Abstracts

Fats and oils

THE ABSENCE OF LIPASE ACTIVITY IN MESOCARP OF THE PALM FRUIT. K.C. OO, *Oleagineux 36*(12):613-616 (1981). The hydrolysis of endogenous triglycerides in mesocarp of ripe palm fruit (slices and undamaged whole fruit) was followed. In studying whether this hydrolysis was due to a lipase enzyme, several attempts were made to detect the presence of lipase activity in aqueous buffered extracts of mesocarp. The results showed that mesocarp tissue contained no active lipase. Lipase activity was also absent from the kernel (ungerminated seed). However, an active lipase was detected in germinating seeds, located in the embryo. This lipase had a pH optimum of approximately 7.4 and was strongly inhibited by fluoride, mercuric chloride and EDTA. It was strongly activated by calcium ions but not by sodium taurocholate.

COMBINING ABILITY ANALYSIS OF CERCOSPORA LEAFSPOT RESISTANCE AND AGRONOMIC TRAITS IN *ARACHIS HYPOGAEA* L. M.A. Hamid, T.G. Isleib, J.C. Wynne and C.C. Green, *Oleagineux 36*(12):605-609 (1981). General and specific combining abilities for yield, oil and protein content, and resistance to *Cercospora arachidicola* Hori (early leafspot) and *Cercospodirium personatum* (Berk. and Curt.) Deighton (late leafspot) were determined using the F_2 generation and parental lines from a full diallel cross of six Virginia-type peanut (*Arachis hypogaea* L.) lines. Variation attributable to general combining ability was about two to five times greater than that for specific combining ability for yield, fruit traits and disease resistances. No significant maternal nor reciprocal effects were observed for any trait indicating that nuclear genes were of primary importance in their inheritance. Genetic correlations suggested that selection for increased yield and early leafspot resistance should be possible. Estimates of GCA effects indicated that 'Florigiant' was the best parent for improving yield and late leafspot. 'NC 3033' for increased oil content and resistance to defoliation, and 'NC 5' for early leafspot resistance. Progeny rows were planted for individual F_2 plants with commercially acceptable pod shape and size selected from families with low average counts for early leafspot lesions. Ratings of early leafspot incidence in this population indicated that the insect-resistant line GP-NC 343 was the best parent for transmitting resistance to early leafspot in conjunction with good pod characters.

SEED GERMINATION AND DEVELOPMENT OF COCONUT PLANTS IN FUNCTION OF NUT POSITION. W. Wuidart and M. de Nucé de Lamothe, *Oléagineux 36*(12): 599-604 (1981). Nut position in the seed bed and nursery has been studied by many research workers, most of whom recommend the horizontal position. The development of a new nursery technique (plastic bag), led us to review this point, as the vertical position has many advantages, including better centering of the seedling, and better plant carriage inside the bag. Three treatments were studied: notched nut in horizontal position, notched in vertical position, and un-notched. The seed bed and nursery were conducted according to I.R.H.O.-recommended techniques. The results show that the nuts germinate faster in horizontal position, but the final percentage is unaffected by treatments. The plants' vegetative development is on the whole better in the vertical position, in spite of lesser initial root development. As delayed germination is a negligeable factor, the use of the vertical position in plastic-bag nurseries is under consideration. However, until such time as the plants' performance in the field is known, its use will be limited to a few special cases where the horizonal position has serious drawbacks.

ENTOMOLOGICAL RESEARCH ON THE OIL PALM IN LATIN AMERICA. P. Genty, *Oléagineux 36*(12): 585-594 (1981). As is the case for all extensive cultures, the creation of large oil palm plantations in the humid tropical zones of South America has profoundly modified the environment. Depending on the trees' age, the entomofauna which developed here varies greatly. In effect, insects which remain scarce so long as there is heavy sunshine and little foliage, multiply greatly on adult crops where they find an environment favorable to their increase. A detailed study of the oil palm environment and its fauna as a whole allowed often-novel means to be properly applied, based on knowledge of each harmful species, both from a biological and a dynamic point of view. Biological studies have also made it possible to detect infectious diseases of the viral type, some of which are used as a specific control method. Some examples of research and results attained in Tropical America are described here, notably the cases of *Sibine fusca, Leptopharsa gibbicarina, Retracrus elaeis* and the lethal Marchitez disease. Among the results obtained, one of the main points was a sharp cutback in use of chlorated or phosphorated pesticides, replaced by low-toxicity products like sulphur or chlordimeform, or even industrial-scale application of biological products like viral diseases or *Bacillus thuringiensis*. It is also worthwhile to show the value of parallel phytopathological and entomological research on *Elaeis guineensis*, which have led to positive solutions to two out of the three lethal oil palm diseases in Tropical America.

ON THE REFINABILITY OF OILS. VI. RELATIONS BETWEEN SPECTROMETRIC CHARACTERISTICS OF REFINED OILS AND ANALYTICAL CHARACTERISTICS OF CORRESPONDING RAW OILS. B. ABOUT SOYBEAN OILS. E. Sambuc, G. Devinat and M. Naudet, *Rev. Franc. Corps Gras 29*(2):69-73, french. RFCG 82-06 (1982). 33 raw soybean oils have been refined in experimental workshop in the same conditions. The direct or derived spectrometric characteristics of refined oils which participate in the prevision of immediate or ending flavor scores have been compared with different analytical characteristics of raw oils by means of statistical analysis. The absorption at 230 nm of refined oils is previsible in the used refining process, by a polynomial first degree relation from carbonyle value, absorption at 230 nm and phosphorus content of raw oils. The trichromatic color of refined oils is previsible by a polynomial second degree relation from carbonyle value, peroxide value and absorption at 230 nm of raw oils. Every analytical test of the raw oil is compared with the corresponding spectrometric characteristic of the refined oil. This study suggests, in the used conditions, the required characteristics of raw oils in order to, after refining, the characteristics of the freshlyy deodorized oil be compatible with the immediate sensorial acceptability threshold.

STUDY ON THE GLYCERIDE STRUCTURE OF THE NEW RAPESEED OIL. J. Rietsch, *Rev. Franc. Corps Gras 29*(2):75-77, french. RFCG 82-07 (1982). The glyceride structure of the new rapeseed oil has been studied. About 55% of linoleic and linolenic acids, and also 27% of oleic acid are in the 2 position of glycerol. Only these three components are practically in this position.

STUDY ON THE UNSAPONIFIABLES OF ALBIZZIA LEBBECK BENTH AND LEUCOENA GLAUCA BENTH OILS. J. Miralles, *Rev. Franc. Corps Gras 29*(2):79-80, french. RFCG 82-08 (1982). The qualitative and quantitative composition of unsaponifiable several fractions: sterols, methyl 4- sterols, triterpenic alcohols and tocopherols for these two Mimosacoe oils has been determinated by thin layer chromatography/gas liquid chromatography.

Biochemistry and nutrition

DRAWBACKS IN THE USE OF 23-NOR-DEOXYCHOLIC ACID STANDARDS. A.F. Attili, M. Angelico, L. Capocaccia, U. Pieche, and A. Cantafora (Istituto di Semeiotica Medica, University of L'Aquila, Istituto di Clinica Medica III, University of Rome, and

Istituto Superiore di Sanita, Rome, Italy) *J. Lipid Res. 23*(1):211-215 (1982). 23-Nor-deoxycholic acid is widely used as internal standard in gas-liquid chromatographic studies of bile acids. Two batches of this compound, submitted to conventional alkaline hydrolysis of bile acid conjugates, were found to be transformed into a product with chromatographic properties different from those of "authentic" 23-nor-deoxycholic acid. To identify this "new" product a comparison was made between chromatographic properties, mass spectra, and NMR spectra of 23-nor-deoxycholic acid before and after alkaline hydrolysis. The results indicate that the "new" compound is the true 23-nor-deoxycholic acid while the product present in the two batches examined is a monoacetate derivative.

HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF BILE SALTS: INDIVIDUAL PROTON ASSIGNMENTS FOR SODIUM CHOLATE IN AQUEOUS SOLUTION AT 400 MHz. S. Barnes and J.M. Geckle (Dept. of Biochem. and Physical Biochem. Section, Comprehensive Cancer Center, Univ. of Alabama in Birmingham, Birmingham, AL 35294) *J. Lipid Res. 23*(1):161-170 (1982). The 400 MHz ^{1}H-nuclear magentic resonance spectrum of sodium cholate in dilute aqueous solution has been successfully resolved using a combination of decoupling, partial relaxation techniques. The individual carbon resonances in the ^{13}C-NMR spectrum of sodium cholate have also assigned. Assignments of individual methylene protons were made by consideration of the molecular structure of sodium cholate and the expected couplings and ^{1}H-nuclear Overhauser enhancement experiments. Verification of the assignments of the methine protons was made by application of single frequency ^{1}H-decoupled ^{13}C-NMR. Variation of pH* from 6.0 to 11.0 did not alter the individual chemical shifts except for those between 2.12 δ and 2.30 δ, originating from the protons on the C_{23} position adjacent to the ionizable carboxyl group. The chemical shifts of the proton resonances were independent of concentration below 5 mM. Above 10 mM (micellar region), the proton chemical shifts were altered slightly and some band broadening occurred. These data are consistent of cholate molecules.

MONOMER-TO-MICELLE TRANSITION OF DIHEXANOYLPHOSPHATIDYLCHOLINE: ^{13}C NMR AND RAMAN STUDIES. R.A. Burns, M.F. Roberts, R. Dluhy, and R. Mendelsohn (Dept. of Chem. Massachusetts Institute of Technology, Cambridge, MA 02139) *J. Am. Chem. Soc. 104*:430-438 (1982). The monomer-to-micelle transition of dihexanoylphosphatidylcholine has been studied by ^{13}C NMR and Raman spectroscopy. Lipid ^{13}C chemical shifts in a variety of solvents indicate that micellization chemical shift differences for many carbons can be successfully modeled as a simple transfer from a strong hydrogen bond donating solvent to a hydrogen bond donor deficient/acceptor solvent. This solvent transfer model fails to predict the micellization shift trends at 3 points in the lipid molecule: the acyl chain peaks, the carbonyls, and the glyceryl backbone methine carbon. The temperature dependence of the phospholipid ^{13}C chemical shifts suggests that the acyl chain micellization shifts are due to conformational changes through the γ effect. This implies a 5% or 7% increase in P_t, for micellar vs monomer lipid. A comparison of the micellization shifts of acyl chain carbons in dihexanoylphosphatidylcholine and its ether-linked analogue *rac*-dihexylphosphatidylcholine suggests similar small ordering effects in both micelles. Solvent transfer and the γ effect cannot account for the ^{13}C micellization chemical shifts of the carbonyl and glyceryl backbond methine carbons. A possible source of these shift discrepancies is a conformational change in the glyceryl backbond between monomer and micelle. 2 regions of the Raman spectrum of dihexanoyl-PC micelles are used to obtain independent structural information about the acyl chains. Data suggest that lateral interactions between the chains in the micelle are disrupted compared to ordered forms that occur at low temperature, thereby leading to reduced intensity for spectral features which depend on interchain interaction for their intensity.

PHYSICAL STUDIES OF D < 1.006 G/ML LYMPH LIPOPROTEINS FROM RATS FED PALMITATE-RICH DIETS. S.B. Clark, D. Atkinson, J.A. Hamilton, T. Forte, B. Russell, E.B. Feldman, and D.M. Small (Biophysics Institute, Boston University School of Medicine, Boston, MA) *J. Lipid Res. 23*:28-41 (1982). At body temperature the stable form of triglycerides rich in saturated fatty acids is crystalline. We examined the physical state of triglyceride-rich lymph lipoproteins from rats fed saturated fat, as a function of temperature. When chylomicrons and very low density lipoproteins were collected, isolated, and examined at 37 C, they were liquid as judged by differential scanning calorimetry, x-ray diffraction analysis, and proton nuclear magnetic resonance spectroscopy, and they appeared spherical by electron microscopy. At 23-26 C, triglyceride began to crystallize in the α form, which transformed to the stable β form at lower temperatures. On cooling from 23 C to 17 C, considerable crystallization occurred and the particle density was increased significantly. When lipoproteins were held at 0-7 C, about 75% of the triglyceride crystallized, distorting the lipoprotein shape. Reheating from 0 C to 37 C left 25% of the triglyceride unmelted. Heating to 58 C was necessary to melt all the crystallized triglyceride and to restore the spherical lipoprotein shape. After complete melting of cooled lipoproteins, the liquid state was maintained on recooling to 27 C, with formation of a metastable particle similar to the nascent lipoprotein. Isolation of lipoproteins contained highly saturated triglyceride at temperatures below 23-26 C results in partial crystallization, alters their physical properties, and may affect their metabolism.

SYNTHESIS OF ISOTOPICALLY LABELED SATURATED FATTY ACIDS. S.K. DasGupta, D.M. Rice, and R.G. Griffin (Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139) *J. Lipid Res. 23*(1):197-200 (1982). An approach to the synthesis of isotopically labeled saturated fatty acids is outlined which is based on the copper-catalyzed coupling of an ω-bromo acid with an isotopically labeled Grignard reagent. The method provides high yields of pure products and offers considerable flexibility in the type of isotopically enriched compound that can be prepared.

PREPARATION OF A FLUORESCENT DERIVATIVE OF CYTOCHROME b_5 AND ITS INTERACTION WITH PHOSPHOLIPIDS. R. Gilmore and M. Glaser (Dept. of Biochem., Univ. of Illinois, Urbana, IL 61801) *Biochemistry 21*(7):1673-1680. A fluorescent derivative of bovine cytochrome b_5 was prepared by using 5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl chloride) in deoxycholate. Reaction conditions were established to specifically label the hydrophobic membrane-binding domain of the protein at a ratio of 0.9 ± 0.1 dansyl group per cytochrome b_5. Fluorescence measurements on the dansyl-labeled protein reflected the state of aggregation of the protein and its binding to lipids. The cytochrome b_5 derivative was a sensitive probe for the detection of phospholipid phase transitions in reconstituted phospholipid vesicles. The rotational relaxation time of the labeled protein was strongly influenced by the phospholipid composition and the cholesterol content of the lipid bilayer, but it was largely insensitive to the integrity of the hydrophilic domain of the protein. When the membrane-binding domain of cytochrome b_5 was bound to phospholipid vesicles, a preferential association with either the gel or the liquid-crystalline phase was not observed. The results suggest that the two domains of cytochrome b_5 undergo predominantly independent motion and that the motion of the dansyl-labeled membrane-binding domain directly reflects the properties of the bulk lipids in the bilayer.

A NONDESTRUCTIVE SPRAY REAGENT FOR THE DETECTION OF PROSTAGLANDINS AND OTHER LIPIDS ON THIN LAYER CHROMATOGRAMS. S.K. Goswami and J.E. Kinsella (Institute of Food Science, Cornell University, Ithaca, NY 14853) *Lipids 16*(10):759-760 (1981). The spray reagent 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt (10 mg/100 ml methanol) is extremely sensitive for locating prostaglandins on thin layer chromatograms. This reagent does not alter the PG, nor interfere with liquid scintillation counting.

ANALOGS OF NATURAL LIPIDS. VII. SYNTHESIS OF CYCLOPENTANOID ANALOGS OF PHOSPHATIDYLCHOLINE. A.J. Hancock, M.D. Lister, and H.Z. Sable (Dept. of Chem. and School of Med., Univ. of Missouri, Kansas City, MO 64110) *J. Lipid Res. 23*(1):183-189 (1982). A series of six analogs of phosphatidylcholine (lecithin) has been synthesized, in which the conformational mobility of the backbone is restricted. The analogs are derivatives of the three diastereoisomeric cyclopentane-1,2,3-triols and were obtained by triisopropylbenzenesulfonyl chloride-mediated condensation isomers of dipalmitoylcyclopentanetriol phosphate (*cyclopentano*-phosphatidic acid) with choline tosylate. The *cyclopentano*-lecithins obtained include the following: 1,2,3/0-(1P); 1,2,3/0-(2P0; 1,2/3-(1P); 1,2/3-(3P); 1,3/2-(2P). The two -2P derivatives are *meso*-forms while the other four derivatives are DL-pairs. Each lecithin analog has been obtained as a stable microcrystalline solid. Elemental analysis indicates that the compounds are hydrated; the data were consistent with the presence of either one, or in two instances, of one-half molecule of water of hydration. The infrared spectra, melting behavior, and chromatographic mobility of each of the analogs resembled those obtained for dipalmitoyllecithin, but the influence upon physical properties of stereochemical differences among the analogs were observed throughout the series.

HEPATIC BILE ACID ELUTION BY ALBUMIN AND BILE ACID CONTENT IN ISOLATED RAT HEPATOCYTES. S. Hashimoto, K. Uchida, and M. Hirata (Shionogi Research Laboratories, Shinogi & Co., Ltd., Fuckushim-ku, Osaka 553, Japan) *Lipids 17*(3):149-

154 (1982). Bile acid contents were determined for isolated rat hepatocytes. During the course of isolating the hepatocytes, perfusion of rat liver with buffer-containing 2% albumin eluted a significant amount of bile acids. The elution was proportional to the volume of the buffer and attributable to albumin in the negligible amount of bile acids, contained 95 ± 12 μg/18 cells of bile acids. The major bile acids were cholic acid (22%), β-muricholic acid (34%) and hyodeoxycholic acid (10%). Levels of the other bile acids were less than 3%. Peak 8, unidentified but presumed to be a trihydroxycholanic acid, accounted for 19%.

