Abstracts

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Fats and oils

A KINETIC AND STRUCTURAL STUDY OF TWO-STEP AGGREGATION AND FUSION OF NEUTRAL PHOSPHOLIPID VESICLES PROMOTED BY SERUM ALBUMIN AT LOW PH. S. Schenkman, P. Soares De Araujo, A. Sesso, F. H. Quina, and H. Chaimovich (Group for Interfacial Studies, Dept. de Bioquimica, Instituto de Quimica, Universidade de Sao Paulo, Caixa Postal 20.780, Sao Paulo, S. P., Brazil) *Chem. Physics Lipids 28* (2):165–180 (1981). The addition of bovine serum albumin (BSA) to 25 ± 5 nm diameter single bilayer phosphatidylcholine (PC) vesicles (SBV) (pH 3.5) gives rise to readily visible transiet turbidity. Studies of this system, employing a series of techniques, including time-dependent turbidity changes, membrane filtration, centrifugation, Sepharose chromatography and freeze fracture electron microscopy demonstrated that the process involves aggregation and fusion of the vesicles. At least three distinct time-dependent steps have been characterized: (1) the rapid initial formation (in approx. 5 min) of large aggregates (responsible for the visible turbidity) composed of SBV interconnected by BSA in its F form. The formation of these aggregates may be reversed by raising the pH or adding excess BSA to the system at this stage; (2) spontaneous collapse of these large aggregates, in an irreversible step, to form a heterogeneous population of vesicles; (3) fusion produces as the final product of the process, a relatively homogeneous population of larger (50 ± 10 nm diameter) vesicles. This system serves as a convenient and simple model system for the detailed study of protein-mediated aggregation and fusion of membranes at the molecular level.

INFRARED AND RAMAN SPECTRA OF SPECIFICALLY DEUTERATED 1,2-DIPALMITOYL-SN-GLYCERO-3-PHOSPHOCHOLINES. S. Sunder, D.G. Cameron, H.L. Casel, Y. Boulanger, and H.H. Mantsch (Whiteshell Nuclear Research Establishment, Atomic Energy of Canada Limited, Pinawa, Manitoba ROE 1L0 and National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada) *Chem. Physics Lipids 28* (2):137–148 (1981). Deuterated methylene groups have been introduced synthetically in selected positions of the *sn*-2 palmitoyl chain of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and deuterated methyl groups in the *sn*-1 and *sn*-2 palmitoyl chains as well as in the *sn*-3 phosphocholine group. The vibrational spectra of seven such deuterium labelled derivatives of the title compound have been studied as the assignment of the C-D stretching vibrations is discussed.

EFFECT OF CHOLESTEROL ON SENSITIVITY OF LECITHIN LIPOSOMES TO SURFACE-ACTIVE SUBSTANCES. B. Yu Zaslavaky, A. A. Borovskaya, Y. A. Davidovich, and S. V. Rogozhin (Institute of Elementoorganic Compounds, Academy of Sciences of the USSR, MOSCOW 117813, U.S.S.R.) *Chem. Physics Lipids 28* (2):181-187 (1981). The effect of cholesterol incorporation into multilamellar egg lecithin liposomes on the liposomes sensitivity toward *N*-acyl derivatives of amino acids was examined. Free energy of intermolecular interaction between lecithin head groups in the bilayer is estimated as 3.8 ± 0.1 kcal/ml.

ENHANCED ACTIVITY OF BRAIN LIPASE IN THE PRESENCE OF ADRENOCORTICOTROPHIC HORMONE. J. Arnaud, O. Nobili and J. Boyer (Service d'Explorations Métaboliques, Hopital de la Conception, 13385 Marseille Cedex 4, France) *Biochim. Biophys. Acta 663*(2):401-7 (1981). The characteristics of the pH-dependent stimulatory influence of adrenocorticotrophic hormone (ACTH) on lipolysis were investigated further. ACTH enhanced 7-30-times the rates of hydrolysis of emulsified trioleoylglycerol by a rat brain lipase, when added to the medium both before and after the enzyme. When lipase activity was inhibited by sodium taurocholate, ACTH fully reversed the inhibition at bile salt concentration up to 2 mM. The reactivation process followed a sharp S-shaped pattern, leveling off at about 10^{-4} M ACTH. With and without bile salt, the stimulatory effect of ACTH culminated at pH 5.75, and was dependent on the presence of trace amount of a water-in-soluble solvent in the substrate emulsion. Taken together, the results suggest that ACTH acts at the lipid-water interface in facilitating the enzyme-substrate interaction. The relevance of the hormonal influence to a colipase-like effect is discussed.

SYNTHESIS OF CARBOXYPHOSPHOLIPIDS. R. Berchtold (Biochemical Laboratory, Mattenhofstrasse 34, CH-3007 Berne Switzerland) *Chem. Phys. Lipids 28*(1):55-60 (1981). The chemical synthesis of dialkylphospholipids containing a carboxyl group is described. This carboxyl group forms amides with substances containing a carboxyl group is described. This carboxyl group forms amides with substances containing free amino groups or certain protective groups.

THERMOTROPIC BEHAVIOR OF BILAYERS FORMED FROM MIXED-CHAIN PHOSPHATIDYLCHOLINES. S.C. Chen and J.M. Sturtevant (Dept. of Chem., Yale Univ., New Haven, CN 06511) *Biochemistry 20*(4):713-8 (1981). The six possible phosphatidylcholines containing two different chains derived from myristic, palmitic, and stearic acids were synthesized, and their bilayer structures were investigated by high-sensitivity differential scanning microcalorimetry. Chain migration during the syntheses caused each of the lipids to contain about 10% of the corresponding positional isomer. A phase diagram for each pair of isomers was constructed to permit estimation of the transition properties of the pure mixed-length phospholipids. The phase transitions of these lipids were found to be similar to those of saturated like-chain phosphatidylcholines. The main transition temperatures and enthalpies fall within the range of those for the like-chain lipids. In each pair of positional isomers, the isomer having the longer chain at position 2 on the glycerol backbone has the higher transition temperature and enthalpy. The transition curves of the pure mixed-chain lipids with myristic acid at position 2 and either palmitic or stearic acid at position 1 exhibited two partially separated peaks for the main transition. No satisfactory interpretation of this unexpected phenomenon has been developed.

THE EFFECT OF THE BILAYER PHASE TRANSITION ON THE CARBONYL CARBON-13 CHEMICAL SHIFT ANISOTROPY. B.A. Cornell (CSIRO Division of Food Research, North Ryde, N.S.W. 2113, Australia) *Chem. Phys. Lipids 28*(1):69-78 (1981). Proton enhanced (PE), natural abundance carbon-13 magnetic resonance spectra have been obtained of the carbonyl groups in hydrated dispersions of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine. A four-fold change in the overall line-width results on passing from the fluid to crystalline phase. The carbonyl resonance provides a sensitive measure of the changes in mobility experienced by the lipid molecule above and below the phase transition temperature. The spectral shapes derived from both the fluid (T = 45 C) and crystalline (T = 15 C) phases indicate that even in the crystalline phase sufficient molecular motion is present to average the chemical shielding tensor. It is suggested that this motion in the $L_{\beta'}$ phase is a result of dislocations and packing faults diffusing in the plane of the bilayer. Because of the small size of the chemical shielding interaction (approx. 3 kHz for ω_ρ = 22.63 MHz) lipid diffusion coefficients of order 10^{-10} cm^2/sec observed in the $L_{\beta'}$ phase [1] are effective in averaging the shielding tensor. A comparison is made with the perturbation suffered by the carbonyl groups in the L_α phase in the presence of substantial amounts of cholesterol or the polypeptide gramicidin A.

THE OXIDATION OF BILAYER DISPERSIONS OF UNSATURATED PHOSPHATIDYLCHOLINES BY DECOMPOSING POTASSIUM PEROXYCHROMATE. J.C. Edwards and P.J. Quinn (Department of Biochemistry, Chelsea College, University of London, London, SW3 6LX, United Kingdom) *Chem. Phys. Lipids 28*(1): 89-97 (1981). The oxidation of aqueous dispersions of unsaturated phosphatidylcholines by products released during the decomposition of potassium peroxychromate has been investigated. The rate and extent of oxidation have been measured by loss of unsaturated fatty acids and related to the rate of decomposition of peroxychromate as monitored by pH titrimetry and chromate analysis. The loss of oleic and linoleic acid from egg lecithin dispersions was similar in systems containing between 0.062 and 2 g peroxychromate and was limited to less than 50% of the total unsaturated residues of the substrate. Studies of the rate of oxidation suggested that the mechanism of reaction involved the progressive oxidation

of the substrate dependent on the continuous supply of relatively short-lived oxidizing species. The use of azide as a singlet oxygen quencher and 2,5-dimehtyl- and 2,5-diphenylfurans as singlet oxygen traps did not prevent oxidation of the phospholipid.

AN IMPROVED METHOD FOR THE PREPARATION OF 1,2-ISOPROPYLIDENE-*SN*-GLYCEROL. H. Eibl (Max-Planck-Institut fur biophysikalisch Chemie, D-3400 Gottingen-Nikolausberg (F.R.G.)) *Chem. Phys. Lipids 28*(1):1-5 (1981). A simple and fast route for the preparation of 1,2-isopropylidene-*sn*-glycerol from D-mannitol in 45% yield is described. The value of optical rotation, $[\alpha]_D^{20}$ + 15.2°, is higher than usual indicating considerable recemization for other procedures. Since 1,2-isopropylidene-*sn*-glycerol serves as general intermediate for the synthesis of glycerides and of phosphoglycerides these lipids contain substantial amounts of the isomer, for instance 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine may consist of up to 15% of 2,3-dipalmitoyl-*sn*-glycerol-1-phosphocholine in earlier preparations.

UPTAKE AND INTERCONVERSION OF CHOLESTEROL AND CHOLESTERYL ESTERS BY *PHYTOPHTHORA CACTORUM*. C.G. Elliot and B.A. Knights (Botony Department, University of Glasgow, Glasgow G12 8QQ, Scotland) *Lipids 16*(1):1-7 (1981). When cholesterol, cholesteryl palmitate and cholesteryl acetate were added individually to sterol-free cultures of *Phytophthora cactorum*, the free sterol was at first taken up more rapidly. By 24 hr, the uptake of esters and free sterol was similar. The 2 esters apparently are taken up by different mechanisms, since much acetate was found in extracts of the mycelium at early harvests, but very little palmitate. In cultures supplemented with a mixture of cholesterol and cholesteryl palmitate, the palmitate-derived cholesterol was preferentially incorporated into the free sterol fraction of mycelial extracts. Cholesteryl palmitate and acetate were both hydrolyzed, and free cholesterol esterified by filtrates of cultures grown on sterol-free medium. Reverse-phase chromatography on hydroxyalkoxypropyl-Sephadex resolved the sterol esters of mycelial extracts into 3 zones, the most polar comprising mainly the linolenate ester, the next linoleate, and the least polar mainly oleate. Linoleate was predominant among the first sterol esters synthesized by the mycelium whether the supplement was free sterol, palmitate or acetate. Later, oleate became predominant.

ELECTRON AND PROTON MAGNETIC RESONANCE STUDIES OF THE EFFECT OF RHODOPSIN INCORPORATION ON MOLECULAR MOTION IN DIMYRISTOYLPHOSPHATIDYLCHOLINE BILAYERS. T.H. Fischer and G.C. Levy (Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306) *Chem. Phys. Lipids 28*(1):7-23 (1981). The effect of rhodopsin incorporation on molecular motion in L-α-dimyristoylphosphatidylcholine (DMPC) bilayers is analyzed with nitroxide ESR and proton NMR techniques. A partial, binary phase diagram for DMPC-rhodopsin is constructed by studying the partitioning of 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo) between polar and hydrophobic domains as a function of temperature and system composition. Proton NMR spin-lattice relaxation measurements show that rhodopsin is associated with a domain of approx. 50 DMPC molecules which have reduced choline methyl mobilities. ESR studies, utilizing nitroxide-labeled fatty acid probes, indicate that rhodopsin immobilizes the outer half of the hydrophobic region in rhodopsin containing DMPC bilayers. Additional ESR studies, involving a nitroxide label placed in the middle of the membrane, as well as proton chain methyl spin-lattice relaxation measurements, indicate only slight rhodopsin-induced immobilization in the central part of the membrane.

THE STEROLS AND FATTY ACIDS FROM PURIFIED FLAGELLA OF *CHLAMYDOMONAS REINHARDI*. M.A. Gealt, J.H. Adler, and W.R. Nes (Department of Biological Sciences, Drexel University, Philadelphia, PA 19104) *Lipids 16*(2):133-6 (1981). Purified flagella of the eukaryotic alga *Chlamydomonas reinhardi* have a sterol composition (55% ergosterol [24β-methylcholesta-5,7,22-*trans*-trien-3β-ol] and 45% 7-dehydroporiferasterol [24β-ethylcholesta-5,7,22,*trans*-trien-3β-ol]) identical to that of the whole algal cell. Fatty acids isolated from *C. Reinhardi* flagella were identified as 16:0, 18:0, 18:1, 18:2 and 18:3. Whole cell fatty acids included 14:0, 16:2 and 16:3 in addition to those found in the flagella. Triunsaturates comprised 22.9% of the flagellar fatty acids and 76.4% of those from the whole cell.

STUDIES ON VITAMIN D (CALCIFEROL) AND ITS ANALOGUES. 21. SOLVOLYTIC RING EXPANSIONS OF VITAMIN D_3. FORMATION OF 1,4-DIHYDROXYL-A-HOMO-19-NOR-9,10-SECOCHOLESTA-5,7-diene. J.M. Gerdes, A.W. Norman, and W.H. Okamura (Depts. of Chem. and Biochem., University of California, Riverside, California 92521) *J. Org. Chem. 46*(3):599-602 (1981). Buffered acetolysis (70 C, 2 h) of the *trans*-benzoyloxy tosylate afforded a 2:1 mixture of A-homoacetoxy benzoates, which upon saponification afforded the A-homo-19-nor analogue of 1α-hydroxyvitamin D_3. Similar acetolysis of the *cis*-benzoyloxy tosylate afforded a 2.8:1 mixture, which upon chromatographic separation and then saponification afforded the *cis*-diols. The benzoyloxy tosylates were prepared in three steps from vitamin D_3. Based on spectral data and mechanistic consideration, the structural and stereochemical assignments are discussed. The three diols failed to exhibit any biological activity in terms of intestinal calcium absorption (ICA) and bone calcium mobilization (BCM) in the chick. Interestingly, the analogue selectively inhibited the BCM activity of natural vitamin D_3, and in addition, it enhanced the normal ICA effect of the active hormone, 1α,25-dihydroxyvitamin D_3.

DIMERIC EPOXY FATTY ACID METHYL ESTERS: FORMATION, CHROMATOGRAPHY AND MASS SPECTROMETRY. J. Gilbert, M.J. Shepherd, J.R. Startin, and J. Eagles (Ministry of Agriculture, Fisheries and Food, Food Laboratories, Haldin House, Queen Street, Norwich NR2 4SX) *Chem. Phys. Lipids 28*(1):61-8 (1981). The formation of dimers is reported from the thermal treatment of a series of epoxy fatty acid methyl esters. These compounds were isolated from the reaction mixture by steric exclusion chromatography and were subsequently characterized by their high resolution electron impact and ammonia chemical ionization mass spectra. The spectra were consistent in each case with the presence of a mixture of four possible positional isomers each containing an ether bridge linking a pair of fatty acid methyl esters across the carbon chains, with a keto group on a carbon adjacent to the bridge on one of the esters.

RAMAN SPECTROSCOPY OF THE THERMAL PROPERTIES OF REASSEMBLED HIGH-DENSITY LIPOPROTEIN: APOLIPOPROTEIN A-I COMPLEXES OF DIMYRISTOYLPHOSPHATIDYLCHOLINE. T. Gilman, J.W. Kauffman and H.J. Pownall (Biomed. Engineering Center, Northwestern University, Evanston, IL 60201) *Biochemistry 20*(3):656-61 (1981). Isolated complexes of apolipoprotein A-I (apoA-I), the major apoprotein of human plasma high-density lipoproteins, and dimyristoylphosphatidylcholine (DMPC) have been prepared and studied by differential scanning calorimetry (DSC) and Raman spectroscopy. DSC studies establish that complexes having lipid to protein ratios of 200, 100, and 50 to 1 each exhibit a broad reversible thermal transition at T_c = 27 C. The enthalpy of lipid melting for each of the three complexes is about 3 kcal/mol of DMPC. Raman spectroscopy indicates that the physical state of lipid molecules in the complexes is different from that in DMPC multilamellar liposomes. A large change was observed in a protein vibrational band at 1340 cm^{-1} for pure vs. complexed apoA-I, indicating that protein hydrocarbon side chains are immobilized by lipid binding.

