analyzed in detail. The acetylenic fatty acid perturbed plasma neutral lipid and phospholipid class concentrations and reduced growth rates. Liver triglyceride concentrations were reduced dramatically in the TYA fed animals, suggesting interference with complex lipid synthesis. Plasma and liver triglycerides were shifted to higher molecular weight species suggesting that TYA affected fatty acid metabolism. The phospholipids showed an accumulation of 18:2 and a fall in 20:4 percentages indicating an inhibition in the conversion of linoleate to arachidonate. All major lipid classes exhibited an increase in 18:1 levels. Analysis of the octadecanoate positional isomers indicated the proportion of oleate increased substantially in all lipid classes whereas vaccenate proportions had fallen dramatically. All of the data collectively suggest that TYA inhibits the elongation of unsaturated fatty acids. A group of rats bearing hepatoma 7288CTC were also fed the TYA diet. Host liver lipids were affected by TYA similar to normal TYA fed animals, but the effects on hepatoma lipids were marginal.

EFFECT OF HEPATOMA ON HOST LIVER, HEART AND LUNG LIPIDS AS TUMOR GROWTH PROGRESSES. R. Wood, A. Zoeller and M. Matocha (Dept. of Biochem. and Biophys., Texas Agric. Exp. Station, Texas A&M Univ. System, College Station, TX 77843) *Lipids 17*(11):771-779 (1982). A large group of rats was transplanted with hepatoma 7288CTC and 4 animals were sacrificed at 3-day intervals for four weeks. Lipid class concentrations, fatty acid class compositions, and the distribution of *cis* octadecenoate positional isomers in the major lipid classes were determined for heart, liver and lung at each time period. The hearts of host animals decreased in dry weight as hepatoma growth progressed. At day 30, heart weights were less than two-thirds of initial weights. Lipid class concentrations changed in all three tissues: cholesterol and free fatty acids increased in liver; triglycerides and cholesterol decreased and then increased in heart; and cholesterol, triglycerides and PC decreased in lung as tumor growth progressed. Hexadecenoate percentages exhibited a progressive decrease in all the lipid classes of heart and liver. Although total octadecenoate percentages showed only minor changes, oleate concentrations generally increased and vaccenate levels decreased in heart and liver lipids as tumor growth progressed. Palmitoleate, precursor of vaccenate, exhibited decreased concentrations early that resulted in decreased vaccenate levels. Decreased palmitoleate concentrations suggest inhibition of the Δ9 desaturase system, but normal oleate concentrations complicate this interpretation. Most of the changes in the lipids were detectable 3-6 days after transplantation, indicating the hepatoma affects the lipid metabolism of the host animal early and well in advance of nutritional stresses.

CHOLESTERYL ESTER HYDROLASE ACTIVITY IN ADRENAL HOMOGENATES FROM NORMAL AND ESSENTIAL FATTY ACID-DEFICIENT FEMALE RATS. A.K. Young and B.L Walker (Dept. of Nutr., College of Biol. Sci., Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1) *Lipids 17*(9):634-638 (1982). Cholesteryl ester hydrolase was assayed in adrenal homogenates from mature female rats fed a control (corn oil-containing) or essential fatty acid (EEA)-deficient diet. Cholesteryl ester of 16:0, 18:0, 18:1, 18:2(n-6), 20:4(n-6) and 22:4(n-6) were used as substrates. In control rats, the unsaturated esters were hydrolyzed more rapidly than the saturated esters and cholesteryl arachidonate was the preferred substrate of the six investigated; cholesteryl oleate elicited the highest activity in the deficient group. Polyunsaturated esters were hydrolyzed at a significantly lower rate by homogenates from EFA-deficient rats than by those from control animals. The esters of 18:1, 18:2(n-6) and 20:4(n-6) were hydrolyzed more extensively in relation to their concentrations in adrenal tissue than were cholesteryl esters of 16:0, 18:0 and 22:4(n-6). This difference was more pronounced in control than in EFA-deficient rats. No simple relationship of adrenal cholesteryl ester hydrolase activity to ester fatty acid structure or to nutritional essentiality was evident.

RATIONALE FOR CHANGES IN THE DIETARY MANAGEMENT OF DIABETES. Barbara R.B. El-Beheri Burgess (M.P.H., R.D., Wesport, CN) *J. Am. Dietet. Assn.*, vol. 81:258 (1982). Although the pathogenesis of cardiovascular disease in diabetes is not completely understood, diabetes is frequently associated with hyperlipidemia, often considered a major determinant of atherosclerosis, and with hyperglycermia, which may function as an independent risk factor. The new higher carbohydrate diets for management of diabetes facilitate reduction in the proportion of fat kilocalories. When total kilocalories are controlled, improvement in glucose tolerance also occurs in individuals with diabetes who have available endogenous or exogenous insulin. It has recently been demonstrated in subjects with diabetes that a mixture of carbohydrate and fiber and a high, rather than low, level of carbohydrate facilitate glycemic control. Includion of fiber-rich foods in meal plans for patients with diabetes augments established modes of therapy, which focus on weight control for Type II diabetes while synchronizing food intake and insulin for Type I diabetes.

EFFECT OF DIETARY ERUCIC ACID ON THE METABOLIZABLE ENERGY VALUE OF THE DIET FOR POULTRY. Ayodhya Prasad and P.V. Rao (Central Avian Research Institute, Izatnagar, U.P. 243122) *Indian J. Nutr. Dietet.*, vol. 18:455 (1981). The role of dietary mustard erucic acid in depressing feed consumption and growth rate in chicks was examined in chicks fed rations containing pure erucic acid replacing groundnut oil in a control diet at 0.605, 1.210 and 1.815 percent. The metabolizable energy content of erucic acid diets was comparable statistically to that of the control, suggesting that the deleterious nutritional responses with the fatty acids were not mediated through a depression in the metabolizability of dietary energy.

FAT AND CHOLESTEROL INTAKES OF WHITE ADULTS IN COLUMBIA, MARYLAND. Katherine M. Salz, R.D., Nancy Z. Haigh, R.D., Gary A Chase, Ph.D. and Peter O. Kwiterovich, Jr., M.D. (Lipid Research-Atherosclerosis Unit, Lipid Research Clinic, Departments of Pediatrics and Medicine, Johns Hopkins University School of Medicine, Baltimore, MD) *J. Am. Dietet. Assn.*, vol. 81: 541 (1982). In a cross-sectional study of white adults with upper-

level socioeconomic status (in Columbia, Maryland), intakes of total, saturated, and polyunsaturated fat and of cholesterol were assessed. The intakes were compared with dietary recommendations made by the Inter-Society Commission for Heart Disease Resources, the American Heart Association, and the Senate Select Committee. Only moderate dietary changes were needed for a menu modification that conforms to the dietary recommendations for men. If the group studied reflects the impact of public health nutrition education, areas in need of attention seem to be a reduction in the amount and a change in the quality of dietary fat.

Fats and oils

A COMPARISON OF SEED PHOSPHATIDES AND SYNTHETIC COMPOUNDS AS ANTIOXIDANTS FOR COW AND BUFFALO GHEE (BUTTER FAT). Narinder Kaur, Pritam S. Sukhija and Iqbal S. Bhatia (Department of Biochemistry, Panjab Agricultural University, Ludhiana, India) *J. Sci. Food Agric.*, vol. 33:576 (1982). The antioxidant capacity of seed phosphatides and synthetic antioxidants when compared in cow ghee was found to be in the order: phosphatidyl ethanolamine > propyl gallate > palmitoyl ascorbate > butylated hyroxy anisole > phosphatidyl choline. Phosphatidyl ethanolamine was found to be the most effective antioxidant. Cow ghee had less peroxide development than buffalo ghee. The ghee prepared at 100°C was more stable against peroxide development compared with that prepared at 50°C. These observations were supported by the analysis of ghee samples for peroxide values and for fatty acids. The phosphatides imparted more antilipolytic activity to ghee than to synthetic antioxidants.

FILM CHARACTERISTICS OF LINSEED EPOXY ESTERS PREPARED FROM NOVOLAC-BASED POLYEPOXIDE RESINS. A.K. Vasishtha and D. Agrawal (Department of Oil and Paint Technology, Harcourt Butler Technological Institute, Kanpur 208002, India) *J. Oil. Col. Chem. Assn.*, vol. 65:276 (1982). Expoxy esters were prepared by reacting linseed oil fatty acids with polyepoxide resins based on phenol-formaldehyde novolac resins. Samples were prepared with polyepoxides of different epoxide equivalents and were compared with those based on bisphenol A; the films of novolac-based epoxy esters were found to have better resistance to alkali and acid. The water resistance of all the epoxy ester films was found to be good.

A STUDY OF SEEDS OF MUSK MELON (*CUCUMIS MELO* L.): A LESSER KNOWN SOURCE OF EDIBLE OIL. Tilak R. Madaan, Tukaram A. More, Brij M. Lal and Valangaman S. Seshadri (Division of Biochemistry and Division of Vegetable Crops and Floriculture, Indian Agricultural Research Institute, New Delhi 110012, India) *J. Sci. Food Agric.*, vol. 33:973 (1982). The oil content and oil characteristics of seeds of 91 accessions of musk melon (*Cucumis melo*), including two allied species collected from different parts of the world, have been studied to assess their variability. Oil content (on a whole seed basis) ranged from 12.5 to 39.1% and iodine values from 106.0 to 124.1. Gas liquid chromatographic analysis showed that only linoleic, oleic, palmitic and stearic acids were present. Unsaturated fatty acids constituted 64.6–88.2% of the total fatty acids present. The proportion of kernel in the seeds of different accessions, varied from 25.0 to 74.1% and contributed significantly to the total oil content of the whole seed.

THE PROTEIN, TRYPSIN INHIBITOR AND LIPID OF THE WINGED BEAN (*PSOPHOCARPUS TETRAGONOLOBUS* (L) DC) SEEDS. Hun-Teik Khor, Nget-Hong Tan and Kai-Choo Wong (Department of Biochemistry, University of Malaya, Kuala Lumpur and Department of Agronomy, Agriculture University of Malaysia, Serdang, Selangor, Malaysia) *J. Sci. Food Agric.*, vol. 33:996 (1982). The protein, trypsin inhibitor and lipid of the seeds from 15 New Guinea and 15 Thai winged bean varieties grown in Malaysia were analyzed. The results show that winged bean seeds have a high protein content, ranging from 27.8 to 47.2% (based on dry seed wt). The trypsin inhibitor contents vary from 1.6 to 3.6 million i.u. 100 g^{-1} of seeds; these trypsin inhibitor activities could be destroyed almost completely by a simple heat treatment. The oil content varies from 15.2 to 27.8% of the dry seed wt. Saturated fatty acids account for 31–37% of the total fatty acids and behenic acid (22:0) alone constitutes about 14–17% of the saturated fatty acid content. Unsaturated fatty acids account for 63–69% of total fatty acids; oleic and linoleic acids together constitute 57–64% of the unsaturated fatty acids.

FATTY ACID COMPOSITION OF *HIBISCUS FICULNEUS* SEED OIL. Sarita Sinha and Sheikh M. Osman (Department of Chemistry, Aligarh Muslim University, Aligarh-202001, India) *J. Sci. Food Agric.*, vol. 33:1010 (1982). The fatty acid composition of seed oil from *Hibiscus ficulneus* (Malvaceae) was analyzed by thin-layer and gas-liquid chromatography. In addition to normal saturated and unsaturated fatty acids, three hydrogen bromide-reactive fatty acids were also identified. These were shown to be epoxyoleic (4.9%), malvalic (4.2%), and sterculic (1.0%) acids. Seed oils of *Vernonia anthelmintica* and *Sterculia foetida* were used as reference standards.

DIRECT SPECTROPHOTOMETRIC DETERMINATION OF IRON IN VEGETABLE OILS. Oi-wah Lau and Chuen-shing Mok (Department of Chemistry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong) *J. Sci. Food Agric.*, vol. 33:1030 (1982). A simple and direct spectrophotometric method for the determination of iron in vegetable oils has been developed. The proposed method is based on the aqueous 1,10-phenanthroline method, where propionic acid is used as solvent for the vegetable oils and the iron(II) 1,10-phenanthroline complex is formed directly in propionic acid with an absorption maximum at 514 nm.

THE ABSENCE OF ENDOGENOUS LIPASE FROM OIL PALM MESOCARP. Michael P. Tombs and J. Morriss Stubbs (Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ) *J. Sci. Food Agric.*, vol. 33:892 (1982). Oil palm (*Elaeis guineensis* Jacq.) fruit mesocarp had higher levels of free fatty acids at the ends of the fruits than in the middle; and microscopy showed that yeasts penetrate the mesocarp via the scars at each end. Very low levels of esterase activity, probably not due to a lipase, were detected in mesocarp extracts by using 4-methyl umbelliferone esters as substrates, but no activity against triacylglycerols could be found. In bruising and storage trials, the only variable that correlated ($P < 0.01$) with free fatty acid level was yeast and mould count. It is concluded that yeasts and moulds are the source of lipolytic activity in oil palm mesocarp, and there is no evidence for an endogenous lipase.

THE ANALYSIS OF POLYUNSATURATED FATTY ACIDS IN MEAT BY CAPILLARY GAS-LIQUID CHROMATOGRAPHY. Andrew J. Sinclair, William J. Slattery and Kerin O'Dea (Department of Agriculture, Veterinary Research Institute, Park Drive, Parkville, Victoria 3052, Australia and Baker Medical Research Institution, Melbourne, Australia) *J. Sci. Food Agric.*, vol. 33: 771 (1982). Polyunsaturated fatty acids (PUFA) of lean meat from domesticated and wild ruminants (cattle, sheep, goat, sambar deer and buffalo) and non-ruminant (pig, horse, and kangaroo) have been examined by capillary gas-liquid chromatography. Ten different PUFA were found in all specimens with linoleic acid accounting for at least 50% of the total, and arachidonic and linolenic acids being the next most abundant. The total PUFA content for the ruminants ranged from 9% in beef to 31% in sambar deer and for the non-ruminants from 25% in pig to 43% in horse. In all species the meat phospholipids (PL) were rich in PUFA (range 24–46% of PL fatty acids), whereas the triglycerides were relatively more saturated (PUFA content range 2–17%). Overall, horse and kangaroo meat had the combination of lowest fat and highest PUFA content, whilst beef and sheep had the highest fat and lowest PUFA content. These results indicate that significant reductions in total fat intake and increases the proportion of polyunsaturated fat in the diet could be achieved without necessarily requiring a diet low in meat.

COMPONENT ACIDS OF SOME ORNAMENTAL SEED OILS. C.D. Daulatabad, R.F. Ankalgi and J.S. Kulkarni (Department of Chemistry, Karnatak University, Dharwad-580 003, India) *J. Food Sci. & Tech.* (India), vol. 19:110 (1982). Seed oils of *Muntingia calabura, Dracaena reflexa, Indigofera wightii*, were found to contain (wt. %) the following acids: capric 2.4, 0.1, nil; lauric 1.4, 0.8, 4.6; myristic 1.9, 1.7, 4.2; palmitic 19.1, 15.0, 29.1; stearic 10.0, 6.1, 12.1; arachidic 1.4, 2.0, 2.2; behenic 1.1, 2.2, 2.5; oleic 18.6, 41.2, 6.6; and linoleic 44.1, 30.8, 38.7, respectively.

MINOR SEED OILS I. COMPONENT FATTY ACIDS OF SOME SEED OILS. C.D. Daulatabad and R.F. Ankalagi (Department of Chemistry, Karnatak University, Dharwad-580 003, India) *J. Food Sci. & Tech.* (India), vol. 19:112 (1982). Seed oils of *Fluggea microcarpa, Ixora parviflora*, and *Diospyros melanoxylon* were found to contain (wt. %) the following acids: capric nil, 1.3, nil; lauric nil, 3.1, 4.6; myristic 5.4, 4.7, 9.9; palmitic 19.8, 11.4, 37.9; stearic 9.7, 11.9, 16.1; arachidic 1.4, 2.9, 5.3; behenic 1.2, 2.0, 5.3; oleic 6.1, 18.7, 19.7 and linoleic 56.4, 44.0, 1.2, respectively.

Abstracts

EXTRACTION AND FRACTIONATION OF LIPIDS AND OTHER NATURAL PRODUCTS WITH LIQUID AND SUPERCRITICAL GASES. E. Stahl, K.W. Quirin and N. Totani, *Rev. Franç. Corps Gras. 29*(6-7):259-263, 1982, french. RFCG 82-20. Varying the operational parameters pressure and temperature allows for adapting the solvent properties of compressed gases in a manner as to facilitate selective separating and fractionating processes. The critical pressure and temperature data of carbon dioxide (Tc = 31.2 C, Pc = 73.8 bars) allow for a gentle extraction of thermally unstable substances. The procedure, if used in the extraction of oilseeds, offers the advantage of the seed proteins not being denaturated with the extracted oils free of phospholipids. Carbon dioxide is plentiful available at reasonable prices; it is not inflammable and free of accompannying material which could be regarded as physiologically dangerous. Carbon dioxide can be gently and completely removed from substrate and extract with little energy and without pollution. The procedure of the high pressure extraction has been tested on a micro and on a pilot scale; it is subject of many patents and patents pending and its use on a technical scale has been introduced.

FATS AND OILS IN ANIMAL NUTRITION. F. Hauzy, *Rev. Franç. Corps Gras. 29*(6-7):265-268, 1982, french. RFCG 82-21. The different factors: economical, technical and nutritional promoting the use of animal fats in animal nutrition are reviewed. The standards of products used according to their nutritional applications are defined, as the tonnages of now used different animal fats. At last, the specifications and their inadequation in relation to needs are criticized; "relaxed" specifications for animal fats and "purist" specifications in the case of used fats in feed of veals.

ON THE REFINABILITY OF OILS. IX. – QUALITY OF REFINED OILS AND CHARACTERISTICS OF RAW OILS. M. Naudet, E. Sambuc and G. Devinat, *Rev. Franç. Corps Gras. 29*(6-7):269-276, 1982, french. RFCG 82-22. The partial results of antecedent studies have been compiled and compared with experimental tasting scores in the case of 35 new rapeseed oils and 33 soybean oils refined in laboratory in established conditions. As consequence, it is possible to foresee, from some carefully selected characteristics of a raw oil, if the refined oil will have or not a predefined tasting score in selecting conditions.

MEASURE OF FOAMING IN BIOLOGICAL AEROBIC MEDIA AND CONTROL OF ANTI-FOAMING POWER. C. Chasseboeuf, G. Lemaire, J. Pore and J.F. Mescle, *Rev. Franç. Corps Gras. 29*(6-7): 277-279, 1982, french. RFCG 82-23. A simple and movable apparatus for dynamically controlling foam has been developed. The superficial and occluded foam is formed by circulation of liquid through a variable flow-pump with possible simultaneous introduction of some air by an immersed diffuser. The temperature of media can be regulated to favour the biological development of studied strains. Two methods are possible: test by circulation with or without aeration.