STRUCTURE AND THERMOTROPIC BEHAVIOR OF PHOSPHATIDYLSERINE BILAYER MEMBRANE. H. Hauser, F. Paltauf, and G.G. Shipley (Depts. of Med. and Biochem., Biophysics Institute, Boston Univ. School of Med., Boston, MA 02118) *Biochemistry 21*: 1061-1067 (1982). The structure and thermotropic properties of a homologous series of diacylphosphatidylserines (PS) in the anhydrous and hydrated state have been examined with low-angle X-ray diffraction and differential scanning calorimetry. In the anhydrous state at low temperatures both acidic PS and its NH_4^+ salts exhibit lamellar bilayer crystal forms that transform to liquid-crystalline hexagonal (type II) structures at higher temperatures. The crystal→liquid-crystal transition temperature increases with increasing chain length, the transition temperature of NH_4^+-PS being higher than that of its corresponding acidic form. In contrast, the transition enthalpies of the acidic PS are higher than those of the NH_4^+ salt forms. Hydrated acidic PS and NH_4^+-PS exhibit reversible lamellar gel→liquid-crystal transitions. In this case the acidic form undergoes this chain length dependent transition at a higher temperature, but with a lower enthalpy change, than the NH_4^+-PS. Both below and above the hydrocarbon chain melting transition, hydrated lamellar bilayer structures are present. The temperature-composition phase diagram of the NH_4^+-dimyristoyl-PS/H_2O system has been studied in detail. The chain melting transition decreases with increasing hydration, reaching a limiting value of 39 C. X-ray diffraction shows that both the bilayer gel structure and the bilayer liquid-crystal form take up water continuously (i.e., no hydration limit), a characteristic of lipid bilayers with a net charge. Electron-density profiles of NH_4^+-dimyristoyl-PS at different hydration levels permit detailed analysis of the structural parameters of the PS bilayer.

2-ACETYL-5-CHLOROPYRROLE IN THE VOLATILE FLAVOR CONSTITUENTS OF COCOA BUTTER. C-T. Ho, Q.Z. Jin, K.N. Lee, and J.T. Carlin (Department of Food Science, Cook College, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903) *J. Agric. Food Chem. 30*:362-364 (1982). The presence of 2-acetyl-5-chloropyrrole in the volatile flavor constituents of cocoa butter was confirmed through synthesis of the authentic compound. This compound was synthesized by chlorination of 2-acetylpyrrole. The structure of the synthesized compound was established by infrared, nuclear magentic resonance, and mass spectrometry. The identification of this compound in the volatile flavor constituents of cocoa butter was confirmed by comparing the mass spectrum and gas chromatographic retention time with those of the authentic sample. 2-Acetyl-5-chloropyrrole is the first chlorinated heterocyclic compound identified in the volatile flavor of foods.

FLUORESCENCE METHOD FOR MEASURING THE KINETICS OF Ca^{2+}-INDUCED PHASE SEPARATIONS IN PHOSPHATIDYLSERINE-CONTAINING LIPID VESICLES. D. Hoekstra (Dept. of Embryology, Carnegie Institute of Washington, Baltimore, MD 21210) *Biochemistry 21*:1055-1061 (1982). The effects of Ca^{2+} and Mg^{2+} on the fluorescence behavior of the phospholipid analogues 1-acyl-2-[6-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino] caproyl] phosphatidylcholine and *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine in small unilamellar vesicles consisting of phosphatidylserine, mixtures of phosphatidylserine/phosphatidylcholine, and mixtures of phosphatidylserine/cholesterol were studied. Fluorescence quenching was observed when Ca^{2+}, but not Mg^{2+}, was added to phosphatidylserine vesicles containing 5 mol % fluorescent lipid. The quenching process, which could be monitored continuously, was virtually complete within 5-6 min at Ca^{2+} concentrations $\geq$ 1.5 mM and resulted in a decrease of fluorescence intensity of approximately 60%. Fluorescence quenching did not occur in the presence of 0.5 mM Ca^{2+}; however, simultaneous addition of 6 mM Mg^{2+} initiated a quenching process similar in rate and extent to that observed at higher concentrations of Ca^{2+} alone. This quenching of 4-nitro-2,1,3-benzoxadiazole (NBD) fluorescence is best explained in term of Ca^{2+}-induced separation of lipid phases that leads to an increase in local concentration of NBD-lipid in the bilayer and hence to self-quenching of NBD fluorescence. The kinetics of Ca^{2+}-induced phase separation were also studied in several mixed lipid systems containing phosphatidylserine. In the case of mixtures of phosphatidylserine/cholesterol, the results indicate the presence of phase-separated regions as an intrinsic property of the vesicles in the absence of Ca^{2+}. Finally, results are presented indicating that the kinetics of phase separation is slow compared to vesicle-vesicle fusion.

HIGH PRESSURE LIQUID CHROMATOGRAPHIC SEPARATION OF MOLECULAR SPECIES OF PHOSPHATIDIC ACID DIMETHYL ESTERS DERIVED FROM PHOSPHATIDYLCHOLINE. J. Y-K. Hsieh, D.K. Welch, and J.G. Trucotte (Dept. of Medicinal Chemistry, College of Pharmacy, Univ. of Rhode Island, Kingston, RI 02881) *Lipids 16*(10):761-763 (1981). A majority of the individual molecular species of phosphatidic acid dimethyl esters derived from multispecies egg yolk and soybean phosphatidylcholines have been separated by reverse-phase high pressure liquid chromatography. Two Partisil-10 ODS columns connected in tandem and the eluents acetonitrile or methanol/water (95:5) were used for molecular species resolution, based on total fatty acyl carbon number and degree of unsaturation.

BIOHYDROGENATION OF UNSATURATED FATTY ACIDS. PURIFICATION AND PROPERTIES OF *CIS*-9, *TRANS*-11-OCTADECADIENOATE REDUCTASE. P.E. Hughes, W.J. Hunter, and S.B. Tove (Department of Biochemistry, North Carolina State University, Raleigh, NC 27650) *J. Biol. Chem. 257*(7):3643-3649 (1982). The enzyme catalyzing the second step in the biohydrogenation pathway of linoleic acid by *Butyrivibrio fibrisolvens cis*-9, *trans*-11-octadecadienoate reductase have been purified to near homogeneity. It has a molecular weight of 60,000 and appears to be single subunit. The purified enzyme contains 2 mol of iron, 10 mol of fructose, and 12 mol of galactose per 60,000 g. The iron, but not the carbohydrate, is required for enzymatic activity. Phosphatidylethanolamine was also found to be associated with the purified enzyme. Unlike the cell extract that can reduce the double bond of the fatty acid with NADH or α-tocopherolquinol as a reductant, the purified enzyme can utilize only α-tocopherolquinol. This indicates that another component of the reduction system exists that couples the production of α-tocopherol to the oxidation of NADH.

THERMODYNAMICS OF DIHEXANOYLPHOSPHATIDYLCHOLINE AGGREGATION. R.E. Johnson, M.A. Wells, and J.A. Rupley (University Department of Biochemistry, University of Arizona, Tucson, Arizona 85721) *Biochemistry 20*(14):4239-4242 (1981). Heats of dilution of aqueous solutions of dihexanoylphosphatidylcholine were determined by use of a flow microcalorimeter to monitor an exponential dilution gradient. Three different models of micelle formation were tested: monomer in equilibrium with micelles of fixed size, with micelles of varied size, or with small aggregates and micelles. The heat of dilution data for low solute concentration would be fit only by assuming the existence of premicellar aggregates. The critical micelle concentration determined calorimetrically is 0.016 ± 0.002 M and is independent of the model. The enthalpy change for transfer of monomer into the micelle is 1.6 ± 0.2 kcal/mol; about one-third of this heat effect is produced in formation of the premicellar aggregation. Comparison of the calorimetric measurements with results obtained by using other methods indicates the complexity of the micellization process.

FREE, ESTERIFIED, AND INSOLUBLE-BOUND PHENOLIC ACIDS. 2. COMPOSITION OF PHENOLIC ACIDS IN RAPESEED FLOUR AND HULLS. K. Krygier, F. Sosulski, and L. Hogge (Institute of Food Technology, Agricultural University of Warsaw, Warsaw, Poland) *J. Agric. Food Chem. 30*:334-336 (1982). The composition of free, esterified, and insoluble-bound phenolic acids in rapeseed cultivars was determined by gas-liquid chromatography and mass spectrometry. Rapeseed hulls contained no free phenolic acids and relatively low levels of soluble ester and bound phenolics. Sinapic acid was the principal phenolic acid released by hydrolysis of the soluble esters in the hulls while protocatechuic acid was the major phenolic acid in the residues. The flours contained 6-98 mg/100 g of free phenolic acids, 768-1196 mg/100 g of phenolic acids from hydrolyzed esters, and no phenolic acids in the residues. Sinapic acid represented a high proportion of the free phenolic acids and 99% of acids released from esters in the flours. Minor phenolic acids included *p*-hydroxybenzoic, vanillic, gentistic, protocatechuic, syringic, *p*-coumaric, and ferulic acids in the various fractions and cultivars.

SEPARATION OF PHOSPHOLIPIDS AND INDIVIDUAL MOLECULAR SPECIES OF PHOSPHOLIPIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. G.M. Patton, J.M. Fasulo, and S.J. Robins (Department of Medicine, Veterans Administration Medical Center, Boston, MA 02130) *J. Lipid Res. 23*:190-196 (1982). Isocratic high-performance liquid chromatography methods are described for separating the major classes of phospholipids and for isolating the individual molecular species of phospholipids. Fraction-

ation of a total lipid extract of rat liver on a silica column resulted in quantitative recoveries of all major phospholipids with preservation of their fatty acid composition. Rat liver phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine were then each chromatographed on a C18 reverse phase column to isolate individual molecular species. Component peaks were identified by their fatty acid composition and quantitated by phosphorus determination. Using this method we found that for each of these phospholipids from 30 to 35 different molecular species can be routinely identified and reproducingly quantitated. A characteristic elution sequence of molecular species permitted their identification based upon their retention times on a reverse phase column.

VITAMIN D COMPOUNDS IN COWS' MILK. L.E. Reeve, N.A. Jorgensen, and H.F. DeLuca (Depts. Biochem. and Dairy Science, College of Ag. and Life Sci., Univ. of Wisconsin-Madison, Madison, WI) *J. Nutr. 112*(4):667-672 (1982). The milk from cows fed normal levels of vitamin D has been found to contain approximately 40 IU per liter of vitamin D activity. A 14-fold increase in dietary vitamin D intake causes only a doubling of the amount of vitamin D in milk. This was determined by measuring stimulation of intestinal calcium transport in the vitamin D-deficient rat. Four vitamin D compounds were then isolated from cows' milk using a combination of conventional chromatography on Sephadex LH-20 and Lipidex 5000 followed by high-performance liquid chromatography. 24,25-Dihydroxycholecalciferol and 1,25-dihydroxycholecalciferol were measured using binding protein assays. One liter of milk contained 27 ng and 4.9 ng, respectively, of these two metabolites. Together these account for about 15% of the vitamin D acitivity. Cholecalciferol was found to be present at a concentration of 281 ng/liter or 11 IU/liter of biological activity. The milk contained 145 ng/liter 25-hydroxycholecalciferol or 29 IU/liter of activity. Therefore the known vitamin D compounds fully account for the biological activity observed in milk. It is therefore clear that no evidence could be found for the existence of a highly active water-soluble form of vitamin D in milk.

SIALOSYLGALACTOSYLCERAMIDE (G_{M4}) IS A MAJOR GANGLIOSIDE IN CHICKEN EMBRYONIC LIVER. M. Saito and A. Rosenberg (Department of Biochemistry and Biophysics, Loyola University Stritch School of Medicine, 2160 South First Avenue, Maywood, IL 60153) *J. Lipid Res. 23*:9-13 (1982). The developmental changes in gangliosides of chicken liver were investigated during embryonic and neonatal life. Sialosylgalactosylceramide (G_{M4}) and sialosyllactosylceramide (G_{M3}) were major gangliosides during the entire period investigated. Sialosylgalactosylceramide (G_{M4}) was detected as the predominant species until 2 days before hatching and then G_{M3} increased to be the major sialoglycolipid. G_{M4} continued to be detectable until at least 2 weeks after hatching. Monogalactosylceramide was detected as the major neutral glycolipid. The fatty acids obtained from monogalactosylceramide showed a similar pattern to that of G_{M4}.

REGIONAL DISTRIBUTION OF GLYCOSYLCERAMIDE-SULFATES IN HUMAN KIDNEY. G.E. Samuelsson (Dept. of Med. Biochem., Univ. of Goteborg, Box 33031, S-400 33 Goteborg, Sweden) *Lipids 17*(3):160-165 (1982). Glycosylceramide-sulfates were prepared separately from human kidney cortex and medulla. Glycosylceramide-sulfates were characterized with respect to long-chain bases, fatty acids, carbohydrates, and sulfuric ester group position. Monogalactosylceramide I^3-sulfates were 3 times more concentrated in medulla compared to cortex, whereas lactosylceramide II^3-sulfates were 3 times more concentrated in cortex compared to medulla. The results were discussed in relation to the possible role of glycosylceramide-sulfates in sodium-potassium ion transport.

A RAPID LIQUID-LIQUID EXTRACTION CLEANUP METHOD FOR THE DETERMINATION OF VOLATILE *N*-NITROSAMINES IN COOKED-OUT BACON FAT. N.P. Sen and S.S. Seaman (Food Research Division, Food Directorate, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2) *J. Agric. Food Chem. 30*:364-367 (1982). A rapid liquid-liquid extraction method is described for the determination of volatile nitrosamines in cooked-out bacon fat. The method consists of partitioning of the nitrosamines between *n*-hexane and an acidic aqueous-methanol mixture containing small amounts of sulfamic acid. An aliquot of the aqueous phase is then extracted with dichloromethane, the dichloromethane extract concentrated, and an aliquot of the concentrated extract analyzed by a GLC-thermal energy analyzer. The average percentage recoveries of *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosopiperidine, *N*-nitrosopyrrolidine, and *N*-nitrosomorpholine when added to cooked-out bacon fat or lard at levels ranging between 5 and 20 ppb were 78.8, 77.8, 89.4, 100.3, and 97.4, respectively. The method has an overall detection limit of 1 ppb for each of the above five nitrosamines. The average levels (uncorrected) of *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine detected in the 11 samples of cooked-out bacon fat were found to be 4.8 and 21.1 ppb, respectively.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND GLASS CAPILLARY GAS CHROMATOGRAPHY OF GEOMETRIC AND POSITIONAL ISOMERS OF LONG CHAIN MONOUNSATURATED FATTY ACIDS. L. Svensson, L. Sisfontes, G. Nyborg and R. Blomstrand (Dept. of Clin. Chem., Huddinge Univ. Hospital, Karolinski Instit., Stockholm, Sweden) *Lipids 17*(1):50-59 (1981). Positional and geometrical isomers of monounsaturated long chain fatty acids were analyzed by the combination of high performance liquid chromatography (HPLC) and glass capillary gas chromatography (GC). A preparative group separation of *cis* and *trans* isomers of the monounsaturated fatty acid methyl esters was achieved according to chain length by reversed-phase HPLC, and using a highly sensitive interference refractive index detector. After collection of the different fractions, they were analyzed for their content of positional isomers using glass capillary GC with Silar-5 CP as stationary phase. The preparative step in the HPLC was also used analytically for the determination of the ratio between the *cis* and *trans* monounsaturated fatty acids. A comparison was made between the results obtained with the HPLC technique and the results of a GLC technique with a packed OV-275 column. There was a good correlation between the 2 techniques with a tendency to higher *trans* values with the HPLC technique (4%). 18:1ω6-*cis* to ω11-*cis* and 18:1ω5-*trans* to ω12-*trans* could be almost quantitatively recovered in the HPLC step. Most of the individual positional isomers of monounsaturated fatty acids of varying chain length could be separated and determined in the glass capillary GC step with the exception of those isomers containing the double bond in a relatively high ω-position. The relative standard deviation of the technique as determined with reference substances was better than 4%. The described technique was applied to the analysis of the isomeric monounsaturated fatty acid content in partially hydrogenated vegetable and marine oils, and about 5 samples a day could be executed.

CHEMICAL SYNTHESIS, BIOLOGICAL ACTIVITY, AND METABOLISM OF 25-HYDROXY-24-OXOVITAMIN D_3. Y. Takasaki, T. Suda, S. Yamada, M. Ohmori, H. Takayama, and Y. Nishii (Dept. of Biochem., School of Dentistry, Showa Univ., 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan) *J. Biol. Chem. 257*:3732-3738 (1982). 25-Hydroxy-24-oxovitamin D_3 (25(OH)24-oxo-D_3), a metabolite of 25-hydroxyvitamin D_3, has been chemically synthesized. The ultraviolet, mass, infrared, and proton nuclear magnetic resonance spectra of the 25(OH)24-oxo-D_3 were identical with those of the natural product isolated from chick kidney incubates. The oxo compound showed biological activity similar to 24,25-dihydroxyvitamin D_3 (24,25)$OH)_2D_3$) in vitamin D-deficient chicks in enhancing intestinal calcium transport and bone calcium mobilization activities. Although 25(OH)24-oxo-D_3 partially restored the impaired eggshell weights of Japanese quails fed a vitamin D-deficient diet, it was much less potent than 25-hydroxyvitamin D_3 or 1α,25-dihydroxyvitamin D_3. In addition, there was no effect on the calcification of medullary bone. When 25(OH)24-oxo[^{3}H]D_3 was incubated with kidney homogenates from vitamin D-deficient chicks, it was metabolized to [^{3}H]-1α,24,25-trihydroxyvitamin D_3 and a metabolite which was eluted in a region between authentic 24*R*,25$(OH)_2D_3$ and 1α,25-dihydroxyvitamin D_3 on high pressure liquid chromatography. In the incubates of kidney homogenates from vitamin D-supplemented chicks, those metabolites were not detected. In vitamin D-supplemented chicks, the recovery of radioactivity in the chloroform phase extracted by the method Bligh and Dyer was only 50%, while that in vitamin D-deficient chicks was 87%. Moreover, the radioactivity eluted in the 25(OH)24-oxo-D_3 fraction from vitamin D-supplemented chicks was only one-fifth of that from vitamin D-deficient birds. The present results indicate that the 24-oxidation of 24,25$(OH)_2D_3$ may be a route of inactivation of vitamin D_3.