CARBAMYL ANALOGS OF PHOSPHATIDYLCHOLINES. SYNTHESIS, INTERACTION WITH PHOSPHOLIPASES AND PERMEABILITY BEHAVIOR OF THEIR LIPOSOMES. C.M. Gupta and A. Bali (Division of Biophysics, Central Drug Research Institute, Lucknow-226001, India) *Biochim. Biophys. Acta 663*(2):506-15 (1981). A novel class of phospholipase-resisting phosphatidylcholine analogs, in which the C-2 ester group or both C-1 and C-2 ester groups have been replaced by carbamyloxy functions, have been synthesized. These lipids were not degraded by phospholipase A_2, while complete hydrolysis occurred with phospholipase C. Ultrasonic irradiation of the aqueous dispersions of the phospholipids in the presence as well as in the absence of cholesterol resulted in the formation of closed bilayer structures as evidenced by negative staining electron microscopy and also by their ability to entrap [^{14}C] glucose. The leakage rates of glucose at 37 C from liposomes of these compounds have also been measured. Liposomes consisting of 1,2-dipentadecanylcarbamyloxy-*sn*-glycero-3-phosphorylcholine were found to be more leaky (2.1%/h) as compared to the liposomes of 1-palmitoyl-2-pentadecanylcarbamyloxy-*sn*-glycero-3-phosphorylcholine (0.5%/h). Moreover, inclusion of cholesterol (33 mol%) into the bilayers of the former phospholipid had no effect on the leakage rate (2.4%/h) while it effectively reduced permeability of the latter (0.22%/h). These phosphatidylcholines are useful for studying the possible role of phospholipases in the capture and lysis of liposomes invivo.

CONVERSION OF ^{14}C-LABELED EICOSAPENTAENOIC ACID (*n*-3) TO LEUKOTRIENE C_5. S. Hammarström (Department of Chemistry, Karolinska Institutet, P.O. Box 60400, S-10401 Stockholm 60, Sweden) *Biochim. Biophys. Acta 663*(2):575-7 (1981). ^{14}C-labeled eicosapentaenoic acid (*n*-3) was converted by mouse mastocytoma cells to 5-hydroxy-6-S-glutathionyl-7,9,11,14,17-eicosapentaenoic acid (leukotriene C_5). The identification was based on comparisons with previously characterized unlabeled material by high-performance liquid chromatography, ultraviolet spectroscopy, and conversion by γ-glutamyl transpeptidase to leukotriene D_5.

A FACILE PROCEDURE FOR THE SYNTHESIS OF SATURATED PHOSPHATIDYLCHOLINES. A. Hermetter and F. Paltauf (Institut fur Biochemie und Lebensmittelchemie, Technische Universitat Graz, Scholgelgasse 9, A-8010 Grax, Austria) *Chem. Phys. Lipids 28*(1):111-5 (1981). 1,2-Dipalmitoyl-, 1,2-distearoyl- and mixed chain 1,2-diacyl-*sn*-glycero-3-phosphocholines were synthesized by amodification of the method of Warner and Benson [7]. Glycerophosphocholine (GPC) cadmium chloride complex was acylated with imidazolides of the corresponding fatty acids in dimethylsulfoxide-tetrahydrofurane (DMSO-THF) using the methylsulfinyl-methide anion as a catalyst. The imidazolides were used for the acylation step without prior isolation and purification. Phosphatidylcholines were obtained in yields over 70% they were free of *sn*-1- and *sn*-2-isomers.

CARBON-13 AND PHOSPHORUS-31 NUCLEAR MAGNETIC RESONANCE STUDIES ON INTERACTION OF CALCIUM WITH PHOSPHATIDYLSERINE. D.L. Holwerda, P.D. Ellis, and R.E. Wuthier (Dept. of Chemistry, Univ. of South Carolina, Columbia, South Carolina 29208) *Biochemistry 20*(2):418-28 (1981). The interaction between Ca^{2+} and phosphatidylserine was studied by ^{13}C and ^{31}P NMR spectroscopy, by IR analysis, by binding constant measurements, and through use of space-filling molecular models. NMR measurements of various salt forms of the lipid were made in two types of organic solvents that allowed sufficient averaging of chemical shift anisotropy and dipolar couplings to yield high resolution spectra. ^{13}C resonances of the polar head-group carbons were broadened relative to those of the acyl chains. This was especially true in samples prepared at neutral pH where ionic interactions appeared to restrict molecular motion. Analysis of the chemical shifts of the various lipid atoms under the differing ionic environments indicates that Ca^{2+} enhanced the deprotonation of both the carboxyl and amino groups and stabilized the entire polar head group against the effects of changing pH. IR data indicate direct involvement of the carboxyl group in Ca^{2+} binding, as evidenced by the appearance of a C=O stretching mode. Binding studies indicated that the phosphate group was the primary binding force but that the carboxyl group also contributes positively. The amino group appears to exert a repulsive effect, which is supported by the chemical shift data which indicate that Ca^{2+} enhances the deprotonation of the amino group. Molecular models indicate direct involvement of the carboxyl and phosphate oxygens and that the amino group must be deprotonated to participate.

RHODOPSIN-PHOSPHOLIPID INTERACTIONS: DEPENDENCE OF RATE OF THE META I TO META II TRANSITION ON THE LEVEL OF ASSOCIATED DISK PHOSPHOLIPID. B.J. Litman, O. Kalisky, and M. Ottolenghi (Department of Biochemistry, University of Virginia Medical School, Charlottesville, Virginia 22908) *Biochemistry 20*(3):631-634 (1981). Solubilization of retinal rod outer segment disk membranes in octyl glucoside was employed to prepare rhodopsin samples with varying amounts of associated disk phospholipid. Flash photolysis studies were carried out on these samples to determine the dependence of the meta I to meta II transition kinetics on the level of associated phospholipid. The rate constant for the formation of meta II increased from 6.9×10^3 to 19.5×10^3 s^{-1} as the molar ratio of phospholipid per rhodopsin fell from 35 to 5. The activation free energy for this process had a linear dependence on the level of phospholipid, with a slope of 24 cal/mol of rhodopsin-associated phospholipid. A variety of evidence suggests that rhodopsin undergoes a reversible conformation change during the meta I to meta II transition. No evidence was found for an enhanced effect on the activation free energy for this conformation change at the level of associated phospholipid which corresponds to the formation of a phospholipid boundary layer around rhodopsin.

A RAMAN SPECTROSCOPY STUDY OF MIXED BILE SALT MONOGLYCERIDE MICELLES. H. Ljusberg-Wahren and K. Larsson (Dept. of Food Technology, Univ. of Lund, Box 740, S-220 07 Lund, Sweden) *Chem. Phys. Lipids 28*(1):25-32 (1981). A Raman spectroscopy study of sodium cholate/monoglyceride mixed micelles is reported, using perdeuterated 1-monostearin. The C-D stretching vibration region of this micellar solution has been compared with different states of the perdeuterated monostearin with known structures: crystals, an aqueous gel phase, aqueous liquid crystalline phases of lamellar and cubic type, the liquid state and an ethanol solution. Also other spectral regions sensitive for conformation of lipid molecules were examined. The results are consistent with the lamellar type of structure proposed by Mazer, Benedek and Carey for lecithin/bile salt mixed micelles.

CHARACTERIZATION OF TWO ORNITHINE-CONTAINING LIPID FROM *ERWINA AROIDEAE*. V.N. Madhavan, J. Done, and J. Vine (Dept. of Biochemistry and Dept. of Pharmacy, The University of Sydney, N.S.W. 2006, Australia) *Chem. Phys. Lipids 28*(1): 79-88 (1981). An apparently pure ornithine-containing lipid (OCL) was isolated from *Erwina aroideae* by solvent extraction and thin-layer chromatography (TLC). However, selective hydrolysis of the lipid under acidic and basic conditions and analysis of hydrolysates by gas chromatography-mass spectrometry (GCMS) showed that two structurally similar OCL were in fact present. These lipids both contained a 3-hydroxyhexadecanoic acid moiety which was linked to ornithine by an amide group formed between the 2-amino group of ornithine and the carboxyl group of the acid. The two lipids, however, differ in the nature of the fatty acid bound through an ester linkage to the hydroxyl group of the 3-hydroxyhexadecanoic acid moiety. One lipid is the ester of hexadecanoic acid whereas the other lipid is the ester of octadecenoic acid. These lipids are present in approximately equal amounts.

STEROL ESTER HYDROLASE IN *FUSARIUM OXYSPORUM*. C. Madhosingh and W. Orr (Chem. and Biology Res. Inst., Res. Branch, Agriculture Canada, Ottawa, K1A 0C6, Canada) *Lipids 16*(2):125-32 (1981). Two electrophoretically different forms of sterol ester hydrolase (EC 3.1.1.13) were obtained from the cytoplasmic extract of the mycelia of *Fusarium oxysporum*. The entities, estimated at 60,000 (I) and 15,000 (II) molecular weights, were obtained in Sephadex G100 column chromatography of the ammonium sulfate precipitate from the cytoplasmic extract. A third form III, 75,000 MW, was obtained from the culture filtrate. The activity of the enzyme was increased by Triton X-100 and was not inhibited by *p*-chloromercuribenzoate (PCMB), a sulfhydryl reagent. The enzymes I and II were inhibited differentially by NaCl. The optimal activities of forms I, II and III occurred at pH 4.8, pH 8.0 and pH 7.0, respectively. The apparent Km values of 7.7×10^{-5} 8.3×10^{-5}, respectively, indicate a similar order of affinity for cholesteryl oleate at pH 7.1. The rate of hydrolysis of cholesteryl esters were in the order: linoleate > oleate > valerate > butyrate > acetate. Cholesteryl benzoate and palmitate were not hydrolyzed. The properties of the microbial enzyme are discussed in relation.

PROTEIN-LIPID INTERACTIONS IN BIOLOGICAL AND MODEL MEMBRANE SYSTEMS. Deuterium NMR OF *ACHOLEPLASMA LAIDLAWII* B, *ESCHERICHIA COLI*, AND CYTOCHROME OXIDASE SYSTEMS CONTAINING SPECIFICALLY DEUTERATED LIPIDS. S.-Y. Kang, R.A. Kinsey, S. Rajan, H.S. Gutowsky, M.G. Gabridge and e. Oldfield (Depts. of Chem. and Microbio., Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801) *J. Biol. Chem. 256*(3):1155-9 (1981). Deuterium nuclear magnetic resonance spectra of *Acholeplasma laidlawii* B (PG9) membranes and lipid extracts enriched biosynthetically in the presence of avidin, with either [14-$^2H_3$]-tetradecan-1-oic acid, [16-$^2H_3$] hexadecan-1-oic acid, [4-$^2H_2$]-, [6-$^2H_2$]-, or [8-$^2H_2$]-tetradecan-1-oic acids, have been recorded at a variety of temperatures. The results indicate that at their growth temperature (37 C) the *A. laidlawii* membrane lipids are ∿90% in a rigid gel-like state. Plasma membranes which had been lyophilized, then rehydrated, behaved in the ^2H-NMR experiment as did fresh plasma membranes. The ^2H-NMR quadrupole splittings ($\Delta\nu_Q$) were very similar for all of the fluid phase spectra recorded. These results indicate that protein has little effect on lipid order in the *A. laidlawii* B membrane system. By contrast, ^2H-NMR spectra of the [6-$^2H_2$]- or [10-$^2H_2$]-hexadecan-1-oic acid-enriched *Escherichia coli* L48-2 cell membranes showed extreme line broadening compared to spectra of their lipid extracts, and $\Delta\nu_Q$ values were slightly decreased. Results with intact *E. coli* cell membranes show essentially the same NMR line shapes as those seen previously with the DMPC-gramicidin A′ system.

LIPIDS OF DERMATOPHYTES. G.K. Khuller, A. Chopra, V.S. Bansal and R. Masih (Dept. of Biochemistry, Postgraduate Institue of Medical Education and Research, Chandigrah-160012, India) *Lipids 16*(1):20-3 (1981). This investigation deals with phosphatides and fatty acid content of *Epidermophyton floccosum*, *Microsporum cookie* and *Trichophyton mentagrophytes* during different phases of growth. Total phosphatide content of these dermatophytes decreased with age, which was reflected in constituent major phosphatide s. The zwitterionic and anionic phospholipids tended to maintain a constant ratio. Short chain fatty acids increased significantly with age in *E. floccosum* whereas these fatty acids represented a minor fraction of the total fatty acids in *M. cookie* and *T. mentagrophytes*. The ratio of saturated to unsaturated fatty acids increased 4-fold during growth in *E. floccosum*, whereas this increase was marginal in *M. cookie*. This ratio decreased in *T. mentagrophytes*.

COMPETITIVE DEPOSITION OF *TRANS*-12- AND *CIS*-9-OCTADECENOATES INTO EGG YOLK LIPIDS. A.C. Lanser and E.A. Emken (Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604) *Lipids 16*(1):15-9 (1981). The deposition of *trans*-12-octadecenoate-12(13)-^3H (12t-18:1-^3H) was compared to *cis*-9-octadecenoate-10-^{14}C (9c-18:1-^{14}C) in the major egg yolk neutral lipids and phospholipids. *trans*-12-Octadecenoate was preferentially incorporated into cholesteryl esters (CE), phospha-

tidylcholines (PC), and phosphatidylethanolamines (PE) but was discriminated against in triglycerides (TG). Isotopic ratios indicate that 5.9 and 5.6 times more 12t-18:1-^{3}H than 9c-18:1-^{14}C was esterified at the 1-acyl position of PE and PC, respectively. The combined 1- and 3-acyl positions of TG and the 2-acyl position of TG, PE and PC were each preferentially esterified with 9c-18:1-^{14}C.

FATTY ACIDS, PART 21: RING OPENING REACTION OF SYNTHETIC AND NATURAL FURANOID FATTY ESTERS. M.S.F. Lie Ken Jie and S. Sinha (Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong) *Chem. Phys. Lipids 28*(1):99-109 (1981). Methyl 2,5-disubstituted C_{18} furanoid fatty ester (vix. methyl 9,12-epoxyocatadeca-9,11-dienoate) was readily converted to methyl 9,12-dioxostearate using mineral or maleic acid. Conversion of the naturally occurring 2,3,5-trisubstituted furanoid fatty ester (vix. methyl 10, 13-epoxy-11-methylocatadeca-10,12-dienoate) to the corresponding methyl 10,13-dioxo-11-methylstearate was much slower in rate under similar reaction conditions. The ease of separating the dioxo derivatives from a mixture of other common fatty esters was demonstrated and the cyclodehydration of the isolated dioxo derivatives to the parent furanoid ester was rapidly achieved using dilute BF_3-methanol complex.

PRESENCE OF THE PHOSPHOLIPID METHYLATION PATHWAY IN MAMMALIAN CULTURED CELLS. M. Maeda, Y. Tanaka and Y. Akamatsu (Dept. of Chemistry, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan) *Biochim. Biophys. Acta 663*(2): 578-82 (1981). Stepwise N-methylation of phosphatidylethanolamine to phosphatidylcholine was examined by measuring incorporation of the radioactive methyl group of S-adenosylmethionine into phosphatidyl-N-monomethylethanolamine, phosphatidyl-N,N'-dimethylethanolamine and phosphatidylcholine by membranes of five mammalian cultured cell lines (BHK-21 cells, Chang liver cells, PC-12h cells, MOPC-31C cells and LM cells). The three successive methylation steps were found in all the cells studied, though the total incorporation and the distribution profile of the radioactivity among the products differed with different cells. Furthermore, increase in the amounts of phosphatidyl-N-monomethylethanolamine and phosphatidyl-N,N'-dimethylethanolamine in the membranes of LM cells cultured in the presence of N-monomethylethanolamine and N,N'-dimethylethanolamine, respectively, resulted in increased methylation of phospholipids. This remarkable enhancement of methylation seems to be a characteristic effect of such modification of membrane phospholipids in mammalian cultured cells.

INTRAMEMBRANOUS PARTICLES AND RIPPLES IN LIPID-CYTOCHROME c BILAYERS. S.K. Malhotra, S. Ross, and J.P. Tewari (Biological Sciences Electron Microscopy Laboratory, University of Alberta, Edmonton, T6G 2E9 Canada) *Chem. Phys. Lipids 28*(1):33-9 (1981). Vesicles made from a mixture of phospholipids (PL) are unilamellar. When such lipid vesicles are incubated with reduced cytochrome *c* multilamellar vesicles (PLC) are visualized by transmission electron microscopy. Both PL and PLC vesicles show a rippling in the freeze-fracture replicas. The rippling effect is likely to be due to the tilting of fatty acid chains. The freeze-fractured faces of PLC vesicles also show 3-4 nm intramembranous particles. Such particles are lacking from the comparable fractured faces of PL vesicles that are produced in the absence of cytochrome *c*. It is suggested that these intramembranous particles result from the presence of cytochrome *c* molecules; therefore, cytochrome *c* molecules may penetrate the lipid bilayer.