ALIEN INCORPORATION IN GROUNDNUT *ARACHIS HYPOGAEA* L. M. Bharathi, U.R. Murty, P.B. Kirti and N.G.P. Rao, *Oléagineux 37*(6):301-306, 1982. Iriploid (*2n* = 30) interspecific hybrids were produced between 9 cultivated varieties of *Arachis hypogaea* L. and *A. chacoense*, a diploid (*2n* = 20) wild species with recorded resistance to leaf spot, rust, thrips and aphids. The hybrids ressembled morphologically the groundnut parent used. The hybrids exhibited varying chiasma frequencies showing the possibility of obtaining desirable segregates. Restitution of chromosomes occurred on the female side. It was concluded that production of triploid hybrids, selection for fertile types and isolation of desirable types provides a rapid method for alien incorporation in groundnut.

OBSERVATION OF VEGETATIVE DEVELOPMENT, FLOWERING AND YIELD CHARACTERISTICS OF THE COCONUT. M. de Nucé de Lamothe, W. Wuidart, *Oléagineux 37*(6):291-300, 1982. All breeders have been acutely aware for some time of the need for a method to describe coconut populations. This article aims at showing what the I.R.H.O. has established in the field, specifying the characters observed and the methodology employed. The first requirement to know a given population is to have enough (100-120) trees planted, and then to observe the characters which may aid the breeder in one area or another from the seed bed to the bearing stage. The characters are separated into 3 groups: vegetative development (germination of seeds, development of the plant and the tree during the non-bearing and bearing stages, precocity of flowering), flowering (male and female stages, inflorescence) and yield (number of bunches and nuts, fruit characteristics). All this data is collected, and the results put together on a record card for each population. A consensus should be arrived at as to the methods and techniques employed, so that results obtained by various Research Centres all over the world can be compared. The authors think that I.B.P.G.R. is the most suitable institution to catalyze efforts to this end.

IMPORTANCE OF BALANCED MANURING ON A YOUNG PALM GROVE IN NORTH SUMATRA. B. Tailliez, *Oléagineux 37* (6):271-281, 1982. In a factorial mineral nutrition trial set up on a North Sumatra palm grove from the time of planting on, five deficiencies, namely N, P, K, Mg and B appeared less than a year after planting. The symptoms which exteriorise each of these deficiencies are described, while the effects of the various fertilizers on nutrition and growth are analyzed. The application of potassium chloride played a role in preventing the appearance of a double precocious deficiency in both potassium and boron; the presence of white spots on young, shorter leaves (incipient little leaf) may be attributed to a boron deficiency, whereas a whole range of yellowings and necroses on the leaves in the middle of the crown would be directly related to potassium deficiency. Urea and rock phosphate, which promote better growth, induce or exacerbate this double deficiency. Generally speaking, there is a close relationship between the symptoms observed, nutrition as evaluated by leaf analysis, and growth of the young trees, but leaf boron levels are no indication as to whether or not this trace element is lacking. Proposals are put forward in an attempt to work out better balanced, more rational fertilizer formulae for young plantations.

IMPROVEMENT OF THE NUTRITIVE VALUE OF OLIVE OIL CAKE BY SODIUM CARBONATE TREATMENT. C. Vaccarino, M.M. Tripodo, A. de Gregorio, F. Salvo et G. Lagana, *Oléagineux 37*(6):307-311, 1982. Caustic soda treatments, proposed for increasing the digestibility of wheat straw and other vegetal wastes, are scarcely effective on deoiled olive husks. On the other hand, they cannot be made on the oily husks before solvent extraction, because caustic soda would destroy all their lipidic content. In the present article the results of an experimental work are reported in which oily olive husks are treated with sodium carbonate. This salt is able to attack to an even greater extent than caustic soda, the bounds which make not "available" both cellulosic materials and proteins contained in olive husks, but does not saponify the glycerides (as it neutralizes only the f.f.a.). In this way, after the subsequent solvent extraction, a double advantage is achieved, i.e. a light colored and neutralized oil and a residue which, after the separation of the stones, may be used as a valuable feedstuff for ruminants. An industrial development of this system seems to be suitable.

CONTRIBUTION OF THE OIL INDUSTRY TO THE QUALITY OF OILMEALS. G. Vermeersch, *Rev. Franç. Corps Gras. 29*(8-9): 311-318, 1982, french, RFCG 82-24. The tonnages of different oilseeds both produced in France and processed in France are reviewed; the schemes for crushing soybean, rapeseed and sunflower are described and also the storage and cleaning in emphasizing the heat treatments; their aims, characteristics and effects and effects on the quality of oilmeals. The peculiar of every oilseed problems: sulfur compounds in rapeseed, antitrypsic factors in soybean are discussed. At last, the researches aimed at the quality of oilmeals are reviewed.

STUDIES ON THE SOLUBILIZATION OF ASCORBYL PALMITATE IN THE VEGETABLE OILS. C.F. Bourgeois, A.M. Czornomaz and P. Pages, *Rev. Franç. Corps Gras. 29*(8-9):319-324, 1983, french, RFCG 82-25. The antioxidant properties of ascorbyl palmitate (A.P.) and other ascorbic acid esters have been known for a long time. Morever, these compounds are able to synergize many other conventional, natural and tocopherols or synthetic, antioxidants. At last, their physiological innocuousness make them very attractive food additives. But, their rather poor solubility in vegetable oils hampered a large use in food technology till now. In this article, it is shown that fair amounts of A.P. (500 ppm) can be solubilized in the sunflower oil which is yet very unsaturated.

THE MARGARINE IN A TURNING-POINT OF ITS HISTORY? M. Fondue, *Rev. Franç. Corps Gras. 29*(8-9):325-328, 1982, french, RFCG 82-26. The history of margarine since its creation is recalled. The conception of this product has been constantly changed in order to correspond to technological, sensorial and nutritional criteria. The present trend seems in favor of low fat margarines, but their commercialization would set many problems.

STUDY ON THE ANTIOXYDANT ACTIVITY AT 75 C of BHA, BHT, TBHQ AND THEIR MIXTURES ON A REFINED CORN OIL AND A VIRGIN OLIVE OIL. C.D. Thomopoulos and H.P. Grigoropoulou, *Rev. Franç. Corps Gras. 29*(8-9):329-331, 1982, french, RFCG 82-27. The antioxidant activity ot TBHQ and of its mix-

tures with 0.05-0.20% BHA and BHT on heated at 75 C refined corn oil and virgin olive oil is studied. The results show that, at equal concentration, TBHQ is more efficient than BHA and BHT used separately and also than its mixtures with BHA and BHT. The mixtures TBHQ + BHA are more efficient than TBHQ + BHT at equal concentration, though alone BHT is more efficient than BHA.

EFFECT OF LONG-TERM FEEDING OF A COCONUT OIL-BASED DIET AND A COMBINED COCONUT OIL-FISH DIET ON SERUM CHOLESTEROL AND FATTY ACID COMPOSITION OF DIFFERENT ORGANS OF ALBINO RATS. P.G. Viswanathan Nair and K. Gopakumar (Dentral Institute of Fisheries Technology, Cochin-682 029) *J. Food Sci. & Tech. (India) 18*:187, 1981. The influence of oil sardine (*Sardinella longiceps*) and coconut oil together on the fatty acid composition of various organs and on the serum cholesterol level of albino rats was studied. Fatty acid pattern of the different organs was affected to varying extents by the dietary fatty acids. The level of arachidonic acid in the liver was found to be independent of the dietary supply of this acid, but appeared to be related to the level of linoleic acid in the diet. Dietary sardine was found to be very effective in lowering the serum cholesterol level even in the presence of a cholesterol-elevating agent such as coconut oil. This effect can be due to the high content of polyunsaturated acids in fish lipids as well as the nature of fish proteins.

TRIACYLGLYCEROL COMPOSITION OF COW MILK FAT. C. Arumughan and K.M. Narayanan (National Dairy Research Institute, Karnal-132 001, India) *J. Food Sci. & Tech. (India) 19*:71, 1982. The triacylglycerols (TG) of cow milk fat were separated first into high molecular weight TGs (HMT), medium molecular weight TGs (MMT), and low molecular weight TGs (LMT). These were further resolved into saturated *trans* monoene, diene and polyene TGs using argentation thin layer chromatography (TLC). The HMT contained mostly the long chain fatty acids whereas most of the short chain acids of the milk fat were concentrated in LMT. The TG species separated on the basis of their degree of unsaturation had differing combination of fatty acids.

SEPARATION OF KERNEL FROM SHELL IN FRUIT-STONE PROCESSING. G.S. Martchouk. *Maslozhir. Prom. 6*:10-02 (1981). The hydroseparation of products from fruit-stone decortication using sodium chloride solution of various densities has been studied. The replacement of kernel-shell separation on aqueous salt solution by a process using an electrostatic field is justified here.

USE OF "NEFRAS 65/70" INSTEAD OF EXTRACTION ESSENCE. G.V. Donskova et al. *Maslozhir. Prom. 11*:40 (1981). The use of this solvent (it is an essence [volatile oil] which boiling limits are in a 65-70 C range) will allow improvement of the quality of the extraction oil and reduction of its loss in the refining, reduction in the occurrence in the oil of coloring matters, resins, waxes, polymerization on oxidation products, improvement in the quality of extraction oil cake, and reduction in the loss of solvent.

SOYBEAN OIL AS A COMPONENT OF LIPIDIC EMULSION. S.N. Volotovskaya et al. *Maslozhir. Prom 12*:16-18 (1981). The results of the use of soybean oil in lipidic emulsion for intravenous injections are given. Lipidic emulsion with soybean oil from subspecies Yantarnaya and Ranniaia-10 meets the requirement related to intravenous preparations. The fatty acid composition of the oils has been shown not to affect the biological properties of lipidic emulsions.

FABRICATION BYPRODUCTS AND PHYTOSTEROLS RESOURCE. L.T. Prokhorova et al. *Maslozhir. Prom. 12*:11-13 (1981). Information about the content and the composition of phytosterols from byproducts of cotton and sunflower treatment (soapstock, deodorization distillate, used destaining earth) are provided. The resources of phytosterol in these products are quantitated.

ANTIOXIDATIVE ACTIVITY OF SUNFLOWER SHELL. L.A. Zhorina, *Maslozhir. Prom. 12*:8-10 (1981). The antioxidative activity of hydrosoluble "melanoidic" (brown) matters in the sunflower shell protecting the seed from natural radiation is shown. The influence of solar activity on this property is demonstrated statistically. Techniques to use those components as antioxidative agents are given.

STATISTICAL STUDIES OF SUNFLOWER SEEDS QUALITY. E.P. Koshevoi et al. *Maslozhir. Prom. 12*:6-8 (1981). Statistical studies of sunflower seed quality indexes have been done and correlations between them proved. It was shown that the index of acid in the seed oil can be used to make homogenous lots of seeds. The sunflower seed repartition according to the index of oil acid content was following a normal distribution, the parameters of which were dependent on the year conditions.

ANTIOXIDATIVE ACTIVITY OF SOME TOCOPHEROL DERIVATIVES. R.K.M. Khafizov et al. *Pishtch. Tekhnol. 6*:40-43 (1981). The relative antioxidative activity of tocopherol derivatives depends on the type of substrate and the concentration of the inhibitor introduced. The optimal concentration of tocopherols for vegetable oils is in a 40-70 mg/g range and for butter over 50 mg/g. Among the tocopherol derivatives, the best inhibitors for vegetable oils are the derivatives, the best inhibitors for vegetable oils are the derivatives of δ- and γ-tocopherols, for butter: propyl-β-tocotrienol and δ-tocopherol.

INFLUENCE OF HYDROTHERMIC TREATMENT ON THE LOCATION OF OIL IN SUNFLOWER SEED CELLS. P.P. Demtchenko et al. *Maslozhir. Prom. 2*:18-20 (1982). The action, on sunflower seed kernel, of temperature, of treatment length and especially of the variation of humidity rate on the particles with overload leads to a modification on cellular structure. The cell wall, the lipidic and proteic inclusions are destroyed. The use of such an intense effector as water steam under 49-98.1 kP pressure is comparable to the mechanical deformation of the kernel with cylinders machines processing. It is not reasonable to go higher on pressure than 98.1 kPa.

AIR COOLING MACHINE. I.E. Bezinglov et al. *Maxlozhir. Prom. 12*:37-39 (1982). In the apparatus for cooling by air, the temperature of hot effluent waters is lowered from 85-90 C down to 40 C and the essence content in these waters is highly decreased. The air consumption to cool down 1 m^3 of hot effluent waters is about 1500 m^3/hr.

SUNFLOWER TREATMENT EFFICIENCY ACCORDING TO THE TECHNOLOGICAL SCHEME. Y.P. Matsouk et al. *Maslozhir. Prom. 2*:29-33 (1982). The increase of 1% on the shell content of the kernel has been shown to decrease at 2-2.5% the potential capacity of the factory. These data have been confirmed at the Vinnitsa oil factory, where switching to process undecorticated sunflower seed lead to a 20% decrease in the factory output.

SYNTHESIS AND PHYSICAL PROPERTIES OF PHOSPHATIDYLCHOLINES CONTAINING ω-CYCLOHEXYL FATTY ACYL CHAINS. T. Endo, K. Inoue, S. Nojima, S. Terashima, and T. Oshima (Department of Health Chemistry, The University of Tokyo, Hongo, Tokyo 113, Japan) *Chem. Phys. Lipids 31*(1):61-74 (1982). Phosphatidylcholines (PCs) with cyclohexyl fatty acids as acyl chains were synthesized from 11-cyclohexyl and 13-cyclohexyl fatty acids and their physical properties were examined. The thermotropic behavior and barrier function of liposomal membranes formed from these PCs were xtudied. These PCs showed about 10 C lower gel-to-liquid crystalline phase transition temperatures (T_c) than the corresponding straight-chain PCs. The properties of mixtures of these cyclohexyl acyl PCs with straight-chain PCs were rather different from those observed with mixtures of straight-chain PCs. Cyclohexyl fatty acyl PCs showed barrier functions even above the T_c unlike the corresponding straight-chain PCs. These results indicate significant differences between the overall packing of cyclohexyl fatty acyl PCs and of the corresponding straight-chain PCs both in the gel state and in the liquid crystalline state. The significance of these cyclohexyl acyl chains in polar lipids, which are abundant in the thermophilic acidophilic bacterium, *Bacillus acidocaldarius*, is discussed.

D-ALANINE ESTER-CONTAINING GLYCEROPHOSPHOGLYCOLIPIDS IN THE MEMBRANE OF GRAM-POSITIVE BACTERIA. W. Fischer (Inst. für Physiologische Chemie, Universitat Erlangen-Nurnberg, Fahrstrasse 17, D-8520 Erlangen, (F.R.G.) *Biochim. Biophys. Acta 711*(2):372-375 (1982). 1,2-Di-*O*-acyl-3-*O*-(6-3(2)-D-alanyl-*sn*-glycerol-1-phospho)-β-D-glucopyranosyl(1-6)-β-D-glucopyranosyl)-glycerol was isolated and characterized from *Bacillus licheniformis*. D-Alanylated glycerophospho- and di(glycerophospho)glycolipids were also identified in *Streptococcus lactis*.

SULFITE-INDUCED OXIDATION AND BROWNING OF LINOLENIC ACID. O. Lamikanra (Procter Dept. of Food Sci., Univ. of Leeds, Leeds LS2 9JT, United Kingdom) *J. Food Sci. 47*(6):2025-2027 (1982). The oxidation and browning of linolenic acid during sulfite oxidation in 80% ethanol were measured by both oxygen uptake and spectrophotometric methods. The addition of a combination of Mn^{++} and glycine accelerated sulfite oxidation, the fatty acid oxidation and its consequent browning. Butylated hydroxytoluene effectively inhibited the reactions, suggesting free radical involvement.

Abstracts

THE EFFECT OF SUNFLOWER OIL ON THE FATTY ACID COMPOSITION OF THE MILK OF COWS FED EITHER A FAT-DEPRESSING DIET OR GRASS SILAGE. J.L. Clapperton (The Hannah Research Institute, Ayr KA6 5HL) *J. Sci. Food Agric.*, vol. 33:741 (1982). Two experiments have been carried out in which different forms of sunflower oil were added to the diet of Ayrshire heifers. In experiment 1, the animals were given a fat-depressing diet of dried, ground grass cubes and flaked maize. When either free sunflower oil or milled, unextracted sunflower seeds were added to this diet, the yield of milk fat and of all fatty acids up to 16:1 was decreased and the yield of all 18-carbon fatty acids was slightly increased. When the sunflower oil was protected by encapsulation in formaldehyde-treated casein, the yield of milk fat was increased, that of the fatty acids up to 16:1 was decreased and that of all the 18-carbon fatty acids, and in particular, of the polyunsaturated fatty acids was increased. In experiment 2, the animals were given a basal diet of grass silage and concentrates to which increasing amounts of protected sunflower oil were added. This tended to increase the yield of milk fat and to reduce that of the fatty acids up to 16:1. The yield of all the 18-carbon fatty acids increased and, in particular, that of the polyunsaturated fatty acids increased progressively as more protected oil was added. It is concluded that it should be possible to produce a milk with any desired proportion of polyunsaturated fatty acids by adding a predetermined amount of protected oil to the diet of the cow.

A COMPARISON OF THE INTERFACIAL BEHAVIOR OF THREE FOOD PROTEINS ADSORBED AT AIR-WATER AND OIL-WATER INTERFACES. Eva Tornberg, Yvonne Granfeldt and Charlotte Håkansson (Department of Food Technology, University of Lund, Box 740, S-220 07 Lund 17, Sweden) *J. Sci. Food Agric.*, vol. 33: 904 (1982). The adsorption behaviour of three food proteins—a soya protein isolate, a sodium caseinate and a whey protein concentrate—at a soya bean oil-water interface has been studied by the drop volume method. The interfacial behaviour has been compared with that at an air-water interface. The kinetics of surface tension decay were evaluated in terms of different rate-determining steps at different ionic strengths and concentrations of the proteins. The ranking order with respect to the surface activity of the proteins adsorbed at an air-water interface was the same as that at a soya bean oil-water interface. In the high concentration range the surface activity of the proteins was higher at an air-water interface than at a soya bean oil-water interface, whereas the reverse was found in the low concentration range. In general, the adsorption of the proteins was more diffusion controlled at an air-water interface than at a soya bean oil-water interface; this suggested that proteins were less folded at the soya bean oil-water interface. A comparison of the rates of the diffusion controlled steps for the proteins at air-water and soya bean oil-water interfaces indicated that the solvation energy gained when caseinates adsorb at the soya bean oil interface was enhanced compared with the other two proteins. This indicated an enhanced loop formation of the casseinate molecules in the oil phase when adsorbing at this interface, as compared with the air-water interface.