MOLECULAR ORGANIZATION IN THE LIQUID-CRYSTALLINE PHASES OF LECITHIN-SODIUM CHOLATE-WATER SYSTEMS STUDIED BY NUCLEAR MAGNETIC RESONANCE. J. Ulmius, G. Lindblom, H. Wennerstrom, L.B-Å Johansson, K. Fontell, O. Soderman, and G. Arvidson (Division of Physical Chemistry 2, Chemical Centre, Univ. of Lund, S-220 7 Lund, Sweden) *Biochemistry 21*(7):1553-1560 (1982). The molecular organization in the hexagonal and lamellar phases of the ternary systems lecithin-sodium cholate-water has been investigated by using a variety of nuclear magnetic resonance techniques. The main findings and conclusions are the following: (i) When calculated on a mole fraction basis, the phase equilibria are insensitive to changes in the alkyl chains of the lecithin. (ii) When incorporated into a lecithin bilayer, cholate exerts a strong perturbation on the lecithin alkyl chain

order, giving a large decrease of the order parameter. (iii) This decrease of the order occurs since the average cross-sectional area per alkyl chain increases probably as a result of cholate placing itself flat on the bilayer surface. (iv) The diffusion of lecithin molecules is approximately equally rapid in the lamellar and hexagonal phases. (v) The hexagonal phase is formed by rodlike aggregates with the polar groups at the surface of the rods and with a continuous hydrocarbon core. The rods are *not* formed by stacking disklike mixed micelles. (vi) With the interpretations of the molecular packing and the phase structures, the observed phase equilibria are in good agreement with current theories of the factors that govern phase behavior in amphiphile-water systems.

PURIFICATION FROM PIG LIVER OF A PROTEIN WHICH PROTECTS LIPOSOMES AND BIOMEMBRANES FROM PEROXIDATIVE DEGRADATION AND EXHIBITS GLUTATHIONE PEROXIDASE ACTIVITY ON PHOSPHATIDYLCHOLINE HYDROPEROXIDES. F. Ursini, M. Maiorine, M. Valente, L. Ferri, and C. Gregolin (Institute of Biol. Chem. Univ. of Padova, 35100 Padova, Italy) *Biochim. Biophys. Acta 710*:197-211 (1982). The cell sap from pig liver contains a protein which protects phosphatidylcholine liposomes and biomembranes from peroxidative degradation in the presence of glutathione. This protein has been assayed by measuring the inhibition of aged phosphatidylcholine liposome peroxidation induced by the Fe^{3+}-triethylenetetramine complex. The peroxidation-inhibiting protein from pig liver has been purified 535-fold to homogeneity with overall recovery of activity of 12%. The protein inhibited peroxidation by Fe^{3+}-triethylenetetraamine following a 15 min preincubation of phosphatidylcholine liposomes in the presence of 5 mM glutathione or 2-mercapthoethanol. The pure protein exhibited glutathione peroxidase activity on hydroperoxide groups of phosphatidylcholine and on cumene and *t*-butyl hydroperoxides. The protein appears to be distinct from the selenoenzyme glutathione peroxidase and from any known glutathione *S*-transferase. The peroxidation was studied also with fresh phosphatidylcholine liposomes and was induced in this case by Fe-ascorbate. To obtain protection by the peroxidation-inhibiting protein and glutathione, preincubation was not necessary, but α-tocopherol, incorporated in the liposomes in the molar ratio 1:250 to phosphatidylcholine, was required. Lipid peroxidation of rat liver mitoplasts and microsomes was blocked when these preparations were incubated in the peroxidizing mixture in the presence of peroxidation-inhibiting protein and glutathione. The protection from Fe^{3+}-triethylenetetramine-induced peroxidation is related apparently to reduction of hydroperoxide groups in polyunsaturated fatty acid residues of phospholipids and to inhibition of free radicals formation by chain branching. Protection from the Fe-ascorbate-induced peroxidation is apparently attributable to the same mechanism.

THE EFFECT OF MALONYL-CoA ON FATTY ACID OXIDATION IN RAT MUSCLE AND LIVER MITOCHONDRIA. J.H. Veerkamp and H.T.B. Van Moerkerk (Department of Biochemistry, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands) *Biochim. Biophys. Acta 710*:252-255 (1982). The effect of malonyl-CoA on palmitate oxidation was compared for skeletal muscle and liver mitochondria from fed rats and rats starved for 18 and 42 hr. The nutritional state did not influence the palmitate oxidation rate by both types of mitochondria. Muscle mitochondria are more sensitive to malonyl-CoA inhibition of palmitate oxidation than are liver mitochondria. Their response is not influenced by the nutritional state, in contrast to that of liver mitochondria. The extent of inhibition was not dependent on the carnitine concentration (above 0.5 mM), but higher at lower palmitate: albumin ratio or palmitate concentration.

NANOSECOND FLUORESCENCE ANISOTROPY DECAYS OF *N*-(9-ANTHROYLOXY) FATTY ACIDS IN DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES WITH REGARD TO ISOTROPIC SOLVENTS. M. Vincent, B. de Foresta, J. Gallay, and A. Alfsen (Etats Lies Moleculaires, 45 rue des Saints-Pères, 75270 Cédex 06 Paris, France) *Biochemistry 21*:708-716 (1982). A set of *N*-(9-anthroyloxy) fatty acids [2-, 7-, 9-, and 12-(9-anthroyloxy)stearic acid (AS) and 16-(9-anthroyloxy)palmitic acid (16-AP)] has been studied by time-resolved and steady-state fluorescence anisotropy measurements in isotropic media and in vesicles of dipalmitoylphosphatidylcholine. The 2 modes of rotation, "in-plane" and "out-of-plane", of the anthroyl ring can be detected by varying the excitation wavelength. In both isotropic solvents, the value of the in-plane rotational rate is of the same order of magnitude as the out-of-plane rate for each of the *n*-(9-anthroyloxy) fatty acids. In propylene glycol, the anthroyl ring motions are similar for all derivatives except for the 16-AP for which the fluorophore rotates at a higher rate. In the liquid paraffinic oil, identical motions of the fluorophore are observed for the 7-, 9-, and 12-AS; the motion for the 16-AP is again faster in this solvent than in propylene glycol in conditions of identical viscosity. When embedded in phospholipid bilayers, these probes report the microenvironment at a graded series of depths from the surface to the center of the bilayer. Studies in dipalmitoylphosphatidylcholine vesicles have been performed at 3 temperatures. The out-of-plane mode of rotation is unhindered. When the in-plane mode of rotation contributes to the anisotropy decay, a hindrance to the motion is observed below the gel to liquid-crystalline transition. In the pretransition temperature range (37 C) the results evidence the existence of structural lipid changes mainly localized in the hydrophobic core of the bilayer. The main transition leads to a complete disappearance of the hindrances on the in-plane rotation.

SOLID-STATE CARBON-13 NUCLEAR MAGNETIC RESONANCE OF THE LECITHIN GEL TO LIQUID-CRYSTALLINE PHASE TRANSITION. R.J. Wittebort, C.F. Schmidt, and R.G. Griffin (Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA) *Biochemistry 20*(14): 4223-4228 (1981). The temperature dependence on the ^{13}C NMR spectra of dipalmitoylphosphatidylcholine (DPPC) which has been ^{13}C labeled at the carbonyl position of the *sn*-2 chain, 2-(1-^{13}C)-DPPC, is reported. In the $L_{\beta'}$ phase an axially summetric spectrum of 112-ppm breadth is observed, and this transforms to an isotropic-like line ($\langle\Delta\sigma\rangle \sim 7$ ppm) in the L_α phase. In the intermediate P_β phase a temperature-dependent superposition of these spectra is observed, which suggests that this phase exhibits microscopic structural and dynamical properties of both the $L_{\beta'}$ and L_α phases. An analysis of the spectral line shapes leads to the conclusion that the appearance of the isotropic-like line in the $P_{\beta'}$ phase is primarily due to a conformational change at the *sn*-2 carbonyl which is complete at the main transition. Increased rates of axial diffusion in the $P_{\beta'}$ phase may contribute to the narrowing.

RELATIVE ACYLGLYCEROL ACYLTRANSFERASE ACTIVITIES IN HOMOGENATES OF ENZYMICALLY DISPERSED RAT JEJUNAL VILLUS AND CRYPT CELLS. A.G.D. Hoffman and A. Kuksis (Banting and Best Dept. of Medical Research, Univ. of Toronto, Toronto, Ontario M5G 1L6, Canada) *Biochim. Biophys. Acta 710*(1):53-62 (1982). The relative acyltransferase activities were compared in homogenates of rat jejunal villus and crypt cells isolated by differential scraping and hyaluronidase dispersion. The contributions of the monoacylglycerol and phosphatidic acid pathways to the higher acylglycerol and phospholipid biosynthesis were assessed using 2-oleoyl-*sn*-[^{3}H]glycerol and [1-^{14}C]palmitic acid as tracers. The stereochemical course of the diacylglycerol biosynthesis was determined by stereospecific analysis. Using 2-oleoyl-*sn*-glycerol as a tracer, the villus cells exhibited four times higher diacylglycerol and 19 times higher triacylglycerol biosynthesis than crypt cells on an equivalent protein basis. Furthermore, while the villus cell homogenates yielded a preponderance (75%) of the 1,2-diacyl-*sn*-glycerols, the crypt cell homogenates formed essentially racemic proportions of 1,2- and 2,3-diacyl-*sn*-glycerols. Both villus and crypt cell homogenates exhibited comparable cyl acceptor and acyl donor concentration dependence and the same cofactor requirements. It is unlikely that these acyltransferase activities in the crypt cell preparation are due to contamination with villus cells, because then more comparable proportions of the enantiomeric diacylglycerols and triacylglycerols would have been anticipated. It is concluded that the crypt cells possess intrinsic monacylglycerol and to a much lesser extent diacylglycerol acyltransferase activities.

INHIBITION OF CHOLESTEROL SIDE CHAIN CLEAVAGE BY ACTIVE SITE DIRECTED ANTIBODY TO CORPUS LUTEUM CYTOCHROME P-450. K. Kashiwagi, A.B. MacDonald, and H.A. Salhanick (Dept. of Population Sci., Harvard School of Public Health, Boston, MA 02115) *J. Biol. Chem. 257*(5):2212-2217 (1982). Goat antibody IgG produced against bovine corpus luteum mitochondrial cytochrome P-450 ($P\text{-}450_{scc}$) associated with cholesterol side chain cleavage (CSCC) was used to compare immunological characteristics of mitochondrial cytochrome P-450s from the bovine adrenal cortex (BAM), bovine corpus luteum (BCLM), and human placenta (HPM). In Ouchterlony double diffusion, anti-$P\text{-}450_{scc}$ produced a single band with BAM and BCLM $P\text{-}450_{scc}$, but not with HPM $P\text{-}450_{scc}$ or BAM $P\text{-}450_{11\beta}$. Appropriate concentrations of this anti-$P\text{-}450_{scc}$ IgG inhibited the conversion of cholesterol to pregnenolone in BCLM and BAM preparations equivalently, but inhibition of placental $P\text{-}450_{scc}$ was considerably less. The addition of BCLM iron sulfur protein and iron sulfur protein reductase to HPM $P\text{-}450_{scc}$ increased CSCC approximately 5-fold. Under these conditions, anti-$P\text{-}450_{scc}$ inhibited CSCC in HPM. Solubilized and

sonicated BCLM preparations were inhibited equivalently but more than whole mitochondria. Addition of anti P-450$_{scc}$ IgG to BAM increased 11β-hydroxylation activity in concentration-dependent fashion. It appears that the cytochrome P-450$_{scc}$s from BAM and BCLM are very similar if not identical, but immunologically different from HPM P-450$_{scc}$. The BAM P-450$_{11\beta}$ is immunologically distinct from BCLM P-450$_{scc}$. The CSCC and 11β-hydroxylation systems of the adrenal are intimately linked because of inhibition of 450$_{scc}$ markedly stimulated 11β-hydroxylation. Finally the inhibition of CSCC activity of BAM, BCLM, and HPMP-450 indicates that the antigenic effect is directed toward the active site.

PALMITATE METABOLISM AND NOREPINEPHRINE SENSITIVITY IN BROWN ADIPOSE, LIVER, AND WHITE ADIPOSE TISSUES OF ZUCKER RATS. T.R. Kasser and R.J. Martin (Dept. of Foods and Nutr., Univ. of Georgia, Athens, GA 30602) *Exptl. Bio. Med. 169*(3):320-325 (1982). Interscapular brown adipose tissue (BAT) *in vitro* utilization of palmitate (0.5 mM) and glucose (5.0 mM) were examined at 0, 0.1, 1.0, and 10.0 μg norepinephrine-bitartrate (NE)/ml. With increasing NE concentrations, *in vitro* palmitate oxidation was stimulated and glucose oxidation was inhibited in BAT of nonobese rats. This characterization of BAT metabolism provided the constraints for our analysis of fatty acid utilization and norepinephrine sensitivity at submaximal response concentrations in BAT of lean and obese Zucker rats. Liver and inguinal white adipose were used for tissue comparisons. In comparison to its lean littermates, it was demonstrated that the obese rat: (1) had heavier interscapular BAT pads, (2) oxidized a similar amount of palmitate to CO_2 per 100 mg of BAT, (3) oxidized more palmitate in response to NE stimulation of BAT, and (4) had slower rates of hepatic ketogenesis and palmitate oxidation. Altered palmitate oxidation or NE sensitivity in BAT was not contributing to the obesity of the "fatty" Zucker rat at 5 weeks of age.

THE EFFECT OF CONTRACEPTIVE STEROIDS ON THE INCORPORATION OF U^{14}C-GLUCOSE INTO PORCINE AORTIC LIPIDS. W.C. Kent, M.E. Soulsby, and J.E. Whitney (Dept. of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205) *Artery 9*(6):425-436 (1981). The *in vitro* effect of 17α-ethinylestradiol (E) and/or medroxyprogesterone acetate (P) was determined on their abilities to alter conversion of glucose to lipid by porcine aorta. The combination of steroidal agents EP/HI (combination at high concentration) at a concentration of 9.5 X 10^{-9} moles/ml caused a significant ($p<0.01$) reduction in ^{14}C incorporation into total lipid. When the concentration of each hormone was reduced by one-half EP/LO (combination at low concentration) the incorporation was also significantly reduced ($p<0.05$). The aforementioned reductions were subsequently found to be the result of depressed incorporation of the substrate glucose for the synthesis of phospholipid (PL), triacylglyceride (TG), a combined fraction of free cholesterol, diacylglyceride and free fatty acid (FC+DG+FFA), and cholesteryl ester (CE). The study suggests that these oral contraceptives, when administered in pharmacological doses, can depress the conversion of glucose into arterial wall lipid.

RELATIONSHIPS BETWEEN PREPARTAL DIETARY CALCIUM AND PHOSPHORUS, VITAMIN D METABOLISM, AND PARTURIENT PARESIS IN DAIRY COWS. T.S. Kichura, R.L. Horst, D.C. Beitz, and E.T. Littledike (Dept. of Anim. Sci., Iowa State Univ. and National Anim. Disease Center Ames, IA 50011) *J. Nutr. 112*(3):480-487, (1982). Twenty Jersey cows were fed one of four prepartal diets: a) low calcium, low phosphorus (LCLP); b) low calcium, high phosphorus (LCHP); c) high calcium, low phosphorus (HCLP); or d) high calcium, high phosphorus (HCHP). Diets were fed for about 4 weeks prepartum. Blood samples were taken periodically, and the collected plasma analyzed for concentrations of calcium, phosphorus, hydroxyproline and 1.25 dihydroxyergocalciferol plus 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$ D). Cows fed the LCLP and LCHP diets, when compared to cows fed the HCLP diet, had: a) greater concentrations of plasma 1,25-$(OH)_2$ D and hydroxyproline prepartum; b) greater plasma calcium concentrations at parturition; and c) less incidence (0 versus 4 cases) of parturient paresis. Thus, low calcium diets, regardless of dietary phosphorus intake, seemed to activate calcium homeostatic mechanisms before parturition by stimulating both bone and gut. Cows fed the HCLP diet had greater plasma calcium concentrations at parturition than did cows fed the HCHP, even though there was no measurable effect on plasma 1,25-$(OH)_2$ D and hydroxyproline concentrations during the prepartal period. It seems possible that the beneficial effect of low dietary phosphorus, when dietary calcium is high, may be result of a prepartal increase in efficiency of absorption of calcium and phosphorus from the gut caused by increased binding of 1,25-$(OH)_2$ D to intestinal receptors.

LYSOLECITHIN ACYLTRANSFERASE AND ALVEOLAR PHOSPHATIDYLCHOLINE PALMITATE IN EXPERIMENTAL ACUTE ALVEOLAR INJURY IN THE DOG LUNG. D.G. Liau, C.R. Barrett, A.L.L. Bell, S.A. Hashim, and S.F. Ryan (Pulmonary Research Group, Dept. of Pathology and Pulmonary Division of the Dept. of Medicine, St. Luke's-Roosevelt Hospital Center) *Biochim. Biophys. Acta 710*(1):76-81 (1982). Lysolecithin acyltransferase (EC 2.3.1. 23) activities in lung homogenates and in subcellular fractions, and fatty acid composition of phosphatidylcholine (PC) in lung lavage were studied in dogs with acute alveolar injury induced by N-nitroso-N-methylurethane. The specific activity in the microsomal fraction was 10 and 3 times higher than those of homogenate and mitochondrial fractions, respectively. Both the lysolecithin acyltransferase activities and the proportions of palmitate in alveolar lavage PC increased during the early phase of injury (days 2-4), and decreased during peak injury (days 6-8). Such correlation was not found during the recovery period (day 15). During recovery, specific and total activities of the enzyme were nearly 2- and 3-fold, respectively, those of controls. Nevertheless, the palmitate proportions in PC were normal, indicating that the increased enzyme activity in vitro was not reflected in increased PC palmitate during recovery. This finding indicates that the enzyme activity per cell was normal during recovery and suggests that the increase in specific and total activities is due to massive regeneration of type II cells and that the enzyme is localized mainly in these cells. The decrease in proportion of palmitate in lavage PC during peak injury may lead to abnormality of surfactant function.