THE EFFECTS OF PHOSPHOLIPIDS ON THE PROPERTIES OF HEPATIC 5'-NUCLEOTIDASE. E.M. Merisko, G.K. Ojakian and C.C. Widnell (Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh Schl. of Med., Pittsburgh, PA 15261) *J. Biol. Chem. 256*(4): 1983-93 (1981). Arrhenius plots of 5'-nucleotidase activity in microsomes or plasma membranes from rat liver exhibited transitions at ∿35 C. The enzyme was purified from homogenates after solubilization in 2% Triton X-100 and 1% sodium deoxycholate. After the initial steps of the purification, the enzyme was recovered in membranes, as judged by both thin section and freeze-fracture electron microscopy, which contained sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine. Phosphatidylcholines containing specific fatty acids all affected the energy of activation of 5'-nucleotidase, and the detergent Sarkosyl, which has been shown to dissociate phospholipids from 5'-nucleotidase. Inhibition of 5'-nucleotidase by concanavalin A followed by reactivation with α-methyl-D-mannoside resulted in linear Arrhenius plots of 5'-nucleotidase activity in membrane fractions, and in lower transition temperatures for the detergent-solubilized enzyme. It is concluded that *in situ*, 5'-nucleotidase interacts with both sphingomyelin and phosphatidylcholine; the first apparently influences the stability of the enzyme and the second, the energy of activation. In addition, the lipid environment of the enzyme seems to be altered as a result of lectin binding.

STRUCTURAL DIMORPHISM OF BILE SALT/LECITHIN MIXED MICELLES. A possible regulatory mechanism for cholesterol solubility in bile? X-ray structure analysis. K. Müller (Institut für Röntgenfeinstrukturforschung, der Österreichischem Akademie der Wissenschaften und des Forschungszentrums Graz, A-8010 Graz, Austria) *Biochemistry 20*(2):404-14 (1981). The three dimensional structure of bile salt/lecithin mixed micelles in 0.15 M saline was derived from X-ray small-angle scattering measurements under various conditions. Two essentially different types of micelles were detected. At bile salt:lecithin molar ratios lower than approximately 2:1, lamellar particles similar to a lecithin bilayer arrangement were found. The thickness of the bilayer is 5.1 nm for mixed micelles having a molar ratio of 1:1. The lateral dimensions of the micelle were found strongly dependent upon molar ratio, increasing as lecithin content increases. In addition, it appeared that under certain incompletely defined conditions vesicular particles having diameters in the region of more than 100 nm occur. A molecular model of this type of micelle has been derived by means of a thorough interpretation of the electron density distribution across the plane of the bilayer. At molar ratios exceeding 2:1, a different type of micelle structure was found. This is a highly isometrical particle of globular shape, probably having a centrosymmetric arrangement of the molecular constituents. The prevalent balance between the two micellar forms may regulate the capacity of bile to transport cholesterol.

DEPLETION AND EXCHANGE OF CHOLESTEROL FROM THE MEMBRANE OF VESICULAR STOMATITIS VIRUS BY INTERACTION WITH SERUM LIPOPROTEINS OR POLY(VINYLPYRROLIDONE) COMPLEXED WITH BOVINE SERUM ALBUMIN. R. Pal, Y. Barenholz, and R.R. Wagner (Dept. of Microbiology and Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908) *Biochemistry 20*(3):530-9 (1981). Cholesterol was depleted from the membrane of vesicular stomatitis virus by exposing virion suspensions to serum lipoproteins enriched with phospholipids. Unlike the reaction of virions with phospholipid vesicles, nonspecific adherence of lipoproteins and exogenous lipids to the envelope of the virus was found to be minimal. The extent of cholesterol depletion was dependent upon the type of phospholipid complexed with interacting lipoprotein; sphingomyelin and dipalmitoyllecithin were found to be highly effective depleters of cholesterol compared to egg phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine. Depletion of cholesterol from the virion membrane resulted in a significant drop in the infectivity of the virus as measured by plating efficiency on L-cell monolayers. Such an effect was not observed when virion cholesterol was exchanged without net reduction in the concentration of viral membrane cholesterol. Part of the loss in infectivity following depletion of cholesterol could be restored by reincorporation of cholesterol in the membrane, thus demonstrating that membrane cholesterol partly contributes to the infectivity of vesicular stomatitis virus.

BINDING OF VITAMIN E IN MAMMALIAN TUMOR CELLS IN CULTURE. K.N. Prasad, D. Gaudreau, and J. Brown (Departments of Radiology and Biochemistry, University of Colorado Health Sciences Center, 4200 East 9th Avenue, Denver, CO 80262) *Proc. Soc. Exp. Biol. Med. 166*(2):167-74 (1981). Radioactive vitamin E (D-α-[5-*methyl*-^{3}H] tocopherol) bound with the cytosol (100,000 g supernatant), pellet (100,000 g pellet), crude nuclear, and purified chromatin fractions from mouse neuroblastoma (NBP_2) and rat glioma (C-6) cells in culture. The level and type of vitamin E binding proteins in the cytosol depended upon the cell type. When the cytosol proteins containing radioactive vitamin E were separated by gel filtration (Sepharose 4B gel), there were five protein peaks in neuroblastoma, three peaks in glioma, and one peak in mouse B-16 melanoma cells which contained bound radioactivity. The level of binding in the neuroblastoma cells was higher than that in glioma cells or melanoma cells. Vitamin E remained bound to the proteins from the cytosols of neuroblastoma and glioma even after denaturation and separation by electrophoresis. This suggests that vitamin E is tightly bound with the cytosol proteins. There was only one vitamin E binding protein in the pellet and nuclear fractions of NB, giolma, and melanoma cells. The significance of vitamin E binding proteins in the mechanism of the effect of vitamin E on mammalian cells in culture is unknown.

PHOSPHORUS NUCLEAR MAGNETIC RESONANCE STUDY OF MEMBRANE STRUCTURE. INTERACTIONS OF LIPIDS WITH PROTEIN, POLYPEPTIDE, AND CHOLESTEROL. S. Rajan, S.-Y. Kang, H.S. Gutowsky and E. Oldfield (Dept. of Chem., Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801) *J. Biol. Chem. 256*(3):1160-6 (1981). Proton-decouples ^{31}P-nuclear magnetic resonance spectra of a series of lipid, lipid-protein, and lipid-chol-

esterol systems have been recorded using the Fourier transform method at 60.7 MHz (corresponding to a magnetic field strength of 3.52 Tesla). Above the gel to liquid crystal phase transition temperature (T_C) of the pure lipid the proteins studied have the effect of slightly decreasing the apparent ^{31}P-chemical shielding anisotropy ($\Delta\sigma$), and in addition they significantly decrease the ^{31}P spin-spin (T_2) and spin lattice (T_1) relaxation times. These results suggest an "immobilization" of the phospholipid headgroup due to protein-lipid (polar group) interaction. Both in the absence and presence of proteins or cholesterol, T_2 relaxation rates are strongly dependent upon the orientation of the phospholipid molecules in the applied magnetic field. Cholesterol has rather little effect on the ^{31}P T_2 or T_1, consistent with the cholesterol molecule simply acting as a "spacer" of the phospholipid polar groups, without interacting with them directly.

STUDIES ON CHOLESTEROL ESTER HYDROLYSIS IN ARTIFICIAL LIPID MIXTURES. M. Shinomiya, K. Shirai, N. Matsuoka, Y. Saito and A. Kumagai (Second Department of Internal Medicine, School of Medicine, Chiba University, Chiba 280, Japan) *Atherosclerosis 38*(3,4):301-7 (1981). Cholesterol ester is present in lipid deposits in atherosclerotic lesions, such as fatty streaks, fibrous plaques and complicated lesions. The possibility of hydrolysis of cholesterol ester in lipid deposits and its mechanism were examined by studying the effects of the various components of lipid deposits on choelsterol ester hydrolysis. Studies were carried out using artificial lipid samples prepared by sonication of mixtures of the components of lipid deposits. Results suggested that phospholipids, especially phosphatidylcholine, play an important role in the hydrolysis and that alteration of lipid components, other than cholesterol esters, influences cholesterol ester hydrolysis in lipid deposits.

ARACHIDONIC ACID RELEASING ACTIVITY IN PLATELET MEMBRANES: EFFECTS OF SULFHYDRYL-MODIFYING REAGENTS. S.T. Silk, K.T.H' Wong, and A.J. Marcus (Divisions of Hematology-Oncology, Depts. of Medicine, New York Veterans Administration Hospital, New York, New York 10010) *Biochemistry 20*(2):391-7 (1981). The effects of sulfhydryl-modifying agents on arachidonic acid releasing activity in isolated platelet membranes were studied. Modification of a few "essential" sulfhydryls was responsible for the rapid inhibition of arachidonic acid releasing activity. Membranes isolated from 5,5′-dithiobis (2-nitrobenzoic acid)-treated intact platelets exhibited less inhibition than membranes similarly treated following isolation. 5,5′-Dithiobis (2-nitrobenzoic acid) (4 mM) inhibited the former by 20% and the latter by 92%. Since no such difference in inhibition was observed with *N*-ethylmaleimide, it is possible that the membrane vesicles are either "inside out" or open to 5,5′-dithiobis (2-nitrobenzoic acid) which does not penetrate membranes. The results are consistent with a model whereby essential sulfhydryls may be located on the inner surface of the platelet membrane. This is also supported by studies where the same 5,5′-dithiobis (2-nitrobenzoic acid) concentration inhibited oxygen consumption of thrombin- or collagen-stimulated platelets by 15-28%. The results suggest that the same enzyme(s) are involved in both arachidonic acid releasing activity in platelet membranes and in arachidonic acid mobilization in stimulated intact platelets.

NOVEL SURFACE PHASE CONTAINING CHOLESTERYL ESTERS. 1. STRUCTURAL CHARACTERITICS DETERMINED FROM SURFACE PRESSURE-AREA MEASUREMENTS. J.M. Smaby and H.L. Brockman (Hormel Inst., Univ. of Minnesota, Austin, MN 55912) *Biochemistry 20*(5):718-23 (1981). The behavior of cholesteryl myristoleate in mixtures with dioleylphosphatidylcholine was investigated at the air-water interface. In addition to the previously described monolayer phase, a second surface phase has been identified. Analysis of surface pressure and molecular area data as a function of composition shows that the molecules in the second phase can exist in two miscible, double-layer states or packing arrangements, only one of which contains lecithin. The mixed double-layer state is preferentially formed and has stoichiometry ranging between 2.0 and 9.5 molecules of cholesteryl ester for each lecithin molecule. The structure of this state resembles a mixed monolayer of pressure-dependent composition and area which is covered by a second layer of cholesteryl esters at 38.2 Å^2/molecule. The cholesteryl myristoleate/lecithin ratio of the layer in contact with the aqueous phase ranges from 0 to 2.8 between 39 and 0 mN/m. The second double-layer state is equivalent to a monolayer of cholesteryl ester at the lipid-water interface, covered by a layer of cholesteryl ester molecules at 38.2 Å^2. Overall, our data show that the presence of lecithin at a lipid-water interface has a definite ordering effect on cholesteryl ester molecules at least 30-50 Å from the interface.

NOVEL SURFACE PHASE CONTAINING CHOLESTERYL ESTERS. 2. NONEQUIVALENCE OF CHOLESTERYL ARACHIDONATE AND THOSE WITH 18-CARBON, *CIS*-UNSATURATED ACYL GROUPS. J.M. Smaby and H.L. Brockman (Hormel Inst., Univ. of Minnesota, Austin, MN 55912) *Biochemistry 20*(4):724-30 (1981). Surface pressure-area isotherms for binary mixtures of cholesteryl octanoate, elaidate, stearate, oleate, linoleate, linolenate, and arachidonate in mixutres with dioleoyllecithin, triolein, oleic acid, and oleoyl alcohol were measured at 24 C. Analysis of the pressure and area characteristics as a function of composition showed that doublelayer surface phase formation is primarily dependent on the structure of the acyl moiety of the cholesteryl ester. Our results show that the polar lipid monolayer separating bulk cholesteryl ester from the aqueous milieu not only solubilizes finite amounts of cholesteryl esters but also can contribute to the organization of lipid adjacent to the monolayer. That such organization is observed with the predominant cholesteryl ester species of blood and aorta suggests a role for double-layer structure in regulating the transport and metabolism of cholesteryl esters in lipoproteins, arterial lipid deposits, and adrenal cortex. The absence of double-layer formation and high monolayer solubility of cholesteryl arachidonate suggest that it should be more abundant than other cholesteryl esters in bilayers and in monolayers surrounding bulk lipid phases.

CALORIMETRIC STUDIES ON SATURATED MIXED-CHAIN LECITHIN-WATER SYSTEMS. NONEQUIVALENCE OF ACYL CHAINS IN THE THERMOTROPIC PHASE TRANSITION. J. Stümpel, A. Nicksch, and H. Eibl (Max-Planck-Institut fur biophysikalische Chemie, D-3400 Gottingen, Federal Republic of Germany) *Biochemistry 20*(3):662-5 (1981). Aqueous dispersions of synthetic lecithins with different fatty acids in positions 1 and 2 of the glycerol molecule were studied by calorimetry. The data show that variation of the acyl chains in different positions of the glycerol backbone either have no influence upon or contribute 0.5 kcal/mol per CH_2 segment to the phase transition enthalpy. Different molecular ordering of the mixed acyl chain lecithins in the bialyer is discussed in light of the results.

PERSISTENCE OF SEGREGATED PHOSPHOLIPID DOMAINS IN PHOSPHOLIPID-LIPOPOLYSACCHARIDE MIXED BILAYERS: STUDIES WITH SPIN-LABELED PHOSPHOLIPIDS. Y. Takeuchi and H. Nikaido (Dept. of Microbiology and Immunology, University of California, Berkeley, CA 94720) *Biochemistry 20* (3):523-9 (1981). Then lipopolysaccharides from *Escherichia coli* B were sonicated together with pure spin-labeled phospholipids without the addition of unlabeled phospholipids, extensive line broadening was observed due to the close proximity of spin-labeled molecules to each other, a result suggesting that spin-labeled phospholipids existed in segregated domains containing few lipopolysaccharide molecules. Such mixed bilayers were incubated under various conditions, and the intermixing of the domains was followed by the decrease in line width. In another series of experiments, spin-labeled phospholipids were diluted with a 100-fold excess of unlabeled phospholipids and then mixed with lipopolysaccharides. These two different lines of approach therefore supported the idea that phospholipid (and most probably lipopolysaccharide) domains in mixed bilayers tend to be rather stable and persist for long periods of time.

THIOBARBITURIC ACID REACTION OF METHYL ARACHIDONATE MONOHYDROPEROXIDE ISOMERS. J. Terao and S. Matsushita (Res. Inst. for Food Sci., Kyoto Univ., Uji, Kyoto, 611, Japan) *Lipids 16*(2):98-101 (1981). Methyl ester of monohydroperoxy eicosatetraenoic acid (MeHPETE) was prepared from methylene blue sensitized photooxidation products of methyl arachidonate. The thiobarbituric acid (TBA) value of MeHPETE was increased by adding ferrous sulfate to the raection mixture. A linear relationship existed between the TBA value and the concentration of MeHPETE when ferrous sulfate was added. By using high performance liquid chromatography, MeHPETE was separated into 5 fractions whose isomeric compositions were determined by gas chromatography-mass spectrometry. The results of the TBA test for each fraction suggest that all of the MeHPETE isomers are positive to the TBA test. It is concluded that each isomer of HPETE formed by peroxidation of arachidonic acid in a biological system can yield TBA-reacting materials during the test reaction.

CELL-INDUCED LEAKAGE OF LIPOSOME CONTENTS. J. Van Renswoude and D. Hoekstra (Lab. of Theoretical Biology, National Cancer Inst., National Institutes of Health, Bethesda, MD 20205) *Biochemistry 20*(3):540-6 (1981). Using the principle of relief of selfquenching of carboxyfluorescein upon leakage of the dye from the interior of lipid vesicles, we investigated the integrity of sonicated small unilamellar vesicles in the presence of isolated hepatocytes, Zajdela ascites hepatoma cells, and plasma membranes of either cell type. We observed that cells as well as plasma membranes induce leakage of carboxyfluorescein from vesicles. Two parameters (initial rate and maximal level of induced leakage) were

determined to quantitate the leakage events and were found to depend on cell density, vesicle concentration, and vesicle lipid composition. We show that leak-inducing activity resides in the plasma membrane and that it can be considerably reduced by treatment of the plasma membranes with neuraminidase or trypsin, suggesting the involvement of cell-surface glycoprotein(s). Release of activity from intact cells and isolated plasma membranes into the medium occurs spontaneously (at a slow rate) but can be facilitated by freezing and thawing; the activity can subsequently be recovered in a soluble form from the medium.