THE ASSOCIATION OF SATURATED AND UNSATURATED TRIGLYCERIDES AND OLEIC ACID WITH WHEAT FLOUR COMPONENTS DURING DOUGH MAKING. Michael J. Warwick and George Shearer (Ministry of Agriculture, Fisheries and Food, Food Laboratory, Colney Lane, Norwich NJ4 7UA) *J. Sci. Food Agric.*, vol. 33:918 (1982). The association of lipids with other flour components after dough making has been examined by aqueous fractionation of flour prior to the extraction of lipids. Glycerol tripalmitate has been shown to be associated with acetic-acid-insoluble protein while glycerol trioleate associates with the glutenin of acetic-acid-soluble protein. Oleic acid divides fairly evenly between these fractions. Changes in lipid and protein distribution in some fractions are associated with the addition of oleic acid to the dough. Some of these changes are at least partially reversed by increasing the triglyceride content of the dough.

THE DISTRIBUTION OF ACYL LIPIDS AND TOCOPHEROLS IN FLOURS MILLSTREAMS. William R. Morrison, Anne M. Coventry and Peter J. Barnes (Food Science Division, Department of Bioscience and Biochemistry, University of Strathclyde 131, Albion Street, Glasgow G1 1SD and RHM Research Ltd., The Lord Rank Research Centre, Lincoln Road, High Wycombe, Bucks HP12 3QR) *J. Sci. Food Agric.*, vol. 33:925 (1982). Kernels from a mixed hard wheat grist were dissected into germ, bran (pericarp, testa and aleurone), and starchy endosperm for direct analysis of tocopherols in lipid extracts by high-performance liquid chromatography. α- and β-tocopherols were almost exclusively in the germ, α-tocotrienol was mostly in the bran, and β-tocotrienol was equally distributed between the bran and the starchy endosperm. Acyl lipids and tocopherols were quantified in 23 millstreams obtained from this grist. Components related to germ and bran (triglyceride, diacylphospholipids, α- and β-tocopherols) and testa (flour colour) showed the highest coefficients of variation whereas endosperm components (glycolipids, *N*-acylphospholipids and β-T-3) showed exceptionally low variation. The quantities of marker tocopherols in the streams were used to calculate the composition of the lipid transferred to the flour from germ and aleurone, and to predict the composition of the basic endosperm, free of aleurone and germ lipids. Low proportions of diacylphospholipids in the lipid transferred to high-grade millstreams indicated the transfer of sepherosome lipid. The low-grade streams exhibited high proportions of phospholipids suggesting additional transfer of germ tissue and aleurine tissue containing membrane lipids. Protein and ash contents of the transferred fraction confirmed that a substantial proportion of the transferred lipid was probably accompanied by protein bodies or by tissue fragments. It is estimated that aleurone contributed less than one-quarter of the transferred lipid in any stream. Hexane-extractable free lipids in four representative streams consisted of almost all the non-polar lipids, 40–67% of the glycolipids, 47–54% of the diacylphospholipids and 30–60% of the lysophospholipids.

EFFECT OF DEGUMMING ON KEEPING QUALITY AND REFINABILITY OF INDIGENOUS SOYBEAN OIL. T. Lakshminarayana, G. Azeemoddin, D. Atchyuta Ramayya and S.D. Thirumala Rao (Oil Technological Research Institute, Anantapur-555 001, India) *J. Food Sci. & Tech.* (India), vol. 19:126 (1982). Crude and degummed soybean oils obtained from *Kalitur* (indigenous black soybean) could be stored well for 210 and 120 days, respectively. Storage showed no adverse effect on the refinability of these oils.

STUDIES ON NIGER *(GUIZOTIA ABYSSINICA)* SEED OIL. Nasirullah, T. Mallika, S. Rajalakshmi, K.S. Pashutaphi, K.N. Ankaiah, S. Vibhakar, M.N. Krishnamurthy, K.V. Nagaraja and O.P. Kapur (Analytical Quality Control Laboratory, Central Food Technological Research Institute, Mysore-13, India) *J. Food Sci. & Tech.* (India), vol. 19:147 (1982). Nine niger seed samples collected from different parts of India were analysed for physico-chemical characteristics and fatty acid composition. Oil content ranged from 30.0 to 32.4%, crude protein from 26.0 to 30.6%, and moisture from 1.7 to 3.0%. Ranges for iodine values, saponification value, free fatty acid percent, butyro-refractometer reading, refractive index, Bellier turbidity temperature and unsaponifiable matter percent were: 112.8 to 129.0, 187.0 to 195.0, 0.2 to 2.0%, 59.5 to 62.2, 1.4655 to 1.4673, 24.5 to 27.8 C and 0.5 to 1.0%, respectively. Oil samples were *trans*-esterified and fatty acid composition determined by gas liquid chromatography. Among saturated fatty acids, palmitic (5.8 to 13.0), stearic (5.0 to 7.5) and arachidic (0.2 to 1.0%) were present, whereas oleic (13.4 to 39.3) and linoleic (45.4 to 65.8%) constituted the major portion of total fatty acids.

BILE ACIDS. LXVII. THE MAJOR BILE ACIDS OF *VARANUS MONITOR*. S.S. Ali, E. Stephenson and W.H. Elliott (Edward A. Doisy Dept. of Biochem., St. Louis Univ. School of Med., St. Louis, MO 63104). *J. Lipid Res. 23*(7):947–954 (1982). The major bile acids of gall bladder bile of *Varanus monitor* have been separated by thin-layer chromatography and shown to be derivatives of taurine. After alkaline hydrolysis, the free acids were separated by thin-layer and partition chromatography. Identification or characterization of the free acids was facilitated by gas-liquid chromatography and gas-liquid chromatography-mass spectrometry of the methyl esters or methyl ester-trimethylsilyl ethers. About 13% of the total bile acids was represented by the C_{24} acids cholic, deoxycholic, allocholic, chenodeoxycholic, and 12-oxo-3α-hydroxy-5β-cholanic acids, of which cholic acid constituted about 50%. The remainder of the bile acids consisted of eight C_{27} acids of which varanic acid was the major constituent; an isomer of varanic acid and 3α,7α,12α-trihydroxy-5β-cholestanoic acid were also identified. By chromatographic behavior and mass spectral fragmentation, the structures of four C_{27} acids with unsaturated side chains were elucidated as follows: 3α,7α-dihydroxy-5β-cholest-23-enoic, 3α,7α-dihydroxy-5β-cholest-24-enoic, 3α,7α,12α-trihydroxy-5β-cholest-23-enoic, and 3α,7α,12α-trihydroxy-5β-cholest-24-enoic acids. Similarly, the structure of the 12-deoxy analog of varanic acid, 3α,7α,-24ϵ-trihydroxy-5β-cholestanoic acid, was suggested for the component that constituted 7% of the total.

DETERMINATION OF BILE ACID MONOMERS IN MICELLAR SOLUTIONS. H.V. Ammon and L.G. Walter (Gastroenterology Section, VA Medical Center, 5000 West National Avenue, Wood, Wisconsin 53193) *Anal. Chem. 54* (12):2079–2082 (1982). A simple method for the determination of monomer concentrations of conjugated bile acids in micellar and mixed micellar solutions is de-

scribed. It utilizes the small exclusion limits of Sephadex G-10 beads. Only bile acids in the monomer phase are able to enter the beads. When small amounts of these beads are incubated in mixed micellar solutions containing ^{14}C-labeled bile acids and ^{3}H-labeled raffinose or sucrose, the monomer concentration of bile acids can be determined from the known concentration of the bile acids in the incubation mixture and the ratio of the markers in the incubation medium and in the beads. Monomer concentrations of taurocholate and taurodeoxycholate were determined for solutions containing 0.01–50 mM bile acids. Phosphatidylcholine reduced the monomer concentration of 5 mM taurodeoxycholate. The results are consistent with the micellar properties of these solutions and predictions about the monomer concentration from thermodynamic calculations.

PHOSPHOLIPID STUDIES OF MARINE ORGANISMS: III. NEW PHOSPHOLIPID FATTY ACIDS FROM *PETROSIA FICIFORMIS*. E. Ayanoglu, R.D. Walkup, D. Sica and C. Djerassi (Dept. of Chem., Stanford Univ., Stanford, CA 94305) *Lipids 17*(9):617–625 (1982). The fatty acyl components of the phospholipids from the sponge *Petrosia ficiformis* consisted predominantly of branched, especially iso and anteiso acids. The two major components of the complex mixture are the hitherto unknown Z,Z-25-methyl-5,9-hexacosadienoic and Z,Z-24-methyl-5,9-hexacosadienoic acids. Other unknown acids are: 7,13,16-docosatrienoic acid, 15-methyldocosanoic acid, 15-methyltricosanoic acid and 24-methyl-5,9-pentacosadienoic acid. Short branched-chain fatty acids, presumably of bacterial origin, are considered to be the possible bio-precursors of these novel phospholipid constituents. The major phospholipids were PE, PC, PG, PS and PI. The distribution of fatty acids among the phospholipid classes was also studied.

FASTING LEVELS OF MONOKETONIC BILE ACIDS IN HUMAN PERIPHERAL AND PORTAL CIRCULATION. I. Björkhem, B. Angelin, K. Einarsson, and S. Ewerth (Depts. of Clin. Chem., Med., & Surgery at Huddinge Univ. Hospital, Karolinska Institutet, S-141 86 Huddinge, Sweden) *J. Lipid Res. 23*(7):1020–1025 (1982). We have determined the approximate concentration of 3-oxo-, 7-oxo-, and 12-oxo-bile acids in human peripheral and portal circulation. These compounds were converted into the corresponding 3α-,7α-, and 12α-hydroxy bile acids by treatment with sodium borodeuteride. The ratio between deuterated and nondeuterated bile acid was determined by combined gas-liquid chromatography-mass spectrometry with use of selected ion monitoring. From the ratio obtained and from the concentration of unlabeled bile acid, the approximate concentration of the different ketonic bile acids could be calculated. This method underestimates 3-oxygenated bile acids by 4–8%, 7-oxygenated bile acids by 2–3%, and 12-oxygenated bile acids by about 25%. The approximate concentration of monoketonic 3,7-oxygenated bile acids was found to be 0.08 ± 0.02 and 0.37 ± 0.25 μmol/l in the peripherol venous serum and the portal venous serum, respectively. The approximate concentration of monoketonic 3,12-oxygenated bile acids was found to be 0.07 ± 0.02 and 0.32 ± 0.12 μmol/l in the peripherol venous serum and the portal venous serum, respectively. The approximate concentration of monoketonic, 3,17,12-oxygenated bile acids was found to be 0.03 ± 0.01 and 0.14 ± 0.05 μmol/l in the peripheral venous serum and in the portal venous serum, respectively. The total concentration of the ketonic bile acids constituted only $9 \pm 1\%$ and $8 \pm 3\%$ of the nonoxidized bile acids in the peripheral venous serum and in the portal venous serum, respectively. Thus it seems less likely that the portal inflow of ketonic bile acids is of significance.

ORGANIZATION OF UNESTERIFIED CHOLESTEROL IN HIGH DENSITY LIPOPROTEINS PROBED BY FILIPIN. L. Blau, R. Bittman, P. Lagocki, R. Byrne and A.M. Scanu (Dept. 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 apolipoprotein 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 apolipoprotein 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.

THE QUANTITATIVE DETERMINATION OF PLASMALOGEN BY ITS REACTION WITH MERCURIC CHLORIDE. E.M. Carey (Dept. of Biochem., The Univ. of Sheffield, Sheffield S10 2TN, United Kingdom) *Lipids 17*(9):656–661 (1982). The alk-1-enyl group of 1-alk-1^{1}-enyl-2-acyl-glycerophospholipids (plasmalogens) rapidly combines with mercuric chloride. At 0 C, there was a 1:1 stoichiometry for Hg binding to the reactive group of plasmalogens. Aldehydes were not released, indicating that the alkenyl ether bond was not cleaved. Hg binding to less reactive double bonds in unsaturated fatty acids was not significant. Quantitative estimation of bound Hg afforded a rapid and sensitive assay for alkenylacyl lipids and gave values similar to those obtained with other methods of analysis. The proportion of plasmalogens in bovine myelin glycerophospholipids and in ethanolamine glycerophosphatide was 35 and 75%, respectively. Plasmalogens account for 23.3% of the total glycerophopholipids of rat erythrocytes.

DIRECT ESTIMATION OF DOLICHYL PHOSPHATE IN RAT LIVER BY HIGH PRESSURE LIQUID CHROMATOGRAPHY. N. Chaudhary, D.J. Freeman, J.W. Rip and K.K. Carroll (Department of Biochem., Univ. of Western Ontario, London, Ontario N6A5C1, Canada) *Lipids 17*(8):558–560 (1982). A method involving reverse-phase high pressure liquid chromatography has been developed for determining the concentration of dolichyl phosphate (Dol-P) in tissues. Individual Dol-P homologs are resolved and amounts as small as 50 ng can be detected. Rat liver was found to contain 2.4 μg Dol-P/g wet weight, or ca. 4% of total liver dolichol. In contrast, rat liver microsomes contained 64 ng Dol-P/mg protein, which is about 40% of total microsomal dolichol. This enrichment in Dol-P is consistent with the role of microsomes as the major site of Dol-P-mediated glycoprotein biosynthesis in liver.

A SIMPLE PROCEDURE FOR RAPID TRANSMETHYLATION OF GLYCEROLIPIDS AND CHOLESTERYL ESTERS. W.W. Christie (Dept. Lipid Biochem. and Enzymology, Hannah Res. Inst., Ayr, Scotland) *J. Lipid Res. 23*(7):1072–1075 (1982). A simple procedure suitable for rapid transmethylation of triacylglycerols, other neutral lipids (including cholesteryl esters), and glycerophospholipids is described. Lipids in diethyl ether solution (50 volumes), in the presence of methyl acetate (1 vol), are reacted with 1M sodium methoxide in methanol (1 vol) at room temperature. Essentially complete transmethylation can occur within a few minutes with no hydrolysis. Glassware and reagent requirements are minimal and samples are ready for gas-liquid chromatography analysis with very little work-up.

TRANSLATIONAL DIFFUSION OF ACETYLCHOLINE RECEPTOR (MONOMERIC AND DIMERIC FORMS) OF *TORPEDO MARMORATA* RECONSTITUTED INTO PHOSPHOLIPID BILAYERS STUDIED BY FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING. M. Criado, W. Vaz, F. Barrantes, T. Jovin (Max-Planck-Institut für biophysikalisch Chemie, D-3400 Göttingen-Nikolausberg, F.R.G.) *Biochemistry 21*(23):5750–5755 (1982). The translational diffusion of fluorescein isothiocyanate labeled acetylcholine receptor from *Torpedo marmorata* reconstituted into phospholipid bilayer membranes with and without cholesterol was examined. The receptor protein was studied in the monomeric state in multibilayers and large paucilamellar liposomes of (a) dimyristoylphosphatidylcholine (DMPC), (b) a 2/5 molar ratio mixture of cholesteryl hemisuccinate and DMPC, and (c) soya bean lipids. The dimeric protein was studied in paucilamellar liposomes and multibilayers of soya bean lipids. In DMPC the translational diffusion coefficient (D_t) for the protein monomer was similar in multibilayers and liposomes at temperatures above 24°C. In multibilayers the fluorescence recovery curves were apparently due to a single diffusing component and a sharp transition occurred at 23°C to multicomponent recoveries. In large paucilamellar liposomes of DMPC, fluorescence recoveries were apparently due to a single diffusing component throughout. D_t for the monomeric acetylcholine receptor showed a monotomic temperature dependence in the cholesteryl hemisuccinate containing DMPC membranes and soya bean lipid membranes over the temperature range (14–37°C). D_t for the dimeric protein in multibilayers of soya bean lipids was almost indistinguishable from that of the monomeric protein.

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SYNTHESIS AND IDENTIFICATION OF BIS (DIACYLGLYCERO) PHOSPHORIC ACID AND BIS (MONOACYLGLYCERO) PHOSPHERIC ACID. Q.Q. Dang, P. Rogalle, R. Salvayre and L. Douste-Blazy (INSERM Unité 101, Biochimie des Lipides, Hôpital Purpan, 31059 Toulouse, France) *Lipids 17* (11):798–802 (1982). Synthesis of bis-(diacylglycero) phosphoric acid from *sn*-1,2-dipalmitoylglycerol and phenylphosphoryl dichloride according to Baer (1) was revised. New data are reported about identification of the intermediate and final products: (a) bis-phosphatidic acid phenyl ester is very slowly visualized by the Zinzade reagent and can escape notice; (b) large amounts of phosphatidic acid chloride phenyl ester are also formed; and (c) very little transacylation from *sn*-1,2-dipalmitoylglycerol to the 1,3-isomer is observed. Hydrolysis of bis-phosphatidic acid to bis-lysophosphatidic acid is much easier using phospholipase A_2 from pig pancreas than from snake or bee venom.

THE (−)[^{3}H]DIHYDROALPRENOLOL BINDING TO RAT ADIPOCYTE MEMBRANES: AN EXPLANATION OF CURVILINEAR SCATCHARD PLOTS AND IMPLICATIONS FOR QUANTITATION OF β-ADRENERGIC SITES. E.M. Dax, J.S. Partilla, and R.I. Gregerman (Gerontology Res. Center, Nat'l Institute on Aging, Nat'l Institutes of Health at Baltimore City Hospitals, Baltimore, MD 21224) *J. Lipid Res. 23* (7):1001–1008 (1982). In rat adipocyte membranes, both β-adrenergic agonists and β-adrenergic antagonists competed with (−)[^{3}H]dihydroalprenolol for high affinity and low capacity binding sites. The antagonists but not the agonists competed with (−)[^{3}H]dihydroalprenolol for lower affinity and higher capacity sites. To characterize the adipocyte β-adrenergic receptor and distinguish it from low affinity, higher capacity sites which were heat-labile and not stereoselective. When isoproternol was used to define the nonspecific binding, saturation studies showed a single binding site with a capacity of ~100 fmol/mg membrane protein. Binding was saturated by 10 nM (−)[^{3}H]-dihydroalprenolol. Approximate K_D's of 2–4 nM were observed. Kinetic analysis of (−)[^{3}H]dihydroalprenolol binding provided an independent measurement of K_D between 0.75 and 1.1 nM. This binding site had the characteristics of a β_1-adrenergic receptor with the potency of isoproterenol > norepinephrine ≥ epinephrine as competitors of binding. The K_D of inhibition of (−)[^{3}H]dihydroalprenolol binding correlated with the Ki of inhibition by antagonists or Da of activation by agonists of glycerol release in isolated adipocytes. These results suggest that β-adrenergic agonists compete with (−)[^{3}H]dihydroalprenolol for the high affinity binding site which represents the physiological site. The use of antagonists to define specific β-binding includes nonspecific site(s) as well as the β-adrenergic state.