DECLINING MORTALITY IN CORONARY HEART DISEASE. F.I. Levy (School of Medicine, Tufts Univ., Boston, MA 02111) *Arteriosclerosis 1*(5):312-325 (1981). Since 1968, there has been a dramatic, unprecedented decline in mortality from cardiovascular disease in the United States, especially from coronary heart disease and stroke. The decline has now been confirmed as real and has been observed in all age, sex, and race groups. Possible causes of the decline in coronary heart disease mortality include the development of the concept of acute coronary care, new drugs, sophisticated surgical techniques such as coronary artery bypass, noninvasive diagnostic methods for earlier disease detection, and the identification of specific cardiovascular risk factors. The decline has been temporarily related to risk factor awareness and reduction and modification (cigarette smoking cessation, hypertension control, diet change, and reduction in cholesterol). Thus, both primary prevention through lifestyle changes and improved treatment regimes have played a role in the decline.

EFFECT OF VITAMIN D ON THE INTESTINAL ABSORPTION OF ^{203}Pb and ^{47}Ca IN CHICKS. H.M. Mykkänen and R.H. Wasserman (Dept. of Physiology, New York State College of Veterinary Med., Cornell Univ., Ithaca, NY 14853) *J. Nutr. 112*:520-527 (1982). The transfer of ^{203}Pb and/or ^{47}Ca across the intestinal epithelium of the chick was investigated, with emphasis given to the functional role of cholecalciferol. ^{203}Pb, after introduction in the intestinal lumen, is rapidly accumulated by the intestinal tissue, and only a fraction of ^{203}Pb is translocated parenterally. Cholecalciferol did not significantly affect the accumulation of ^{203}Pb by intestinal tissue but did accelerate ^{203}Pb movement across the basal-lateral membrane. Cholecalciferol both decreased ^{47}Ca tissue levels and increased ^{47}Ca absorption. In rachitic chicks, the rate of absorption of ^{203}Pb was greater in the distal than in the proximal segments of the intestine; after cholecalciferol repletion, the degree of absorption in all segments was similar, indicating the order of cholecalciferol effectiveness as duodenum>jejunum>ileum. An acute dose of 1,25-$(OH)_2D_3$ to rachitic chicks also enhanced both ^{203}Pb and ^{47}Ca absorption, but the time course and pattern of absorption of these metal cations differed. The time at which the absorption of ^{203}Pb peaked and returned to base-line occurred sooner than for ^{47}Ca. Also the back-flux of ^{47}Ca was enhanced by cholecalciferol, whereas no effect on the back-flux of ^{203}Pb was noted. These studies show that cholecalciferol and 1,25$(OH)_2D_3$ affects both the ^{203}Pb and ^{47}Ca absorptive processes, but the nature of these responses are not identical, suggesting differences in the transport path or the macromolecular interactions of these metal ions during the course of absorption, or both.

BIOSYNTHESIS OF PLATELET-ACTIVATING FACTOR. I. EVIDENCE FOR AN ACETYL-TRANSFERASE ACTIVITY IN MURINE MACROPHAGES. E. Ninio, J.M. Mencia-Huerta, F. Heymans, and J. Benveniste (INSERUM u 200, 32 rue des Carnets, 92140 Clamart and Laboratoire de Pharmacologie Moleculaire, Universite Paris VII, 1 place Jussieu, 75005 Paris, France) *Biochim. Biophys. Acta 710*:23-31 (1982). Platelet-activating factor (PAF-acether; 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) is released from murine peritoneal adherent cells by inflammatory and non-inflammatory stimuli. We have found, in extracts from these cells, an enzyme activity that synthesized PAF-acether from synthetic lyso-PAF-acether by transferring the acetyl moiety of acetyl-coenzyme A onto the lyso-PAF-acether molecule. The enzyme is

stabilized by 1 mM dithiothretitol, is calcium-dependent, has an apparent K_m of 172 μM for acetyl-CoA and is active in a 6-8 pH range. When the acetyl-CoA substrate is replaced by propionyl-CoA, an ether lipid is produced which turns out to be as potent an aggregating agent as PAF-acether. IN all cases, the products of the reaction were characterized by their behaviour in platelet-aggregation tests and their high-pressure liquid chromatography (HPLC) elution profiles. The precise definition of this acetyl-transferase is of primary importance for the development of new pharmacological agents capable of modulating a potent platelet-aggregating factor.

ESSENTIAL AND NONESSENTIAL FATTY ACID OXIDATION IN MICE BEARING EHRLICH ASCITES CARCINOMA. M. Ookhtens and N. Baker (Tumor-Lipid Laboratory, Research Service, Veterans Administration Wadsworth Medical Center, Los Angeles, CA and the Department of Medicine, UCLA School of Medicine, Los Angeles, CA) *Lipids* 17(2):65-71 (1982). We tested the hypothesis that mobilized (essential) free fatty acids (FFA) are spared from oxidation in cancer-bearing animals. We injected tracers [1-^{14}C] linoleate, [1-^{14}C] palmitate and NaH^{14}CO intravenously as single rapid doses in separate groups of mice bearing Ehrlich ascites tumor (EAT) and controls, and measured breath $^{14}CO_2$. The data from NaH^{14}CO injections were used to develop kinetic, compartmental models of the HCO_3-CO systems. These models were integrated with our earlier model of plasma FFA turnover for control and EAT-bearing mice. The integrated multicompartmental models were then fitted to breath $^{14}CO_2$ data from mice injected with tracer FFA to compute the rates of FFA oxidation. FFA were not spared from an oxidative fate in our cancer-bearing vs normal animals; moreover, essential FFA were not preferentially spared from oxidation compared to nonessential FFA in the cancer-bearing mice.

DIETHYLSTILBESTROL TREATMENT INCREASES THE AMOUNT OF CHOLINE KINASE IN ROOSTER LIVER. H.B. Paddon, C. Vigo, and D.E. Vance (Dept. of Biochem., Univ. of British Columbia, Vancouver, B.C. V6T 1W5 (Canada)) *Biochim. Biophys. Acta* 710(1):112-115 (1982). Studies have been performed on the mechanism by which diethylstibestrol stimulates the activity of choline kinase in livers from cockerels. The enzyme was purified 700-900-fold by affinity chromatography. The increased enzyme activity could not be accounted for by diethylstilbestrol alteration of the kinetic constants of the enzyme. Rabbit antibody was raised to the purified enzyme. Titration studies with antiserum demonstrated a 2-fold increase in the amount of choline kinase in diethylstilbestrol-treated cytosol, which correlated with a 2-fold elevation in the activity of the enzyme. We conclude that diethylstilbestrol stimulates the activities of choline kinase in cockerel liver by promoting a corresponding increase in the amount of enzyme.

TISSUE SITES OF CATABOLISM OF RAT AND HUMAN LOW DENSITY LIPOPROTEINS IN RATS. R.C. Pittman, A.D. Attie, T.E. Carew and D. Steinberg (Department of Medicine, University of California, San Diego, La Jolla, CA 92093) *Biochim. Biophys. Acta* 710:7-14 (1982). We have determined the sites of degradation of low density lipoprotein in rats using covalently linked [^{14}C] sucrose as tracer. On degradation, ^{14}C is trapped intracellularly as a cumulative measure of the amount of protein catabolized by each tissue. [^{14}C] Sucrose-labeled rat low density lipoprotein (*d*1.02-1.05 g/ml) was cleared from the plasma at a rate (0.092±0.003 h^{-1}) similar to that for ^{125}I-labeled LDL (0.096±0.22 h^{-1}). Tissues were examined for total ^{14}C content 24 hr after injection of ^{14}C-labeled lipoprotein. At death, animals were perfused thoroughly to remove trapped plasma. Recovery of ^{14}C in tissues was 100±23% of catabolized ^{14}C-labeled lipoprotein (calculated from plasma decay kinetics). In three test tissues, leakage of ^{14}C over 5 days was less than 10%/day; leakage from liver was 10%/day, predominantly into bile; ^{14}C content of kidney increased slightly. Thus, ^{14}C trapping was adequate. The ^{14}C-labeled lipoprotein was catabolized 66.8±2.5% by liver. No other organ catabolized more than 8%. Liver, adrenal and ovary were the most active per unit wet weight, followed by spleen. Urinary excretion, in 24 hr, was 3% and biliary excretion was 7% of catabolized. Human low density lipoproteins were similarly examined with similar results; this similarity may be due to exchange of rat apolipoproteins onto human lipoprotein in the circulation.

GLYCOLIPIDS AND THEIR DEVELOPMENTAL PATTERNS IN CHICK THIGH AND LEG MUSCLES. M. Saito and M. Rosenberg (Dept. Biochem. and Biophys., Loyola Univ. Stritch School of Med., 2160 South First Ave., Maywood, IL 60153) *J. Lipid Res.* 23(1): 3-8 (1982). Five major gangliosides and two major neutral glycolipids were isolated from chick thigh and leg muscles using Unisil column chromatography and preparative thin-layer chromatography. They were identified by gas-liquid chromatography as G_{D3}, G_{M3}, and N-acetyl glucosamine-containing mono- and disia-logangliosides. Two major neutral glycolipids were found and identified as monogalactosylceramide and digalactosylceramide. The quantification of each ganglioside by thin-layer chromatography was done usng a direct densitometric method, and the pattern of changes of glycolipids during development was investigated. In the embryonic period, G_{D3} and the N-acetyl-glucoseamine-containing disialoganglioside decreased; G_{M3} increased during embryonic life and became the major post-natal ganglioside. The two neutral glycolipids also increased rapidly after hatching.

PLASMA APOLIPOPROTEIN A-I ABSENCE ASSOCIATED WITH A MARKED REDUCTION OF HIGH DENSITY LIPOPROTEINS AND PREMATURE CORONARY ARTERY DISEASE. E.J. Schaefer, W.H. Heaton, M.G. Wetzel, and H.B. Brewer, Jr. (Building 10, Room 7N-117, National Institutes of Health, Bethesda, MD 20205) *Arteriosclerosis* 2(1):16-26 (1982). A 45-year-old woman with corneal opacification and severe coronary artery disease was noted to have the following plasma lipid levels (mg/dl, ±SD): total cholesterol 111±13, triglyceride 62±6, very low density lipoprotein cholesterol 4±1, low density lipoprotein cholesterol 106±14, and high density lipoprotein (HDL) cholesterol 1±1 (normal, 50±14). Her two offspring and one brother were found to have HDL cholesterol values (mg/dl) of 23,20, and 20, respectively. Their percentage of cholesterol in the esterified form in the patient's plasma was normal at 70%. Lipoprotein electrophoresis showed no alpha lipoprotein band, and no HDL was detectable when plasma was subjected to analytic ultracentrifugation. Only trace amounts of lipids were noted within the HDL density region following preparative ultracentrifugation. Mean plasma apolipoprotein (apo) A-II, apoB, and apo C-II plasma levels were 13.8%, 130.6%, and 26.6% of normal, respectively. The ratio of apo B to cholesterol within LDL was elevated. Apo A-I, the major HDL protein constituent, was immunologically undetectable in this patient's plasma. A decreased HDL cholesterol concentration has been associated with premature coronary artery disease. These data indicate that plasma apo A-I absence results in a striking reduction in HDL, is associated with premature coronary artery disease, and represents a new distinct disease entity.

PURIFICATION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR, AN ACIDIC GLYCOPROTEIN OF 164,000 MOLECULAR WEIGHT. V.J. Schneider, U. Beisiegel, J.L. Goldstein, and M.S. Brown (Depts. of Molec. Genetics and Internal Medicine, Univ. of Texas Health Sci. Center at Dallas, Dallas, TX) *J. of Biol. Chem.* 257 (5):2664-2673 (1982). This paper describes a rapid two-step procedure for the purification of the low density lipoprotein receptor from bovine adrenal cortex membranes. After solubilization with nonionic detergents, the receptor adheres tightly to a DEAE-cellulose column at pH 6. Following elution from DEAE-cellulose, detergent is removed, leaving the receptor in a soluble form. The receptor is eluted with suramin, a newly-found inhibitor of low density lipoprotein-receptor interactions. This procedure yields a single protein with a molecular weight of 164,000. The same protein is also isolated when the crude DEAE-cellulose fraction is applied to an immunoaffinity column containing a monoclonal antibody directed against the receptor. The 164,000-dalton receptor protein has an acidic isoelectric point of 4.6, which rises to 4.8 after extensive treatment with neuraminidase. The purified receptor retains all of the binding properties of the receptor of intact cells and crude membranes.

LIPID COMPOSITION OF TRACHEOBRONCHIAL SECRETIONS FROM NORMAL INDIVIDUALS AND PATIENTS WITH CYSTIC FIBROSIS. A. Slomiany, V.L.N. Murty, M. Aono, C.E. Synder, A. Herp, and B.L. Slomiany (Gastroenterology Research Laboratory, Dept. of Medicine, New York Medical College, Research Center, Metropolitan Hospital, New York, NY 10029) *Biochim. Biophys. Acta* 710(1):106-111 (1982). The lipid composition of tracheobronchial secretions from normal individuals and patients with cystic fibrosis was investigated. Lipids were extracted from the dialyzed and lyophilized samples, and fractionated on silicic acid columns into neutral lipids, glycolipids and phospholipids. The lipids contained in each fraction were separated into individual components by thin-layer chromatography and quantified. The secretions of patients with cystic fibrosis were found to contain about 30% more lipids than that of normal individuals and exhibited elevated levels of cholesterol, phospholipids and glycosphingolipids. The level of free fatty acids and glycerolglucolipids was higher in the normal secretions. The phospholipids of cystic fibrosis secretions exhibited higher content of sphingomyelin and phosphatidylserine, while the normal samples contained more lysophosphatidylcholine. The glycosphingolipids of both types of samples consisted mainly of glucosyl- and lactosylceramides. The major glyceroglucolipid of the normal tracheo-bronchial secretions was tetraglucosyl glyceroglucolipid, whereas hexa- and octaglucosyl glyceroglucolipids were the predominant compounds of the cystic fibrosis secretions.

IN VITRO PROSTAGLANDIN SYNTHESIS BY VARIOUS RAT RENAL PREPARATIONS. J. Sraer, W. Siess, L. Moulonguet-doleris, J.-P. Oudinet, F. Dray and R. Ardaillou (INSERUM U 64,

Hopital Tenon, 75970 Paris 20) *Biochim. Biophys. Acta 710*:45-52 (1982). Prostaglandin synthesis by eight different structures from the rat kidney was measured in vitro after incubation with [^{14}C]-arachidonic acid using high-performance liquid chromatography followed by RIA with four specific anti-prostaglandin antibodies. Prostaglandin production by the whole cortex and cortical tubules was very low. The order of abundance for isolated glomeruli was thromboxane B_2 > prostaglandin E_2 > prostaglandin $F_{2\alpha}$ > 6 keto-prostaglandin $F_{1\alpha}$. Masangial cells synthesized prostaglandin E_2 at a markedly high rate, and in decreasing order: prostaglandin $F_{2\alpha}$, thromboxane B_2 and 6 keto-prostaglandin $F_{1\alpha}$. The same order of abundance was observed for epithelial cells. The papilla synthesized essentially prostaglandin E_2 and prostaglandin $F_{2\alpha}$, whereas the main product for the outer medulla was 6 keto-prostaglandin $F_{2\alpha}$. Cultured interstitial cells synthesized mainly prostanglandin E_2 and to a lesser extent prostaglandin $F_{2\alpha}$. Unidentified peaks eluting between 6 keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 were also observed chiefly with glomeruli but they were absent with the medullary preparations. They disappeared after incubation with indomethacin or aspirin and represented for glomeruli the greatest percentage of conversion of [^{14}C] arachidonic acid. These results show that the prostanoid profile varies markedly with different regions and cells of the rat kidney.

PROPERTIES OF A SOLUBILISED AND RECONSTITUTED PREPARATION OF ACYL-COA: CHOLESTEROL ACYL-TRANSFERASE FROM RAT LIVER. K.E. Suckling, G.S. Boyd and C.G. Smellie (Department of Biochemistry, University of Edinburgh Medical School, Hugh Robson Building, George Square, Edinburgh, EH8 9XD, United Kingdom) *Biochim. Biophys. Acta 710*:154-163 (1982). Rat liver acyl-CoA:cholesterol acyltransferase activity was released from the microsomal fraction by treatment with Triton X-100. After fractionation with polyethylene glycol 6000, the solubilised preparation was reconstituted in liposomes of different lipid composition by an octyl glucoside dilution method. The activity of the reconstituted system was dependent on the amount of cholesterol used in the liposomes and could also be stimulated by transfer of cholesterol into the reconstituted system from other membranes. The results are consistent with the hypothesis that substrate supply and the fluidity of the membrane contribute in the regulation of the rate of cholesterol ester formation.