EFFECT OF PHAGOCYTOSIS AND IONOPHORES ON RELEASE AND METABOLISM OF ARACHIDONIC ACID FROM HUMAN NEUTROPHILS. C.E. Walsh, L.R. Dechatelet, M.J. Thomas, J.T. O'Flaherty and M. Waite (Dept. of Biochem., Bowman Gray Schl. of Med., Wake Forest Univ., Winston-Salem, NC 27103) *Lipids 16*(2): 120-4 (1981). Challenge of human neutrophils prelabeled with [^{3}H] arachidonate and [^{14}C] palmitate or [^{14}C] stearate with opsonized zymosan or the Ca^{2+} ionophores A23187 or Ionomycin caused the release of [^{3}H], but [^{14}C], fatty acid. With the ionophores, but not zymosan, considerable conversion of the [^{3}H] arachidonate to hydroxyeicosate-traenoates occurred. Although various isomers were recovered, the 5-hydroxyeicosatetraenoate appeared to be the major product. In these experiments, no [^{14}C] products were detected such as lysophospholipid, diglyceride or monoglyceride. Although no definitive statement can be made about the mechanism of release of arachidonate, our data are most easily interpreted as the result of the action of a phospholipase A_2.

THE COMPOSITION OF THE SKIN SURFACE LIPIDS OF THE GERBIL. D. Yeung, S. Nacht and R.E. Cover (Dept. of Dermatological Research, Vick Toiletry Research Division, Richardson-Merrell Inc., Mount Vernon, NY 10553) *Biochim. Biophys. Acta 663*(2):524-35 (1981). The skin surface lipids of the gerbil were found to consist sterol esters (10%), wax diesters (36.3%), triacylglycerol (26.1%), free fatty alcohols (8.8%), free fatty acids (5.4%), cholesterol (8.4%) and polar lipids (5%). The wax diesters, identified as Type II, were made up of saturated 1,2-diols with odd carbon number, esterifeid with two molecules of unsubstituted fatty acids with even carbon number. Both the triacylglycerols and the free fatty acid fractions had saturated and unsaturated components. The free and esterifeid sterols were all cholesterol. The sterol esters contained saturated monoenoic and dienoic fatty acids, with both straight- and branched-chain components. The fatty alcohols were all straight-chain in structure, mostly of even carbon number. Comparison of these results with those previously reported for other species, indicates that the gerbil skin surface lipids are unique in that they contain diacyl alkane diols and fatty alcohols, both of which consist exclusively of saturated components.

INFLUENCE OF STAGE OF LACTATION ON THE TRIACYLGLYCEROL COMPOSITION OF BUFFALO MILK FAT. C. Arumughan and K.M. Narayanan (National Dairy Res. Inst., Karnal 132001, India) *Lipids 16*(3):155-64 (1981). Milk fats obtained from colostrum and early, middle and late lactation samples of buffalo milk were analyzed for their triacylglycerol (TG) compositions. Each milk fat was first separated by thin layer chromatography (TLC) into high, medium and low molecular weight TG. The TG fractions thus obtained were further segregated by argentation TLC, according to their degree of unsaturation into saturated, *trans*-monoene, *cis*-monoene, diene and polyene species. With progressive lactation, the major changes from colostral fat were an increase in lower fatty acids and decline in oleic acid. This caused, in turn, marked variation in saturated TG and diene TG and, to a smaller extent, in polyene TG. Monoene TG, both *cis* and *trans*, remained practically constant throughout. These trends were largely reversed toward the end of lactation.

THE RELATIONSHIP BETWEEN DIETARY PHYTOSTEROLS AND THE STEROLS OF WILD AND CULTIVATED OYSTERS. C.J. Berenberg and G.W. Patterson (Department of Botany, University of Maryland, College Park, MD 20742) *Lipids 16*(4):276-8 (1981). Wild oysters (*Crassostrea virginica*) contained cholesterol, 24-methyl-cholestra-5, 22-dienol, 24-methylenecholesterol, 22-dehydrocholesterol, 24-methylcholesterol, 24-ethylcholesterol, 24-norcholesta-5, 22-dienol, 24-ethylcholesta-5, 22-dienol and fucosterol. The same species was cultivated on a defined diet of *Thalassiosira pseudonana* and *Isochrysis* sp. The dietary algae were cultured and their sterol compositions were analyzed by gas chromatography and mass spectroscopy. *T. pseudonana* and *Isochrysis* sp. had 24-methylenecholesterol and 24-methyl-cholesta-5, 22-dienol as their major sterols. The sterol composition of the cultivated oysters revealed the predominance of cholesterol (19%), 24-methylcholesta-5, 22-dienol (21%) and 24-methylenecholesterol (46%). Therefore, oysters must be able to bioconvert phytosterols to cholesterol, concentrate dietary cholesterol, or synthesize cholesterol de novo.

ENZYMATIC SYNTHESIS/HYDROLYSIS OF CHOLESTERYL OLEATE IN SURFACE FILMS. INHIBITION BY LECITHIN AND ITS REVERSAL BY BILE SALTS. S.G. Bhat and H.L. Borckman (The Hormel Institute, University of Minnesota, Austin, MN 55912) *J. Biol. Chem. 256*(6):3017-23 (1981). The synthesis/hydrolysis of cholesteryl oleate as catalyzed by porcine pancreatic cholesterol esterase has been studied in lipid films at the air-buffer interface. With only reactants and products initially present at the interface, equilibrium is rapidly attained. The equilibrium constant for the reaction is independent of pH, initial composition, and surface pressure. Lecithin, if present in molar excess relative to the sum of free and esterified cholesterol, is inhibitory. Inhibition is associated with division of the substrate into reactive and unreactive pools which are not exchangeable. Bile salts and other surfactants reverse the inhibition at concentrations one-tenth their critical micelle concentrations. Presumably this occurs through formation of a surfactant surface excess at the lipid-water interface which disrupts the unreactive lecithin-substrate complex. The adsorption of cholesterol esterase to oleic acid monolayers is first order with respect to enzyme and is saturable. At saturation, the enzyme forms a close packed monolayer at the lipid-water interface with a molecular area of 4510 A^2. Adsorption of cholesterol esterase to lecithin monolayers is less than one-tenth that to oleic acid monolayers and is proportional to subphase enzyme concentration.

LACTOPEROXIDASE-CATALYZED IODINATION OF ARACHIDONIC ACID: FORMATION OF MACROLIDES. J.M. Boeynaems, D. Reagan, and W.C. Hubbard (Departments of Pharmacology and Chemistry, Vanderbilt University, Nashville, TN 37232) *Lipids 16*(4):246-9 (1981). In the presence of iodide and hydrogen peroxide, lactoperoxidase catalyzed the conversion of arachidonic acid into several iodinated products; the major one was previously identified as an iodo-δ-lactone. Two minor and less polar products have now been characterized as 15-iodo-14-hydroxyeicosatrienoic acid, ω-lactone and 14-iodo-15-hydroxyeicosatrienoic acid, ω-lactone, on the basis of ^{125}I incorporation, mass spectrometry, proton magnetic resonance spectroscopy and chemical modifications.

HYDROPHOBIC AND ELECTROSTATIC INTERACTIONS OF MYELIN BASIC PROTEIN WITH LIPID. PARTICIPATION OF N-TERMINAL AND C-TERMINAL PORTIONS. J.M. Boggs, D.D. Wood and M.A. Moscarello (Res. Inst., Hosp. for Sick Children, Toronto, ON, Canada M5G 1X8) *Biochemistry 20*(5):1065-73 (1981). The basic protein of myelin may be involved in association of the cytoplasmic surfaces of myelin by a bridging mechanism whereby the N-terminal half of the protein binds to one surface and the C-terminal half binds to the opposing surface. The feasibility of this mechanism was investigated by studying the ability of the N-terminal and C-terminal fragments, prepared by cleavage of the protein at the tryptophanyl residue 116, to interact both electrostatically and hydrophobically with bilayers of acidic lipids. Both fragments, the N-terminal two-thirds and the C-terminal one-third, and the intact protein had a qualitatively similar effect on the lipid-phase transition temperature, and fatty acid chain packing suggesting that they interact hydrophobically with lipid in a similar way. The C-terminal fragment had quantitatively less effect, however. The amount of lipid protected from Mn^{2+} binding by the intact protein, N-terminal fragment, and C-terminal fragment was 26 ± 5, 17 ± 3, and 11 ± 1.5 molecules of lipid per molecule of protein, respectively. Since the number of positively charged amino acids per mole of protein at pH 7.4 is 31, 20, and 11, respectively, this suggests that nearly all of these residues participate in binding to acidic lipid head groups. It is possible for each half of the protein to bind to opposing lipid bilayers or cytoplasmic surfaces in myelin through similar hydrophobic and electrostatic interactions, suggesting that a bridging mechanism is quite possible.

FRACTIONATION AND CHARACTERIZATION OF EDIBLE TALLOW. D.M. Bussey, T.C. Ryan, J.I. Gray, and M.E. Zabik (Department of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824) *J. Food Sci. 46*(2):526-30 (1981). Tallow or fractionated tallow could be utilized as suitable replacements for expensive imported fats. Detergent (aqueous) fractionation is applicable to beef tallow and involves the addition of water containing an inorganic salt (Na_2SO_4) and a surface active agent, sodium dodecyl sulfate (SDS), to a partly crystallized oil. Centrifugation yields olein and stearin layers. Investigation of four principal process variables revealed that olein production was maximized with (1) 5 hr of crystallization, (2) minimum stirring during crystallization, (3) subsequent addition of SDS to fractions, and (4) a 4:1 electrolyte to detergent ratio. A comparison with olein yields for current fractionation methods for tallow revealed that the modified procedure was more efficient.

FLUORESCENCE QUENCHING IN MODEL MEMBRANES. 3. RELATIONSHIP BETWEEN CALCIUM ADENOSINETRIPHOS-

PHATASE ENZYME ACTIVITY AND THE AFFINITY OF THE PROTEIN FOR PHOSPHATIDYLCHOLINES WITH DIFFERENT ACYL CHAIN CHARACTERISTICS. M. Caffrey and G.W. Feigenson (Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853) *Biochemistry 20*(7):1949-61 (1981). The dependence of function and lipid binding affinity of an integral transport protein on the fatty acyl chain characteristics of a membrane-forming phospholipid have been determined. When a newly developed fluorescence quenching technique is used for examining lipid-protein interactions in membranes, the Ca^{2+}ATPase from rabbit sarcoplasmic reticulum is found to bind with equal affinity a large variety of phosphatidylcholines used to reconstitute the protein into enzymatically active vesicles, regardless of fatty acyl chain length or details of unsaturation. In parallel with the lipid binding studies, we have measured the sensitivity of the catalytic activity of the Ca^{2+}ATPase to the fatty acyl chain characteristics of the phosphatidylcholine membranes in which the enzyme was reconstituted. The enzyme appears to be sensitive only to the effective fatty acyl chain length, which determines the thickness of the bilayer in which the protein is inserted and displays little sensitivity to such details of unsaturation as degree, position, and isomeric type. Both ATP hydrolyzing and Ca^{2+} transporting activities of the enzyme were similarly affected by bilayer thickness, and maximum activity was observed in membranes of intermediate thickness. A freeze-thaw method was used to reconstitute the Ca^{2+}ATPase, and the vesicles so obtained have been characterized by gel permeation chromatograpy, density gradient centrifugation, and electron microscopy (thin section).

REGULATION OF CHOLESTEROL BIOSYNTHESIS IN ENUCLEATED CELLS. W.K. Cavenee, H.W. Chen and A.A. Kandutsch (Jackson Laboratory, Bar Harbor, Maine 04609) *J. Biol. Chem. 256*(6):2675-81 (1981). In cells that had been physically enucleated after treatment with cytochalasin B (cytoplasts) levels of cholesterol synthesis and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity were nearly constant over a 6-h period of time. The ratio of the inactive to the active form of the reductase was unaltered by enucleation and did not change when the cytoplasts were incubated at 37 C. The addition to the medium of 25-hydroxycholesterol or serum, agents which specifically suppress the reductase activity in nucleated cells, or of cycloheximide, a general inhibitor of protein synthesis, did not affect cholesterol synthesis or reductase activity in the cytoplasts. In contrast compactin rapidly suppressed reductase activity and cholesterol synthesis. 3-Hydroxy-3-methylglutaryl-CoA reductase activity was stable when protein synthesis was blocked by cycloheximide, indicating that degradation of the reductase did not occur in the cytoplasts. The stability of 3-hydroxy-3-methylglutaryl-CoA reductase activity levels in cytoplasts appeared, therefore, to be due to the absence of both synthesis and degradation of the reductase.

CONVERSION OF 3T3-L1 FIBROBLASTS TO FAT CELLS BY AN INHIBITOR OF METHYLATION: EFFECT OF 3-DEAZAADENOSINE. P.K. Chiang (Lab. of Gen'l and Comparative Biochem., Nat'l Institute of Mental Health, Bethesda, Maryland 20205) *Science 211* (4487):1164-1166 (1981). 3-deazaadenosine, an inhibitor of methylation, increased the frequency of conversion of 3T3-L1 fibroblasts to fat cells in a dise-dependent manner. Once converted, the 3T3-L1 fat cells retained their adipose morphology and accumulated triglycerides even when 3-deazaadenosine was removed from the culture medium. 3-Deazaadenosine may perturb cellular methylation and thereby lead to an increase in the frequency of differentiation of 3T3-L1 fibroblasts to fat cells.

PHOTOACTIVATABLE CARBENE-GENERATING PHOSPHOLIPIDS: PHYSICAL PROPERTIES AND USE IN DETECTION OF PHASE SEPARATIONS IN LIPID MIXTURES. W. Curatolo, R. Radhakrishnan, C.M. Gupta, and H.G. Khorana (Departments of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139) *Biochemistry 20*(5):1374-8 (1981). Phospholipids which carry photoreactive carbene-generating groups have been cross-linked with a variety of acceptor phospholipids in binary and ternary mixtures, and the results have been correlated with the physical behavior of the lipids involved. In equimolar mixtures of these lipids with dipalmitoyllectithin (DPL), phase separation occurs below characteristic temperatures as has been previously observed for mixtures of saturated and unsaturated phospholipids. Photolysis of mixtures of PCI or PCII with saturated or unsaturated phospholipids results in the formation of covalently linked phospholipid dimers. Photolysis-dependent dimer formation was studied in mixtures of DPL and dioleyllecithin (DOL) which exhibit temperature-dependent phase separation and also in mixtures of DPL and dimyristoyllecithin (DML) which do not exhibit phase separation. Covalent cross-linking to PCII to each of the lipids in these mixtures was quantitated as a function of temperature. The photoreactive phospholipid was excluded from gel phases in both the DPL/DOL and KPL/KML systems and reacted preferentially with the liquid-crystalline phospholipid in the phase-separated DPL/DOL system. Thus, the photochemical labeling approach is sensitive to the presence of phase separations and has the capability of identifying the major components of each phase.

FRACTIONATED EDIBLE BEEF TALLOW AS A DEEP-FAT FRYING MEDIUM FOR FRENCH FRIES. C.L. DeFouw, M.E. Zabik, and J.I. Gray (Department of Food Science & Human Nutrition, 139 Food Science Building, Mich. State University, East Lansing, MI 48824) *J. Food Sci. 46*(2):452-6 (1981). Edible beef tallow fractionated by detergent (SDS) at controlled temperatures, unfractionated tallow, blends of tallow with corn and soybean oil, and a commercial frying oil were compared as deep-fat frying media. French fries were fried in the oil for 20 consecutive fryings. Oils were tested physically and chemically and French fries were evaluated both objectively and by a taste panel. Results showed that color of the oils became darker and viscosity, peroxide value, and refractive indices increased progressively. Sensory scores showed no significant differences ($p<0.05$) in general acceptability in fries prepared with any of the oils, indicating that fractionated tallow does have potential for use as a deep-fat frying medium.

INVESTIGATIONS OF EDIBLE OILS FOR VOLATILE NITROSAMINES. W. Fiddler, J.W. Pensabene, and W.I. Kimoto (USDA-SEA Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118) *J. Food Sci. 46*(2):603-605 (1981). Twenty-one commercial edible oils and five margarine samples were purchased locally and analyzed for nitrosamines (NAs) by a gas-liquid chromatograph interfaced with a Thermal Energy Analyzer. N-nitrosodimethylamine (NDMA), at levels of 0.22-1.01 ppb, was the only volatile apparent NA detected. These levels were found in 7/7 corn, 6/6 olive, 2/2 sunflower, 1/3 soybean, 2/3 undesignated oils, and 4/5 margarine samples. The other three oils and one margarine sample showed no NDMA at the lower limit of 0.1 ppb. These levels of nitrosamine detected in the edible oils tested were much lower than the levels previously reported.