LONG-CHAIN PHENOLS: XX. SYNTHESIS OF OXIDATIVE DEGRADATION PRODUCTS FOR THE METHYLATED COMPONENT PHENOLS OF *ANACARDIUM OCCIDENTALE* AND OTHER PHENOLIC LIPIDS: CONFIRMATION OF THE STRUCTURE OF THE PARENT PHENOLS AND OF RELATED MATERIAL. A.A. Durrani, G.C. Sun, and J.H.P. Tyman (Schl. of Chem., Brunel University, Uxbridge, Middlesex U B8 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 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.

MECHANICAL CALORIMETRY OF LARGE DIMYRISTOYLPHOSPHATIDYLCHOLINE VESICLES IN THE PHASE TRANSITION REGION. E. Evans and R. Kwok (Dept. of Academic Pathology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5) *Biochemistry 21* (20):4874–4879 (1982). Micromechanical tests have been used to determine the equilibrium changes in area of a dimyristoyl-phosphatidylcholine (DMPC) bilayer vesicle as produced by variable bilayer tensions at discrete temperatures in the range of 20–30°C. The micromechanical tests involved aspiration of a large vesicle with a small suction pipet. The data yielded values for the elastic area compressibility in the liquid state (~30°C) of 0.007–0.008 cm/dyn, in the solid state (~20°C) of less than 0.001 cm/dyn, and in the coexistence region (~24.2°C) of 0.03–0.05 cm/dyn. Likewise, the thermal area expansivity values ranged from (4–6) × 10^{-3}/°C in the coexistence region to (5–8) × 10^{-4}/°C in the solid state. The results of the analysis gave an expectation value for the transition temperature of 24.2°C, the statistical width of the transition of 0.3°C, and the heat of the transition of about 7 ± 0.7 kcal/mol.

QUANTITATIVE MEASUREMENT OF PROSTAGLANDINS E_2 AND E_3 BY SELECTED ION MONITORING. A. Ferretti, V.P. Flanagan, and J.M. Roman (Agricultural Research Service, Beltsville Human Nutrition Research Center, Lipid Nutrition Laboratory, Beltsville, MD 20705) *Lipids 17* (11):825–830 (1982). A method for the simultaneous quantitative analysis of prostaglandin E_2 (PGE_2) and PGE_3 is described. The PG were analyzed by selected ion monitoring as the methyl ester-TMS ether derivatives of PGB_2 and PGB_3, respectively. The internal standard for the quantification of both species was [3,3,4,4−$^2H_4$]PGE_2. A linear response over the range 0.6–50 ng (1.7–143 pmoles) was demonstrated for PGE_3. The chromatographic conditions used (2% SP-2330 column) afforded nearly baseline separation of the prostaglandins. New standard curves for PGE_3 must be developed each time the ion source parameters are changed. In a typical calibration run, the instrumental precision, expressed as coefficient of variation, ranged from 1.1 to 7.2% for PGE_2 (3 to 100 ng injected) and from 1.6 to 11.1% for PGE_3 (1.5–50 ng injected). The method was applied to the PG analysis of rat renomedullary tissues. The recovery of synthetic PGE_2 added to medullary homogenates was 100.5 ± 1.7% (mean ± SEM, n = 9), and the recovery of PGE_3 was 91.3 ± 1.4% (n = 9).

PREPARATION OF *CIS,CIS,CIS*-5,8,11-EICOSATRIENOIC ACID FROM ARACHIDONIC ACID. A. Ghosh, M. Koley, and J. Dutta (Department of Chemistry, Bose Institute, 93/1 A.P.C. Road, Calcutta 700 009, India) *Lipids 17* (4):214–216 (1982). Arachidonic acid was reduced by hydrazine to yield isomeric eicosatrienoic acids with other products. Methyl *cis,cis,cis*-5,8,11-eicosatrienoate was isolated from the products by silver ion chromatography and preparative gas liquid chromatography in 8% yield. The structure was confirmed by spectral studies and oxidative degradation.

MORPHOLOGY AND FATTY ACID COMPOSITION OF RETICULOCYTES FROM PHENYLHYDRAZINE-TREATED RATS. S.C. Goheen, E.C. Larkin and G.A. Rao (Hematology Research Laboratory, Veterans Administration Medical Center, Martinez, CA 94553) *Lipids 17* (9):594–597 (1982). Reticulocytosis was induced in rats by injecting phenylhydrazine, a potent oxidizing agent. Red cell morphology was analyzed by scanning electron microscopy. The majority of red cells from rats given injections of phenylhydrazine were types 2 and 3 echinocytes. Stomatocytes were also observed, but pitted lobular reticulocytes were not detected. Echinocytes have not previously been observed in reticulocyte populations. In the reticulocytes, the relative levels of 16:1 and 18:1 were significantly greater than in erythrocytes. These differences in monoenoic acids may be due to the presence of endoplasmic reticulum, the site of desaturase activity in reticulocytes. Of all the fatty acids, the polyunsaturates are the most susceptible to attack during peroxidation. However, the polyunsaturated fatty acid composition of reticulocytes was similar both to that of erythrocytes and to reported values of young erythrocytes isolated by density. Therefore, it is unlikely that lipid peroxidation caused the formation of echinocytes.

ANATOMICAL DISTRIBUTION OF STEROLS IN OYSTERS (*CRASSOSTREA GIGAS*). D.T. Gordon and N. Collins (Seafoods Laboratory, Oregon State University, 250-36th Street, Astoria, OR 97103) *Lipids 17* (11):811–817 (1982). Oysters (*Crassostrea gigas*) contain at least 8 predominant sterols as determined by gas liquid chromatography and a modified Liebermann-Burchard reaction. These sterols and the average amount found in mg/100 are: C_{26}-sterol (22-*trans*-24-norcholesta-5, 22-diene-3β-ol), 19.1; 22-dehydrocholesterol, 15.1; cholesterol, 46.8; brassicasterol, 27.2; $\Delta^{5,7}$-sterols (i.e., 7-dehydrocholesterol) 22.5; 24-methylenecholesterol 29.1; 24-ethylcholesta-5,22-diene-3β-ol, 1.2; and 24-ethylcholesta-5-en-3β-ol, 12.7. The distribution of these sterols appears uniform (r^2 = 0.938) between 5 major organs of the oyster. The percent body mass vs percent total sterol in these 5 organs are: mantle 44.1–41.4; visceral mass 30.3–36.7; gills 13.2–11.7; adductor muscle 8.3–3.7; and labial palps 4.2–6.5. The possible sources of these sterols are discussed.

RHODOPSIN-PHOSPHOLIPID RECONSTITUTION BY DIALYSIS REMOVAL OF OCTYL GLUCOSIDE. M.L. Jackson and B.J. Litman (Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22908) *Biochemistry 21* (22):5601–5608 (1982). Recombinant membranes were prepared from phospholipid-free rhodopsin and egg phosphatidycholine (PC) under a wide variety of conditions employing an octyl β-D-glucoside (OG) dialysis procedure. Two bands were consistently observed after sucrose density centrifugation of these recombinants. The major band, which was protein rich, had a molar phospholipid:protein ratio that was in the range of 30:1 to 50:1, even when the molar phospholipid:protein ratio of the solubilized solution prior to OG removal was as high as 300:1. Similar results were obtained when

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dioleoyl-PC, 1-palmitoyl-2-oleoyl-PC, disk lipids, or diphytanoyl-PC was used instead of egg PC. These results can be explained in terms of a lower stability of the OG-phospholipid micelles relative to the OG-phospholipid-rhodopsin micelles. Of the phospholipids that were used in the OG dialysis procedure, only saturated dimyristoyl-PC produced a protein-rich recombinant band with a phospholipid: protein ratio close to that of the initial solubilized solution. In contrast to the results obtained by using OG, when solubilized disks supplemented with egg PC were reconstituted from sodium cholate or dodecyltrimethylammonium bromide, the resulting recombinant membranes had initial and final phospholipid:protein ratios which were similar.

THE BIOSYNTHESIS OF OLIGOSACCHARIDE-LIPIDS. ACTIVATION OF MANNOSYLTRANSFERASE II BY SPECIFIC PHOSPHOLIPIDS. J.W. Jensen and J.S. Schutzbach (Department of Microbiology and the Diabetes Research and Training Center, The University of Alabama in Birmingham, Birmingham, AL 35294) *J. Biol. Chem. 257* (15):9025–9029 (1982). Mannosyltransferase II catalyzes transfer directly from GDP-mannose to an oligosaccharide-lipid resulting in the formation of the α-1,3-mannoyl linkage in Manα1-3-(Manα1-6)Manβ-GlcNAcβ-P-P-lipid. The enzyme has been solubilized and purified 660-fold from rabbit liver microsomes. Enzyme activity was reconstituted in the presence of specific phospholipids and evidence for the formation of an enzyme-phospholipid complex was obtained from kinetic studies, by the stabilization of the enzyme by phosphatidylethanolamine, and by co-sedimentation of the enzyme with phospholipid. Maximal activity was restored only when the enzyme was reconstituted in the presence of synthetic or naturally occurring phosphatidylethanolamine which contained unsaturated acyl chains. Investigation of the phospholipid specificity required for activation of the enzyme and results obtained with phospholipid vesicles of mixed composition suggest that the enzyme may have a requirement for phospholipids that can associate to form nonbilayer lipid structures in aqueous environments.

STEREOSPECIFICITY OF PREMATURE HUMAN INFANT LINGUAL LIPASE[1]. R.G. Jensen, F.A. Dejong, R.M. Clark, L.G. Palmgren, T.H. Liao and M. Hamosh (Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06268; and Department of Pediatrics, Georgetown University Medical School, Washington, DC 20007) *Lipids 17* (8):570–572 (1982). The lingual lipase in gastric aspirates from premature infants was found to be partially stereospecific for *sn*-3 esters of synthetic enantiometric triacylglycerols containing 18:1 and 16:0. The *sn*-3 ester was hydrolyzed about 4 times faster than the acid at the *sn*-1 position with no difference in rates between 18:1 and 16:0. The *sn*-2 was also hydrolyzed to some extent.

MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY: A BREAKTHROUGH IN THE ASSESSMENT OF PHYSIOLOGICAL VITAMIN K LEVELS. M.F. Lefevere, A.P. DeLeenheer, A.E. Claeys, I.V. Claeys and H. Steyaert (Laboratoria voor Medische Biochemie en Klinische Analyse, Rijksuniversiteit Gent, Krijgslaan 271, B-9000 Gent, Belgium) *J. Lipid Res. 23* (7):1068–1072 (1982). Owing to the lack of sensitivity and/or selectivity of the existing chemical assays, vitamin K deficiency has always been diagnosed indirectly by measuring its effect on blood coagulation. We used our recently developed multidimensional liquid chromatographic assay for what is, to the best of our knowledge, the first systematic investigation of physiological vitamin K levels in human blood. It allowed the unequivocal demonstration of *trans*-phylloquinone (vitamin K_1) and its quantification down to a level of 0.5 nanogram per milliliter of serum (ng/ml). In healthy adults, a mean serum concentration of 2.6 ng/ml was found, with a normal range of 0.9 to 7.8 ng/ml. These values apparently are distributed in a log-normal way.

ASPERGILLUS NIGER VAN TIEGHAM MANNOSYLATION: POLYPRENYLPHOSPHATE MANNOSULTRANSFERASE SPECIFICITY. R. Letoublon, B. Mayet, J. Frot-Coutaz, and R. Got (Lab. de Biol. et Technologie des Membranes, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France) *J. Lipid Res. 23* (7): 1053–1057 (1982). *Aspergillus niger* van Tieghem microsomes contain an enzyme that catalyzes mannose transfer from GDP-mannose to polyprenylphosphate. The studies of the specificity of this enzyme for both the sugar donar (nucleoside diphosphate sugar) and the acceptor (polyprenylphosphates that were made available to the enzyme by means of the fusion of acceptor-loaded liposomes with the microsomal membranes) gave the following results. *i)* All the polyprenylphosphates from C_{15} to C_{120} were acceptors except retinylphosphate. *ii)* The specificity of the enzyme for both the sugar and the base is very strict.

COMPOSITION AND BIOSYNTHESIS OF STEROLS IN SELECTED MARINE PHYTOPLANKTON. D.S. Lin, A.M. Ilias, W.E. Connor, R.S. Caldwell, H.T. Cory and G.D. Daves, Jr. (Northwestern Aquatic Sciences, Inc., Marine Research Laboratory, P.O. Box 1437, Newport, OR 97365) *Lipids 17* (11):818–824 (1982). Six species of phytoplankton, *Pseudoisochrysis paradoxa, Isochrysis galbana, Monochrysis lutheri, Platymonas suecica, Thalassiosira fluviatilis* and a *Chaetoceros* species, were cultured in the laboratory and their sterol contents analyzed utilizing digitonin precipitation, thin layer and gas chromatography and gas chromatography-mass spectrometry. A total of 7 sterols were found in phytoplankton. The occurrence of these sterols, cholest-5-en-3β-ol, cholest-5,22-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol, 24-methylcholest-5-en-3β-ol, 24-methylcholesta-5,22-dien-3β-ol, 24-ethylcholest-5-en-3β-ol and 24-ethylcholast-5,22-dien-3β-ol, differed slightly among the various phytoplankton species. Cultures of *P. paradoxa* biosynthesized both of the sterols found in this species when incubated in the presence of ^{14}C- or ^{3}H-mevalonic acid for 0.5–9 days. These sterols were cholesterol and 24-methylcholesta-5,22-dien-3β-ol. Since 5 of the sterols found in the phytoplankton commonly occur in mollusks which feed on plytoplankton, it is likely that at least some of the tissue sterols in mollusks are of dietary origin.

ANTIBODY INTERACTION WITH A MEMBRANE-BOUND FLUORESCENT LIGAND ON SYNTHETIC LIPID VESICLES. R. Luedtke and F. Karush (Dept. of Microbiol., Univ. of Pennsylvania Schl. of Med., Philadelphia, PA 19104) *Biochemistry 21* (23):5738–5744 (1982). The interaction of membrane-bound ligand with bivalent and monovalent fragments of monoclonal antibody was studied by fluorescence and precipitation analysis using synthetic lipid vesicles. The ligand *Nε*-[5-(dimethylamino)-naphthyl-1-sulfonyl] lysine was linked to the hydrophobic anchor dipalmitoylphosphatidylethanolamine and ranged between 0.01 and 1 mol % of the membrane components. The effects of cholesterol on the specific interaction were observed over the range of 0–50 mol %. A precipitation assay was developed to evaluate various factors related to the cross-linking of small unilamellar vesicles by bivalent antibody. The cholesterol content was critical for this process as demonstrated by the increased efficiency of precipitation over the range of 0–40 mol % of this component. Fluorescence analysis yielded the parallel finding of increased accessibility of the ligand to the antibody with greater cholesterol content. Increased surface density of the ligand also was found to enhance the intervesicle interaction. Finally, a comparison of the kinetics by fluorescence analysis of the binding of monovalent and bivalent fragments indicated that the bivalent interaction involved primarily the cross-linking of vesicles in accord with published findings of the interaction of monoclonal antibody with cell membrane antigens.

ESTOLIDE TRIGLYCERIDES OF *TREWIA NUDIFLORA* SEED OIL. R.V. Madrigal and C.R. Smith, Jr. (Northern Regional Res. Center, Agr. Res. Service, U.S. Dept. of Agr., Peoria, IL 61604) *Lipids 17* (9):650–655 (1982). The seed oil of *Trewia nudiflora* is known to contain glycerides of α-kamlolenic (18-hydroxy-*cis*-9, *trans*-11,*trans*-13-octadecatrienoic) acid. We have shown that a large part of these glycerides contain estolides in which the hydroxyl group of α-kamlolenic acid is esterified to a molecule of another acid, either a hydroxy acid or an ordinary fatty acid. By preparative thin layer chromatography, we isolated a series of tri-, tetra-, penta- and hexaacyl glycerols. By lipolysis and gas chromatography-mass spectrometry, we isolated and characterized estolid-linked fatty acids containing two acid molecules.

COMPOSITION AND METABOLISM OF PHOSPHOLIPIDS OF HUMAN LEUKOCYTES. G.V. Marinetti and K. Cattieu (Dept. of Biochem., Univ. of Rochester Med. Center, Rochester, NY 14642) *Chemistry and Physics of Lipids 31* (2):169–177 (1982). Human mononuclear (MN) and polymorphonuclear (PMN) leukocytes were analyzed for their phospholipid, triglyceride, cholesterol and fatty acid content. The phospholipid/cholesterol ratio was 1.24 for both cells. MN cells contain more phosphatidylcholine (PC), but less phosphatidylserine (PS), phosphatidylethanolamine (PE) and sphingomyelin (SPH) than PMN cells when expressed as percent of total phospholipid. When expressed on the basis of lipid content per cell, MN cells contain less PS, PE and SPH but more triglyceride than PMN cells. PMN cells incorporate palmitic, stearic, linoleic and linolenic acids into their phospholipids, triglycerides or cholesterol esters. The incorporation into triglycerides was highest for all fatty acids. Of the phospholipids, the incorporation was highest into PC. Labeled fatty acids also were found in proteins which had been dilipidized by exhaustive extraction with organic solvents. These represent tightly or covalently bound fatty acids. The incorporation of [^{3}H]palmitic acid into this protein fraction is stimulated by insulin.

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STEREOSPECIFIC SYNTHESIS OF 16α-HYDROXY-17-OXO STEROIDS BY CONTROLLED ALKALINE HYDROLYSIS OF CORRESPONDING 16-BROMO 17-KETONES AND ITS REACTION MECHANISM. M. Numazawa, M. Nagaoka and Y. Osawa (Medical Foundation of Buffalo, Inc., Buffalo, New York 14203) *J. Org. Chem. 47*(21):4024-4029 (1982). Synthesis of 16α-hydroxy-17-oxo steroids 3, 5b, and 6b and 3β, 16α-hydroxy-5-17-oxoandrosten-3-yl sulfate (7) from 16α-bromo-17-oxo steroids 1, 5a, and 6a and the reaction mechanism of the controlled alkaline hydrolysis are described. Treatment of the bromo ketones with NaOH in aqueous DMF gave the 16α-hydroxy 17-ketones stereoselectively in 95% yield without formation of other ketols. The sodium salt of 3-sulfate 7 was also obtained in one step in 85% yield from the corresponding bromo ketone (1a). Isotope-labeling experiments and time-course studies showed that equilibration between the 16-bromo epimers 1 and 2 precedes the formation of 3, in which the true intermediate is 2 and not 1, and that the ketol 3 is formed by the direct S_N2 displacement of the 16β-bromine. The 16β-morpholino derivative 8 obtained by reaction of 1 with morpholine was shown to be an isomerized product of the 16α isomer which is produced also by S_N2 displacement of the 16β-bromine. The mechanism of ketol rearrangement of 3 to the 17β-hydroxy-16-oxo compound 4 was found to involve a hydration to the carbonyl function. The new hydration-dehydration mechanism is proposed for the ketol rearrangement.