ENZYMATIC DEOXYGLUCOSYLATION OF CERAMIDES BY MICROSOMES OF BHK-21 CELLS. THE EFFECT OF DEOXYGLUCOSE TREATMENT AND HERPES VIRUS INFECTION. Y. Suzuki and H.A. Blough (Division of Biochem. Virology and Membrane Research, Dept. of Ophtalmology, Univ. of Pennsylvania School of Medicine, Scheie Eye Inst., Philadelphia, PA 19104) *Biochim. Biophys. Acta 710*(2):221-229 (1982). The microsomal fractions of cultured hamster fibroblasts (BHK-21 cells) catalyze the incorporation of glucose from UDPglucose or of deoxyglucose from UDP deoxyglucose into a reaction mixture with liposomes consisting of ceramide and phosphatidylcholine. The microsomal fractions also catalyze the transfer of glucose from UDPglucose to enogenous acceptors. The specific activity of ceramide deoxyglucoside or ceramide glucoside formation was significantly higher when microsomal preparations obtained from deoxyglucose-treated or herpesvirus-infected BHK-21 cells were used as the glucosyltransferase source. Deoxyglucose was incorporated from UDPdeoxyglucose into hydroxy- and nonhydroxy-fatty acid-containing ceramides at approximately the same rate. Competitive inhibition of deoxyglucosylation of ceremides by UDPglucose suggests that both reactions were catalyzed by the same enzyme, viz. UDPglucose: ceramide glucosyltransferase. This inhibition of glycosphingolipid synthesis may account, in part, for the inhibitory effect of deoxyglucose on lipid-containing viruses.

THE TURNOVER OF MOLECULAR SPECIES OF PHOSPHATIDYLINOSITOL IN EHRLICH ASCITES TUMOR CELLS. K. Waku, T. Shibata, H. Kato, K. Tsutsui and Y. Nakazawa (Faculty of Pharmaceutical Sciences, Teikyo University, Tsukuigun, Sagamiko, Kanagawa 199-01) *Biochim. Biophys. Acta 710*:39-44 (1982). It has been shown previously that $^{32}P_i$ is incorporated into phosphatidylinositol 30 times faster than into the other phospholipid classes in Ehrlich ascites tumor cells, whereas [1-^{14}C] glycerol is incorporated at almost the same rate. It was therefore suggested that there is a recirculating system (phosphatidylinositol→diacylglycerol→phosphatidic acid→CDP-diacylglycerol→phosphatidylinositol) of phosphatidylinositol in Ehrlich ascites tumor cells. In this work, $^{32}P_i$ or [1-^{3}H] glycerol was injected into the peritoneal cavity of mice bearing Ehrlich ascites tumor cells from which the lipids were extracted after selected periods. Phosphatidylinositol was prepared and fractionated in the form of dimethylphosphatidic acid into six molecular species by $AgNO_3$-impregnated TLC. The specific radioactivities of the fractionated species were determined. $^{32}P_i$ was incorporated into diene molecular species and [1-^{3}H] glycerol into monoene species with a higher rate than the other species and both precursors were incorporated into tetraene species rather slowly. $^{32}P/^3H$ values appeared to be at almost the same for each molecular species, although monoene species showed slightly lower values. These results suggest that there could be a recirculating of the phosphorylinositol moiety in each of the molecular species of phosphatidylinositol.

BIOSYNTHESIS OF DOLICHOL AND CHOLESTEROL DURING EMBRYONIC DEVELOPMENT OF THE CHICKEN. T.K. Wong and W.J. Lennarz (Dept. of Physiological Chemistry, The Johns Hopkins University School of Medicine, 725 Wolfe Street, Baltimore, MD 21205) *Biochim. Biophys. Acta 710*:32-38 (1982). The polyisoprenol dolichol, in its phosphorylated form, serves as the lipid intermediate in *N*-linked glycoprotein synthesis, and shares a portion of its biosynthetic pathway with cholesterol. The synthesis of dolichol and cholesterol was followed over the course of development by incubating chicken embryos with [^{3}H] acetate for 24-hr intervals. Dolichol and cholesterol were isolated from the non-saponifiable lipids and identified by gel filtration and thin-layer chromatography. It was found that the rate of acetate incorporation into these two compounds per g of embryo decreased from days 8 to 14 of development. In the whole embryo, over the course of development the ratio of the rates of acetate incorporation into dolichol to that into cholesterol decreased markedly. These results suggest that mechanisms exist for controlling the differential rates of dolichol and cholesterol synthesis during development. When labeled organs from day 10 embryos were analyzed, it was found that the ratio of the rates of synthesis of dolichol to cholesterol in brain was about 14-fold lower than that in liver and heart. Similar values for the rates of synthesis of these two compounds were obtained when isolated organs were labeled in vitro. These data suggest that these three organs are capable of synthesizing dolichol and cholesterol de novo, and that each organ independently regulates the rates of synthesis of dolichol and cholesterol.

EFFECTS OF DIET COMPOSITION AND ADRENALECTOMY ON THE LIPOGENIC RESPONSES OF RATS TO STARVATION-REFEEDING. B.H. Williams, and C.D. Berdanier (Univ. of Georgia, Dept. of Foods, and Nutr., Dawson Hall, Athens, GA 30602) *J. Nutr. 112*(3):534-541 (1982). The interacting effects of diet and glucocorticoid (GC) on the tritium incorporation into lipid and glucose-6-phosphate dehydrogenase activity in starved-refed rats was studied. Male Sprague-Dawley rats were intact, adrenalectomized (ADX), or ADX and given GC and fed either ad libitum or not fed for 48 hours and refed either a 65% glucose diet, 65% sucrose diet, 65% protein diet or a 40% fat diet. No diet differences in rates of ^{3}HOH incorporation into total lipids were observed and ad libitum-fed rats. ADX lowered lipogensis and this effect was diet dependent. Sucrose-fed, glucose-fed and protein-fed ADX rats had lower rates of lipogenesis than their intact controls. Starvation-refeeding increased lipogenesis in all groups of intact rats except those fed the 40% fat diet. The magnitude of the response was diet dependent. Sucrose-fed rats had greater responses than fat-fed rats. The diet effect was dependent on the presence of the adrenals and GC. Thus, the large increase in liver lipid associated with starvation-refeeding is contingent on the composition of the diet and the presence of the adrenals.

EFFECTS OF ETHIONINE FEEDING ON FATTY LIVER AND PLASMA LIPOPROTEIN FRACTIONS IN RATS. F. Yokota, Y. Igarashi, and R. Suzue (The National Inst. of Nutr., 1, Toyamo-cho, Shinjuku-ku, Tokyo, 162 Japan) *J. of Nutr. 112*(3):405-405 (1982). We studied the changes in the lipid metabolism of rats fed a 0.5% DL-ethionine-containing diet. On day 3, hepatic lipid and cholesterol were markedly elevated in male and female rats; their plasma total cholesterol and plasma lecithin cholesterol acyltransferase (LCAT) activity levels were lower than in the controls. On day 7 and 14, hepatic lipid and cholesterol were near the control level, while plasma cholesterol and LCAT activity, especially in male rats, were higher than in the controls. Electrophoresis of the plasma lipoprotein complexes revealed a progressive decrease of the alpha fraction, irrespective of sex. These DL-ethionine-induced changes may be due to changes in hepatic adenosine triphosphate lavels leading to the inhibition of protein biosynthesis.

INTERACTION OF METAL IONS WITH PHOSPHATIDYLCHOLINE BILAYER MEMBRANES. H. Akutsu and J. Seelig (institute for Protein Research, Osaka Univ., Suita, Osaka 565, Japan) *Biochemistry 20*:7366-7373 (1981). The interaction of mono-, di-, and trivalent metal ions with bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was investigated with deuterium and phosphorus magnetic resonance. The measurements of the residual deuterium quadrupole splitting provided a sensitive handle to monitor directly the binding of ions. For the α segment of the choline group changes in the quadrupole splitting of up to 9 kHz were observed. The ion concentrations were varied between 5 mM and 2M. The fol-

lowing conclusions could be derived. (1) Addition of metal ions led to a structural change at the level of the polar groups. (2) The strength of interaction increased with the charge of the metal ion in the order $Na^{+}<Ca^{2+}<La^{3+}$. However, distinct differences were also noted between ions of the same charge. Furthermore, the strongly hydrophobic tetraphenylammonium ion induced almost the same change as La^{3+}. (3) The variation of the quadrupole splittings with ion concentration exhibited a plateau value at high concentrations of La^{3+}. The titration curves of DPPC with Ca^{2+} and La^{3+} could be described in terms of a Langmuir adsorption isotherm with an interaction potential. (4) The addition of NaCl considerably enhanced the binding of Ca^{2+} and La^{3+}. (5) The ion-induced conformational changes were qualitatively similar for all ions investigated. The quadrupole splittings of DPPC observed in the presence of chloroform or cholesterol and the variation of the quadrupole splittings with temperature could also be summarized in a linear plot that was different from that obtained for metal ion binding. This suggests the existence of at least two kinds of structural responses of the polar head groups to external perturbations.

GENETIC VARIATION IN FOWL SEMEN CHOLESTEROL AND PHOSPHOLIPID LEVELS AND THE RELATIONSHIPS OF THESE LIPIDS WITH FERTILITY OF FROZEN-THAWED AND FRESH SEMEN. G.A. Ansah and R.B. Buckland (Dept. of Animal Sci., Macdonald Campus of McGill Univ., Ste. Anne de Bellevue, Quebec, Canada H9X 1C0) *Poultry Sci. 61*:623-637 (1982). We studied the influence of five and six generations of selection for duration of fertility of frozen-thawed fowl semen on spermatozoa and seminal plasma cholesterol and phospholipid levels, their ratios, and their relationships with fertility of frozen-thawed and fresh semen. Males from the selected and control line were used. Cholesterol and phospholipid levels were determined in 2 trials for spermatozoa and in a single trial for seminal plasma per generation. The selected line had significantly ($P<.01$) higher fertility for both frozen-thawed semen and fresh semen. Spermatozoa cholesterol level, cholesterol to phospholipid ratio, and seminal plasma cholesterol and phospholipid levels were significantly ($P<.05$) lower in the selected line than in the control line; spermatozoa phospholipid to cholesterol ratio was significantly ($P<.05$) higher in the selected line but spermatozoa phospholipid level and seminal plasma liquid ratios were not changed significantly ($P>.05$). The heritability estimates of spermatozoa cholesterol and phospholipid levels ranged from zero to .66 and zero to .81, respectively. The phenotypic correlations of spermatozoa and seminal plasma cholesterol with fertility of frozen-thawed semen were negative as were the phenotypic correlations of seminal plasma cholesterol with fertility of fresh semen.

DISTRIBUTION OF α-TOCOPHEROL IN HUMAN PLASMA LIPOPROTEINS. W.A. Behrens, J.N. Thompson, and R. Madere (Bureau of Nutritional Sciences, Sir F. Banting Bldg., Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2) *Am. J. Clin. Nutr. 35*:691-696 (1982). Lipoproteins were removed from human plasma by ultracentrifugation at a density of 1.225. Three classes of lipoproteins were then separated by 4% Agarose-column chromatography: very low density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). A sensitive high performance liquid chromatography method with a fluorescence detector was used to estimate α-tocopherol in plasma and in column eluates. Total plasma tocopherol was not signficantly different in males (n=6) and females (n=6) and almost all of the vitamin was recovered in the isolated lipoproteins. Although LDL and HDL were the main carriers of α-tocopherol in both males and females, more tocopherol was found in LDL than in HDL in males but the opposite was true in females. The distribution of α-tocopherol in males was: VLDL, 8%; LDL, 59%; and HDL, 33% whereas that in females was VLDL, 2%; LDL, 42%; HDL, 56%. The distribution of protein in lipoprotein from males was: VLDL, 4%; LDL 37%; and HDL, 59% and in females: VLDL, 2%; LDL, 25%; and HDL, 73%. The α-tocopherol concentration (expressed as μg α-tocopherol/mg protein) in lipoproteins differed little between the sexes. The values in males were: VLDL, 7.0; LDL, 4.3; and HDL, 1.5, in females: VLDL, 3.9; LDL, 4.7; and HDL, 2.1. The data suggest that the different distribution of α-tocopherol in plasma lipoproteins in males and females is due to the different levels of proteins in those lipoprotein fractions. Overall, tocopherol and protein levels were highly correlated in HDL, a lower correlation was found in LDL.

TRIGLYCERIDE AND CHOLESTEROL METABOLISM IN PRIMARY HYPERTRIGLYCERIDEMIA. U. Beil, S.M. Grundy, J.R. Couse, and L. Zech (Center for Human Nutrition, Univ. of Texas Health Science Center, 5323 Harry Hines Boulevard, Dallas, TX 75235) *Arteriosclerosis 2*(1):44-57 (1982). To determine mechanisms of elevated plasma triglycerides (TG) in patients with primary hypertriglyceridemias, simultaneous studies were carried out on kinetics of very low density lipoprotein-triglycerides (VLDL-TG) and synthesis of cholesterol and bile acids. Sixteen hypertriglyceridemic patients with familial combined hyperlipidemia (FCHL) and 12 patients with poorly classified, primary hypertriglyceridemia were studied. The mean value for transport (synthesis) of VLDL-TG in patients with FCHL was about twice normal. It appeared that the major cause of hypertriglyceridemia in FCHL was an elevated production of VLDL-TG. However, the height of the plasma TG in FCHL patients also was influenced by individual clearance capacities for VLDL-TG, and fractional clearance rates in several seemed particularly low. Synthesis rates for cholesterol and/or bile acids were high in several patients with FCHL, suggesting simultaneous overproduction of VLDL-TG and sterols; however, increased synthesis of both was not observed in all the patients. Most patients with poorly classified hypertriglyceridemia had overproduction of VLDL-TG. In these patients, increased synthesis of cholesterol and bile acids was infrequent. Our results indicate that abnormally high production of VLDL-TG seemed to be the major factor in causing primary hypertriglyceridemia, but that clearance capacity can play an important role in determining the severity of TG elevation.

LIPID HYDROLYSES CATALYZED BY PANCREATIC CHOLESTEROL ESTERASE. REGULATION BY SUBSTRATE AND PRODUCT PHASE DISTRIBUTION AND PACKING DENSITY. S.G. Bhat and H.L. Brockman (Hormel Inst., Univ. of Minnesota, Austin, Minnesota 55912) *Biochemistry 21*(7):1547-1552 (1982). The role of oleic acid in the regulation of the hydrolysis of cholesteryl oleate in lipid films at the air-buffer interface was investigated by using initial rate techniques. A small quantity of enzyme is rapidly adsorbed to substrate containing films; however, a much greater, although slower, adsorption occurs if oleic acid is present. The rate constant for the slow adsorption is independent of the phase distribution of cholesteryl oleate but is markedly dependent upon both the concentration oleic acid head groups and the acyl chain packing density in the film. Adsorption is controlled by two ionizable groups, one of which may be the carboxyl group of oleic acid. In contrast to adsorption, catalysis by the surface excess of enzyme is pH independent between 5.5 and 7.5 and is relatively specific for substrate in the monlayer phase. The second-order rate constants for the hydrolysis of cholesteryl oleate in the monolayer phase and the interfacial layer of the double-layer phase are 27 and 2 $cm^2 s^{-1}$-$fmol^{-1}$. These results indicate that adsorption and catalysis occur at functionally if not physically, distinct sites on the protein. The adsorption of enzyme to a hydrolysis product, oleic acid, constitutes a form of product activation which presumably helps keep it at the interface during intraluminal fat digestion. The catalytic properties of the adsorbed enzyme suggest that substrate in the appropriate physical state at the lipid-water interface.

CERAMIDE STRUCTURE OF SPHINGOMYELIN FROM HUMAN MILK FAT GLOBULE MEMBRANE. J-F. Bouhours and D. Bouhours (Laboratoire de Biochimie des Membranes, LBTM-CNRS, Universite Claude Bernard, 43 Boulevard du 11 Novembre 1918, 6962 69622 Villeurbanne cedex, France) *Lipids 16*(10):726-731 (1981). Sphingomyelin was purified from human milk fat globule membrane and submitted to phospholipase C to yield ceramide. The structure of this cermide was investigated by gas liquid chromatographic analyses of its components, fatty acids, and sphingoid bases. The structure of the native ceramide was confirmed by direct-inlet mass spectrometry. It was shown to contain a major base C_{18}-sphingosine associated with a high proportion (60%) of C_{20}, C_{22}, C_{24}, and $C_{24:1}$ nonhydroxylated fatty acids. As these very long-chain fatty acids might be of nutritive importance, the concentration of sphingomyelin in human milk and its distribution in cream and skim milk were established.

RELATIONSHIPS IN DIFFERENT PARTS OF THE NEPHRON BETWEEN ENZYMES OF GLYCEROL METABOLISM AND THE METABOLITE CHANGES WHICH RESULT FROM LARGE GLYCEROL LOADS. H.B. Burch, A.E. Hays, M.D. McCreary, B.R. Cole, M.M.-Y. Chi, C.N. Dence, and O.H. Lowry (Dept. of Pharmacology, Washington Univ., School of Medicine, St. Louis, MO 63110) *J. Bio. Chem. 257*(7):3676-3679 (1982). Glycerol metabolism in 9 defined parts of rat nephron was studied by measurement of glycerol kinase and observation of effects of large glycerol loads on related metabolites and on ATP and total adenylate. Glycerol kinase with glycerol as substrate was highest in the proximal convoluted tubule, slightly lower in straight portions, 20 to 25 times lower in distal segments, and almost indetectable elsewhere. With dihydroxyacetone as substrate, enzyme distribution differed little except for 30% lower activity in proximal convoluted segments. Methods of measurement with 10-40 ng of freeze-dried tissue are described. Glycerol loads produced large accumulations of glycerol 3-phosphate in proximal segments and in distal convoluted tubules. In the control nephron, fructose bisphosphate plus triose phosphates was 10 times higher in distal straight and convoluted segments than in proximal segments. Increases with glycerol loads were limited to proximal straight tubules (2-fold) and the thin limb area (60%). Glucose-6-P increased

3-fold in the late portion of the proximal straight tubule but not elsewhere. ATP and total adenylate were markedly depleted in both proximal and distal segments even where not phosphorylated intermediates accumulated. Levels of P_i were decreased in whole cortex and medulla following glycerol loads.