PHYSICAL PROPERTIES OF CHOLESTERYL ESTERS HAVING 20 CARBONS OR MORE. G.S. Ginsburg and D.M. Small (Biophysics Institute, Housman Medical Research Center, Departments of Medicine and Biochemistry, Boston University Medical Center, Boston, MA 02118) *Biochim. Biophys. Acta 664*(1):98-107 (1981). By polarizing microscopy and differential scanning calorimetry we observed that the relative stability of the smectic and cholesteric mesophases of cholesteryl esters of acyl chain length of 20 carbons or more depends on the length of the acyl chain and its degree of unsaturation. Significantly, the addition of a single double bond to the acyl chain of a fully saturated cholesteryl ester which exhibits no mesophases (e.g., cholesteryl behenate ($C_{22:0}$) and cholesteryl lignocerate ($C_{24:0}$)) yields an ester which displays an unusually stable smectic mesophase, but no cholesteric mesophase. In fact, increasing unsaturation was found to have a destabilizing effect on the cholesteric phase. Similarly, a decrease in thermal stability of the cholesteric mesophase was observed with increasing chain length in a series of *cis*-monounsaturated cholesteryl esters, while the thermal stability of the smectic mesophase increased in the same series. X-ray scattering data are presented on the smectic mesophase of cholesteryl erucate ($C_{22:1}$) and cholesteryl nervonate ($C_{24:1}$). Significant differences in molecular packing of these two monounsaturated $\omega = 9$ cholesteryl esters in the crystalline state are demonstrated by preliminary X-ray scattering experiments.

CONVERSION OF 5,8,11-EICOSATRIENOIC ACID TO LEUKOTRIENES C_3 AND D_3. S. Hammarström (Dept. of Chemistry, Karolinska Institutet, P.O. Box 60400, S-10401 Stockholm, Sweden) *J. Biol. Chem. 256*(5):2275-2279 (1981). 5,8,11-Eicosatrienoic acid was converted by mouse mastocytoma cells stimulated with ionophore A23187 to two slow reacting substances. These were characterized by spectroscopy and by chemical and enzymatic degradations as two geometrical isomers of 5-hydroxy-6-S-glutathionyl-7,9,11-eicosatrienoic acid (E,E,Z; leukotriene C_3 and E,E,E; 11-*trans*-leukotriene C_3). Corresponding cysteinylglycine compounds (leukotriene D_3 and 11-*trans* leukotriene D_3) were obtained from the leukotriene C_3 isomers by treatment with kidney γ-glutamyl transpeptidase. The biological effects of leukotrienes C_3 and D_3, on the isolated guinea pig ileum, were approximately the same as of leukotrienes derived from arachidonic acid.

SEPARATION OF THE APOPROTEIN COMPONENTS OF HUMAN VERY LOW DENSITY LIPOPROTEINS BY ION-PAIRED, REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. W.S. Hancock, C.A. Bishop, A.M. Gotto, D.R.K. Harding, S.M. Lanplugh, and J.T. Sparrow (Department of Chemistry, Biochemistry and Biophysics, Massey University, Palmerston North, New Zealand) *Lipids 16*(4):250-9 (1981). A number of crude apolipoprotein samples isolated from human very low density lipoproteins (VLDL) were analyzed by reversed phase high per-

formance liquid chromatography. The mobile phase consisted of a 1% solution of the polar ion-pairing reagent triethylammonium phosphate. A slow, nonlinear gradient of acetonitrile (37-42%) was used to elute the apolipoproteins. The order of elution was as follows: apolipoprotein C_X, apolipoprotein C-I, apolipoprotein C-III_2, apolipoprotein C-III_1, apolipoprotein C-III_Q and apolipoprotein C-II. This order is consistent with the known polarity of the proteins, i.e., the most nonpolar, apolipoprotein C-II, was the last to be eluted, whereas apolipoprotein C-I, with the lowest nonpolar surface area eluted first. The recovery of the individual apolipoproteins was 80-95% and the individual peaks were characterized by amino acid analysis, UV absorption spectra and chromatography of pure protein standards.

NOVEL COMPLEX POLAR LIPIDS FROM THE METHANOGENIC ARCHAEBACTERIUM *METHANOSPIRILLIUM HUNGATEI*. S.C. Kushwaha, M. Kates, G.D. Sprott, and I.C.P. Smith (Dept. of Biochem., Univ. of Ottawa, Ottawa, Canda KIN 9B4) *Science 211* (4487):1163-4 (1981). The methanogenic archaebacterium *Methanospirillum hungatei* contains two unusual phosphoglycolipids that account for 64 percent of the total cellular lipids. These lipids are derivatives of the dibiphytanyl diglycerol tetraether, previously identified in methanogens. One of the free hydroxyls of this tetraether is esterified with glycerophosphoric acid, and the other is linked glycosidically to a discaccharide. The two phosphoglycolipids may function as covalently bonded lipid bilayers to impart stability and rigidity to methanogen membranes.

THE USE OF SP2340 GLASS CAPILLARY COLUMNS FOR THE ESTIMATION OF THE *TRANS* FATTY ACID CONTENT OF FOODS. E. Lanza and H.T. Slover (Nutrient Composition Laboratory, Beltsville Human Nutrition Research Center, Human Nutrition, Science and Education Administration, United States Department of Agriculture, Beltsville, MD 20705) *Lipids 16*(4):260-7 (1981). Glass capillary gas chromatography (GCGC) on 100-m and 60-m SP2340 columns was used for quantitation of the *trans* unsaturated fatty acids in shortenings and fast foods. The separation of the *cis* and *trans* octadecenoates on GCGC was evaluated by preparatory argentation thin layer chromatography. In addition, the *trans* content of shortening samples obtained by GCGC was compared to *trans* content determined by infrared analysis.

PRODUCTION OF OIL AND PROTEIN FOOD PRODUCTS FROM RAW PEANUTS BY AQUEOUS EXTRACTION AND ULTRAFILTRATION. J.T. Lawhon, L.J. Manak, K.C. Rhee, and E.W. Lusas (Food Protein Research & Development Center, Texas A&M University, College Station, TX 77843) *J. Food. Sci. 46*(2):391-5 (1981). Two significant protein isolation techniques (the Aqueous Extraction Process and the Membrane Isolation Process) were combined to obtain a new processing strategy for producing oil and protein food products from raw peanuts. Three varieties of peanuts were processed to obtain either a low-fat isolate or a full-fat product and an oil cream using aqueous extraction, centrifugation and industrial ultrafiltration membranes. Proximate and amino acid analyses and nitrogen solubility profiles were made on spray-dried products. Mean membrane permeation rates achieved exceeded 70 gallons per sq ft per day (gfd). Protein products possessed high nitrogen solubility, a bland taste, and were desirably light in color.

BINDING OF DIVALENT CATIONS TO DIPALMITOYLPHOSPHATIDYLCHOLINE BILAYERS AND ITS EFFECT ON BILAYER INTERACTION. L.J. Lis, V.A. Parsegian, and R.P. Rand (Brock University, St. Catharines, Ontario, Canada L2S 3A1) *Biochemistry 20*(7):1761-1770 (1981). We have confirmed that $CaCl_2$ swells the multilayer lattice formed by dipalmitoylphosphatidylcholine (DPPC) in an aqueous solution. Specifically, at room temperature 1 mM $CaCl_2$ causes these lipid bilayers to increase their separation, d_W, from 19 Å in pure water to >90 Å. $CaCl_2$ concentrations >40 mM cause less swelling. We have measured the net repulsive force between the bilayers in 30 mM $CaCl_2$ at T = 25 C (below the acyl chain freezing temperature). For interbilayer separations between 30 and 90 Å, the dominant repulsion between bilayers is probably electrostatic; Ca^{2+} binds to DPPC lecithin bilayers, imparting a charge to them. The addition of NaCl to $CaCl_2$ solutions decreases this repulsion. For d_X < 20 Å, the bilayer repulsion appears to be dominated by the "hydration forces" observed previously between both neutral and charged phospholipids. From the electrostatic repulsive force, we estimate the extent of Ca^{2+} binding to the bilayer surface. The desorption of bound Ca^{2+}, apparent when bilayers are pushed together, is more rapid than one would expect if an association constant governed Ca^{2+} binding. The association affinity does not appear to be a fixed quantity but rather a sensitive function of ionic strength and bilayer separation.

SULFITE-INDUCED LIPID PEROXIDATION. M.C.C. Lizada and S.F. Yang (Dept. of Vegetable Crops, Univ. of California, Davis, CA 95616) *Lipids 16*(3):189-94 (1981). Sulfite initiated the peroxidation of linoleic acid and linolenic acid emulsions via a free radical mechanism. Peroxidation of these fatty acids required oxygen and sulfite and occurred with concomitant oxidation of sulfite to sulfate. In reaction mixtures containing linoleic acid, the formation of conjugated diene equaled the formation of hydroperoxide. In reaction mixtures containing linolenic acid emulsions, thiobarbituric acid reactive materials were also formed. Peroxidation was pH-dependent; peroxidation of linoleic acid proceeded between pH 4 and 7, but linolenic acid peroxidation was significant only if pH was belo pH 6. The linoleic acid hydroperoxides thus formed were reduced and methylated to methyl hydroxystearate. Analysis of methyl hydroxystearate by gas chromatography-mass spectrometry indicated that sulfite-induced peroxidation was significant only if pH was below pH 6. The linoleic acid hydroperoxides thus formed were reduced and methylated to methyl hydroxystearate. Analysis of methyl hydroxystearate by gas chromatography-mass spectrometry indicated that sulfite-induced peroxidation gave rise to the 9- and 13-hydroperoxy isomers. In addition to the hydroperoxides, sulfite adducts were detected. Hydroquinone, butylated hydroxytoluene and α-tocopherol effectively inhibited both sulfite oxidation and hydroperoxide formation. Conjugated diene formation also was inhibited by 4-thiouridine, suggesting that the reaction is mediated by the sulfite radical. No significant inhibition was observed with the addition of superoxide dismutase, catalase, or the hydroxyl radical scavengers, mannitol or *t*-butanol. A possible mechanism is presented to account for sulfite-induced peroxidation of linoleic acid.

FLUORESCENCE QUENCHING IN MODEL MEMBRANES. 1. CHARACTERIZATION OF QUENCHING CAUSED BY A SPIN-LABELED PHOSPHOLIPID. E. London and G.W. Feigenson (Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853) *Biochemistry 20*(7):1932-8 (1981). A new method is described to evaluate contact between fluorophors and lipids in model membranes. This method utilizes a nitroxide spin-labeled phosphatidylcholine to quench the fluorescence from a variety of membrane-bound molecules by a static process. The distance dependence of the fluorescence quenching is analyzed in terms of simple models. The analysis shows that quenching of diphenylhexatriene, *p*-terphenyl, and molecules containing tryptophan arises only from spin-labeled phospholipid that is in contact with the fluorophor.

FLUORESCENCE QUENCHING IN MODEL MEMBRANES. 2. DETERMINATION OF THE LOCAL LIPID ENVIRONMENT OF THE CALCIUM ADENOSINETRIPHOSPHATASE FROM SARCOPLASMIC RETICULUM. E. London and G.W. Feigenson (Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853) *Biochemistry 20*(7):1939-48 (1981). Fluorescence quenching by spin-labeled phospholipid is used to determine the affinities of different phospholipid species to an intrinsic membrane protein, the Ca^{2+}-ATPase of sacroplasmic reticulum. The phospholipids in contact with the Ca^{2+}ATPase are examined in a reconstituted system in which the enzyme is incorporated into a model membrane of defined phospholipid composition. The local phospholipid environment of the protein is considered to be governed at each phospholipid binding site by an equilibrium: lipid A + (lipid B = protein) ⟷ lipid B + (lipid A - protein). Phospholipid binding constants to the Ca^{2+}ATPase can be obtained from an analysis of fluorescence quenching data. The binding constants for a number of phospholipid species are nearly identical when the phospholipids are in the liquid-crystal state. However, temperature or Ca^{2+}-induced phase separation of phospholipid induces striking changes in the composition of the phospholipids in contact with the Ca^{2+}ATPase, relative to the overall composition of the membranes. The implications of these results with respect to the control of local phospholipid environment by intrinsic membrane proteins and the nature of the phospholipid binding sites on the proteins are discussed. General applicability of this type of fluorescence quenching study to the problem of lipid-protein interactions in membranes is considered, and this method is compared to other techniques.

COVALENT BINDING OF PEROXIDIZED PHOSPHOLIPID TO PROTEIN: III. REACTION OF INDIVIDUAL PHOSPHOLIPIDS WITH DIFFERENT PROTEINS. H. Nielsen (Inst. of Medical Biochem., Univ. of Aarhus, Aarhus, Denmark) *Lipids 16*(4):215-22 (1981). Various peroxidized phospholipids were reacted with proteins under N_2. In all cases, phospholipid is bound covalently to the proteins whose molecular size is increased. Both the amount of bound phospholipid and the increase in molecular size of the protein depends on the nature of the phospholipid. Ultraviolet (UV) absorption of the proteins is increased in qualitatively similar ways. Their difference spectra, which show a gradual increase in absorption from 400 nm toward shorter wavelength, differ from that of malon-

aldehyde-protein complexes. The various complexes of proteins and peroxidized phospholipids have similar fluorescence spectra showing two excitation maxima at 310-320 nm and at 340-350 nm, respectively, and emission maximum at ca. 400 nm. This is different from both fluorescence spectra of malonaldehyde-protein complexes and fluorescence spectra reported for proteins after reaction with peroxidized polyunsaturated fatty acids. Amino groups of the proteins are consumed in the reaction with peroxidized phospholipids. Blocking the amino groups decreases the binding of phospholipid considerably. Besides amino groups, other structures of the protein molecule react with the peroxidized phospholipids. The similar features of UV absorption, fluorescence, decrease of amino groups, and covalently bound phospholipid phosphorus of the various complexes suggest that they are formed by common types of reactions. The reactions seem to be different from those generally believed important between peroxidized lipid and protein. Important reacting species are compounds other than malonaldehyde.

FATTY ACIDS AND STEROLS ASSOCIATED WITH CITRUS ROOT MYCORRHIZAE. H.E. Nordby, S. Nemec, and S. Nagy (U.S. Citrus and Subtropical Products Laboratory, Southern Region, U.S. Dept. of Agriculture, Science and Education Administration, Winter Haven, Florida 33880) *J. Agric. Food Chem. 29*(2):396-401 (1981). The structures of four fatty acids present in citrus roots infected with VA-mycorrhizal fungus but not in noninfected roots were shown to be 11*c*-16:1, 6*c*,9*c*,12*c*-18:3, 8*c*,11*c*,14*c*-20:3, and 5*c*,8*c*,11*c*,14*c*-20:4. Compositional differences of these four acids between seedlings infected with three *Glomus* species were slight. These acids were present in the triglycerides and to a lesser extent in three phospholipids and one glycolipid of the fibrous roots. They were absent in the tap roots and leaves. Supplemental phosphorus failed to produce these acids in seedlings grown in noninoculated soil. Levels of these acids were lower in mature trees than in seedlings. Lipids from *Glomus mosseae* chlamydospores contained 11*c*-16:1 as well as a 20:5 fatty acid and almost exclusively a 24-methylcholesterol in its free sterol fraction.

UNEVEN DISTRIBUTION OF PALMITOYL CARNITINE IN SOLUTION BECAUSE OF MIGRATION TO AIR/WATER INTERPHASE. S.V. Pande (Lab. of Intermediary Metabolism, Clinical Res. Inst. of Montreal, Montreal, P.Q., H2W 1R7, Canada) *Biochim. Biophys. Acta 663*(3):669-73 (1981). Standard solutions of palmitoyl carnitine could not be prepared in water because, even at below critical micelle concentrations, palmitoyl carnitine did not distribute uniformly in solutions. Evidence indicates that palmitoyl carnitine prefers to leave the bulk phase to segregate readily at the water/aid and water/apolar interphases. Thus, in metabolic and kinetic studies, the actual concentration of long-chain acyl carnitines available for reactions at any instant can be drastically different from that calculated from the amounts added.

EVALUATION OF NEW METHODS FOR THE ASSESSMENT OF USED FRYING OILS. A.J. Paradis and W.W. Nawar (Department of Food Science & Nutrition, University of Massachusettes, Amherst, MA 01003) *J. Food Sci. 46*(2):449-51 (1981). Three methods recently introduced for quality assessment of frying oils were compared. These were: column chromatography of polar components, dielectric constant measurement, and GLC of triglyceride dimers. For a given frying operation all three methods correlated well with each other and with time of frying, while large differences were obtained for samples of unknown history. The dielectric readings were found to represent a net balance of opposing effects from two groups of compounds. The GLC method was found to be simpler and more specific than the column technique.