SPHINGOLIPIDS IN IMMATURE AND MATURE SOYBEANS. M. Ohnishi and Y. Fujino (Dept. of Agricultural Chemistry, Obihiro, Univ. of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080, Japan) *Lipids 17*(11):803-810 (1982). Ceramides and cerebrosides were isolated from immature and mature soybeans, and structures of the constituents were investigated. As component fatty acids, normal, 2-hydroxy and 2,3-dihydroxyacids were found in ceramides, whereas only normal and 2-hydroxy acids were identified in cerebrosides. The principal fatty acid component was 2-hydroxylignoceric acid in ceramides, and 2-hydroxypalmitic acid in cerebrosides. Sphingoids in ceramides consisted mainly of trihydroxy bases, with 4-hydroxy-*trans*-8-sphingenine being predominant. In contrast, cerebrosides contained mainly dihydroxy bases, the principal constituent being *trans*-4,*trans*-8-sphingadienine. The only sugar in cerebrosides was glucose. The constituents of the two sphingolipids was similar to each other in immature and mature seeds. Possible metabolic relations of plant sphingolipids, based on composition, are discussed.

THE CRYSTAL STRUCTURE OF CHOLESTERYL DIHYDROGEN PHOSPHATE. I. Pascher and S. Sundell (Dept. of Structural Chem., Faculty of Med., Univ. of Göteborg, Box 33031, S-400 33 Göteborg, Sweden) *Chemistry and Physics of Lipids 31*(2):129-143 (1982). Crystals of cholesteryl dihydrogen phosphate grown from 1,4-dioxane solution are monoclinic, space group C2 with $a = 24.40$, $b = 6.27$, $c = 40.86$ Å and $\beta = 102.7°$. The asymetric unit contains two molecules of cholesteryl phosphate CP and one dioxane molecule of the solvent. The CP molecules pack tail to tail in a bilayer structure. Within the layer they are arranged in double rows with their phosphate groups linked to ribbons by hydrogen bonds. Laterally the double strands of phosphate groups are separated by rows of dioxane molecules. The dioxane serves as hydrogen bond acceptor and as a spacer molecule that compensates the differences in cross-sectional area of the cholesteryl residue (38.4 Å^2) and the phosphate group (24 Å^2). In the cholesterol matrix the CP molecules joined to double rows have packing content with the smooth side of their skeleta and interdigitate with their annular methyl groups with those of molecules of the adjacent double rows. The branched cholesteryl side chains facing the bilayer center are loosely packed and show considerable disorder and/or thermal motion.

NITROXIDE SPIN-LABELED ANALOGS OF THE NON-IONIC DETERGENT TRITON X-100. R.B. Roman and J.F.W. Keana (Dept. of Chem., Univ. of Oregon, Eugene, OR 97403) *Chemistry and Physics of Lipids 31*(2):161-168 (1982). Two nitroxide spin labeled analogs of the non-ionic detergent, Triton X-100, have been synthesized. Electron spin resonance spectra of these compounds in solution, in egg lecithin multilayers, and in aqueous dispersion of dimyristoylphosphatidylcholine vesicles are described.

PHOTOSENSITIZED OXIDATION OF METHYL LINOLENATE. SECONDARY PRODUCTS. W.E. Neff, E.N. Frankel and D. Weisleder (Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture, 1815 North University Street, Peoria, IL 61604) *Lipids 17*(11):780-790 (1982). Previous studies of secondary oxidation products by high-pressure liquid chromatography (HPLC) of autoxidized methyl oleate, linoleate and linolenate and photosensitized-oxidized linoleate are extended to photosensitized-oxidized linolenate. Photosensitized-oxidized linolenate was fractionated by silicic acid chromatography with diethyl ether/hexane mixtures. Selected silicic acid chromatographic fractions were separated by polar phase HPLC and characterized by thin layer and gas liquid chromatography and by ultraviolet, infrared, nuclear magnetic resonance and mass spectrometry. Secondary products from the photosensitized oxidation mixtures (containing 8.2 to 29.0% monohydroperoxides) included keto- and epoxy-dienes (0.4-1.6%), hydroperoxy epidioxides (0.8-4,9%), hydroperoxy bicyclic monones (0.1-0.3%), dihydroperoxides (1.0-5.6%), and hydroperoxy bisepidioxides (0.7-1.6%). Some of these secondary products are new and unique to photosensitized oxidation. Cyclization of the 10-, 12-, 13- and 15-hydroperoxides of linolenate would account for their lower relative concentration than that found for the 9- and 16-hydroperoxides. Dihydroperoxides may be derived from monohydroperoxides by singlet oxygenation or free radical oxidation. The hydroperoxy bis-epidioxides may be formed by further serial cyclization of the hydroperoxy epidioxides from 10- and 15-monohydroperoxides. Dihydroperoxides, hydroperoxy epidioxides and hydroperoxy bis-epidioxides are suggested as important flavor precursors in oxidized fats.

COMPARATIVE STUDY ON THE PROPERTIES OF SATURATED PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE BILAYERS: BARRIER CHARACTERISTICS AND SUSCEPTIBILITY TO PHOSPHOLIPASE A_2 DEGRADATION. P.C. Noordam, A. Killian, R.F.M. Oude Elferink and J. De Gier (Dept. of Biochem., State Univ. of Utrecht, Transitorium 3, Padualaan 3, 3584 CH Utrecht (The Netherlands) *Chemistry and Physics of Lipids 31*(2):191-204 (1982). Comparative studies on bilayer systems of saturated phosphatidylcholines and phosphatidylethanolamines revealed a maximum in ionic permeability in phosphatidylcholine bilayers at the temperature of the gel to liquid-crystalline phase transition but such an increase in permeability was not detectable in bilayers of phosphatidylethanolamine. Furthermore, it was found that at the phase transition temperature the phosphatidylcholine bilayers are subject to rapid hydrolysis by pancreatic phospholipase A_2 whereas phosphatidylethanolamine bilayers are not. These differences are discussed in view of detailed information on the molecular organization in the gel and liquid crystalline phases of the two phospholipid classes.

AN INFRARED-SPECTROSCOPIC STUDY OF REVERSED MICELLAR SOLUTIONS OF SODIUM *N*-OCTANOATE AND WATER IN *N*-DECANOL. J.B. Rosenholm, J. Sjöblom and J.-E. Österholm (Dept. of Physical Chem., Åbo Akademi, Porthansgatan 3-5, SF-20500 Åbo 50 Finland) *Chemistry and Physics of Lipids 31*(2):117-127 (1982). The association process to reversed micelles in the system water/sodium *n*-octanoate/*n*-decanol is studied by means of wave number shifts in the fundamental infrared region. It is found that the antisymmetric vibration band (σ_3), and the scissors vibration band (σ_2) of water, together with the antisymmetric stretching vibration band of the ionised carboxylic groups (σ_{COO^-}) are capable of detecting and visualising changes in the micellar association equilibria. The information is primarily qualitative, but some rough quantitative estimations are also made. A comparison between the intensities of the narrow σ_3-line corresponding to unassociated water OH-oscillators discernible at high dilutions in decanol, and the broad σ_3-band of the associated species reveals that the fraction of unassociated OH-groups in water is low, perhaps only a few percent.

EFFECTS OF BENZYL ALCOHOL ON PHOSPHATIDYLCHOLINE LAMELLAR PHASE WITH DIFFERENT WATER CONTENTS. T. Shibata, Y. Sugiura and S. Iwayanagi (Inst. of Physical and Chem. Res., Wako, Saitama 351 and Faculty of Technology, Gunma Univ., Gunma 376, Japan) *Chemistry and Physics of Lipids 31*(2):105-116 (1982). Effects of benzyl alcohol (BA) on the bilayer thickness d_1 and the fluidity of egg phosphatidylcholine (PC) lamellar phase with various water contents have been studied by X-ray diffraction and the proton spin-lattice relaxation rate. At lower water contents, BA causes d_1 to decrease and the rate of molecular motions to increase. By contrast, with increasing BA at excess water, d_1 remains nearly unchanged, though the rate of motions increases. Hydration experiment for egg phosphatidylcholine lamellae with BA at a 1:1 molar ratio shows that in the range from 15% to 30% water, d_1 decreases to the value of the fully hydrated sample without BA and is nearly constant above 30% water. The value at full hydration is suggested to be a lower limit of the bilayer thickness, the chain is in the unperturbed state. It is in an extended structure at lower water contents. This

leads to the difference in the effect of BA on the bilayer thickness at different water contents.

PERMEABILITY OF BILAYERS COMPOSED OF MIXTURES OF SATURATED PHOSPHOLIPIDS. M. Singer (Dept. of Med., Queen's Univ., Kingston, Ontario K7L 3N6, Canada) *Chemistry and Physics of Lipids 31* (2):145–159 (1982). Liposomes composed of an equimolar binary mixture of phospholipids were formed from a series of saturated phosphoatidylcholines (PC) and phosphatidylethanolamines (PE). Mixtures were chosen such that the two phospholipids differed either in terms of head group alone, chain length alone, or both head group and chain length. Cation effluxes, both with and without ionophores (nigericin and valinomycin) were measured over a range of temperatures that encompassed the regions of phase separation for these different lipid mixtures. There was a good correlation between the temperatures at which permeability maxima and phase separation occur. For phospholipid mixtures with the same acyl chain but different head groups (PC vs. PE), the PC component 'controls' permeability pattern particularly if the chain lengths are sufficiently different. Lipids differing in both head group and chain length give rise to more complex permeability patterns. The results of the present study are interpreted in terms of a model in which one of the lipid components of the mixture may specifically congregate at defects between co-existing phases and thus 'regulate' permeability.

PHYSICAL STUDIES OF CELL SURFACE AND CELL MEMBRANE STRUCTURE. DEUTERIUM NUCLEAR MAGNETIC RESONANCE STUDIES OF *N*-PALMITOYLGLUCOSYLCERAMIDE (CEREBROSIDE) HEAD GROUP STRUCTURE. R. Skarjune and E. Oldfield (Central Research Laboratories, 3M Co., St. Paul, MN 55144) *Biochemistry 21* (13):3154–3160 (1982). Deuterium Fourier-transform nuclear magnetic resonance spectra of *N*-palmitoyl[2,3,4,6,6-$^2H_5$]glucosylceramide, *N*-palmitoyl[1-^2H]-glucosylceramide, *N*-palmitoyl[6,6-$^2H_2$]glucosylceramide have been obtained at 55.3 MHz (corresponding to a magnetic field strength of 8.5 T) for lipids as multilamellar dispersions in excess water at 90°C, above the gel to liquid-crystal phase transition temperature (T_c = 82°C). Spectra were also obtained for these same lipids dispersed with 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, and cholesterol, all in excess water at 90°C. In each system studied, the polar head group projects essentially straight up from the bilayer surface into the aqueous region, thereby permitting maximum hydration of the four glucose hydroxyl groups by bulk water molecules.

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC ANALYSIS OF SEED OILS CONTAINING CONJUGATED UNSATURATED ACIDS. A.P. Tulloch (National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada S7N 0W9) *Lipids 17* (8):544–550 (1982). ^{13}C Nuclear magnetic resonance spectroscopy has been used in a nondestructive investigation of conjugated unsaturated acids in seed oil triacylglycerols. Spectra of seven seed oils, from *Punica granatum, Cucurbita palmata, Jacaranda mimosifolia, Centranthus ruber, Catalpa bignonioides, Chilopsis linearis* and *Calendula officinalis*, containing among them six isomeric trienoic acids, *cis,trans,cis-* and *trans,-trans,cis-8,10,12-*, *cis,trans,cis-*, *cis,trans,trans-*, *trans,trans,cis-* and *trans,trans,trans-*9,11,13-octadecatetrienoic acid, and of the oil of *Impatiens balsamina* containing *cis,trans,trans,cis-9,11,13,15*-octadecatetraenoic acid, have been examined. Structures of component acids were derived from shifts of double bond carbons and of carbons close to the double bond systems. Compositions of the oils were obtained from signal intensities. Results were similar to those obtained by older methods. Only oil of *Centranthus ruber* contained more than one major conjugated acid; both *cis,trans,trans-* and *trans,trans,trans-*9,11,13-octadecatrienoic acids were found. The latter acid is now thought to occur naturally.

THE EFFECT OF HYDRATION ON THE MOBILITY OF PHOSPHOLIPIDS IN THE GEL STATE. A PROTON NUCLEAR MAGNETIC RESONANCE SPIN ECHO STUDY. F. Volke, K. Arnold and K. Gawrisch (Dept. of Physics, Karl-Marx Univ., DDR-7010 Leipzig and Dept. of Biol., Humboldt Univ., DDR-1040 Berlin) *Chemistry and Physics of Lipids 31* (2):179–189 (1982). Proton nuclear magnetic resonance (NMR) dipolar echo studies are presented for the gel state of dipalmitoylglycerophosphocholine (dipalmitoyl-GPC)-heavy water dispersions. The mobility and the mean order of the chains and the head group of dipalmitoyl-GPC were determined for different water concentrations and temperatures. For smaller than 5 mol D_2O per mol dipalmitoyl-GPC the molecule undergoes temperature- and hydration-dependent restricted rotational oscillations about the long axis of the molecule. For hydration numbers equal or larger than 5 mol D_2O per mol dipalmitoyl-GPC the molecules rotate effectively about their long axes and intermolecular dipolar interactions between proton groups of neighbour molecules are averaged. The onset of the lateral diffusion of dipalmitoyl-GPC is observed which averages out all intermolecular dipolar interactions. Deviations of the individual segments of the chains from the all-*trans* state have to be considered. The widely accepted model that the dipalmitoyl-GPC molecules rotate about their long axes with stiff all-*trans* chains should be modified. The polar head groups of dipalmitoyl-GPC effectively rotate about the bilayer-normal and restricted rotations about single bonds in the head group are allowed. An order parameter of about 0.6 for the head group was obtained for fully hydrated dipalmitoyl-GPC molecules at ambient temperature.

GLASS CAPILLARY GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF WAX ESTERS, STERYL ESTERS AND TRIACYLGLYCEROLS. S.G. Wakeham and N.M. Frew (Dept. of Chem., Woods Hole Oceanographic Institution, Woods Hole, MA 02543) *Lipids 17* (11):831–843 (1982). Complex mixtures of wax esters, steryl esters and triacylglycerols isolated from representative biological and geochemical samples have been analyzed using combined high resolution gas chromatography and electron impact and chemical ionization quadrupole mass spectrometry. These low volatility neutral lipids containing up to 65 carbons were chromatographed intact on 15–20 m high-temperature (upper limit: 370 C) persilylated SE-52 and SE-30 glass capillary columns. Discrimination effects due to adsorptive losses and degradation were minimized using a nonvaporizing on-column injector and a direct all-glass capillary connection (370 C) to the quadropole mass spectrometer. Structural information regarding the fatty acid and alcohol moieties was found to be maximal for methane-CI spectra in the case of wax and steryl esters, whereas EI spectra were most useful in interpreting triacylglycerol structures. Principal features of the EI and CI fragmentation patterns are discussed. The molecular composition of complex mixtures of these lipids is reconstructed for selected samples.

STUDIES ON THE SUBSTRATE SPECIFICITY OF PURIFIED HUMAN MILK LIPOPROTEIN LIPASE. C.S. Wang, A. Kuksus and F. Manganaro (Lab. of Lipid and Lipoprotein Studies, Oklahoma Medical Research Found., Oklahoma City, OK) *Lipids 17* (4): 278–284 (1982). The fatty acid specificity of purified human milk lipoprotein lipase was studied using the C_{18} to C_{54} (total acyl carbon number) saturated and the C_{54} mono-, di- and triunsaturated monoacid triacylglycerols. Kinetic determinations indicated that the medium-chain triacylglycerols were better substrates than long- or very short-chain saturated triacylglycerols. The unsaturated triacylglycerols were hydrolyzed at rates comparable to that of tricaprylin with triolein having the highest rate of hydrolysis of the unsaturated species tested. The enzyme attacked the primary ester bond much more readily than the secondary ester bond. The purified human milk lipoprotein lipase showed a perferential stereospecific lipolysis of the *sn*-1-position of the triacylglycerol molecule.

AUTOXIDATION OF MODEL MEMBRANE SYSTEMS: COOXIDATION OF POLYUNSATURATED LECITHINS WITH STEROIDS, FATTY ACIDS, AND α-TOCOPHEROL. H. Weenen and N.A. Porter (Paul M. Gross Chemical Laboratories, Duke University, Durham, North Carolina 27706) *J. Am. Chem. Soc. 104* (19): 5216–5221 (1982). The autoxidation of diL-PC and 1S,2A-PC in aqueous emulsion with several cosubstrates was investigated. Cholesterol, 7-dehydrocholesterol, linoleic acid, and α-tocopherol were cosubstrates in the autoxidation of dilinoleoylphosphatidylcholine (diL-PC). The distribution of the products, *tc* and *tt* diene hydroperoxides, was determined and evaluated. It was concluded that cholesterol has a lower H atom denoting ability (K_D) and 7-dehydrocholesterol a much higher K_D than diL-PC. Linoleic acid when mixed with diL-PC, diP-PC, or a mixture of the two was found to behave analogous to a mixture of just the two lecithins. The cooxidation of diL-PC with α-tocopherol in the bilayer, a very efficient antioxidant. 1-Stearoyl-2-arachidonoylphosphatidylcholine (1S-2A-PC) bilayer autoxidation gave a product distribution very similar to arachidonic acid near autoxidation. However the products from cooxidation of a 1S,2A-PC bilayer with α-tocopherol unexpectedly did not include the 5-hydroperoxy eicosatetraenoic acid isomer (5-HPETE), although the 12, 15, 11, 9, and 8 isomers were present in almost equal amounts.

CARBON-13 AND DEUTERIUM NUCLEAR MAGNETIC RESONANCE STUDY OF THE INTERACTION OF CHOLESTEROL WITH PHOSPHATIDYLETHANOLAMINE. A. Blume and R.G. Griffin (Francis Bitter Natl. Magnet Lab., Massachusetts Inst. of Technology, Cambridge, MA 02139) *Biochemistry 21*(24):6230-6242 (1982). Mixtures of dipalmitoylphosphatidylethanolamine (DPPE) and cholesterol (CHOL) have been studied with solid-state

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^{13}C and ^{2}H nuclear magnetic resonance (NMR) techniques. DPPE was ^{13}D labeled at the carbonyl group of the sn-2 chain, and ^{2}H was introduced at the 4 position of the sn-2 chain and the 1 position of the ethanolamine head group. The ^{13}C and ^{2}H spectra of each labeled lipid were studied as a function of temperature and CHOL concentration, and the results indicate three distinguishable temperature-composition regions. In region I, which occurs at low temperatures and CHOL concentrations, the ^{13}C and ^{2}H spectra are similar to those observed for pure DPPE in its gel phase. In region II, which occurs at higher temperatures or CHOL concentrations, the sn-2 ^{13}C==0 spectra of DPPE/CHOL mixtures display two components, indicating the coexistence of two conformationally and dynamically inequivalent DPPE molecules. In region III, which occurs at high temperatures and CHOL concentrations, both the ^{13}C and ^{2}H spectra are those expected of liquid-crystalline lipid. The NMR results are compared to, and found to be different from, those obtained with calorimetric investigations. It is suggested that these differences are due to the small domains present in DPPE/CHOL mixtures that lead to phase transitions of low cooperativity. Some metastability of the DPPE/CHOL system was observed at high CHOL concentrations and low temperatures.