EFFECTS OF CHOLESTYRAMINE ON LOW DENSITY LIPOPROTEIN BINDING SITES ON LIVER MEMBRANES FROM RABBITS WITH ENDOGENOUS HYPERCHOLESTEROLEMIA INDUCED BY A WHEAT STARCH-CASEIN DIET. Y-S. Chao, T-Y. Yamin, and A.W. Alberts (Merck Institute for Therapeutic Research, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065) *J. Biol. Chem.* 257(7):3623-3627 (1982). Rabbits fed a wheat starch-casein diet develop a marked hypercholesterolemia with a lipoprotein distribution similar to that of humans. Approximately 76% of the total cholesterol is carried in the low density lipoprotein (LDL) fraction ($1.006<d<1.063$ g/ml). Inclusion of 1% cholestyramine in the diet prevents the increase in plasma cholesterol. The cholestyramine effect is mediated through an increased fractional catabolic rate of ^{125}I-LDL. In order to determine the potential role of hepatic LDL receptors in the removal of LDL from the plasma, binding of ^{125}I-LDL and ^{125}I-β-VLDL (β-migrating very low density lipoproteins) to hepatic membranes prepared from livers of rabbits fed the wheat starch-casein diet with or without cholestyramine supplementation was investigated. Membranes from livers of the cholestyramine-supplemented animals exhibit high levels of specific EDTA-sensitive binding of either of the ^{125}I-labeled lipoproteins. Very little EDTA-sensitive binding occurs on liver membranes from wheat starch-casein-fed rabbits that have not been treated with cholestyramine. These results indicate that the hypercholesterolemia in rabbits associated with the wheat starch-casein diet is wholly or partially the results of a decreased number of specific hepatic LDL receptors and thus a decreased catabolism of plasma cholesterol. The response of the liver to the inclusion in the diet of the bile acid sequestrant, cholestyramine, is to maintain or increase the number of specific LDL binding sites, thus promoting catabolism of plasma cholesterol.

EFFECTS OF ZINC DEFICIENCY AND CASTRATION ON FATTY ACID COMPOSITION AND DESATURATION IN RATS. S. Clejan, M. Castro-Magana, P.J. Collipp, E. Jonas, and V.T. Maddaiah (Nassau County Med. Center, Dept. of Med., Div. of Cardiol.; and Dept. of Pediatrics and SUNY at Stony Brook, East Meadow, NY 11554) *Lipids* 17(3):129-135 (1982). The effects of zinc deficiency and testosterone on fatty acid composition of plasma lipids and microsomes of liver, intestine and testes were studied. The activities of fatty acid desaturase (Δ6 and Δ5) in rat liver and testes were also measured. A significant decrease in the level of arachidonic acid was observed in plasma of normal rats fed the ainc-deficient diet. Castration significantly decreased arachidonic acid but increased 20:3 fatty acid, which is negligible in normal rats. Testosterone and zinc administration restored arachidonic acid to normal values. Zinc deficiency does not significantly change the fatty acid profile in liver, but castration decreased both arachidonic 22:6 fatty acid. Intestinal mucosal microsomes showed that the predominant fatty acid in this tissue, palmitic acid, is independent of zinc status, whereas polyunsaturated fatty acids 18:2 and 20:4 were decreased by zinc-deficient diet or castration. Zinc deficiency sharply decreased 22:5 fatty acid and to some extent, other polyunsaturated fatty acids in testis microsomes. These changes in fatty acids are in agreement with increased Δ9 desaturation and decreased Δ5 desaturase activity. In testes, both Δ6 and Δ5 desaturase activities are decreased in zinc deficiency. It appears that zinc influences Δ6 desaturase activity. The data suggest that zinc deficiency may be one of the important factors in the causation of polyunsaturated fatty acid deficiency, which, in turn, may induce serum hypertriglyceridemia.

EFFECTS OF ADRENALECTOMY AND DEXAMETHASONE ON HEPATIC LIPID METABOLISM. T.G. Cole, H.G. Wilcox, and M. Heimberg (Dept. of Pharmacology, Univ. of Missouri School of Medicine, Columbia, MO 65212) *J. Lipid Res.* 23:81-91 (1982). The influence of adrenalectomy and dexamethasone on hepatic free fatty acid metabolism was studied in isolated perfused livers from male rats. Adrenalectomy 1 week prior to perfusion did not affect uptake of oleate, output of triglyceride, or rate of ketgenesis compared to sham-operated match-fed controls. Livers from dexamethasone-treated rats (0-2 mg/kg per day for 7 days) removed less oleate from the perfusate, esterified more to total and very low density lipoprotein (VLDL) triglyceride, and oxidized less to ketone bodies, compared to match fed controls; additional studies with [1-^{14}C]oleate confirmed these findings. The output of glucose by livers from dexamethasone-treated rats was also stimulated. The output of VLDL triglyceride was correlated with output of total perfusate triglyceride (r=0.77, P<0.001). Prior to perfusion, dexamethasone livers accumulated more triglyceride than did control livers. Adrenalectomy did not affect the concentration of plasma free fatty acid or blood ketones and glucose; however, the plasma concentration of triglyceride was elevated. Dexamethasone increased the concentration of plasma free fatty acid, total triglyceride, and VLDL protein, triglyceride, phospholipid, and free cholesterol. No changes were observed in the concentration or composition of plasma low density lipoprotein (LDL) lipids. The concentration of plasma high density lipoprotein (HDL) protein and lipid, and plasma apoA-I, tended to increase; the ratio of total HDL cholesterol to LDL cholesterol was elevated with dexamethasone treatment. The observations suggest that augmented synthesis and secretion of VLDL triglyceride contribute to glucocorticoid-induced hypertriglyceridemia.

1,25-DIHYDROXYVITAMIN D_3 RECEPTORS AND FUNCTIONS IN CULTURED PIG KIDNEY CELLS (LLC PK_1). REGULATION OF 24,25-DIHYDROXYVITAMIN D_3 PRODUCTION. K. Colston and D. Feldman (Dept. of Med., Standord Univ. School of Med., Stanford, CA 94305) *J. Biol. Med.* 257(5):2504-2508 (1982). Although 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) regulates the renal metabolism of 25-hydroxyvitamin D_3 (25-OH-D_3), the mechanism is not well understood. The established pig kidney cell line, LLC PK_1, was used to study this feedback regulation. These cells possess a receptor for 1,25-$(OH)_2D_3$ with a sedimentation coefficient of 3.2 S. Scatchard analysis of 1.25-$(OH)_2D_3$ binding to cell cytosol yielded an apparent K_d of 0.12 nM and an N_{max} of 26 fmol/mg of cytosol protein. LLC PK_1 cells respond to 1,25-$(OH)_2$-D_3 by changes in the metabolism of 25-OH-D_3. When incubated with 25-OH-[^{3}H]D_3, homogenates of untreated cells did not produce detectable 1,25-$(OH)_2$[^{3}H]D_3. However, after treatment of cell monolayers with 1,25-$(OH)_2D_3$ for 8 h, homogenates converted substantial 25-OH-[^{3}H]D_3 substrate to 24,25-$(OH)_2$[^{3}H]D_3. The appearance of this 24-hydroxylase activity in response to 1,25-$(OH)_2D_3$ was time- and dose-dependent. Half-maximal levels of enzyme activity were achieved with 0.13 nM k,25-$(OH)_2D_3$, a concentration almost identical to the K_d of the 1,25-$(OH)_2D_3$ receptor. The stimulation of 24-hydroxylase activity was shown to be an induction event; treatment of monolayers with 13 nM 1,25-$(OH)_2D_3$ for a 4-h pulse was sufficient to induce maximal activity assayed at 9 h. The presence of the transcriptional inhibitor, actinomycin D, during the 4-h pulse abolished the induction of 24-hydroxylase activity. These results demonstrate for the first time the presence of both 1,25-$(OH)_2D_3$ receptors and stimulation of 24-hydroxylase activity within the same established mammalian kidney cell line.

CHARACTERIZATION OF CHYLOMICRON REMNANT BINDING TO RAT LIVER MEMBRANES. A.D. Cooper, S.K. Erickson, R. Nutik, and M.A. Shrewsbury (Dept. of Med., Standord Univ. School of Med., Stanford, CA 94305) *J. Lipid Res.* 23:42-52 (1982). The binding of chylomicron remnants to rat liver membranes was investigated using radioiodinated lipoproteins. The specific activity of binding increased in parallel with increased enrichment in plasma membrane markers. The yield of receptor activity, however, decreased with enrichment. Accordingly, a partially purified plasma membrane preparation was used for routine studies. Binding was saturable, with half maximal binding achieved at 4.6 μg tetramethylurea-precipitable protein per ml. The rate of binding was time- and temperature-dependent. It could be inhibited only moderately by 10 mM EDTA. Chylomicron remnants appeared to bind to the membrane as a unit. The bound particle was richer in apoproteins of 20,000-50,000 molecular weight relative to low molecular weight apoproteins than the particles that were not bound. Lipoprotein particles containing only human apoB did not bind to liver membranes nor did they compete for the remnant binding site. Rat lipoproteins of d 1.019-1.063 g/ml did compete for remnant binding. When they were separated into apoB-rich (LDL) or apoE-rich (HDL_C) fractions by block electrophoresis, the apoE-rich fraction was a more potent competitor. ApoE purified and reconstituted into dimyristoyl phosphatidylcholine vesicles was a potent competitor for the remnant binding site. Vesicles containing ^{125}I-labeled apoE bound to the membranes, and they could be displaced by unlabeled remnants. Dimyristoyl phosphatidylcholine vesicles themselves did not compete with either remnants or apoE-phospholipid vesicles.

IN VITRO MODULATION OF THE DISTRIBUTION OF NORMAL HUMAN PLASMA HIGH DENSITY LIPOPROTEIN SUBFRACTIONS THROUGH THE LECITHIN:CHOLESTEROL ACYLTRANSFERASE REACTION. W.H. Daerr and H. Greten (Medizinische Kernklinik und Poliklink, Universitats-Krankenhaus Eppendorf, 2000 Hamburg 20, Germany) *Biochim. Biophys. Acta* 710:128-133 (1982). The effect of the lecithin:cholesterol acyltransferase reaction on the chemical composition, morphology and distribution of normal human plasma high density lipoprotein (HDL) subclasses was studied in vitro. Incubation of plasma in the presence of polyenephosphatidylcholine (PPC) resulted in a 45 ± 11% (n=6) decrease in unesterified cholesterol after 20 h. This effect was abolished by prior heating of the plasma at 56 C or by the addi-

tion of diisopropyl fluorophosphate (DIFP). Plasma triacylglycerol levels were constant. Analysis of the plasma lipoproteins by zonal ultracentrifugation and isopycnic equilibrium banding revealed a bimodal distribution of the HDL of native plasma and both heat-inactivated or DIFP-treated samples. Following the lecithin:cholesterol acyltransferase reaction essentially all of the HDL material had flotation characteristics typical of HDL_2. There were no apparent changes in the distribution of the lipoproteins of $d<1.063$ g/ml. The newly formed HDL were poor in PC and unesterified cholesterol but rich in cholesteryl ester, sphingomyelin and lyso-PC. The HDL apolipoprotein pattern was unaltered. HDL morphology was not affected by the lecithin:cholesterol acyltransferase reaction. Similar results were obtained in the absence of PPC. However, under these conditions the total phospholipid content of the HDL was reduced and lyso-PC was not demonstrable as a product of the lecithin:cholesterol acyltransferase reaction after 20 h. The results are interpreted to indicate that lecithin:cholesterol acyltransferase is involved in the transformation of HDL_3 into HDL_2.

INTRAHEPATIC ASSEMBLY OF VERY LOW DENSITY LIPOPROTEIN. R.A. David, M.M. McNeal, and R.L. Moses (Cell Biology Unit, Dept. of Physiology, Louisiana State Univ. Med. Center, New Orleans, LA 70119) *J. Biol. Chem. 257*(5):2634-2640 (1982). Cultured rat hepatocytes maintained in a chemically defined medium were used to examine the mechanism through which cholesterol feeding alters the core lipid components of *de novo* synthesized very low density lipoprotein (VLDL). Hepatocytes obtained from rats fed a cholesterol-rich diet secreted VLDL which contained less triacylglycerol (-30%) and more cholesterol ester (+4-fold) compared to hepatocytes from chow-fed controls. These changes were similar to changes observed in serum VLDL obtained from donor rats. Cholesterol feeding also markedly affected the concentration of hepatocyte neutral lipids. Transmission electron microscopy showed a dramatic accumulation of non-membrane-bound lipid in hepatocytes from cholesterol-fed rats. Biosynthetic studies using [^{3}H]glycerol and [^{3}H]$_2$O demonstrated that hepatocytes from cholesterol-fed rats synthesized more [^{3}H]triacylglycerol, yet they secreted 23 and 46% less, respectively, into the medium. The possibility that cholesterol ester competes with triacylglycerol for secretion as a component of the VLDL core was investigated by adding mevalonic acid to the culture medium to increase cholesterol ester synthesis. After 22 h, mevalonic acid inhibited [^{3}H]triacylglycerol secretion by 80% and caused a slight accumulation of [^{3}H]triacylglycerol within the hepatocyte. Additional studies in which mevalonic acid and oleic acid were added to the culture medium to stimulate the synthesis of cholesterol ester and triacylglycerol showed that the mole ratio of these two core lipids in secreted VLDL varied as a linear function of the same mole ratio of hepatocyte neutral lipids. These results show that the neutral lipid composition within the hepatocyte dictates the core lipid composition of *de novo* synthesized VLDL.

EFFECTS OF DIETARY LONG-CHAIN FATTY ACIDS ON THE BIOSYNTHESIS OF UNSATURATED FATTY ACIDS IN THE RAT. R. De Schrijver and O.S. Privett (Hormel Institute, Univ. of Minn., Austin, MN 55912) *J. Nutr. 112*(4):619-626 (1982). Menhaden oil (ME) was included in semisynthetic diets to study the effect of long-chain fatty acids, mainly 20:5n3 and 22:6n3, on the biosynthesis of polyunsaturated fatty acids and on the 6- and 9- desaturase activities in liver microsomes. Five experimental diets, with the following fat supplements, were fed to male rats from weaning for a period of 33 weeks: 5% safflower oil (SAF) + 10% hydrogenated coconut oil (HCO), 5% SAF + %% HCO +5% ME, 5% SAF + 10% ME, 15% HCO and 5% HCO + 10% ME. The last two diets were deficient in linoleic acid. The three nondeficient diets contained similar amounts of linoleic acid. Including ME in the diets depressed the 6- and 9- desaturase activities, especially in the linoleic acid-deficient rats. The syntheses of 20:4n6, 22:4n6 and 22:5n6 were depressed. These effects were related to the preferential accumulation of dietary 20:5n3 and 22:6n3 in the liver microsomes, as compared to the n6 fatty acids. It is hypothesized that dietary 20:5n3 and 22:6n3 increase the minimum requirement for linoleic acid in the diet.

SOLUBILIZATION, PARTIAL PURIFICATION, AND RECONSTITUTION IN PHOSPHATIDYLCHOLINE-CHOLESTEROL LIPOSOMES OF ACYL-COA: CHOLESTEROL ACYLTRANSFERASE. B.M. Doolittle and T. Chang (Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire) *Biochem. 21(4)*:674-679 (1982). Acyl-CoA:cholesterol acyltransferase (ACAT) was solubilized from pig liver microsomes with a combination of 1:1% deoxycholate and 1 M potassium chloride. This solubilized activity was then reconstituted in lipid vesicles by diluting the extract into a solution of phosphatidylcholine, cholesterol, and sodium cholate, followed by dialysis. The reconstituted activity was shown to be dependent upon cholesterol in the reconstitution mixture and also shown to vary with changes in the phospholipid headgroup: phosphatidylethanolamine was most active, phosphatidylcholine was next, and phosphatidylserine or phosphatidylinositol was inhibitory. The reconstituted activity showed a migration pattern of ficoll gradients that was distinct from that of the unreconstituted enzyme and similar to that of phospholipid-cholesterol liposomes. These methods provide a technique to assay the ACAT activity in defined lipid environment. The solubilized ACAT fraction was further purified by ammonium acetate fractionation and sepharose 4B column chromatography. The entire purification procedure yielded a 150-fold increase in ACAT specific activity with 40% of the original activity recovered.

STIMULATION OF LIPID PEROXIDATION *IN VIVO* BY INJECTED SELENITE AND LACK OF STIMULATION BY SELENATE (41333). J.J. Dougherty and W.G. Hoekstra (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison, Madison, WI 53706) *Proc. Soc. Exp. Bio. Med. 169*(2):209-215 (1982). Male weanling rats were raised on diets based on torula yeast which were deficient in vitamin E and selenium, or supplemented with these substances. They were injected intraperitoneally with sodium selenite at 2 mg Se/kg or with sodium sulfite to give the same level of sodium. Following injection, lipid peroxidation *in vivo* was estimated by monitoring the produftion of ethane. In the hour following injection, vitamin E- and selenium-deficient rats injected with selenite produced 15 times as much ethane as did controls injected with sulfite. All rats in this group died from 1 to 4 hr after injection. Rats fed diets supplemented with selenite showed only a two- to threefold stimulation of ethane production by selenite and 3/4 survived. Rats fed diets supplemented with vitamin E did not produce more ethane in response to selenite injection and 3/4 survived. All four rats supplemented with both vitamin E and selenium survived without showing increased ehtane production. Thus, the increased vulnerability of vitamin E- and selenium-deficient rats to acute selenite toxicity may involve peroxidation *in vivo*. Rats fed diets supplemented with vitamin E could survive at least twice as much selenite as rats deficient in selenium and vitamin E.