CHANGES IN FATTY ACIDS AND SENSORY QUALITY OF FRESH WATER PRAWN (*MACROBRACHIUM ROSENBERGII*) STORED UNDER FROZEN CONDITIONS. S.K. Reddy, W.K. Nip, and C.S. Tang (Dept. of Food Science & Human Nutrition, University of Hawaii, Honolulu, HI 96822) *J. Food Sci. 46*(2):353-6 (1981). The effects of frozen storage and packaging methods on the oxidation of fatty acids and rancidity development in fresh-water prawn *Macrobrachium rosenbergii* were studied. The lipids of these fresh prawns contained 23% saturated, 46% monounsaturated, and 31% polyunsaturated fatty acids. The fatty acids, especially the unsaturated ones decreased during frozen storage for 6 months at -18 C, regardless of the packaging procedure employed. No objectionable rancid flavor was detected in these prawns during the 6-month frozen storage study.

METABOLISM OF STEROLS BY ANAEROBIC *SACCHAROMYCES CEREVISIAE*. B.C. Sekula and W.R. Nes (Dept. of Biological Sciences, Drexel Univ., Philadelphia, PA 19104) *Lipids 16*(3):195-8 (1981). Anaerobically grown *Saccharomyces cerevisiae* retained the ability to transfer a C_1-group to the C-24 position of a $\Delta^{24(25)}$-sterol and to reduce the $\Delta^{24(28)}$-bond of a 24-methylenesterol. Both desmosterol and 24-methylenecholesterol yielded 24β-methylcholesterol. However, when the substituent at C-24 was enlarged to a 24-ethylidene group (fucosterol), reduction of the $\Delta^{24(28)}$-bond did not occur. In no cases was a Δ^{7}- or a Δ^{22}-bond introduced. Because the $\Delta^{24(28)}$-bond was reduced in the absence of the Δ^{22}-bond, the Δ^{22}-bond is not an obligatory requirement for reduction.

PHOSPHATIDATE AND OXIDIZED FATTY ACIDS ARE CALCIUM IONOPHORES. STUDIES EMPLOYING ARSENAZO III IN LIPOSOMES. C. Serhan, P. Anderson, E. Goodman, P. Dunham and G. Weissmann (Marine Biological Lab., Woods Hole, MA 02543) *J. Biol. Chem. 256*(6):2736-41 (1981). Liposomes which have entrapped the metallochromic dye, arsenazo III, constitute a sensitive assay system for ionophoresis of divalent cations. By this means we have compared known calcium ionophores (A23187, ionomycin) with membrane phospholipids, fatty acids, prostanoids, and retinoids. Added at micromolar concentrations to preformed multilamellar liposomes both A23187 and ionomycin, as well as phosphatidic acid and products derived from linoleic acid, linolenic acid, and two eicosatrienoic acids provoked Ca influx. A variety of other phospholipids, fatty acids, prostanoids, retinoids and glyceryl ether phosphorylcholines ("platelet-activating factors") were without effect. Phosphatidic acid and oxidized fatty acids translocated divalent cations selectively, demonstrating the same rank order as A23187 or ionomycin: Mn > Ca > Sr >> Mg. Liposomes with phosphatidic acid or oxidized trienoic acids preincorporated at 1-5 mole % of total lipids also permitted translocation of Ca but not Mg. Reduction of ionopheretic fatty acids or ionomycin with stannous chloride abolished their ionophoretic activity. Release of Ca from liposomes which had entrapped arsenazo III-Ca complexes into a medium rich in EGTA permitted calculation of efflux induced by ionophores. Data suggest that phosphatidic acid and oxidized di- and trienoic fatty acids, which act as calcium ionophores in model bilayers, could serve as "endogenous ionophores" in cells.

CHROMATOGRAPHIC SEPARATION OF THE STEREOISOMERS OF α-TOCOPHEROL. H.T. Slover and R.H. Thompson, Jr. (Nutrient Composition Laboratory, Beltsville Human Nutrition Research Center, SEA/USDA, Beltsville, MD 20705) *Lipids 16*(4):268-75 (1981). The diastereoisomers of 2-*ambo*-α-tocopherol were completely separated as TMS ethers by gas chromatography on a 115 m × 0.25 mm glass capillary column coated with SP2340, at a column temperature of 195 C. In the same way, *all-rac*-α-tocopherol was separated into four peaks, corresponding to the four racemates present, and having the same retention ratios as the four diastereoisomers of 4′-*ambo*-8′-*ambo*-α-tocopherol (produced by the hydrogenation of natural α-tocotrienol). Retention data and relative peak areas for the diastereoisomers of the synthesized α-tocopherols and several commercial products were determined. Limited data on the isomers of other tocopherols also are reported.

EFFECT OF FAT AND MICROFLORA ON HEPATIC, SMALL INTESTINAL AND COLONIC HMG CoA REDUCTASE, CYTOCHROME P_{450} AND CYTOCHROME B_5. P. Smith-Barbaro, D. Hanson and B.S. Reddy (Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY 10595) *Lipids 16*(3):183-8 (1981). High levels of dietary fat caused a significant reduction in HMG CoA reductase activity in the liver of germ-free rats whereas significantly elevated small intestinal enzyme activity was observed. Dietary fat had no significant effect on HMG CoA reductase activity in any tissue studied in the conventional rat. No significant change in colonic HMG CoA reductase activity was observed between any of the experimental groups. Rats fed a high-fat diet tended to exhibit higher cytochrome P_{450} levels in all tissues studied, regardless of the presence of intestinal microflora.

A COMPARISON OF SPIN PROBE ESR, ^{2}H- AND ^{31}P-NUCLEAR MAGNETIC RESONANCE FOR THE STUDY OF HEXAGONAL PHASE LIPIDS. M.G. Taylor and I.C.P. Smith (Div. of Biological Sciences, National Res. Council of Canada, Ottawa, K1A 0R6, Canada) *Chem. Phys. Lipids 28*(2):119-36 (1981). We have investigated the feasibility of the various possible magnetic resonance probes of lipids which form non-bilayer phases. As a model system we have used equimolar mixtures of phosphatidylethanolamine (PE) and cholesterol, which exhibit a thermotropic transition from a bilayer to a hexagonal phase. Variable temperature electron spin resonance (ESR) spin probe spectra were obtained using random dispersion and oriented lipid systems. ^{31}P- and ^{2}H-nuclear magnetic resonance (NMR) studies were carried out using a deuterated PE. The ESR spin probe4 in the dispersions show essentially no effect attributable to the phase transition. However, there are large, reversible effects in the temperature-dependent behaviour for the oriented system. The orientation dependence of the spectra above the transition temperature indicate that the hexagonal phase lipids may spontaneously assume a macroscopic organization on a flat

surface. Taken together, the ^{2}H and ^{31}P data indicate that the structure of the headgroup in PE is quite similar in both the bilayer and hexagonal phase. ^{2}H-NMR should be very useful in probing the structural and dynamic characteristics of lipids in non-bilayer phases.

STEROL BIOSYNTHESIS IN THE OYSTER, CRASSOSTREA VIRGINICA. S-I Teshima and G.W. Patterson (Department of Botany, University of Maryland, College Park, MD 20742) *Lipids* 16(4):234-9 (1981). Sterol biosynthesis in the oyster, *Crassostrea virginica*, was examined by injection of [2-^{14}C]acetate and [2,3-^{3}H]-lanosterol. The oyster incorporated [2-^{14}C]acetate into squalene, 4,4'-dimethylsterols, 4-monomethylsterols, cholesterol, desmosterol, isofucosterol, and 24-methylenecholesterol, and also converted [2-^{3}H]lanosterol to cholesterol. Therefore, the oyster was concluded to synthesize cholesterol, desmosterol, isofucosterol, and 24-methylenecholesterol from acetate via squalene, probably using the lanosterol pathway.

TRANSLATIONAL MOBILITY OF GLYCOPHORIN IN BILAYER MEMBRANES OF DIMYRISTOYLPHOSPHATIDYLCHOLINE. W.L.C. Vaz, H.G. Kaptiza, J. Stümpel, E. Sackmann, and T.M. Jovin (Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, Federal Republic of Germany) *Biochemistry* 20(5): 1392-6 (1981). The translational diffusion of the integral membrane sialoglycoprotein from erythrocyte membranes, glycophorin, incorporated into bilayer membranes of dimyristoyl-phosphatidylcholine at a protein/lipid molar ratio of 1:4500 was examined by using the fluorescence redistribution after photobleaching technique. A plot of the diffusion coefficient vs. temperature shows a sharp decrease in the rate of diffusion at about 15 C. This sharp diffusion transition is at a temperature some 9 C lower than the calorimetrically measured lipid gel-liquid crystalline phase transition temperature of the system. The difference between the diffusion transition temperature and the lipid phase transition temperature is attributed to a localized fluidizing effect of the protein upon the gel phase lipid. The value of the diffusion coefficient above 15 C was found to be (1-2) $\times$ 10^{-8} cm^2s^{-1}, and below 15 C it was lower than about 5 $\times$ 10^{-11} cm^2s^{-1}. The fluorescence recovery in the bleached area as a consequence of diffusional redistribution appeared to be due to a single diffusing species at temperatures above 15 C and due to more than one diffusing species below this temperature.

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DETERGENCY POWER OF A COMPOSITION CONTAINING ENZYMES AND SODIUM PERBORATE. L. Bolinski et al. *Maslozhir. Prom.* 1980(4), 29-31. (Rev. Franc. Corps Gras)

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THE HYPERFREQUENT TECHNIQUE FOR THE MEASURING OF THE HUMIDITY OF SUNFLOWER SEEDS. G.K. Rybalko et al. *Maslozhir. Prom.* 1980(4), 19-20. (Rev. Franc. Corps Gras)

Biochemistry and nutrition

1,25-DIHYDROXYVITAMIN D IN MALE, NONSPAWNING FEMALE, AND SPAWNING FEMALE TROUT. L.V. Avioli, Y. Sonn, D. Jo, T.H. Nahn, M.R. Haussler, and J.S. Chandler (Division of Bone and Mineral Metabolism, Washington University School of Medicine and The Jewish Hospital of St. Louis, St. Louis, MO 63310) *Proc. Soc. Exp. Biol. Med. 166*(2):291-3 (1981). The concentration of circulating 1,25-dihydroxyvitamin D was measured by means of a specific radioreceptor assay in male, nonspawning female, and spawning female trout. Male trout showed serum levels of $1,25(OH)_2D$ which were comparable to those of weanling rats and infant children and higher than levels found in female trout. 1,25-dihydroxyvitamin D levels in nonspawning and spawning female trout were comparable, despite significant higher levels of circulating calcium and phosphate in the spawning trout. The study documents the existence of 1,25-dihydroxyvitamin D in the blood of teleost fish for the first time.

CHANGES IN PLASMA LIPIDS AND LIPOLYTIC ACTIVITY DURING RECOVERY FROM EXERCISE OF UNTRAINED RATS. H.A. Barakat, D.S. Kerr, E.B. Tapscott and G.L. Dohm (Biochemistry Dept. East Carolina University Medical School, Greenville, North Carolina 27834) *Proc. Soc. Exp. Biol. Med. 166*(2):162-6 (1981). The activities of lipoprotein lipase in adipose tissue and heart homogenates, and epinephrine-stimulated lipase of adipose tissue were determined at the end of an exercise bout and during recovery from exercise of untrained rats. Concurrently, triglyceride and free fatty acid concentrations of plasma were measured. Lipoprotein lipase activity in adipose tissue homogenates was depressed at the end of the exercise bout and remained depressed 24 hr later. Heart lipoprotein lipase activity was significantly elevated 24 hr after the exercise bout. Basal activity of epinephrine-stimulated lipase (without epinephrine) was significantly elevated at the end of the exercise bout, remained elevated for 6 hr, but returned to control levels 12 hr later. In the presence of epinephrine, however, the activity of the enzyme was elevated at the end of the bout and throughout the 18-hr period of recovery after the exercise bout. Plasma triglyceride concentrations tended to decrease with exercise and the subsequent periods of rest with significant differences observed for the 12- and 18-hr-rested groups. Free fatty acid levels were significantly elevated immediately after exercise but returned to control levels 6 hr after the exercise bout. These findings show that certain changes in lipid metabolism resulting from a single bout of exercise, persist for some time after termination of the exercise bout.

POSTPRANDIAL EXCHANGE OF APOLIPOPROTEIN C-III BETWEEN PLASMA LIPOPROTEINS. S.I. Barr, B.A. Kottke, and S.J.T. Mao (Atherosclerosis Research Unit, Division of Cardiovascular Diseases, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901) *Am. J. Clin. Nutr. 34*(2):191-8 (1981). Three healthy male subjects were given a high fat meal after fasting for 12 h. Blood samples were drawn at hourly intervals over 6 h. The plasma triglyceride levels reached peak values within 3 to 6 h postprandially. Plasma cholesterol concentration, however, remained constant in the three subjects as well as in a fasting normal subject. Apolipoprotein C-III (apoC-III), a major apoprotein in triglyceride-rich lipoprotein particles and known to be exchangeable between plasma high density lipoproteins (HDL) and triglyceride-rich particles, was studied in regard to its net transfer between lipoproteins during meal absorption and postabsorptive lipolysis. ApoC-III levels in total plasma as quantitated by radioimmunoassay were stable regardless of the increase of plasma triglycerides. When triglyceride levels increased after a meal, apoC-III in the d. < 1.063 lipoprotein fraction increased concomitantly, while apoC-III in HDL decreased. The converse was observed during lipolysis as plasma triglycerides fell. In any case, apoC-III levels in d. < 1.063 lipoproteins were positively correlated with plasma triglycerides ($r = 0.82$, $p < 0.01$) after the meal. The finding suggests that the apoC-III concentrations in HDL are very dynamic in vivo. ApoC-III can transfer from HDL to triglyceride-rich particles during meal absorption and can transfer from triglyceride-rich particles to HDL during postabsorptive lipolysis.

EXPRESSING DIETARY VALUES FOR FAT-SOLUBLE VITAMINS: CHANGES IN CONCEPTS AND TERMINOLOGY. J.G. Bieri and M.C. McKenna (Room 5N-102, Building 10, National Institutes of Health, Bethesda, Maryland 20205) *Am. J. Clin. Nutr. 34*(3,4):289-295 (1981). The 9th ed. of the Recommended Dietary Allowances has abandoned the use of international units for expressing the food values for vitamins A, D, and E, and has set forth expressions based on absolute weights, including vitamin K. These changes will necessitate a revision in the manner in which both experimental and applied nutritionists deal with these vitamins. In this review, the background and rationale for these changes are presented in an effort to aid investigators in their transition to this new concept. Changes in the nomenclature of vitamin E are also explained.

REDUCED INTESTINAL ABSORPTION OF VITAMIN E BY LOW DIETARY LEVELS OF RETINOIC ACID IN RATS. J.G. Bieri, A. Wu and T.J. Tolliver (Laboratory of Nutrition and Endocrinology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, MD 20205) *J. Nutr. 111*(3):458-67 (1981). It was observed that rats fed a low dietary level of retinoic acid had markedly lower plasma concentrations of α-tocopherol than did rats fed the same amount of retinol. In this report, the possible mechanism by which retinoic acid alters vitamin E metabolism has been investigated. Weanling male rats were fed a complete purified diet with either retinol or retinoic acid at 4 mg/kg diet; plasma and tissues were analyzed after 2-5 weeks. The plasma α-tocopherol concentration in rats ingesting retinoic acid was one-half that of rats ingesting retinol, and this difference also occurred in the liver and adipose tissue. Similar effects occurred in chicks. Retinoic acid did not affect the rate of decrease in endogenous α-tocopherol in normal rats fed a vitamin E-free diet for 3 weeks. In rats with mesenteric lymph cannulas, dietary retinoic acid caused a reduced absorption of ^{3}H-labeled α-tocopherol. In chicks fed retinoic acid, plasma and liver radioactivity 2.5 hours after an oral dose of ^{3}H-α-tocopherol was one-fifth that of chicks fed retinol. More oxidation of α-tocopherol occurred during absorption in rats fed retinoic acid than in those fed retinol, as evidenced by more α-tocopherylquinone in the collected lymph. We postulate that dietary retinoic acid reduces the intestinal absorption of α-tocopherol and may also

promote its oxidation.