MOLECULAR DYNAMICS AND CONFORMATION IN THE GEL AND LIQUID–CRYSTALLINE PHASES OF PHOSPHATIDYLETHANOLAMINE BILAYERS. A. Blume, D.M. Rice, R.J. Wittebort, and R.G. Griffin (Francis Bitter Natl. Magnet Lab., Massachusetts Inst. of Technology, Cambridge, MA 02139) *Biochemistry 21* (24):6220-6230 (1982). Solid-state deuterium and carbon-13 nuclear magnetic resonance (NMR) spectra have been used to study the molecular dynamics and conformation of dipalmitoylphosphatidylethanolamine (DPPE) in both the gel (L_β) and liquid-crystalline (L_α) phases. For this purpose DPPE was labeled with ^{13}C in the carbonyl group of the sn-2 chain and with ^{2}H at three different position-4,8, and 12-of the sn-2 chain, at the 2 position of the glycerol backbone, and at the 1 position of the ethanolamine head group. The ^{13}C carbonyl and ^{2}H chain spectra indicate that in the gel phase the DPPE molecules diffuse about their long axes at rates of 10^5-10^6 s^{-1} and acyl chains aproximate an all-trans conformation. The glycerol backbone spectra suggest that the backbone is in a gauche conformation in the gel state, rather than a trans conformation such as found in single crystals. The head group spectra in the gel phase are broad, featureless lines of about 20-kHz width. At the $L_\beta \rightarrow L_\alpha$ phase transition several changes take place: the chains disorder, and fast long-axis rotational diffusion begins, which results in the sharp, axially symmetric L_α phase ^{2}H spectra, which are a factor of 2 narrower than those observed in the L_β phase; the head group spectra also sharpen substantially at the transition, although their total width remains approximately constant; the ^{2}H spectra of the glycerol backbone labeled DPPE narrow by a factor of about 4, and we believe this is due to a conformational change in this region of the molecule. Consistent with this interpretation is the fact that the power pattern exhibited by the sn-2 ^{13}C==0 in the L_β phase collapses to an isotropic-like line at the phase transition.

PHASE EQUILIBRA, MOLECULAR CONFORMATION, AND DYNAMICS IN PHOSPHATIDYLCHOLINE/PHOSPHATIDYLETHANOLAMINE BILAYERS. A. Blume, R.J. Wittebort, S.K. Das Gupta, and R.G. Griffin (The Francis Bitter Natl. Magnet Lab., Massachusetts Inst. of Technology, Cambridge, MA 02139) *Biochem. 21*(24):6243-6253 (1982). Solid-state ^{13}C and ^{2}H NMR experiments have been used to examine the phase equilibria and dynamic structure of binary mixtures of diplamitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine (DPPE). The experiments rely on changes in the ^{13}C and ^{2}H spectra of *sn*-2 ^{13}C==0 labeled and ^{2}H chain and head group labeled lipids at phase boundaries. In particular, broad powder patterns are observed in the gel state, and these patterns narrow dramatically in the liquid-crystalline phase. In the two-phase region a superposition of the gel-phase and liquid-crystallinephase spectra is observed, which results from the presence of large domains in the DPPC/DPPE system. The appearance of the liquid-crystalline component and the disappearance of the gel component permit the solidus and liquidus curves of the phase diagram to be located accurately. Furthermore, a comparision of the temperature dependence of the ^{13}D and ^{2}H spectra shows the phase transition mechanism to be a function of composition. This result, together with other evidence, supports the hypothesis that the gel-state lattice changes from the $L_{\beta'}$ or $P_{\beta'}$ to the L_β configuration with increasing DPPE content. Finally, a detailed examination of the composition dependence of the spectra provides evidence for nonideal mixing

STEROLS IN MARINE INVEREBRATES. 33. STRUCTURES OF FIVE NEW 3β-(HYDROXYMETHYL)-*A*-NOR STERANES: INDIRECT EVIDENCE FOR TRANSFORMATION OF DIETARY PRECURSORS IN SPONGES. L. Bohlin, U. Sjöstrand, G. Sodano, C. Djerassi (Dept. Chem., Stanford Univ., Stanford, CA 94305) *J. Org. Chem. 47*(27)-5309-5314 (1982). Sixteen 3β-(hydroxymethyl)-*A*-nor steranes, of which five are new, have been found in the Red Sea sponge *Acanthella aurantiaca* (Family Axinellidae), which contains no sterols with conventional skeletons. The new structures were elucidated by 360-MHz ^{1}H NMR and mass spectral analysis. The 360-MHz ^{1}H NMR spectra of all *A*-nor sterols are summarized as an aid to the future rapid analysis of mixtures containing this class of marine sterols. The sterochemistry in the 3-position was proved by synthesis of 3α-(hydroxymethyl)-*A*-nor-5α-cholestane, which has different physical properties than the corresponding 3β-compound.

THE TEMPERATURE-DEPENDENT INTERFACIAL INACTIVATION OF PORCINE PANCREATIC LIPASE: EFFECT OF COLIPASE AND BILE SALTS. B. Borgström (Dept. of Physiological Chem., Univ. of Lund, Lund, Sweden) *Biochimica et Biophysica Acta 712*(3):490-497 (1982). This paper confirms and extends the previous observation that colipase and bile salts stabilize pancreatic lipase against inactivation at its water/substrate interface. It is shown that colipase and bile salts above their critical micellar concentration offer better protection than either of them alone. Colipase has no effect on the catalytic efficiency of lipase against an emulsified substrate in the absence or presence of bile salts. Its reported activation of pancreatic lipolysis at high temperatures in the absence of bile salts is, most likely, fully explained by its protective effect on lipase inactivation. Colipase at high concentrations relative to lipase inhibits the enzyme activity in a competitive fashion. The temperature-dependent surface inactivation of lipase has certain consequences for the methodology of lipase activity determination.

FATTY ACID COMPOSITION OF HUMAN PLASMA LIPOPROTEIN PHOSPHATIDYLINOSITOLS. W.C. Breckenridge and F.B. St. C. Palmer (Dept. of Biochem., Dalhousie Univ., Halifax, Nova Scotia, B3H 4H7, Canada) *Biochim. Biophys. Acta 712*(3):707-711 (1982). Phosphatidylinositol (PI) was the only anionic phospholipid found consistently in human plasma lipoproteins. High density lipoproteins (HDL) contained a greater proportion of PI (2.6% of the phospholipids) than did either the low density (LDL) or the very low density (VLDL) lipoproteins (1.6 and 1.7%, respectively). Lipoprotein PI was enriched in stearic and arachidonic acids when compaed to phosphatidylcholines from the same fractions. The fatty acid composition of the lipoprotein PI, although the same in all lipoprotein fractions, had less arachidonic acid than platelet PI and less palmitic acid than erythrocyte PI. The data suggest that significant exchange of PI between lipoproteins and cell membranes is not likely, whereas PI exchange among the different classes of lipoproteins is possible.

STUDIES OF FAMILIAL TYPE III HYPERLIPOPROTEINEMIA USING AS A GENETIC MARKER THE APOE PHENOTYPE E2/2. J.L. Breslow, V.I. Zannis, T.R. SanGiacomo, J.L.H.C. Third, T. Tracy, and C.J. Glueck (Metabolism Div., Children's Hospital Med. Center, and the Dept. of Pediatrics, Harvard Med. Schl., Boston, MA 02115) *J. Lipid Res. 23*(8):1224-1235 (1982). Clinical symptoms, lipoprotein patterns, and apoE phenotypes were determined in 17 individuals with type III hyperlipoproteinemia (type III HLP) and in their relatives and spouses. The apoE phenotype E2/2 occurred in 15 type III HLP probands (88%) and the apoE phenotype E4/2 was found in 2 probands. In each of the families studied, the apoE phenotype inheritance was compatible with a model we previously proposed in which apoE is determined at a single genetic locus with three common alleles. In addition, when compared to a control group in the general population, the whole group of relatives had normal cholesterol and HDL cholesterol levels, slightly low LDL cholesterol levels, and almost twice elevated triglyceride levels. In summary, a) a very strong but not invariate association exists between type III HLP and the apoE phenotype E2/2 with some type III HLP individuals having the apoE phenotype E4/2; b) apoE phenotype inheritance is determined by three alleles at a single genetic locus; c) relatives of type III HLP probands, no matter what their apoE phenotype, have on the average nearly twofold elevated plasma triglyceride levels compared to a control population; and d) non-proband type III HLP individuals with the apoE phenotype E2/2 have been indentified. As a group these individuals, particularly the males, show a tendency to express type III HLP, but clearly genetic or environmental factors other than the apoE phenotype E2/2 are required for the full phenotype expression of this disease.

[^{14}C] N-ETHYLMALEIMIDE LABELING OF THE PLASMA MEMBRANE [H^+]-ATPASE OF *NEUROSPORA CRASSA* R. J. Brooker and C.W. Slayman (Dept. of Human Genetics and Physiology, Yale Univ. Schl. of Med., New Haven, CT 06510) *J. Biol. Chem. 258*(1): 222-226. Treatment of the purified plasma membrane [H^+]-ATPase

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of *Neurospora crassa* with the sulfhydryl reagent N-ethylmaleimide (NEM) leads to a marked inhibition of ATPase activity. Several lines of evidence indicate that inhibition is caused by the reaction of NEM with a single cysteine residue on the M_r=104,000 polypeptide. (1) Inhibition by NEM follows pseudo-first order kinetics. (2) MgADP protects against NEM inactivation and at the same time decreases the incorporation of [^{14}C] NEM into the M_r=104,000 polypeptide. When "protectable" [^{14}C] NEM incorporation is plotted as a function of inhibition of ATPase activity, a linear relationship is observed with a slope of 0.8. (3) Labeling of the ATPase [^{14}C] NEM can be restricted to the protectable site by pretreatment with cold NEM in the presence of MgADP, followed by an incubation with [^{14}C] NEM in the absence of nucleotides. When this is done, and the enzyme is subjected to tryptic mapping and autoradiography, a single radioactive peptide fragment is found. The protectable site may yield information about the role of cysteine in the ATPase mechanism, and should also serve as a useful point of reference in enzyme mapping studies.

MINIMAL SIZE PHOSPHATIDYLCHOLINE VESICLES: EFFECTS OF RADIUS OF CURVATURE ON HEAD GROUP PACKING AND CONFORMATION. C.G. Brouillette, J.P. Segrest, T.C. Ng, and J.L. Jones (Dept. Pathology, Univ. Alabama, Medical Center, Birmingham, AL 35294) *Biochem. 21*(19): 4569-4575 (1982) Egg phosphatidylcholine samll unilamellar vesicles ranging from 150 to 270 Å in diameter have been studied by proton nuclear magnetic resonance (400 MHz) to investigate the relationship between phosphatidylcholine head group conformation and small changes in the vesicle radius of curvature. Our results demonstrate that the choline *N*-methyl chemical shift is a sensitive indicator of head group surface area. We infer from our results that (1) the inner monolayer head group packing significantly influences the size limitations of a small unilammelar vesicle, (2) the inner phosphatidylcholine *N*-methyl chemical shift is indicative of the vesicle radius, and (3) the chemical shift of a phosphatidylcholine *N*-methyl residing in a planar bilayer will be very similar to that in an outer monolayer of a small unilamellar vesicle.

SUBSTRATE SPECIFICITY OF BACTERIAL GLYCEROPHOSPHOLIPID: CHOLESTEROL ACYLTRANSFERASE. J.T. Buckley (Dept. of Biochem. and Microbio., Univ. of Victoria, Victoria, British Columbia V8W 2Y2, Canada) *Biochemistry 21*(26):6699-6703 (1982). The substrate specificity of a bacterial analogue of the plasma enzyme lecithin: cholesterol acyltransferase (LCAT) has been examined with small unilamellar liposomes and Trition mixed micelles. In contrast to LCAT, the microbial enzyme is capable of using all of the naturally occurring phospholipids as acyl donors. In general reaction rate depends more on the length or degree of unsaturation of the acyl chains than on the nature of the phosphlipid head group. Among a series of disaturated phosphatidylcholines in liposomes, dilauroylphosphatidylcholine is the preferred acyl donor. Like LCAT, the enzyme will catalyze acyl transfer by using other alcohols in addition to cholesterol. Of saturated straight chain primary alochols l-decanol is the preferred acyl acceptor. Cholesterol, however, is a far better acceptor than any non-sterol alochol tested. Other steroids with equatorial hydroxyls at position C-3 and trans-fused A:B rings will also act as acceptors whereas those steroids with axial hydroxyls at C-3 or cis-fused rings are inhibitors of acyl transfer. The ability of steroids to act as acyl acceptors may be due to the nature of their interaction with the phospholipid acyl donor.

COMPOSITION AND DISTRIBUTION OF LIPIDS OF GOAT'S MILK. J. Cerbulis, O.W. Parks, and H.M. Farrell, Jr. (Eastern Regional Res. Center, Philadelphia, PA 19118) *J Dairy Sci 65*(12):2301-2307 (1982). Fresh commercial goats' milks were examined for their lipid contents and distribution of these lipids among milk fractions. Whole milk, skim milk (produced by centrifugation at 330 and 2000 X *g*), and cream were studied. Petroleum ether (free lipids) and chloroform methanol (2:1) (bound lipids) were used successively to extract the lipids from all milk fractions. Average total lipid content for five bulk milk samples was 5.0 ± 1.2%, Lipid fractions of whole milk and cream contained 97 to 99% free lipid and 1 to 3% bound lipid, respectively. Free lipid was 96.8% triglyceride, whereas bound lipids contained neutral lipid, glycolipid, and phospholipid, In this respect, goats' milk resembled cows' milk. This free lipid, investigated in detail by gas chromatography, was shown similar in triglyceride distribution and fatty acid content to whole goats' milk triglyceride. Quantitative data for the triglyceride distribution in all fractions are given and differ from published data for fresh goats' milk.

BIPHASIC MODULATION OF PLATELET PHOSPHOLIPASE A_2 ACTIVITY AND PLATELET AGGREGATION BY MEPACRINE (QUINACRINE). A.C. Chan, E.T. Pritchard, J.M. Gerrard, R.Y.K. Man, and P.C. Choy (Dept. of Foods and Nutrition, Oral Biol., Pediatrics, Physiology and Biochem., Univ. of Manitoba, Winnipeg, Manitoba, R3E 0W3, Canada) *Biochim. Biophys. Acta 713*(1)170-172 (1982). The modulation of rat platelet phospholipase A_2 (phosphatide 2-acylhydrolase, EC 3.1.1.4) and rat platelet aggregation by mepacrine was investigated. The 2-acyl specificity of phospholipase A_2 acitivity was confirmed by using 1-[^{14}C] palmitoyl-2-[^{3}H] arachidonylphosphatidylcholine as substrate. Under optimal pH, phospholipase A_2 activity was not affected by aspirin but was inhibited by indomethacin. Contrary to previous reports, a biphasic modulatory role of mepacrine on phospholipase A_2 activity and platelet aggregation was demonstrated. The data suggest that platelet aggregation is mediated vis phospholipase A_2.

ACTIVATION OF D-β-HYDROXBUTYRATE APODEHYDROGENASE USING MOLECULAR SPECIES OF MIXED FATTY ACYL PHOSPHOLIPIDS. P. Churchill, J.O. McIntyre, H. Eibl, and S. Fleischer (Dept. of Molecular Bio., Vanderbilt Univ., Nashville, TN 37235) *J. Biol. Chem. 258*(1)208-214 (1983). D-β-Hydroxybutyrate apodehydrogenase is a lipid requiring enzyme with a specific requirement of lecithin for enzymatic function. The purified enzyme which is devoid of lipid can be reactivated with lecithin or mixtures of natural phospholipid-containing lecithin. However, it is mitochondrial phospholipid which activates the enzyme optimally and with kinetic parameters similar to that of the native membrane-bound enzyme. Mitochondrial phospholipid consists of three classes of phospholipid (lecithin: phosphatidylethanolamine:diphosphatidyglycerol in ratio of approximately 2:2:1 by phosphorus); each class consists of a multiplicity of different molecular species due to diversity in the fatty acyl substituents. In this study, we have synthesized defined molecular species of mixed fatty acyl phospholipids to evaluate whether multiplicity of phospholipid molecular species are essential for optimal reactivation. We find that:1) ternary mixtures of single molecular species of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylpropan-1,3-diol in the liquid crystalline state mimic the optimal reactivation of the exzyme obtained with mitochondrial phospholipids; 2) although some negatively charged phospholipid appears necessary for optimizing the efficiency of activation, diphosphatidylglycerol can be replaced by phosphatidylpropan-1,3-diol, another negatively charged phospholipid; and 3) biphasic Arrhenius plots can be correlated with the liquid crystalline and gel states of the phospholipid.

COMPUTERIZED RAPID HIGH RESOLUTION QUANTITATIVE ANALYSIS OF PLASMA LIPOPROTEINS BASED UPON SINGLE VERTICAL SPIN CENTRIFUGATION. J.T. Cone, J.P. Segrest, B.H. Chung, J.B. Ragland, S.M. Sabesin, A. Glasscock (Depts. of Pathology, Biochem., and Microbio., Univ. of Alabama in Birmingham Med. Center, Birmingham, AL 35294) *J. Lipid Res. 23*(6)923-935 (1982). A method has been developed for rapidly quantitating the cholesterol concentration of normal and certain variant lipoproteins in a large number of patients (over 240 in one week). The method employs a microcomputer interfaced to the vertical autoprofiler (VAP) described earlier (Chung et al. 1981. *J. Lipid Res.* 22:1003-1014). Software developed to accomplish rapid on-line analysis of the VAP signal uses peak shapes and positions derived from prior VAP analysis of isolated authentic lipoproteins HDL, LDL, and VLDL to quantitate these species in a VAP profile. Variant lipoproteins VHDL (a species with density greater than that of HDL_3), MDL (a species, most likely Lp(a), with density intermediate between that of HDL and LDL), and IDL are subsequently quantitated by a method combining difference calculations with curve shapes. The procedure has been validated qualitatively by negative stain electron microscopy. gradient gel electrophoresis, strip electrophoresis, chemical analysis of the lipids, radioimmunoassay of the apolipoproteins, and measurement of the density of the peak centers. It has been validated quantitatively by comparison with Lipid Research Clinic Methodology for HDL-, LDL-, and VLDL-cholesterol, and for MDL- and IDL-cholesterol by comparision of the amounts of MDL or IDL predicted to be present by the method with that known to be present following standard addition to whole plasma. These validations show that the method is a rapid and accurate technique of lipoprotein analysis suitable for the routine screening of patients for abnormal amounts of normal or variant lipoproteins, as well as for use as a research tool for quantitation of changes in cholesterol content of six or seven different plasma lipoprotein fractions.