RATES OF DEPLETION OF LINOLEIC ACID FROM FAT DEPOTS OF SELECTED LINES OF MICE DIFFERING IN GROWTH RATE AND ADIPOSITY. E.J. Eisen, A.L. Cartwright, K.M. Weller, and K.J. Smith (Dept. of Animal Socience, North Carolina State Univ., Raleigh, NC 27650) *Lipids 17*(3):136-148 (1982). Rates of depletion and half-lives of linoleic acid from epididymal, subcutaneous and retroperitoneal fat pads and the residual body were compared among 5 genetically diverse strains of mice. Rates of depletion and half-lives of linoleic acid were obtained on a fat-free diet following an enrichment period of feeding a diet high in linoleic acid. The M16 mice have an increased capability of synthesizing fat from carbohydrates as shown by a continued increase in fat depot weights on the fat-free diet. Rates of depletion of linoleic acid were significantly different among lines in each of the 4 depots. Ranking of lines for depletion rates were similar among the 3 discrete depots, but a more rapid rate of depletion was observed in subcutaneous and retroperitoneal fat depots than in the epididymal fat depot. Rates of depletion in line M16 were slower than in the ICR control line. Line H_6 had a slower rate of depletion than line L_6. Line L_6 deviated more from the C_2 control than did line H_6, indicating an asymmetric correlated selection response. The decreased depletion rate of linoleic acid in fat tissue of M16 and H_6 mice suggests the possibility that the turnover rates of fatty acids have been reduced in these mice suggests that selection for small body size has substantially increased the rate of fat turnover. The experiment demonstrates that genetic differences among lines in fat turnover have accrued as correlated responses to selection for growth rate.

INHIBITION OF PERMEABILITY-DEPENDENT CA^{2+} RELEASE FROM MITOCHONDRIA BY *N*-ACYLETHANOLAMINES, A CLASS OF LIPIDS SYNTHESIZED IN ISCHEMIC HEART TISSUE. D.E. Epps, J.W. Palmer, H.H.O. Schmid and D.R. Pfeiffer (The Hormel Institute, Univ. of Minnesota, Austin, MN 55912) *J. Biol. Chem. 257*:1838-1391 (1982). Long chain *N*-acylethanolamines which accumulate in large amounts in the infarcted areas of canine myocardium are shown here to inhibit the development of increased inner membrane permeability in heart mitochondria produced by Ca^{2+} plus Ca^{2+}-releasing agents such as oxalacetate, *N*-ethylmaleimide, and palmitoyl coenzyme A. The inhibition is concentration-dependent, requiring approximately 30 μM for a half-maximal effect with *N*-oleoylethanolamine. Higher levels of the compound inhibit energy-dependent Ca^{2+} accumulation, maximal rates of succinate oxidation and the development of membrane potential. Half-maximal effects for these activities are seen at approximately 120 μM. Inhibition of Ca^{2+} uptake appears to be a secondary consequence of inhibited energy production rather than an effect on the Ca^{2+} uniporter *per se*. Inhibition of succinate oxidation is noncompetitive with respect to succinate concentration. *N*-

Acylethanolamine has analogous actions on liver mitochondria. At lower levels, a stimulation rather than inhibition of Ca^{2+}-dependent permeability increase is observed. This difference is due to the action of a hydrolase degrading the amide linkage. The resulting accumulation of free fatty acids in liver mitochondria can lead to the synthesis of intromitochondrial acylcoenzyme A which increases the sensitivity to Ca^{2+} and other Ca^{2+}-releasing agents. A survey of tissue homogenates revealed that hydrolysis of *N*-oleoylethanolamine occurs most rapidly in liver but does not occur in heart. The actions of *N*-acylethanolamine on mitochondria and the requirements for the biosynthesis of these compounds could function to protect cells subjected to ischemic insult and perhaps to injury by other agents which provoke large increases in intracellular Ca^{2+} levels.

X-LINKED ICHTHYOSIS: INCREASED BLOOD CHOLESTEROL SULFATE AND ELECTROPHORETIC MOBILITY OF LOW-DENSITY LIPOPROTEIN. E.H. Epstein, Jr., R.M. Krauss, and C.H.L. Shackleton (Department of Dermatology, San Francisco General Hospital, Medical Center, San Francisco, CA 94110) *Science 214*(6):659-660 (1981). Plasma cholesterol sulfate concentration is increased in patients with recessive X-linked ichthyosis, a disease in which steroid sulfatase activity is absent. In these patients, cholesterol sulfate is found primarily in the low-density lipoprotein fraction in plasma, and the electrophoretic mobility of these lipoproteins is greatly increased.

DIFFERENT POOLS OF ESTERIFIED ARACHIDONIC ACID IN RABBIT KIDNEY MEDULLA: RELATIONSHIP TO CA^{2+}-STIMULATED PROTAGLANDIN BIOSYNTHESIS. A. Erman, R. Azuri, and A. Rax (Dept. of Biochem. George S. Wise Center of Life Sciences, Tel-Aviv Univ., Tel-Aviv, Israel) *Lipids 17*(3):119-123 (1982). We investigated the effect of Ca^{2+} ions on renal medulla metabolism of endogenous esterified arachidonic acid in contrast to that of radioactive arachidonate incorporated into medullary lipids. Some striking differences between the release of unlabeled prostaglandin E_2 and of ^{14}C-labeled protaglandin E_2 and arachidonic acid were seen in incubations in absence or presence of Ca^{2+} ions. The first Ca^{2+}-sensitive pool is characterized by a higher arachidonate turn over rate and incorporates more rapidly added radioactive arachidonate. The acylhydrolase activity which release arachidonate from this pool is not efficiently coupled to prostaglandin endoperoxide synthase. In contrast, the second Ca^{2+}-sensitive lipid pool has a slower arachidonate turnover rate and, consequently, a slower incorporation of added ^{14}C-acid. The acylhydrolase activity associated with this pool is more tightly coupled to prostaglandin endoperoxide synthase, so that a higher portion of released arachidonate is converted to prostaglandin E_2.

3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE FROM RAT INTESTINE: SUBCELLULAR LOCALIZATION AND IN VITRO REGULATION. F.J. Field, S.K. Erickson, M.A. Shrewsbury, and A.D. Cooper (Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305) *J. Lipid Res. 23*:105-113 (1982). The subcellular localization of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in rat intestine was reinvestigated. Highly enriched fractions of endoplasmic reticulum and mitochondria were prepared from mucosal cells. The highest specific activity of HMG-CoA reductase was located in the endoplasmic reticulum fraction with recovery of 25% of the total activity. The mitochondria had low specific activity and low recovery of reductase activity relative to whole homogenate 92-2%). Despite attempts to maximize cell lysis, much of the activity (about 60%) was recovered in a low speed pellet which consisted of whole cells, nuclei, and cell debris as determined by light microscopy. Taken together, the evidence strongly suggests that much of the cellular HMG-CoA reductase activity is present in the endoplasmic reticulum fraction and that mitochondria have little or no intrinsic HMG-CoA reductase. The in vitro regulation of intestinal microsomal HMG-CoA reductase was studied. The intestine possesses a cytosolic HMG-CoA reductase kinase-phosphatase system which appears to be closely related to that present in the liver. Intestinal reductase activity in microsomes prepared from whole mucosal scrapings was inhibited 40-50% by the presence of 50 mM NaF in the homogenizing buffer. It was less susceptible to the action of the kinase than liver reductase. The effects of NaF were reversed by incubation with partially purified intestinal or liver phosphatases.

INCREASED APOPROTEIN B IN VERY LOW DENSITY LIPOPROTEINS OF PATIENTS WITH PERIPHERAL VASCULAR DISEASE. G. Franceschini, A. Bondioli, M. Mantero, M. Sirtori, G. Tattoni, G. Biasi, and C.R. Sirtori (Univ. of Milan, Via A. Del Sarto, 21, 20129 Milan, Italy) *Arteriosclerosis 2*:74-80 (1982). Lipoprotein compositional studies were carried out in 20 patients with atherosclerotic peripheral vascular disease. Twelve of these patients were normolipidemic, the other eight, hypertriglyceridemic. Ten normolipidemic and 10 hypertriglyceridemic age-matched subjects were used as controls. High density lipoprotein cholesterol levels were markedly reduced in the hypertriglyceridemic subjects. A decreased relative content of apo C-II in very low density lipoproteins in the hypertriglyceridemic subjects, as compared to the normolipidemics, was detected by isoelectric focusing. Apoprotein B levels in very low and low density lipoproteins were determined by electroimmunodiffusion and selective precipitation with tetramethylurea. All the patients with peripheral vascular disease showed an increased apo B content in very low density lipoproteins (VLDL) as compared to controls. A significant correlation between VLDL cholesterol and apo B levels was detected both in peripheral vascular disease patients and in controls; however, two distinct populations could be clearly separated. The data suggest a possible discriminatory power of VLDL-apo B levels in patients with peripheral vascular disease independent from other lipoprotein and lipid parameters.

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, Universita di Modena, Via Campi 287, 4110 Modena, Italy) *Biochim. Biophys. Acta 710*: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 of the present study was to investigate the changes in plasma and urinary lipoproteins during the administration of puromycin aminonucleoside (20 mg/kg for 7 days) and the subsequent development of nephrotic syndrome. Since massive albuminuria occurred after 6 days of treatment, the time-course study was divided into 2 stages: pre-nephrotic stage (day 1-5) and nephrotic stage (day 6-11). In the 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.

EFFECTS OF PROTEIN MODIFICATION PROCEDURES ON THE INTERACTION BETWEEN 25-HYDROXYVITAMIN D AND THE HUMAN PLASMA BINDING PROTEIN FOR VITAMIN D AND ITS METABOLITES. M. Kawakami and D.S. Goodman (Dept. of Med., Columbia Univ. College of Physicians and Surgeons, New York, NY 10032) *Biochemistry 20*:5881-5887 (1981). Studies were conducted to explore the effects of protein modification procedures on the interaction between 25-hydroxyvitamin D_3 [$25(OH)D_3$] and the human plasma transport protein for vitamin D and its metabolites (DBP). DBP is identical with the human plasma group-specific component (Gc) protein. The effects of progressive modification of lysine and of arginine residues, by reductive methylation and with and with cyclohexanedione, respectively, of iodination of tyrosine residues, of reduction and alkylation of disulfide bonds, and of exposure to 6 M guanidine hydrocholoride were investigated. Effects on binding properties were compared with those on DBP immunoreactivity. Progressive modifications of lysine, arginine, and tyrosine residues all resulted in progressive decreases in the binding activity of DBP for $25(OH)D_3$ without affecting immunoreactivity. The patterns of loss of binding activity differed among these 3 kinds of modifications. Thus, modification of 6 (of a total of 14) arginine residues did not affect binding activity, whereas further arginine modifications resulted in a progressive decrease in binding. Iodination of DBP beyond 1 atom of iodine per molecule of protein led to a fairly marked decline in binding activity, whereas a more gradual decrease in binding was seen with progressive lysine modification. In the presence of 6 M guanidine hydrochloride, DBP displayed virtually no binding activity for $25(OH)D_3$. The effects of guandine on the association of DBP with its ligand were, however, completely reversible. Reductive alkylation of disulfide bonds profoundly decreased both the $25(OH)D_3$ binding activity and the immunoreactivity of DBP. The results suggest that a limited number of disulfide bonds play a major role in maintaining the stable, 3-dimensional structure of DBP.

CHANGES OF FATTY ACID COMPOSITION OF PHOSPHOLIPIDS IN LIVER MITOCHONDRIA AND MICROSOMES OF THE RAT DURING GROWTH. A. Keranen, P. Kankare, and M. Hallman (Dept. of Med. Chem., Univ. of Helsinki, Sitavuorenpenger

10 A, SF-00170 Helsinki 17, Finland) *Lipids 17*(3):155-159 (1982). The fatty acid patterns of rat liver mitochondrial and microsomal phospholipids were analyzed from term fetuses, 1 and 4 days old, and adult rats. The main fatty acids of phosphatidylethanolamine and -choline were stearic and palmitic acids, although the patterns differed slightly. The fatty acid composition of corresponding phospholipids in mitochondria and microsomes was similar. The fatty acid pattern of cardiolipin was dominated by linoleic acid. The most consistent feature of the developmental changes in the fatty acid patterns of all phospholipids studied was a decrease in the relative amount of monounsaturated fatty acids. The percentages of saturated fatty acids in phosphatidylethanolamine and -choline increased during neonatal development. It is suggested that the high levels of fetal monounsaturated fatty acids were due to low availability of polyunsaturated fatty acids.

A COMPARATIVE STUDY OF SERUM LIPIDS BETWEEN BELGIUM AND KOREA. H. Kesteloot, C.S. Lee, H.M. Park, C. Kegels, J. Geboers, L. Math, J.H. Claes, and J.V. Joossens (Academisch Ziekenhuis Sint-Rafaël, 3000 Leuven, Belgium) *Circulation 65*:795-799 (1982). Serum cholesterol values are markedly lower in Korea than in Belgium in both males and females. This is attributed to the much lower consumption of saturated fat in Korea. A mean population serum cholesterol value of about 160 mg/dl appears to be compatible with excellent general health and with the absence of ischemic heart disease or other atheromatous diseases. The influence of age, height and weight on cholesterol between Belgium and Korea is qualitatively similar but quantitatively different. High-density lipoprotein cholesterol values are lower in Korea than in Belgium, particularly in females. Differences in the HDL cholesterol level thus cannot explain the low prevalence of ischemic heart disease in Korea.

EFFECT OF FLOW-INDEPENDENT REDUCTION OF METABOLISM ON REGIONAL MYOCARDIAL CLEARANCE OF ^{11}C-PALMITATE. R.A. Lerch, S.R. Bergmann, H.D. Ambos, M.J. Welch, M.M. Ter-Pogossian, and B.E. Sobel (Cardiovascular Division, Washington Univ. School of Med., 660 S. Euclid Ave., St. Louis, MO 63110) *Circulation 65*:731-738 (1982). Recent studies with sequential positron-emission tomography have demonstrated that early clearance of activity from myocardium after i.v. carbon-11 (^{11}C)-palmitate is decreased in regions of ischemia. To determine whether the reduced clearance is a reflection of decreased washout of labeled substrate or its metabolites, or a reflection of decreased metabolism labeled fatty acid, we characterized the effects of restricted oxygen supply on regional ^{11}C clearance rates in vivo under two conditions: hypoxia without concomitant reduction of flow and hypoxia induced by reduction of flow (ischemia). In 21 open-chest dogs, the left anterior descending coronary artery (LAD) was cannulated and perfused by an extracorporaal bypass system. In each dog two regional time-activity curves were recorded with a β-detector probe after intracoronary injection of ^{11}C-palmitate. In control dogs no intervention was imposed between the 2 studies. In the experimental dogs, oxygen supply was reduced 15 minutes before the second injection of ^{11}C-palmitate by either reducing LAD flow by an average of 76% or by perfusing the LAD bed at normal flow rate with venous blood, resulting in an average reduction in oxygen content of 66%. Myocardial blood flow in the LAD-perfused region determined based on washout of $H_2{}^{15}O$ did not change in either group, but decreased an average of 64% in the ischemia group. Clearance of ^{11}C-activity after extraction of ^{11}C-palmitate by myocardium is consistently reduced in regions rendered hypoxic despite persistence of perfusion, supporting the hypostesis that the metabolic attenuation induced by hypoxia or ischemia per se can be detected in patients based on sequential and quantitative analysis of regional time-activity curves obtained by positron-emission tomography.

MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES. 27.[1] ISOLATION, STRUCTURE ELUCIDATION, AND PARTIAL SYNTHESIS OF 25-METHYLXESTOSTEROL, A NEW STEROL ARISING FROM QUADRUPLE BIOMETHYLATION IN THE SIDE CHAIN. L. Niang Li, U. Sjöstrand, and C. Djerassi (Department of Chemistry, Stanford University, Stanford, California 94305) *J. Org. Chem 46*(19):3867-3870 (1981). A novel C_{31} sterol, 25-methylxestosterol, resulting from quadruple biomethylation in the side chain has been isolated as a trace constituent of the sterol fraction from a Carribbean sponge (*Xestospongia* sp.). Its structure (1,24-methylene-25,26,27-trimethylcholesterol) has been elucidated by spectroscopic methods and confirmed by partial synthesis. A biosynthetic route leading to 1 is proposed that is consistent with the hypothesis of stepwise biomethylations and with earlier discoveries of "extended" side chains among marine sterols.

AQUEOUS LIPID PHASES OF RELEVANCE TO INTESTINAL FAT DIGESTION AND ABSORPTION. M. Linström, H. Ljusberg-Wahren, K. Larsson, and B. Borgström (Department of PHysiological Chemistry, University of Lund, P.O. Box 750, S-220 07 Lund 7, Sweden) *Lipids 16*(10):749-754 (1981). The phase behavior of monoglyceride/water systems, with oleic and linoleic acid as the dominating fatty acid residues, was investigated. Increased solubilization of triglycerides (oil) or oleic acid in the cubic liquid-crystalline phase formed by monoglyceride and water resulted in the formation of a reversed hexagonal liquid-crystalline phase followed by an L2-phase. The liquid-crystalline phases have different dispersion properties compared to each other in dilute micellar bile salt solutions. The cubic phase is found to be easily dispersed. The relevance of aqueous lipid phases other than micellar is discussed in relation to intestinal lipid digestion and absorption.