ANTILIPIDEMIC ACTIVITY OF 4-OXO-FUNCTIONALIZED ETHYL 6-CHLOROCHROMAN-2-CARBOXYLATE ANALOGS AND A RELATED TRICYCLIC LACTONE IN THREE RAT MODELS. R.C. Cavestri, J.A. Minatelli, J.R. Baldwin, W. Loh, D.R. Feller, H.A.I. Newman, C.L. Sober and D.T. Witiak (Division of Medicinal Chemistry and Pharmacology, College of Pharmacy and the Dept. of Pathology, Division of Clinical Chemistry, College of Medicine, The Ohio State University, Columbus, OH 43210) *Lipids 16*(1):30-6 (1981). The synthesis of ethyl *cis*-6-chloro-4-hydroxychroman-2-carboxylate and 6-chloro-4-hydroxychroman-2-carboxylic acid lactone are reported. The antilipidemic properties of these compounds in 3 rat models were compared to the activity obtained for the previously synthesized related analogs ethyl 6-chlorochroman-2-carboxylate, ethyl 6-chlorochromanone-2-carboxylate and clofibrate. The biologically most interesting analog, ethyl 6-chlorochroman-2-carboxylate like clofibrate, was an effective antitriglyceridemic and anticholesterolemic agent in Triton WR-1339 hyperlipidemic rats, sucrose-fed hyperlipidemic rats and chow-fed normolipemic rats. Ethyl 6-chlorochromanone-2-carboxylate was found to be active only after 7 days of administration to sucrose-fed rats. In sucrose-fed, male Sprague-Dawley rats, the comparative effects of these analogs on various hepatic drug parameters also were carried out. Consistent with previous findings, results obtained with these compounds provide evidence showing that changes in hepatic HMG-CoA reductase activity bear no relationship to serum cholesterol lowering in the sucrose-fed model.

THE CONTRASTING EFFECTS OF A DIETARY SOYA LECITHIN PRODUCT AND CORN OIL ON LIPOPROTEIN LIPIDS IN NORMOLIPIDEMIC AND FAMILIAL HYPERCHOLESTEROLEMIC SUBJECTS. M.T. Childs, J.A. Bowlin, J.T. Ogilvie, W.R. Hazzard, and J.J. Albers (Northwest Lipid Research Clinic, Dept. of Medicine, and School of Nutritional Sciences, Univ. of Washington, Seattle, WA 98195) *Atherosclerosis 38*(1,2):217-28 (1981). The effect of 36 g per day of an oral soya lecithin product (LEC), supplementing an ad libitum diet, on the plasma lipoprotein lipid levels of 12 subjects with normolipidemia and 6 with hypercholesterolemia was evaluated and compared with the effect of 30.5 g corn oil (CO), containing polyunsaturated fatty acids (PUFA) equivalent to those in the LEC, to determine whether the PUFA content of the LEC was responsible for changes in the lipoprotein lipids. High density lipoprotein cholesterol (HDL-CH) was increased by LEC, but was not changed by CO. Low-density lipoprotein cholesterol (LDL-CH) was reduced significantly by CO and reduced to a lesser extent by LEC. Total cholesterol (T-CH) was not modified as a result of taking LEC, because of opposite HDL- and LDL-CH responses, but was reduced as a result of taking CO normolipidemics. The T-CH/HDL-CH ratio was decreased by approximately the same amount as a result of taking LEC and CO. The triglycerides were generally reduced in normolipidemics taking either supplement but were increased in hypercholesterolemics taking LEC and not changed in those taking CO. The hypercholesterolemics also increased their body weight when taking LEC. We conclude that LEC may increase HDL-CH by a mechanism independent of its PUFA content, whereas the PUFA content of both LEC and CO may alter the LDL-CH levels.

LYMPHATIC ABSORPTION OF NONVOLATILE OXIDATION PRODUCTS OF HEATED OILS IN THE RAT. N. Combe, M.J. Constantin and B. Entressangles (Unité de Biochimie–ITERG, Institut de Chimie Biologique, Universitéde Provence, Place Victor-Hugo, 13331 Marseille Cédex 3, France) *Lipids 16*(1):8-14 (1981). The lymphatic absorption of nonvolatile oxidation products (NVOP) formed during heating of fats was studied. Heated colza or soybean oils or synthetic triglycerides containing a definite aromatic or alicyclic fatty acid were fed to thoracic duct-cannulated rats. Tritium-labeled triolein was added to each dietary fat, as an internal standard, in order to calculate the percentage of lymphatic absorption of the ingested NVOP. Results show that 4% of the total polymeric acids, 53% of the total oxidized monomeric acids and 96% of the total cyclic monomeric acids were recovered in the lymphatic lipids. Gas liquid and quantitative thin layer chromatography of these 3 classes indicated that, within a NVOP class, the various constituents did not present the same absorption rate. The lymphatic absorptions of individual oxidized monomers were between 25 and 93%. Concerning the polymer fraction, the lymphatic recovereis were 2% (nonpolar dimers), 6.8% (polar dimers) and 12% (polar oligomers). Aromatic acids were absorbed to a lesser degree (50-60%) than cyclohexenic acids (91-98%).

ARTERIAL EFFECTS OF PALMITIC, LINOLEIC AND ACETOACETIC ACID. P. Constantinides and M. Kiser (Pathology Department, Louisiana State University Medical School, Shreveport, LA 71130) *Atherosclerosis 38*(3,4):309-19 (1981). The ultrastructural effects of a single brief intra-arterial infusion of palmitic, linoleic and acetoacetic acid on the arterial endothelium of the rat were investigated, and the following results obtained: (1) *Palmitic acid*, infused at a concentration of 4 mM/1, damaged the arterial lining by producing large cytoplasmic clefts and occasional blebbing and lysis of the endothelial cells. By contrast, *Linoleic acid*, infused at the same concentration, had no damaging effects on arterial endothelium. (2) *Acetoacetic acid* damaged the arterial wall when infused at concentration of 0.2 mM/1 or higher by inducing extreme swelling and loss of cristae of the mitochondria in arterial endothelium and myocytes. The above results raise the possibility that (a) high saturated fatty acid diets may promote atherosclerosis not only by inducing hypercholesterolemia but also by injuring the arterial lining, and (b) diabetes may promote atherosclerosis not only by inducing hyperlipemia but also by damaging the arterial wall during periods of uncontrolled ketoacidosis.

STIMULATION AND INHIBITION OF PGI_2 SYNTHETASE ACTIVITY BY PHOSPHOLIPIDS (PL), CHOLESTEROL ESTERS (CE), UNESTERIFIED FATTY ACIDS (UFA) AND LIPOPROTEINS (LDL AND HDL). W. Förster, J. Beitz and P. Hoffmann (Department of Pharmacology and Toxicology, Martin Luther University, Halle-Wittenberg, 402 Halle, Germany) *Artery 8*(5):494-500 (1980). We investigated the influence of different lipids on the transformation of PGH_2 to PGI_2 by the microsomal fraction of pig aorta. Phospholipids and cholesterol esters obtained from animals fed prenatally a linoleic acid diet stimulated the PGI_2 formation. However, when obtained from animals fed prenatally a linoleic acid deficient diet, phospholipids and cholesterol esters inhibited PGI_2 production. Unesterified fatty acids are potent inhibitors at 1 mM. The degree of inhibition was dependent on the length of the carbon chain and the number of double bonds in the UFA molecule. We found further that lipoproteins modified the PGI_2 formation at physiological concentrations. The amount of high density lipoprotein cholesterol had a positive correlation with the activity of PGI_2 synthetase, whereas the amount of low density lipoproteins cholesterol had a negative one.

AEROBIC EXERCISE EFFECTS ON LIPOPROTEINS AND TISSUE LIPIDS IN YOUNG PIGS. W.A. Forsythe, E.R. Miller, B. Curry and M.R. Bennink (Dept. of Food Science and Human Nutr., Nichigan St. Univ., East Lansing, MI 48824) *Atherosclerosis 38*-(3,4):327-37 (1981). Young castrated male pigs were assigned to an exercise or non-exercise group (8 pigs/group). Initial weights were 26 ± 1 kg and 28 ± 3 kg for the non-exercised and exercised groups, respectively. Following a 3-week training period, the aerobic exercise regimen consisted of running 5.3 km/h for 9 min and 4.8 km/h for 20 min on alternate days on a treadmill for 10 weeks. These levels of exercise increased heart rate to approximately 70% of maximum heart rates obtained by a pretraining maximal stress test. During the first 5 weeks of the experiment (3 weeks of training and 2 weeks of exercise) the pigs were fed a low-fat diet with no added cholesterol. For the remainder of the study, the pigs consumed a high-fat diet with added cholesterol (0.05% of diet). After 10 weeks of exercise, the exercised pigs had 16% less plasma total cholesterol and 21% less unesterified cholesterol and the percentage of cholesterol associated with high density lipoprotein (HDL) increased 33% compared to non-exercised pigs. The relative percentages of low density lipoproteins (LDL) and HDL were also affected by exercise with a 12% decrease in LDL levles and a 16% increase in HDL levels in the exercised pigs. Exercise caused plasma molar lecithin:cholesterol acyltransferase activity to decrease by 26%. Body composition and relative heart weights were similar for both groups at the end of the study. These results show that an aerobic exercise program can significantly affect plasma cholesterol and lipoprotein concentrations.

EFFECT OF REMAINING FAMILY MEMBERS ON FATNESS PREDICTION. S.M. Garn. S.M. Bailey, M.A. Solomon, and P.J. Hopkins (Center for Human Growth and Development, University of Michigan, Ann Arbor, Michigan 48109) *Am. J. Clin. Nutr. 34*(2):148-53 (1981). As shown first by stepwise multiple correlations and then by family "sets" the probability that a parent or a child will be obese is a direct function of the fatness level of remaining family members. For a four-member nuclear family the probability that one member will be obese is well below chance expectancy (i.e., 12.6%) if the remaining three members are all lean and far higher (i.e., 40.7%) if the remaining family members are all obese. While mothers follow family line fatness expectancy, there is an excess of obese mothers in lean nuclear families, consistant with the inverse relationship between adult female fatness and socioeconomic status.

THE PREVENTION OF ALCOHOLIC FATTY LIVER USING DIETARY SUPPLEMENTS: DIHYDROXYACETONE, PYRUVATE AND RIBOFLAVIN COMPARED TO ARACHIDONIC ACID IN PAIR-FED RATS. S.C. Goheen, E.E. Pearson, E.C. Larkin and G.A. Rao (Hematology Res. Lab., Veterans Admin. Med. Center, Martinez, CA 94553) *Lipids 16*(1):43-51 (1981). Male

Sprague-Dawley rats were fed for 30 days a high-fat liquid ethanol dieth with dihydroxyacetone, pyruvate and riboflavin added as supplements (AMA-). Plasma triglyceride (TG) levels were 6-fold greater in these rats than in those fed alcohol with and without the supplements (AA-). The liver TG content in rats fed the AMA- diet was similar to that of rats fed a control diet (CA-) in which alcohol was replaced with isocaloric amounts of dextrose. Livers of rats fed the AA- diet had 3 times more TG than controls. Alcohol ingestion also enhanced the hepatic content of cholesteryl estesr (CE) and phospholipid (PL). The fatty acid compositions of TG, CE and PL from livers of rats fed the AMA- diet were similar to those of corresponding lipids from rats fed the control diet (CA-) but differed from compositions when fed the alcohol diet (AA-). Regardless of the diet fed, TG had the same fatty acid composition in plasma and liver. The same was true of PL fatty acid composition. However, the fatty acid composition of CE differed between liver and plasma. Dihydroxyacetone, pyruvate and riboflavin did not prevent alcohol-induced fatty liver when 20:4 was included in the AMA- diet. Our results confirm that dietary dihydroxyacetone, pyruvate and riboflavin prevent alcohol-induced fatty liver, and show that this effect may result from increased moblilization of fat from liver.

EFFECT OF FEEDING RATS SUCROSE IN A HIGH FAT DIET. J. Hallfrisch, L. Cohen and S. Reiser (Carbohydrate Nutrition Laboratory, Beltsville Human Nutrition Research Center, Human Nutrition, Science and Education Administration, U.S. Department of Agriculture, Beltsville, MD 20705) *J. Nutr. 111*(3):531-536 (1981). Rats were fed ad libitum a 40% fat diet containing either 30% sucrose or 30% starch by weight for 8-9 weeks. Insulin levels during a meal tolerance test were significantly greater in rats fed sucrose than in rats fed starch, but serum glucose levels were not affected by diet and tended to decrease as time after the meal increased. Insulin levels during an oral glucose tolerance test were significantly greater in the rats fed sucrose. Serum glucose levels were not affected by diet. Body weights and epididymal and perirenal fat pad weights were higher in rats fed sucrose than in rats fed starch. Serum triglyceride and cholesterol levels were not different. These results show that relatively low sucrose levels in a high fat diet can produce higher insulin levels than starch before and after either a glucose load or a meal. This relative insulin resistance is symptomatic of onset diabetes.

INCORPORATION OF *CIS*-OCTADECENOIC ACIDS INTO THE RAT LIVER MITOCHONDRIAL MEMBRANE PHOSPHOLIPIDS AND ADIPOSE TISSUE TRIGLYCERIDES. C.-E. Høy and G. Hølmer (Dept. of Biochem. and Nutr., The Technical Univ. of Denmark, Building 224, 2800 Lyngby, Denmark) *Lipids 16*(2): 102-108 (1981). The incorporation of the dietary *cis* 18:1(n-12) and *cis* 18:1(n-10) into liver mitochondrial membrane phospholipids and adipose tissue triglycerides was studied in 4 groups of rats fed diets containing 10 weight percent (wt%) of fat with the following contents of octadecenoic acids: 50% *cos* 18:1(n-12) + 9% *cis* 18:1(n-9); 25% *cis* 18:1(n-12) + 32% *cis* 18:1(n-9); 50% *cis* 18:1-(n-10) + 10% *cis* 18:1(n-9); or 54% *cis* 18:1(n-9). Dietary linoleic acid was 3 wt% in all 4 groups. In the mitochondrial membranes, the isomeric octadecenoic acids were primarily incorporated into the 1-position of phosphatidylcholines and phosphatidylethanolamines at the expense of saturated fatty acids. The maximal incorporations observed in the 1-position of phosphatidylethanolamines were 4.8% 18:1(n-12) and 8.9% 18:1(n-10). No effects on the contents of polyunsaturated fatty acids in the phospholipids were seen. In the adipose tissue, the isomeric octadecenoic acids were incorporated at a level of 13% *cis* 18:1(n-12) or 23% *cis* 18:1(n-10), paralleled by a reduction in the content of oleic acid.

COMPARATIVE STUDY OF CHYLOMICRON AND FATTY ACID UTILIZATION IN SMALL INTESTINE AND HEART. W.C. Hülsmann, W.A.P. Breeman, H. Stam and W.J. Kort (Dept. of Biochemistry I and Laboratory of Esperimental Surgery, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, Netherlands) *Biochim. Biophys. Acta 663*(2):373-9 (1981). Chylomicrons were isolated from the urine of rats after a surgical procedure in which the cysterna chyli was connected with the right ureter. The fatty acids of the chylomicrons served as a respiratory substrate for rat heart and not for rat small intestine during in vitro vascular perfusions.The reason for the absence of chylomicron utilization in small intestine was found to be the virtual absence of lipoprotein lipase from this organ. Both heart and small intestine oxidized oleate complexed to albumin. Increasing the molar ratio of fatty acid to albumin from 3 to 6 did not affect the rate of fatty acid oxidation in heart, but increased fatty acic oxidation in small intestine.

FATTY ACID COMPOSITION OF HUMAN COLOSTRUM AND MATURE BREAST MILK. R.A. Gibson and G.M. Kneebone (Paediatric Unit, Flinders Medical Centre, Bedford Park, South Australia 5042, Australia) *Am. J. Clin. Nutr. 34*(3,4):252-7 (1981). The fatty acid composition of human milk obtained on individual samples from 120 mothers early (day 3 to 5) and later (day 40 to 45) in lactation were determined by argentation thin-layer and gas chromatographic procedures. In comparison with mature milk, human colostrum was characterized by a lower percentage of saturated fatty acids including medium chain length acids, a higher percentage of their long chain polyunsaturated derivatives. It is concluded that in view of their levels in breast milk, the polyenoic derivatives of linoleic and linolenic acids must be taken into account when assessing infant foods.

TYPE III HYPERLIPOPROTEINEMIA: DEFECTIVE METABOLISM OF AN ABNORMAL APOLIPOPROTEIN E. R.E. Gregg, L.A. Zech, E.J. Schaefer, and H.B. Brewer, Jr. (Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205) *Science 211*(4482):584-585 (1981). The apolipoprotein E isolated from plasma of individuals with type III hyperlipoproteinemia (HLP shows an abnormal pattern when it is examined by isoelectric focusing. Compared to apolipoprotein E from normal subjects, apolipoprotein E isolated from subjects with type III HLP had a decreased fractional catabolic rate in vivo in both type III HLP patients and normal individuals. The delayed catabolism of apolipoprotein E in type III HLP patients may be responsible for the lipid and lipoprotein abnormalities characteristic of these patients.