DETERMINATION OF TOTAL TOCOPHEROLS IN GRAINS, GRAIN PRODUCTS, AND COMMERCIAL OILS, WITH ONLY ALIGHT SAPONIFICATION, AND BY A NEW REACTION WITH CUPRIC ION. E. Contreras-Guzman and F.C. Strong III (Faculdade de Alimentos a Agricola, Universidade Estandual de Campinas, 13100 Campinas, SP, Brasil) *J. Agric. Food Chem. 30*(6):1109-1112

(1982). This work describes a sequence of techniques for the extraction, purification, and chemical determination of tocopherols in grains, edible oils, and byproducts of oil refining. It is shown that nontocopherol reducing substances can be removed by gentle treatment with alcoholic KOH, with only slight saponification. Total tocols are determined by a new reaction with cupric ions and complexation with 2,2'-biquinoline (cuprione). It is carried out in a two-phase system, in which the upper phase (heptane) contains the lipids and the lower phase (80% ethanol) the Cu^{2+} ions and cuprione. The reaction occurs when the two phases are mixed by shaking for 2.5 min. The complex, $Cu(cuprione)_2^+$, forms in the 80% ethanol, and in this medium the molar absorptivity, expressed as a function of α-tocopherol, is 14490 L mol^{-1} cm^{-1} at 545 nm. The two-phase system permits control of the time of reaction, eliminating the influence of liposoluble pigments remaining in the heptane phase.

FATTY ACID AND VITAMIN E CONTENT OF NUTRIMAIZ, A SUGARY/OPAQUE-2 CORN CULTIVAR. E. Contreras-Guzman, F.C. Strong III, and W.J. da Silva (Inst. de Biologia, Univ. Estadual de Campinas, Caixa Postal 6121, 13100 Campinas, SP, Brazil) *J. Agric. Food Chem. 30*(6):113-117 (1982). Comparative analyses were carried out on Nutrimaiz (a corn cultivar with Sugary/Opaque-2 endosperm), derived from Opaque-2 and Sugary varieties, on its progenitors, and on a common corn (Maya XII Normal) for tocopherols, tocotrienols, and fatty acids. A shorter procedure was developed for extracting nonsaponifiable material (containing the tocols) after direct saponification of the sample, instead of first extracting the lipids. Individual tocopherols and tocotrienols were separated by TLC on silica gel, extracted, and determined by a new reaction with cupric ion and a complexing agent, either cuproine or bathocuproine. Fatty acids were determined by GLC of their methyl esters. Nutrimaiz was found to be more similar to Sugary than to Opaque-2 in total lipids, fatty acids, and total tocols, indicating that the Sugary gene is epistatic over that of Opaque-2 for these traits. Results expressed on a dry basis showed that all varieties increased in total lipids and total tocols in going from the fresh to the mature state.

STEROLS IN MARINE INVERTEBRATES. 32. ISOLATION OF 3β(HYDROXYMETHYL)-*A*-NOR-5α-CHOLEST-15-ENE, THE FIRST NATURALLY OCCURRING STEROL WITH A 15-16 DOUBLE BOND. M.L. Eggersdorfer, W.C.M.C. Kokke, C.W. Crandell, J.E. Hochlowski, C. Djerassi (Dept. Chem., Stanford Univ., Stanford, CA 94305) *J. Org. Chem. 47*(27):5304-5309 (1982). A new A-nor sterol, 3β-(htdroxymethyl)-A-nor-5α-cholest-15-ene, with an unusual unsaturation in the Δ^{15} position, has been found in the Pacific sponge *Homaxinella trachys* together with ten other *A*-nor sterols. The structure was elucidated by 360-MHz ^1H NMR, ^{13}C NMR, and mass spectral analysis as well as by chemical interconversion and comparison with synthetic 5α-cholest-15-en-3β-ol. ^{13}C NMR spectra of the title compound and of various 3β-(hydroxymethyl)-A-nor-5α-cholestanes were assigned as an aid in future structure elucidation of this class of sterols.

EFFICIENT ENZYMATIC HYDROLYSIS OF POLYPRENYL PYROPHOSPHATES. H. Fujii, T. Koyama and K. Ogura (Chem. Res. Inst. of Non-Aqueous Solutions, Tohoku Univ., Sendai 980, Japan) *Biochim. Biophys. Acta 712*(3)716-718 (1982). A method of efficient enzymatic hydrolysis of polyprenyl pyrophosphates was established. Polyprenyl pyrophosphates such as all-*trans*-octaprenyl and all-*trans*-non-aprenyl (solanesyl) pyrophosphate can be hydrolyzed completely to the corresponding primary alcohols by the action of potato acid phosphatase in the presence of 60% (v/v) methanol.

EFFECTS OF REACTION CONDITIONS AND REACTANT CONCENTRATIONS ON POLYMERIZATION OF LYSOZYME REACTED WITH PEROXIDIZING LIPIDS. J.A. Funes, U. Weiss, and M. Karel (Dept. of Nutr. and Food Sci., Massachusetts Inst. of Technology, Cambridge, MA) *J. Agric. Food Chem. 30*(6):1204-1208 (1982). Previous studies have shown that lysozyme undergoes deteriorative changes when exposed to peroxidizing lipids. In the present study we show the influence of concentration of protein and lipids and of the degree of unsaturation of the lipid on these changes. We also determined the effects of freeze-drying of aqueous emulsions containing lysozyme and methyl fatty acid esters. Lysozyme polymerization and loss of biological activity are promoted by higher protein-lipid concentration and higher degree of lipid unsaturation. Freeze-drying promotes protein polymerization in aqueous emulsions containing lysozyme and peroxidizing methyl fatty acid esters and decreases lipid hydroperoxide and malonaldehyde concentrations. These results suggest that reactant concentration induced by freeze-drying promotes hydroperoxide decomposition and facilitates free radical transfer reactions between lipids and proteins. However, freeze-drying does not affect the rate of loss of enzyme activity of the protein.

DYNAMICS OF FATTY ACIDS IN PHOSPHOLIPID VESICLES USING SPIN RELAXATION OF PROTON-COUPLED CARBON-13 SPECTRA. M. Fuson and J. Prestegard (Chem. Dept., Yale Univ., New Haven, CT 06511) *J. Am. Chem. Soc. 105*(2):168-176 (1983). Spin relaxation of a ^{13}C-enriched methylene at the 2 position of myristric acid dissolved in small unilamellar vesicles has been examined under proton-coupled conditions. The data obtained are analyzed using a formalism that considers both autocorrelation and cross-correlation dipolar spectral densities leading to an improved definition of motional properties of a fatty acid chain in a membrane environment. Several motional models are tested for consistency with the data. Among suitable models, one that includes axial diffusion, rotational bond isomerization, and rapid wobbling tures well below the solid→liquid transition, the lipoproteins contained a significant fraction (~33%) of *liquid* triglycerides which were relatively enriched in unsaturated fatty acyl chains. For model systems containing mixtures of solid and liquid triglycerides, the temperature dependence of line widths of fatty acyl resonances demonstrated that solid triglycerides decreased the mobility of the liquid triglycerides. A similar temperature dependcnce for the lipoprotein resonances suggested the solid and liquid species are co-mixed in individual lipoprotein particles within a purified subfraction. Temperaturedependent line width and intensity changes were observed for the phospholipid-choline methyl resonance in lipoprotein spectra and were apparently independent of the core transition.

EFFECT OF DETERGENTS ON STEROL SYNTHESIS IN A CELL-FREE SYSTEM OF YEAST. S. Hata, T. Nishino, N. Ariga, and H. Katsuki (Dept. of Chem., Faculty of Sci., Kyoto Univ., Kyoto Univ., Kyoto 606, Japan) *J. Lipid Res. 23*(6):803-810 (1982). In order to obtain information about the reactivity of enzymes in sterol synthesis of yeast, the effects of some detergents were investigated. Among the detergents tested, Trition X-100 was found to exert a unique ation, and its effect on the incorporation of ^{14}C-labeled acetate, mevalonate, farnesyl pyrophosphate, or *S*-adenosyl-L-Methionine into squalene, 2,3,-oxidosqualene, and sterols in a cell-free system was examined. Triton X-100 showed virtually no effect on the enzyme activities in the reactions from acetyl CoA to franesyl pyrophosphate, but it had a marked effect on reactions from farnesyl pyrophosphate to ergosterol. Evidence was obtained suggesting that Triton X-100 apparently activated squalene synthetase (EC 2.5.1.21) but inhibited squalene epoxidase (EC 1.14.99.7) and Δ^{24}-sterolmethyltransferase (EC 2.1.1.41). The activity of epoxidase was protected from the inhibition by increasing the concentration of cell-free extract or by the prior addition of lecithin liposomes to the reaction mixture. The inhibition of methyltransferase was partially reversed by treatment with bio-beads Sm-2, but that of epoxidase was not reversed by the treatment.

AUTOMATED GEL PERMEATION SYSTEM FOR RAPID SEPARATION OF INDUSTRIAL CHEMICALS AND ORGANOPHOSPHATE AND CHLORINATED PESTICIDES FROM FATS. M.L. Hopper (Total Diet Research Center, Food and Drug Administration, Kansas City, MO 64106) *J. Agric. Food Chem. 30*(6):1038-1041 (1982). A gel permeation chromatography (GPC) system for the rapid separation of industrial chemicals and organophosphate and chlorinated pesticides from fats has been developed. The system uses Bio-Beads SX-3 with a methylene chloride-*n*-hexane (50:50 v/v) solvent system. This gives good recoveries for a wide range of industrial chemicals and pesticides. Less than 1% fat remained in the pesticide fraction.

SOLUBILIZATION OF PHOSPHATIDYLCHOLINE BILAYERS BY OCTYL GLUCOSIDE. M.L. Jackson, C.F. Schmidt, D. Lichtenberg, B.J. Litman, and A.D. Albert (Dept. of Biochem., Univ. of Virgina, Schl. of Med., Charlottesvile, VA 22908) *Biochem. 21*(19):4576-4582 (1982). The solubilization of large, unilamellar egg phosphatidylcholine vesicles by the nonionic detergent octyl glucoside (OG) was investigated by nuclear magnetic resonance (NMR), fluorescence anisotropy, turbidity, electron microscopy, and centrifugation followed by compositional analysis. The solubilization process is well described by the three-stage model previously proposed for other detergents. In stage I, the OG partitions between the bilayer and aqueous phases with a molar partition coefficient of 59 ± 6. The presence of OG in the bilayers produces a small "fluidizing" effect, as indicated by changes in the NMR and fluorescence anisotropy parameters. A rearrangement that forms a large mixed bilayer occurs in the latter part of stage I. Stage II, the conversion of detergent-saturated bilayers into mixed micelles, begins at a ratio of total OG concentration minus the critical micelle concentration to total phosphatidylcholine concentration of approximately 1.5 and continues until this ratio reaches about 3.0. The correction for the critical micelle concentration of the OG is necessary for comparison of experimental results obtained at different lipid concentrations. The mixed bilayer-mixed micelle interconversion is quantified by the

centrifugation experiments and by ^{31}P NMR. The agreement between the two methods is excellent. Adventages of the NMR method are discussed. In stage III, which was not studied in detail here, all of the phosphatidylcholine is present as mixed micelles. Evidence is presented that the various structures present in the dispersions are in equilibrium with one another during these experiments.

UNIFYING DESCRIPTION OF THE EFFECT OF MEMBRANE PROTEINS ON LIPID ORDER. VERIFICATION FOR THE MELITTIN/DIMYRISTOYLPHOSPHATIDYLCHOLINE SYSTEM. F. Jahnig, H. Vogel, and L. Best (Max-Planck-Inst. fur Bio., 7400 Tubingen, BRD) *Biochem. 21*(26):6790-6798 (1982). The effect of of the director axis proves most appealing. This model assigns a short correlation time to bond isomerization, 4×10^{-11}s, and a rather long correlation time to axial diffusion, 2×10^{-7}s. A small C-H order parameter of -0.1 is predicted. This can be compared to order parameters obtained on multilayer membranes. The results are discussed in terms of implications for the effect of vesicle curvature and presence of membrane protein on acyl chain motion.

STRUCTURAL STUDIES OF PLANT MEMBRANE LIPID DISPERSIONS SUBJECTED TO OXIDATION IN THE PRESENCE OF DECOMPOSING PEROXYCHROMATE. D. Galanopoulou, W.P. Williams, and P.J. Quinn (Dept. of Biochem. and Biophys., Chelsea College,Univ. of London, London SW3 6LX U.K.) *Biochim. Biophys. Acta 713*(2):315-322 (1982). Total membrane polar lipid extracts of broad bean (*Vivia faba*) leaves have been dispersed in phosphate buffer and oxidised in the presence of decomposing potassium peroxychromate. Changes in the organisation of the membrane structures formed by these lipids resulting from the oxidation of unsaturated fatty acyl residues were assessed by different biophysical methods. Fluorescence polarisation values of 1,6-diphenylhexatriene intercalated into oxidised dispersions indicated considerable restriction was not due to the removal of unsaturated residues by oxidation leaving predominantly gel-phase lipid. This view was confirmed by wide-angle, X-ray diffraction studies, which showed diffuse reflections in control and oxidised preparations centred around 0.46nm as compared to a sharp reflection at about 0.41nm in a hydrogenated sample, and electron microscopic studies. Freeze-fracture and negatively stained dispersions of unoxidised lipids show typical lamellar structures with inverted micelles of lipid sandwiched within bilayer structure. Oxidation of the lipid destroys the bilayer arrangement and leaads to a generally amorphous structure. These results are discussed in terms of the role of oxidation in senescing plant tissue.

SEPARATION AND QUANTITATION OF SUBCLASSES OF HUMAN PLASMA HIGH DENSITY LIPOPROTEINS BY A SIMPLE PRECIPITATION PROCEDURE. L.I. Gidez, G.J. Miller, M. Burstein, S. Slagle, and H.A. Eder (Dept. of Biochem., Med., and Community Health, Albert Einstein College of Med., Bronx, NY 10461) *J. Lipid Res. 23*(8):1206-1223 (1982). Studies in recent years have suggested that measurement of high density lipoprotein (HDL) subclasses may provide significant information beyond that provided by measurement of total HDL. However, conventional methodology for separation of HDL subclasses involves various types of ultracentrifugation that are time-consuming, costly, and not suitable for many clinical or epidemiological studies. We have developed a simple precipitation method for the separation of HDL subclasses in human plasma. The chemical compositions of HDL_2 and HDL_3 isolated by the precipitation method were very similar to those of HDL_2 and HDL_3 isolated by preparative ultracentrifugation. The concentration of HDL_2 cholesterol was 40% higher in normal women than in normal men. In men with coronary heart disease, total HDL was decreased by 28%, HDL_2 was decreased by 44%, while HDL_3 was 19% lower. A similar pattern of change was found in women with coronary heart disease. In other conditions where total HDL either increased or decreased, the change in HDL_2 was always proportionately greater than the change in total HDL. HDL_3 showed relatively less change, and in some instances its concentration was unchanged. Thus HDL_2 is the more variable component and may be a more meaningful index of altered HDL metabolism.

H NMR STUDIES OF LYMPH CHYLOMICRA AND VERY LOW DENSITY LIPOPROTEINS FROM NONHUMAN PRIMATES. J.A. Hamilition, D.M. Small and J.S. Parks (Biophys. Inst., Dept. of Med. and Biochem., Boston Univ. Schl. of Med., Boston, Massachusetts 02118) *J. Bio. Chem. 258*(2):1172-1179 (1983). H NMR spectroscopy at 200 MHz was used to study triglyceride crystalline→liquid transitions which occurred on heating between 10 and 50 C in very low density lipoprotein and subfractionated chylomicron particles from nonhuman primates fed a saturated fat (butter fat) diet. Model system studies of pure triglycerides (triolein, tripalmitin and a 1:1 mixture) and emulsion particles consisting of these triglycerides with a surface of egg phosphatidylcholine showed that high resolution spectra were obtained only from liquid triglycerides. In lipoprotein spectra, changes in ^1H NMR peak intensities and line widths accompanied the solid→liquid transition of the constituent triglycerides. Peak areas of fatty acyl resonances were proportional to the percentage of melted triglyceride determined by differential scanning calorimetryl NMR peak area measurements showed that the calorimetric transition involved the melting of relatively greater numbers of saturated fatty acyl chains than unsaturated chains; at temperamelittin on lipid order in dimyristoylphosphatidylcholine bilayers is investigated by means of Raman spectroscopy and fluorescence anisotropy using diphenlhexatriene as fluorescence probe. In the fluid lipid phase, the Raman results indicate a slight increase in the conformational order of the lipid chains, and the fluorescence anisotropy results indicate a considerable increase in the figid body orientational order of the lipid chains. These results are contrasted with the reported decrease of the deuterium magnetic resonance order parameter. A consistent interpretation of the complete set of experimental data is presented according to which proteins induce a tilt of the preferred axes of lipid orientation and increase the orientational order with respect to these axes. The values of the tilt angle and the orientational order parameter at the surface of proteins are determined from the experimental data within a continuum model of lipid-protein interaction. The same value are obtained for melittin, Ca/Mg-ATPase, and cytochrome *c* oxidase, suggesting that different membrane proteins affect the lipid order in the same way.

NANOSECOND ROTATIONAL MOTIONS OF APOLIPOPROTEIN C-I IN SOLUTION AND IN COMPLEXES WITH DIMYRISTOYLPHOSPHATIDYLCHOLINE. A. Jonas, J.-P. Privat, P. Wahl, and J.C. Osborne, Jr. (Dept. of Biochem. and Schl. of Basic Med. Sci., Univ. of Ill., Urbana, IL 61801) *Biochem. 21*(24):6205-6211 (1982). Human apolipoprotein C-I Iapo C-I) in solution, in monomeric and oligomeric form, and in micellar complexes with dimyristoylphosphatidylcholine (DMPC), below and above the phase transition temperature of DMPC, was investigated with steady-state and time-resolved fluorescence methods. The environment of the Trp residue of apo C-I, in each physical state, was evaluated from fluorescence spectra and their changes upon KI quenching. Rotational correlation times of Trp residues were obtained from fluorescence anisotropy decay measurements. Static fluorescence anisotropy was determined as a function of temperature for the Trp residues of apo C-I in all physical states and for diphenylhexatriene dissolved in apo C-I·DMPC complexes. It was found that the Trp residues of apo C-I in solution are exposed from 75 to 88% to the aqueousmedium, depending on the state of self-association. On the other hand, the Trp residues in apo C-I·DMPC complexes are only 42-45% exposed to KI quenching through an environment distinct from water. Apolipoprotein C-I in all its physical forms had two rotational correlation times associated with Trp motions: a longer one dependent on the size and flexibility of the entire particle and a very short one in the range from 0.2 to 0.4 ns. The later correlation times correspond to local Trp residue motions. These Trp motions were not significantly affected by a transition from the gel to the liquid-crystalline state of the lipid in apo C-I·DMPC complexes, suggesting that there is no coupling between the local motions of lipids and those of Trp side chains of apo C-I.