CHANGES IN PHASE TRANSITION TEMPERATURE OF PHOSPHOLIPIDS INDUCED BY ENDOTOXIN. M-S. Liu, T. Onji, and N.E. Snelgrove (Dept. of Physiology and Pharmocology, Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27103) *Biochim. Biophys. Acta 710*:248-251 (1982). The effects of endotoxin (lipopolysaccharide from *Salmonella minnesota* Re 595) on the phase transition temperature (T_m) of various phospholipids were studied. Endotoxin had no effect on the T_m and the width of the phase transition of dipalmitoyl-*sn*-3-phosphatidylcholine. Endotoxin at 100 μg/ml increased the T_m of dipalmitoyl-*sn*-3-phosphatidylethanolamine by 1.1°C ($P<0.01$) and narrowed the range of transition from 4.5 to 2.6 °C; the endotoxin-induced changes in the T_m and the transition range were abolished by the presence of 0.25 mM $CaCl_2$. Endotoxin increased the T_m of dipalmitoyl-*sn*-3-phosphatidic acid by 1.1 ($P<0.01$), 1.2 ($P<0.01$), and 3.1 ($P<0.01$) °C at 25, 50, and 100 μg/ml, respectively. Furthermore, the width of phase transition of phosphatidic acid was narrowed from 6.5 to 4.0° C by endotoxin at 100 μg/ml. The endotoxin-induced changes in the T_m and the transition range of phosphatidic acid were not affected by the presence of EDTA (1 mM) or $CaCl_2$ (0.05-0.1 mM). These results suggest that endotoxin decreases the fluidity of negatively charged phospholipids such as phosphatidic acid and phosphatidylethanolamine. A change in the physical properties of membrane lipid bilayers induced by endotoxin may have an adverse effect on the function of biological membranes.

PROPERTIES AND METABOLIC FATE OF TWO VERY LOW DENSITY LIPOPROTEIN SUBFRACTIONS FROM RHESUS MONKEY SERUM. L. Lusk, J. Chung, and A.M. Scanu (Depts. of Medicine andBiochem., Univ. of Chicago, Pritzker School of Medicine, Chicago, IL 60637) *Biochem. Biophys. Acta 710*(2):134-142 (1982). Physical, chemical and physiological approaches were used to examine the properties of two very low density lipoproteins, VLDL-I and VLDL-II which were isolated by agarose column chromatography from the serum of rhesus monkeys fed either Purina Chow or one of four hyperlipidemic diets containing 0.5-20% cholesterol suspended in either coconut oil, peanut oil, mixed coconut oil and butter fat or lard. In the coconut oil-fed hyperlipidemic animals, the majority of the apolar lipids of VLDL-I was represented by cholesteryl ester. The small percentage of triacylglycerol (15%) had a fatty acid composition which resembled that of the fatty acid in each of the diets. In turn, VLDL-II had a triacylglycerol-rich core and differed from VLDL-I in apolipoprotein distribution. Both VLDLs were hydrolyzed in vitro by milk lipoprotein lipase by first-order kinetices although VLDL-I exhibited a slightly slower reaction rate. When an oral dose of [^{3}H] retinol was given to one of the animals, both VLDLs became labeled but the specific activity of VLDL-I was six times higher than that of VLDL-II and the other lipoproteins. We conclude that VLDL-I represents a cholesteryl ester-rich lipoprotein probably of intestinal origin, whereas VLDL-II may be a particle of hepatic derivation modified by its interaction with the other plasma lipoproteins.

IMMUNOSPECIFIC TARGETING OF LIPOSOMES TO CELLS: A NOVEL AND EFFICIENT METHOD FOR COVALENT ATTACHMENT OF FAB' FRAGMENTS VIA DISULFIDE BONDS. F.J. Martin, W.L. Hubbell, and D. Papahadjopoulos (Cancer Research Institute and Dept. of Pharmacology, Univ. of CA, San Francisco, CA 94143) *Biochemistry 20*(14):4229-4238 (1981). An efficient method for covalently cross-linking 50K Fab' antibody fragments to the surface of lipid vesicles is reported. Coupling up to 600 μg of Fab'/μmol of phospholipid (about 6000 Fab' molecules per 0.2-μm vesicle) is achieved via a disulfide interchange reaction between the thiol group exposed on each Fab' fragment and a pyridyldithio derivative of phosphatidylethanolamine present in low concentration in the membranes of preformed large unilamellar vesicles. The coupling reaction is efficient, proceeds rapidly under mild conditions, and yields well-defined products. Each vesicle-linked Fab' fragment retains its original antigenic specificity and full capacity to bind antigen. We have used Fab' fragments, coupled to vesicles by this method, to achieve immunospecific targeting of liposomes to cells in vitro. Vesicles bearing antihuman erythrocyte Fab' fragments bind quantitatively to human erythrocytes (at multiplicities up to 5000 0.2-μm vesicles per cell) while essentially no binding is observed to sheep or ox red blood cells. Vesicle-cell binding is stable

over a pH range from 6 to 8 and is virtually unaffected by the presence of human serum (50%). Cell-bound vesicles retain their aqueous contents and can be eluted intact from cells by treatment with reducing agents (dithiothreitol or mercaptoethanol) at alkaline pH.

REACTIONS OF β-(2-FURYL)PROPIONYL COENZYME A WITH "GENERAL" FATTY ACYL-COA DEHYDROGENASE. J.T. McFarland, M-Y. Lee, J. Reinsch, and W. Raven (Lab for Molecular Biomedical Research, Univ. of Wisconsin, Milwaukee, WI 53201) *Biochemistry 21*:1224-1229 (1982). We have prepared a new pseudosubstrate for the "general" acyl-CoA dehydrogenase, β-(2-furyl) propionyl-CoA. This substrate reacts with enzyme to yield *trans*-β92-furyl)acryloyl-CoA which absorbs maximally at 340 nm, the isobestic point for oxidized and fully reduced flavin. FPCoA is a better substrate than butyryl-CoA but not as good a substrate as octanoyl-CoA. By observing the rate of formation of FACoA and comparing it with the rate of formation of the semiquinone of electron-transfer flavoprotein, we have established a 2:1 stoichiometry for this reaction. The reaction of FPCoA with acyl-CoA dehydrogenase is a biphasic first-order reaction when either flavin reduction or FACoA formation is observed. However, observation of FACoA production reveals a new oxygen-dependent production of enoyl-CoA product which is not reflected in the reaction profile of the FAD of fatty acyl-CoA dehydrogenase. This reaction requires oxygen, produces H_2O, and can therefore be characterized an "oxidase" reaction. The reaction is zero order and is linearly dependent upon enzyme concentration. The charge transfer product complex of FACoA and acyl-CoA dehydrogenase is not stable and completely dissociates. The zero-order rate constant characterizing the production of FACoA is also $2X10^{-2}$. Oxygen reacts with reduced acyl-CoA dehydrogenase at a rate of dissociation of the charge transfer product complex. Another electron acceptor, crotonyl-CoA, reacts in a transhydrogenation reaction at the same rate of charge transfer product complex dissociation. The normal electron acceptor, ETF, reacts with a much larger rate constant. This establishes the fact that normal electron transfer to ETF occurs within the charge transfer product complex. The charge transfer product complex formed upon reduction of acyl-CoA is essential in assuring the transfer of electrons to ETF.

INHIBITORS OF STEROL SYNTHESIS. L.R. Miller, T.N. Pajewski, and G.J. Schroepfer, Jr. (Depts. of Biochem. and Chem., Rice Univ. Houston, TX 77001) *J. Biol. Chem. 257*(5):2412-2419 (1982). 5α-Cholest-8(14)-en-3β-ol-15-one, a potent hypocholesterolemic agent is a potent inhibitor of sterol synthesis in cultered mammalian cells and causes a reduction in the levels of 3-hydroxy-3-methyl-glutaric acid (HMG)-CoA reductase activity in the same cells. Preincubation of 5α-cholest-8-(14)-en-3β-ol-15-one, at concentration of 10^{-4} M, with the 10,000 X *g* supernatant fraction of rat liver homogenate preparations had no effect on the activities of acetate thiokinase, cytosolic acetoacetyl-CoA thiolase and HMG-CoA synthase. Identical findings on the latter two enzymes were also made in 48,000 X *g* supernatant fraction of rat liver homogenate preparations. Similarly, direct addition of the $\Delta^{8(14)}$-15-ketosterol (10^{-4} M) to rat liver microsomes, under a variety of conditions had no effect of the level of HMG-CoA reductase activity. 5α-Cholest-8(14)-en-3β-ol-15-one and 5α-cholesta-6,8(14)-dien-3β-ol-15-one (at 10^{-4} M) had no effect on the distribution of radioactivity in nonsaponisiable lipids derived from labeled acetate and mevalonate, respectively, in 10,000 X *g* supernatant fraction of rat liver homogenate preparations. These findings are in contrast to those made recently with another 15-oxygenated sterol (14α-ethyl-5α-cholest-7-ene-3β, 15α-diol) which caused, at 10^{-6} M, a marked accumulation of labeled lanosterol and 24,25-dihydrolanosterol in cell-free homogenate preparations of rat liver. 5α-Cholest-8(14)-en-3β-ol-15-one (10^{-4} M) caused slight (12-15%) inhibition of the synthesis of digitonin-precipitable sterols from labeled acetate, but not from mevalonate, upon incubation with cell-free homogenate preparations of rat liver. The effects of a number of other C_{27} 15-oxygenated sterols on the synthesis of digitonin-precipitable sterols from labeled acetate and/or mevalonate have also been investigated.

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ETHER LIPID CONTENT AND FATTY ACID DISTRIBUTION IN RABBIT POLYMORPHONUCLEAR NEUTROPHIL PHOSPHOLIPIDS. H.W. Mueller, J.T. O'Flaherty, and R.L. Wykle (Dept. of Biochem., Bowman Gray School of Med. of Wake Forest Univ., Winston-Salem, NC 27103) *Lipids 17*(2):72-77 (1982). This study was undertaken to determine if rabbit neutrophils contain sufficient ether-linked precursor for the synthesis of 1-*O*-alkyl-2-acetyl-*sn*-glycerol-3-phosphocholine by a deacylation-reacylation pathway. The phospholipids from rabbit peritoneal polymorphonuclear neutrophils were purified and quantitated, and the choline-containing and ethanolamine-containing phosphoglycerides were analyzed for ether lipid content. Choline-containing phosphoglycerides, ethanolamine-containing phosphoglycerides, and sphingomyelin were the predominant phospholipid classes, with smaller amounts of phosphatidylserine and phosphatidylinositol. The choline-linked fraction contained high amounts of 1-*O*-alkyl-2-acyl- and 1,2-diacyl-*sn*-glycero-3-phosphocholine with a trace of the 1-*O*-alk-1′-enyl-2-acyl species. The ethanolamine-linked fraction contained high amounts of 1-*O*-alk-1′-enyl-2-acyl- and 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine and a low quantity of the 1-*O*-alkyl-2-acyl species. The predominant 1-*O*-alkyl ether chains found in the *sn*-1 position of the choline-linked fraction were 16:0, 18:0, 18:1, 20:0, and 22:0. The major 1-*O*-alk-1′-enyl ether chains found in the *sn*-1 position of the ethanolamine-linked fraction were 14:0, 16:0, 18:0, 18:1, and 18:2. This work shows that there is ample precursor present to support the synthesis of a platelet activating factor by a deacylation-reacylation pathway.

UTILIZATION OF LONG-CHAIN FREE FATTY ACIDS IN WHITE AND RED MUSCLE OF RATS. G. Okano and T. Shimojo (Dept. of Phys. Ed. and Dept. of Biochem., Sapporo Medical College, Sapporo 060 (Japan)) *Biochim. Biophys. Acta 710*(2):122-127 (1982). Utilization of long-chain fatty acids was studied in white and red muscle slices prepared from quadricepts femoris muscle of rats, using radioactive palmitate and linoleate as precursors. After 2 h incubation, red muscle oxidized more palmitate to CO_2 and converted more of it to tracylglycerol and phospholipid compared with white muscle. The metabolic fate of palmitate incorporated into skeletal muscle fibers varied with muscle fiber types. In white muscle, 22% of incorporated palmitate was oxidized and 34% was converted to triacylglycerol and 43% to phospholipid. The percent distribution of radioactivity among CO_2, triacylglycerol and 43% to phospholipid, in red muscle was 44%, 22% and 34%, respectively. Percent distribution of the radioactivity in phospholipids was similar in white and red muscle. Predominant labeling with radioactive palmitate was found in phosphatidylcholine. Incubations with linoleate decreased the rates of oxidation and esterification of the fatty acid in both types of muscle compared with those with palmitate. The labeling profiles of radioactivity in muscle phospholipids with radioactive ethanolamine, phosphatidylinositol, phosphatidylserine, cardiolipin and phosphatidylglycerol compared with palmitate. Palmitate had faster rates of incorporation into phosphatidylcholine and sphingomyelin than linoleate.

OXYGENATION OF ARACHIDONIC ACID BY HEPATIC MONOOXYGENASES ISOLATION AND METABOLISM OF FOUR EPOXIDE INTERMEDIATES. E.H. Oliw, F.P. Guengerich, and J.A. Oates (Dept. of Pharmacology and Biochem and Center in Environmental Toxicology, Vanderbilt Univ., School of Medicine, Nashville, TN 37232) *J. Bio. Chem. 257*(7):3771-3781 (1982). [^{3}H] Arachidonic (eicosatetraenoic acid was incubated with rabbit liver microsomes, NADPH, and 1 mM 1,2-epoxy-3,3,3-trichloropropane, an epoxide hydrolase inhibitor, for 15 min at 37 C. The metabolites were separated by reverse phase high performance liquid chromatography and two epoxides, 11(12)oxido-5,8,14-eicosatrienoic acid and 14(15)oxido-5,8,11-eicosatrienoic acid, were identified by gas chromatography-mass spectrometry. Hepatic cytochromes P-450 purified from rabbits and rats treated with phenobarbital metabolized arachidonic acid to these two epoxides, as well as 5(6)oxido-8,11,14-eicosatrienoic acid and the corresponding *vic*-diols as the major products. Cytochromes P-450 purified from rabbits and rats treated with β-naphthoflavone mainly formed (m-1)- and ω-hydroxy arachidonic acids, while the four epoxides and the *vic*-diols were formed in small amounts. Synthetic [^{14}C] 14(15)oxido-, 11(12)oxido-, 8(9)oxido-, and 5(6)oxidoeicosatrienoic acid were enzymatically converted to *vic*-diols by hepatic and renal cortical microsomal and cytosolic fractions and by purified liver microsomal epoxide hydrolase. Liver microsomes oxygenated the epoxides to many polar products in the presence of NADPH. All eight trihydroxy acids, formed by ω- or (ω-1)-hydroxylation of the four *vic*-diols, four *vic*-diol dicarboxylic acids, and other metabolites were identified by gas chromatography-mass spectrometry.

DECREASED ERYTHROCYTE MEMBRANE FLUIDITY AND ALTERED LIPID COMPOSITION IN HUMAN LIVER DISEASE. J.S. Owen, K.R. Bruckdorfer, R.C. Day, and N. McIntyre (Dept. of Biochem. and Chem., Royal Free Hospital School of Med., Univ. of London, 8 Hunter St., London WC1N 1BP, England) *J. Lipid Res. 23*:124-132 (1982). Abnormal plasma lipoproteins in patients with liver disease are associated with characteristic changes in erythrocyte membrane lipid composition. The membranes are enriched in cholesterol and phosphatidylcholine and both the cholesterol/phospholipid and phosphatidylcholine/sphingomyelin molar ratios are increased. Phospholipid fatty acid composition is also abnormal; the proportions of arachidonic acid and stearic acid are decreased and that of palmitic acid raised. In this study we have examined the effects of these membrane lipid abnormalities on membrane fluidity. Erythrocyte membrane fluidity was assessed in 30 patients with a variety of liver diseases and in 25 normal subjects using the hydrophobic, fluorescent probe 1,6-diphenylhexa-1,3,5-triene and the values were related to their lipid composition. Membrane fluidity was significantly decreased in the patient erythrocytes (lipid order parameter, $S_V[37°C] = 0.713 \pm 0.018$, mean ±S.D. compared to 0.686 ± 0.008 in the normal subjects, $P < 0.001$) and correlated significantly with the cholesterol/phospholipid ratio ($r = 0.88$, $P < 0.001$). The fluidity of lipid extracts from the membranes of patient erythrocytes was also decreased, suggesting that decreased membrane fluidity was mainly a consequence of altered lipid composition rather than protein abnormalities. Incubation of patient erythrocytes for 20 hr with normal, heated plasma removed the excess cholesterol without affecting the phosphatidylcholine/sphingomyelin ratio or phospholipid fatty acid composition; following incubation the fluidity of these membranes was similar to that of normal membranes.

LYSOPHOSPHATIDYLCHOLINE-CHOLESTEROL COMPLEX. L.S. Ramsammy and H. Brockerhoff (New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314) *J. Biol. Chem. 257*(7):3570-3574 (1982). Lysophosphatidylcholine (lysoPC) and cholesterol at 1:1 molar ratio form multilamellar and, on sonication, unilemellar liposomes in water. Calorimetric scannings of varied mixtures in water give evidence for the existence of a 1:1 complex of the lipids. The permeability of the 1:1 bilayer against glycerol, at 22-42 C, is lower than that of phosphatidylcholine-cholesterol bilayers; the energy of activation of permeation is 73% higher. This implies a low groundstate of the entropy of activation of permeation. Unilamellar lysoPC-cholesterol 1:1 liposomes, isolated by gel exclusion chromatography, are able to incorporate no more than 8 to 10% excess lysoPC and no additional cholesterol at all. Their physical parameters such as outer radius (14.8 nm) and bilayer thickness (4.2 nm) are between those reported for phosphatidylcholine vesicles and phosphatidylcholine-cholesterol vesicles. The oustide-inside distribution of lysoPC in the small vesicles (determined by ^{31}P-NMR) is 2.0. A comparison of ^{13}C-NMR spectra of lysoPC (in ^2H_2O) and lysoPC-cholesterol vesicles shows that in the vesicles the signals for the carboxyl carbon of lysoPC as well as those for carbons 1 and 2 (and partly, 3) of *sn*-glycerol have been suppressed, and indication of motional restriction in this region of the molecule. The low groundstate of the entropy of activation of permeation, and the immobilization of the glycerol moiety of the lysoPC, argue for a high degree of structural organization in the "hydrogen belt" regions of the lysoPC-cholesterol bilayer, and for lipid-lipid complexing via hydrogen bonding in these regions.