FACTORS AFFECTING THE INTEGRITY OF HIGH DENSITY LIPOPROTEINS IN THE ULTRACENTRIFUGE. S.T. Kunitake and J.P. Kane (Cardiovascular Res. Inst. and the Dept. of Med., Univ. of California, San Francisco, CA 94143) *J. Lipid Res. 23*(6): 936-940 (1982). Because of reported losses of apolipoproteins from high density lipoproteins during ultracentrifugation, we studied several factors that could affect the integrity of these lipoprotein complexes. Alteration of terperature, rotor configuration, and composition of the tubes had little effect on loss of apolipoprotein A-I. Interestingly, the high ionic strengths commonly used in ultracentrifugal isolation of these lipoproteins were associated with the smallest loss of apolipoprotein A-I. Losses increased substantially as the ionic strength of the medium was decreased. After repeated ultracentrifugation. apolipoprotein A-I content of high density lipoproteins approached a limiting value of approximately 65% of the original serum value, but no apolipoprotein A-II was lost. Our results imply that the binding environments of these two apolipoproteins in high density lipoproteins differ. Further, they imply that apoliporotein A-I may exist in more that one type of environment or in more than one form in high density lipoproteins.

MILK CASEIN: INHIBITOR OF LIPOXYGENASE-CATALYZED LIPID PEROXIDATION. S. Laakso and E-M. Lilius (Dept. Biochem., Univ. of Turku, 20500 Turku 50, Finland) *J. Agric. Food Chem. 30*(5):913-916 (1982). Various milk products inhibited lipoxygenase-catalyzed lipid peroxidation in a model system consist-

ing of linoleic acid, one of the two purified soybean isozymes, and the inhibitor to be tested. The inhibition by milk was not dependent on its fat content, pasteurization, dialyzation, or heating. The inhibitory effect was associated with the isoelectrically precipitated casein fraction. This effect was verified with a commercial casein product. However, on the basis of kinetic grounds neither did the absorption of substrate to casein nor did calcium cause the inhibition. Luminometric measurements showed that casein inhibition was accompanied by a loss of chemiluminescence emission. This suggests that casein either has radical trapping properties or changes in the mode of attack of the enzyme so that such radicals are not formed. The inhibition was not limited to the purified enzymes but a 50% reduction of oxygenation in various plant homogenates was achieved by 0.06-3.2% (w/w) casein supplementation.

HIGH-PERFORMANCE LIQUID CHROMATORGRAPHY OF LONG-CHAIN NEUTRAL GLYCOSPHINGOLIPIDS AND GANGLIOSIDES. W.M.F. Lee, M.A. Westrick, and B.A. Macher (Cancer Res. Inst. and Dept. of Med, Univ. of California, San Francisco, CA 94143) *Biochimica et Biophysica Acta 712*(3):498-504 (1982). We describe a method for analyzing the perbenzoyl derivatives of both neutral glycosphingolipids and gnagliosides with a single high-performance liquid chromatography system. Use of the system, combined with endo- and/ of exoglycosidase treatment of glycosphingolipids, provides a sensitive method for obtaining structural information on these compounds. This system has two advantages over previously published chromatography procedures: (i) it uses a commercially available column, and (ii) this single column can be used to analyze gangliosides and their neutral glycosphingolipid products generated by neuraminidase treatment. With this method, we have studied 24 different glycosphingolipids, containing one to ten sugars and one or two sialic acid residues, and have demonstrated its usefulness in evaluating the gangliosides present in human leukocytes.

MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES. 31. ISOLATION AND STRUCTURE ELUCIDATION OF 23*H*-ISOCALYSTEROL, A NATURALLY OCCURRING CYCLOPROPENE. SOME COMPARATIVE OBSERVATIONS ON THE COURSE OF HYDROGENOLYTIC RING OPENING OF STEROIDAL CYCLOPROPENES AND CYCLOPROPANES. L.N. Li, H-t. Li, R.W. Lang, T. Itoh, D. Sica, and C. Djerassi (Department of Chemistry, Stanford University, Stanford, CA 94305) *J. Am. Chem. Soc. 104*(24):6726-6732 (1982). A new naturally occurring cyclopropene, 23*H*-isocalysterol, and a novel steroidal cyclopropane, 23,24-dihydrocalysterol, were isolated from the sponge *Calyx niceaensis*. Structure elucidation was accomplished by both NMR and mass spectroscopic analyses. Catalytic hydrogenation and detailed NMR analysis of the products led to the determination of the absolute configuration of calysterol (28*R*), 23*H*-isocalysterol (23*R*), and 23,24-dihydrocalysterol (23*S*,24*S*,28*R*). Partial synthesis of several hydrogenolysis products as well as a synthetic approach to 23,24-dihydrocalysterol are also reported.

STABILIZATION OF CAROTENE AND XANTHOPHYLL IN ALFALFA LEAF PROTEIN CONCENTRATES. C. Lyon and G. Kohler (Western Regional Res. Center, Agric. Res., U.S.D.A., Albany, CA 94710) *J. Agric. Food Chem. 30*(5):934-937 (1982). Carotene and xanthophyll are valuable constituents of alfalfa leaf protein concentrate (Pro-Xan), which is now prepared commercially. The effects of moisture content, pH, pelleting, inert atmosphere storage, and the addition of an antioxidant, fat, or alfalfa-soluble solides on the storage stability of these carotenoids were investigated. Earlier work on storage at low temperatures and in the dark is discussed. Greatest stability is obtained by addition of the antioxidant ethoxyquin (0.05%), storage in an inert atmosphere, and, if economically feasible, cold storage. Increases in stability, particularly of carotene, are also obtained by drying to a lower moisture content, addition of fat, or addition of the soluble solids remaining after separation of the alfalfa protein. It is essential that any added fat be stabilized with antioxidants and free of peroxides that promote the oxidation and destruction of carotenoids.

STEREOSPECIFIC REMOVAL OF THE D_R HYDROGEN ATOM AT THE 10-CARBON OF ARACHIDONIC ACID IN THE BIOSYNTHESIS OF LEUKOTRIENE A_4 BY HUMAN LEUKOCYTES. R.L. Maas, C.D. Ingram, D.F. Taber, J.A. Oates, and A.R. Brash (Dept. of Med., Vanderbilt Univ. Schl. Med., Nashville, TN 37232) *J. Biol. Chem. 257*(22):13519-13525 (1982). Arachidonic acid, sterospecifically labeled with ^3H in the L_S or D_R configuration at the 10-carbon and admixed with material labeled with ^{14}C at the 3-carbon was prepared and used to investigate the mechanism of leukotriene A_4 biosynthesis in human leukocytes. 5-hydroxyeicosatetraenoic acid and a further transformation product of it, 5,15-dihydroxyeicosatetraenoic acid, were enriched in ^3H relative to ^{14}C following incubation with arachidonic acid labeled with ^3H in the D_R configuration at the 10-carbon. This enrichment is proposed to represent a primary kinetic isotope effect and indicates that removal of the D_R hydrogen at the 10-carbon of 5(*S*)-hydroperoxyeicosatetraenoic acid is the rate-limiting step in leukotriene A_4 biosynthesis from 5(*S*)-hydroperoxyeicosatetraenoic acid.

THE MOLECULAR SPECIES COMPOSITION OF INDIVIDUAL DIACYL PHOSPHOLIPIDS IN HUMAN PLATELETS. V.G. Mahadevappa and B.J. Holub (Dept. of Nutr., College of Biological Sci., Univ. of Guelph, Guelph, Ontario, NIG 2W1 (Canada)) *Biochim. Biophys. Acta 713*(1):73-79 (1982). The molecular species composition of the individual idacyl phospholipids was determined in human platelets. The l-acyl (16:0, 18:0, etc.) homologues of the various 2-acyl (16:0, 18:1, 18:2, 20:4, etc.) species of the phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol were assessed by the use of thin-layer and gas-liquid chromatography in combination with specific lipases for establishing the positional distribution of the fatty acyl chains and generating the diacylglycerol derivatives. A marked heterogeneity was found in the complement of individual molecular species associated with the different platelet phospholipids. Our results indicate that the mechanisms involved in the release of arachidonate for prostaglandin and thromboxane synthesis would need to possess a profound degree of selectivity if any single molecular species of a given platelet phospholipid were the source of the released arachidonic acid.

ONE-STEP SCREENING METHOD FOR THE POLYMORPHISM OF APOLIPOPROTEINS A-I, A-II, and A-IV. H-J. Menzel R-G. Kladetzky, and G. Assmann (Zentrallaboratorium der Med. Einrichtungen der Westfälischen Wilhelms Univ., Münster, FRG) *J. Lipid Res. 23*(6):915-922 (1982). Apolipoprotein A-I exhibits a polymorphism that can be easily investigated in native serum by a simple method involving incubation of serum in the presence of decylsulfate and β-mercaptoethanol and subsequent isoelectric focusing. From six to eight proteins can be separated in a pH gradient from 4 to 6 and thus patients with apolipoprotein A-I variants can be distinguished from normal persons. This method also permits monitoring for polymorphic forms of apoA-II and apoA-IV as well as detection of C apolipoproteins. To verify the identity of the different apolipoproteins. a two-dimensional electrophoresis technique was applied, with as SDS system for the second dimension. In addition, monospecific antibodies for apolipoproteins A-I, A-II, and A-IV were used for the immunological identification. The method described here led to the discovery of three different familial apolipoprotein A-I variants.

LOCALIZATION OF TRITIATED 1,25-DIHYDROXY VITAMIN D_3 IN FETAL RAT MANDIBLE. KIDNEY. AND INTESTINE. R.J. Midgett, C.M. Friedman and P.E. Schneider (Depts. of Physiology and Pedodontics, Louisiana State Univ., Schl. of Dentistry, 1100 Florida Ave., New Orleans, LA 70119) *Acta Vitaminol. Enzymol. 4*(3):233-236 (1982). In the fetal rat the specific uptake of this hormone appears in the kidney and in the mandible-tooth germ, but not by the intestine. Furthermore, uptake by the two former tissues was greatly influenced by gestational age. Localization in the mandible and tooth germ increased from approximately 50 DPM/mg wet weight of tissue on day seventeen of gestation to over 300 DPM/mg wet weight on the nineteenth day. A similar, but not as dramatic, increase occurred in the kidney. Thus, these data demonstrate: (1) the development of the specific uptake of 1,25-dihydroxy vitamin D_3 in the kidney and bone with gestational age, and (2) the absence of specific receptive sites in the fetal intestine.

THE OXYGENATED MYCOLIC ACIDS OF *MYCOBACTERIUM FORTUITUM, M. FARCINOGENES* AND *M. SENEGALENSE*. D. Minnikin, S. Minnikin, M. Goodfellow (Dept. of Organic Chem. and Dept. of Microbiol., University, Newcastle upon Tyne, NE1 7RU, U.K) *Biochim. Biophys. Acta 712*(3):616-620 (1982). Previous studies showed that whole-organism acid methanolysates of representatives of *Mycobacterium fortuitum, Mycobacterium farcinogenes* and *Mycobacterium senegalense* contained characteristic mixtures of polar oxygenated mycolic acid methyl esters. These polar mycolates were absent in alkaline methanolysates of these organisms, being replaced by a single oxygenated mycolic acid methyl ester. This latter mycolate had the chemical and spectroscopic properties of a long-chain epoxide and was converted, on acid methanolysis, to the characteristic components present in whole-organism acid methanolysates. Spectroscopic analyses, supported by chemical transformations and degradations, showed that the homologous series of epoxymycolic adics present in these bacteria had carbon skeletons (main component C_{79}) similar to those previously found in mycobacterial keto, methoxy and wax-ester mycolates. The characteristic

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functional group in these epoxymycolic acids is an unusual *trans* epoxy group with a methyl branch on the adjacent carbon remote from the mycolate 2-alkyl-branched, 3-hydroxy acid unit.

CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROMETRY OF GLOBOTRIAOSYLCERAMIDE. H.A. Nunez and C.C. Sweeley (Dept. of Biochem., Michigan State Univ., East Lansing, MI 48824) *J. Lipid Res. 23*(6):863-867 (1982). Resonances in the carbon-13 natural abundance, proton-decoupled, 90.5 MHz nuclear magnetic resonance spectrum of globotriaosylceramide were assigned to specific carbon nuclei. The chemical shifts were rationalized in terms of the number of sugar residues, the sugar ring structures, the positions and anomeric configurations of the intersugar linkages, and the approximate degree of unsaturation of the alkyl chains of the ceramide moiety.

REACTION OF NITROGEN DIXOIDE WITH ALKENES AND POLYUNSATURATED FATTY ACIDS: ADDITION AND HYDROGEN ABSTRACTION MECHANISMS. W.A. Pryor, J.W. Lightsey, and D.F. Church (Department of Chemistry, Louisiana State Univeristy, Baton Rouge, LA 70803) *J. Am. Chem. Soc. 104*(24):6685-6692 (1982). The reactions of nitrogen dioxide in a carrier gas (nitrogen, oxygen, or air) with cyclohexane and a series of mono-, di-, and trienes is reported at NO_2 concentrations ranging from 70 ppm to 50%. A complete product analysis was made with cyclohexene, and these data allow the calculation of the fraction of the NO_2 that reacts by addition to the double bond or by abstraction of an allylic hydrogen. At high concentrations of NO_2, addition is the predominant process, in agreement with the literature. However, below 10000 ppm (1%), hydrogen abstraction predominates. We suggest this is because of competition between a reversible addition and an irreversible H-abstraction step, much as is the case for the well-known bromine atom reaction system. In fact, a kinetic analysis shows that the ratios of rate constants for addition and abstraction are similar for both NO_2 and the bromine atom. A less direct method (analysis of water formed) was used to estimate the addition to abstraction ratio for other alkenes and for esters of unsaturated fatty acids; these data are in agreement with the cylohexene data. The autoxidation of unsaturated fatty acid esters initiated by NO_2 also was studied, and kinetic chain lengths and autoxidizability ratios are given.

SIMULTANEOUS DETERMINATION OF VITAMINS A AND E IN DIETETIC PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. E. Sanzini, G. Bellomonte (Laboratorio degli Alimenti - Istituto Superiore di Sanita, V.le Regina Elena 299-00161 Roma, Italia). A simple method for the simultaneous determination of vitamins A and E in dietetic products has been developed. The analysis involves saponification and injection directly into a high performance liquid chromatograph using a Lichrosorb RP 18 column. Operating conditions were: solvent, sistem methanol-water (98:2), flow rate 1.2 mL/min, temperature 30 C, UV detector 290 nm. The presence of α-tocopherol acetate as internal standard eliminates the inconvenience of using calibrated standards of vitamins for every analysis.

ISOLATION OF HUMAN SERUM HDL_1 BY ZONAL ULTRACENTRIFUGATION. C. Schmitz and G. Assmann (Zentrallaboratorium der Medizinischen Einrichtungen der Westfalischen Wilhelms-Universitat, Domagkstrasse 3, 440 Munster, Germany) *J. Lipid Res. 23* (6):903-910 (1982). High density lipoprotein subfraction-1 (HDL_1) is thought to interact with the high-affinity apoprotein B, E receptors of peripheral cells and may act as a modulator of LDL binding and uptake. In the present study the concentration and composition of HDL_1 in normal and hypercholesterolemic sera were studied using zonal ultracentrifugation. Our data support the concept that HDL_1 formation occurs during LCAT-mediated HDL_3/HDL_2 interconversion in vitro.

PURIFICATION AND PROPERTIES OF THE FATTY ACID SYNTHETASE COMPLEX FROM THE MARINE DINOFLAGELLATE, *CRYPTHECODINIUM COHNII*. U. Sonnenborn and W.-H. Kunau (Arbeitsgruppe für Bioorganische Chem., Inst. für Phys. Chem., Ruhr-Univ. Bochum, D-4630 Bochum 1 F.R.G) *Biochimica et Biophysica Acta 712*(3):523-534 (1982). De novo biosynthesis of fatty acids in the heterotrophic marine dinoflagellate, *Crypthecodinium cohnii*, has been studied in vitro. Fatty acid synthetase was located in the cytosol and its activity was dependent on acetyl-CoA, malonyl-CoA, $NADPH_2$ and $NADH_2$. The enzyme was purified 100-fold using ion-exchange chromatorgraphy on DEAE-Sephadex A-25, adsorption to hydroxyapatite and gel filtration on Sepharose 4B columns. Very active endogenous proteases were separated from the fatty acid synthetase at the first step of purification. The purified enzyme had a molecular weight of about 400,000, as judged from gel filtration, sucrose density gradient centrifugation and polyacrylamide gel electrophoresis under non-denaturing conditions. Polyacrylamide gel electrophoresis under denaturing conditions in the presence of SDS and urea revealed one major protein band of M_r 180,000, suggesting that the enzyme is composed of two multifunctional subunits of apparently identical molecular weight. Reaction products of the *C. cohnii* fatty acid synthetase are free fatty acids due to the presence of a thioesterase activity in the purified enzyme complex. The main product is palmitate. Docosahexaenoic acid (C22:6, *n*-3), the major fatty acid component of *C. Cohnii* lipids, is not directly synthesized by the enzyme.

PRECISION SCANNING CALORIMETRY OF BILE SALT-PHOSPHATIDYLCHOLINE MICELLES. C.H. Spink, K. Muller, J.M. Sturtevant (Dept. of Chem., Yale Univ., New Haven, CT 06511) *Biochemistry 21*(25):6598-6605 (1982). Precision scanning calorimetry has been used to examine the thermal behavior of mixed micelles formed between bile salts and dipalmitoylphosphatidylcholine (DPPC). It was found through equilibrium dialysis that considerably less taurocholate than taurodeoxycholate is incorporated into mixed micelles with DPPC at a given bile salt concentration. Accounting for these concentration differences provides a means for more direst analysis of changes in the thermal transitions with mole ratio and dilution for the two bile salt components. Resolution of the thermal transitions into several component contributions is employed as an aid to interpretation of the differential scanning calorimetry curves. The curve resolutions lead to estimates of van't Hoff and calorimetric enthalpies of the individual contributions. The results of the curve resolutions, along with the behavior of the total enthalpies of the transitions, are consistent with a transformation in micellar structure occurring when the actual micellar composition is a mole ratio of bile salt to DPPC of about 1 to 1. The transformation region is near that found from X-ray evidence and is thought to correspond to a change from disk-shaped to spherical micelles.

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