EFFECT OF HIGH FAT/HIGH ERUCIC ACID DIET ON PHOSPHATIDATE SYNTHESIS AND PHOSPHATIDATE PHOSPHATASE IN THE SUBCELLULAR FRACTIONS OF RAT HEART AND LIVER. K.J. Kako and S.D. Peckett (Dept. of Physiology, Faculty of Health Science, School of Medicine, University of Ottawa, Ottawa, Ontario) *Lipids* 16(1):23-9 (1981). Rats of weaning age were fed for a period of 1, 3 or 6 weeks either a control diet or a semisynthetic diet containing 20% by weight of either mustard seed oil (1/3 of the total fatty acids were comprised of erucic acid) or corn oil (2/3 of the total fatty acids consisted of linoleic acid). Mitochondrial and microsomal fractions were isolated from the hearts and livers of these rats, and the rate of acylation of *sn*-[U-^{14}C] glycerol 3-phosphate (P) was examined using palmitoyl-CoA or erucoyl-CoA as the acyl donor. In addition, activities of phosphatidate phosphatase of the mitochondrial, microsomal and soluble fractions were assayed. Studies on the acylation of glycerol 3-P with palmitoyl-CoA demonstrated that feeding of the high fat/high erucic acid diet for 1, 3 or 6 weeks significantly increased the rate of formation of monoacylglycerol 3-P by the cardiac subcellular fractions as compared to the control. These results suggest that acylation of glycerol 3-P by the mitochondria cannot be attributed to the action of the contaminating microsomal enzymes.

THROMBOXANE B_2 BIOSYNTHESIS AND PHOSPHOLIPIDS HYDROLYSIS IN PLATELETS FROM HYPERCHOLESTEROLEMIC RABBITS. H. Kawaguchi, T. Ishibashi and Y. Imai (Dept. of Biochem., School of Medicine, Hokkaido University, Sapporo 060 Japan) *Lipids 16*(1):37-42 (1981). Thromboxane B_2 biosynthesis from arachidonic acid was increased in platelets from hypercholesterolemic rabbits. The enzymic activity of phosphalipase A_2 which released arachidonic acid, the precursor for the biosynthesis of thromboxane B_2, showed hardly any change in hypercholesterolemic platelets. Phospholipase C and diglyceride lipase activities also were not changed in platelets from hypercholesterolemic rabbits. Furthermore, phospholipid concentration in platelets were not increased in this state. Thus, we conclude that the supply of precursor for thromboxane B_2 biosynthesis was not increased in platelets from hypercholesterolemic rabbits as compared to controls. These results suggest that the enzyme activity of thromboxane B_2 biosynthesis may be enhanced in platelets from hypercholesterolemic rabbits.

HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN HAEMORRHAGIC HYPERLIPIDAEMIA IN RABBITS. Y. Kertula, T.H. Weber and P. Tanner (Aurora Hospital, Minerva Institute for Medical Research, and Medix Laboratories Ltd., Helsinki, Finland) *Atherosclerosis 38*(3,4):321-6 (1981). The effects of repeated bleeding (15 ml/day/kg b.wt. on 3 consecutive days) on plasma lipids, especially on high density lipoprotein (HDL)-cholesterol were studied in rabbits. As a result of the bleeding, plasma triglycerides (TG) increased from a mean of 0.61 (SD range 0.34-1.09) mmol/L to 2.86 (1.16-7.12) mmol/L ($P < 0.02$). Plasma total cholesterol (TC) increased from 1.4 ± 0.5 S(D) mmol/L to 2.0 ± 0.5 mmol/L. The concentration of HDL-cholesterol, however, decreased from 0.72 ± 0.16 mmol/L to 0.48 ± 0.23 mmol/L ($P < 0.02$). The HDL-cholesterol/TC ratio decreased from 0.57 ± 0.17 to 0.29 ± 0.15 ($P < 0.05$). In connection with the haemorrhagic hyperlipidaemia a negative correlation ($P < 0.02$) appeared between plasma TG and

HDL-cholesterol. It is concluded that hypoxia, being the primary cause of haemorrhagic hyperlipidaemia, may be one factor regulating HDL-cholesterol concentrations and thereby possibly the development of atherosclerosis.

CHOLESTEROL VEHICLE IN EXPERIMENTAL ATHEROSCLEROSIS. PART 18. COMPARISON OF NORTH AMERICAN AFRICAN AND SOUTH AMERICAN PEANUT OILS. D. Kritchevsky, S.A. Tepper, D.A. Scott, D.M. Klurfeld, D. Vesselinovitch and R.W. Wissler (The Wister Institute of Anatomy and Biology, Philadelphia, PA 19104) *Atherosclerosis 38*(3,4):291-9 (1981). Peanut oils were obtained from the United States (NAPNO), Africa (APNO), and South America (SAPNO) and their effects on atherosclerosis in rabbits fed 2% cholesterol were determined. The major differences among the oils were in the content of oleic (NAPNO, 48.9%; APNO, 58.6%; SAPNO, 36.4%) and linoleic (NAPNO, 29.6%; APNO, 21.7%; SAPNO, 41.1%) acids. In a series of 4 experiments it was found that South American peanut oil was 7% more atherogenic than African peanut oil and 18% more atherogenic than North American peanut oil. American peanut oil was 14% more atherogenic than corn oil (CO). South American peanut oil gave highest serum and liver lipid levels. The differences in atherogenicity may be due to the structure of the triglycerides of the various peanut oils.

THE ROLE OF LIPID PEROXIDATION DURING CHRONIC AND ACUTE EXPOSURE TO ETHANOL AS DETERMINED BY PENTANE EXPIRATION IN THE RAT. R.E. Litov, D.L. Gee, J.E. Downey and A.L. Tappel (Dept. of Food Science and Technology, University of California, Davis, CA 95616) *Lipids 16*(1):52-7. Weanling rats were fed one of 3 diets containing 0, 11 or 200 international units (IU) dl-α-tocopherol acetate/kg diet for 4 weeks. Following this period, the drinking water was replaced with an 18% solution of ethanol (v/v). An isocaloric D-glucose solution was substituted for the drinking water of a control group of rats fed the vitamin-E-deficient diet for 4 weeks. The 4 treatment groups were maintained on the diet and drinking regimen for 20 weeks. Basal levels of expired pentane were determined at weeks 0, 1, 3, 5, 7 and 9. Chronic ethanol consumption did not influence basal pentane production during the 9-week treatment. Basal levels of expired pentane were affected by dietary vitamin E. Rats supplemented with vitamin E had basal pentane levels less than one-half of the level of rats fed a vitamin-E-deficient diet. After 14 weeks of treatment, the 2 groups of rats fed a vitamin-E-deficient diet were administered p.o. an acute dose of 6 g of ethanol/kg body wt. Pentane expired above basal levels during the following 4-hr period correlated with the amount of hepatic triglycerides determined at the conclusion of the experiment. The etiology of ethanol toxicity is a complex and multifactorial system made up of many biological variables that influence lipid peroxidation. The appropriate choices of experimental designs and methods are important in examining the role of lipid peroxidation.

EFFECT OF BLENDING AND LEVEL OF INCLUSION ON THE METABOLIZABLE ENERGY OF TALLOW AND TOWER RAPESEED SOAPSTOCKS. A. Jabbar Muztar, S. Leeson, and S.J. Slinger (Dept. of Nutrition and of Animal Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1) *Poultry Sci. 60*(2): 365-72 (1981). Experiments were conducted with prime tallow (PT) and Tower rapeseed soapstocks (TSS) to determine the possible synergism between these fats by the total collection and chromicoxide (Cr_2O_3) apparent metabolizable energy (AME) methods as well as the true metabolizable energy (TME) assay and to examine the effect of inclusion level on the metabolizable energy (ME) values of fats. Graded levels of 0, 3, 6, and 9% of each of PT, TSS, and a 1:1 blend of the two were from a linear regression of the ME of the diets and the levels of fat inclusion. The total collection and Cr_2O_3 methods gave essentially the same AME values for PT as well as for TSS when these fats were fed separately. The 1:1 blend of these fats gave a somewhat higher value with the index method than with the total collection procedure. A positive synergistic effect of blending PT with TSS was observed in all three methods. Increases in the observed ME of the mixture over the calculated ME were 3.94, 5.26, and 5.72%, respectively, for the total collection, Cr_2O_3, and TME assays. When calculated by difference, the ME values of the two fats and the blend varied widely with inclusion level in all three assay procedures. The implications of use of a single level assay to determine the ME content of fats are discussed.

INFLUENCE OF DIETARY UNSATURATED AND SATURATED FAT ON THE PLASMA LIPOPROTEINS OF MOGOLIAN GERBILS. R.J. Nicolosi, J.A. Marlett, A.M. Morello, S.A. Flanagan and D.M. Hegsted (Nutrition Division, Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772) *Atherosclerosis 38*(3,4):359-71 (1981). Lipid and apoprotein moieties of the plasma lipoproteins of Mongolian gerbils (*Meriones unguiculatus*) were compared in animals fed semipurified diets containing either coconut oil (COC) or safflower oil (SAF). COC-induced hypercholesterolemia was associated with elevations in very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Saturated fat feeding also resulted in the saturation of fatty acids of triglycerides and cholesterol esters of VLDL and LDL, but had little effect on HDL fatty acids. Lipoprotein composition and size were not influenced by the type of dietary fat, suggesting that the hypercholesterolemia with saturated fat feeding was due to the circulation of a greater number of lipoprotein particles. The apoproteins of gerbil lipoproteins has molecular weights comparable to other animals. The relative amounts of apoproteins, particularly the apoC peptides, increased with dietary fat saturation.

ELASTIN-LIPID INTERACTION IN THE ARTERIAL WALL. PART 2. IN VITRO BINDING OF LIPOPROTEIN-LIPIDS TO ARTERIAL ELASTIN AND THE INHIBITORY EFFECT OF HIGH DENSITY LIPOPROTEINS ON THE PROCESS. A. Noma, T. Takahashi andT. Wada (Division of Clinical Biochemistry, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan) *Atherosclerosis 38*(3,4):373-82 (1981). The mechanism of lipoprotein binding to arterial elastin, and the inhibitory effect of high density lipoprotein (HDL) on the in vitro complex formation between plasma low density lipoprotein (LDL) and elastin were studied. The binding of LDL-cholesterol, phospholipids and triacylglycerols to delipidated elastin increased progressively with time over 24 h of incubation. The results of a kinetic study on lipoprotein-cholesterol concentration in the incubation medium, suggest that the ability to bind cholesterol to elastin decreases in the following order: very low density lipoprotein (VLDL), LDL, intermediate density lipoprotein (IDL) and HDL, and that the capacities to bind to a fixed amount of elastin decrease in the order: LDL, IDL, VLDL and HDL. When a definite amount of LDL was incubated with elastin in the presence of increasing concentrations of HDL, the binding of lipids to elastin progressively decreased. On the other hand, no release of cholesterol, bound to elastin during preincubation with LDL, could be detected in additional incubations with HDL, apoHDL or apoHDL-phospholipid complex.

CLONING OF GENES INVOLVED IN MEMBRANE LIPID SYNTHESIS. EFFECTS OF AMPLIFICATION OF PHOSPHATIDYLSERINE SYNTHASE IN *ESCHERICHIA COLI*. A. Ohta, K. Waggoner, K. Louie and W. Dowhan (Department of Biochemistry and Molecular Biology, University of Texas Medical School and the University of Texas Graduate School of Biomedical Sciences, Houston, Texas 77025) *J. Biol. Chem. 256*(5):2219-25 (1981). The CDP-1,2-diacyl-*sn*-glycerol: L-serine O-phosphatidyltransferase of *Escherichia coli* catalyzes the committed step in the synthesis of the major phospholipid (phosphatidylethanolamine) of this organism. A hybrid plasmid (pLC34-44) has been isolated carrying the structural gene (*pss*) for the phosphatidylserine synthase which directs the 15- to 20-fold overproduction of the enzyme. We have now constructed several smaller derivations of pLC34-44 and characterized them with respect to restriction endonuclease digestion and the position of the *pss* gene locus. In addition we have measured the copy number of these plasmids present *in vivo* and found a direct relationship between the gene dosage and level of active gene product. The construction of a hybrid plasmid containing the structural gene carried in the vector pBR322 along with the NOP region of λC1857 has allowed the induction of phosphatidylserine synthase to levels in excess of 100-fold over that found in wild type cells. The enzyme from such high overproducing cells has been purified to near homogeneity in high yield by a new purification scheme described herein. Amplification of phosphatidylserine synthase activity by 15- to 20-fold caused an increase in the relative rate of synthesis of phosphatidylethanolamine when compared to phosphatidylglycerol *in vivo*.

DIET AND HIGH DENSITY LIPOPROTEINS. P. Oster, G. Schlierf, C.C. Heuck, S. Hahn, H. Szymanski and B. Schellenberg (Klinisches Institut fur Herzinfarktforschung an der Medizinischen Universitatsklinik, Heidelberg, Heidelberg, West Germany) *Lipids 16*(2): 93-7 (1981). The acute and subacute effects of different diets on serum high density lipoprotein (HDL) cholesterol concentration and/or HDL composition are described. In obese normolipemic, hypercholesterolemic (type II) and hypertriglyceridemic (type IV) patients, low HDL cholesterol values remained low during total starvation for 2 weeks. Prudent diets in patients with type II and IV hyperlipoproteinemia had no significant effects on HDL cholesterol values remained low during total starvation for 2 weeks. Product diets in patients with type II and IV hyperlipoproteinemia had no significant effects on HDL cholesterol within 3 weeks, whereas in normal individuals, a high carbohydrate diet given for 10 days caused significant decreases in HDL cholesterol with concommitant increases in HDL triglycerides as compared to a high-fat diet. The HDL triglyceride fatty acid composition changed in healthy volunteers during the day, depending on the type of ingested fat. The

data demonstrate the possibility of acute manipulations of HDL in normal patients and the difficulties of normalizing low HDL cholesterol levels in patients by dietary means. Additional information on the function of HDL is desirable before the therapeutic manipulation of HDL cholesterol or other constituents of HDL can be evaluated regarding their effectiveness in the prevention of ischemic vascular disease.

ELEVATION OF SERUM HDL AND HDL CHOLESTEROL IN CHOLESTEROL-FED MALE RABBITS TREATED WITH ESTROGEN. T.I. Pynadath and S. Chanapai (Kent State University, Kent, OH 44242) *Atherosclerosis 38*(3,4):255-65 (1981). The effect of cholesterol feeding and estrogen administration on serum lipoproteins and lipoprotein lipid composition was investigated in male rabbits. The lipoprotein fractions were separated by preparative ultracentrifugation and the purity of each fraction was determined by polyacrylamide disc gel electrophoresis. The amount of cholesterol, cholesterol ester, triglycerides and phospholipids was determined in each of the lipoprotein fractions. It was found that treatment of the cholesterol-fed animals with estrogen resulted in an increase of their serum HDL, HDL cholesterol, HDL phospholipids and HDL triglycerides. This increase was accompanied by a decrease in serum VLDL, VLDL cholesterol and VLDL phospholipids. Since increased HDL and HDL cholesterol have been known to be associated with lower coronary heart disease, the increased serum HDL and HDL cholesterol resulting from estrogen treatment might account for the reported lower incidence of coronary heart disease in young women than in young men and the protective effect of estrogen against diet-induced and spontaneous atherosclerosis in animals.

PUBERTY ONSET IN MALES AND FEMALES FED A HIGH FAT DIET. J.A. Ramaley (Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, NE 68105) *Proc. Soc. Exp. Biol. Med. 166*(2):294-6 (1981). Balanopreputial separation and vaginal opening and ovulation were observed in males and females as markers of sexual maturation. Animals fed a high fat diet containing the same protein content as a low fat diet showed normal pubertal onset. Both males and females fed a diet reduced in protein consisting of a mixture of dry chow and Crisco in a ratio of 2:1 showed delayed pubertal onset although their total daily caloric intake was similar to the animals fed a high fat diet with adequate protein. It is concluded that puberty onset in both males and females is sensitive to changes in dietary intake.

FENOFIBRATE THERAPY OF HYPERLIPOPROTEINAEMIA—A DOSE–RESPONSE STUDY AND A COMPARISON WITH CLOFIBRATE. S. Rossner and L. Oro (Departments of Internal Medicine, Karolinska Hospital and King Gustaf V Research Institute, Stockholm, Sweden) *Atherosclerosis 38*(3,4):273-82 (1981). Fenofibrate is an efficient serum lipid-lowering drug with few clinical side effects. The drug was further evaluated in a study comprising 56 patients, which combined a dose–response trial with a subsequent comparison between the optimal fenofibrate dose and a clofibrate dose of 2 g/day. When the fenofibrate dose was gradually increased (200–300–400 mg/day), a reduction of the elevated lipoproteins within each type of hyperlipoproteinaemia was found. During the dose–response part of the therapy a transient serum creatinine rise was observed, which disappeared at the 400 mg/day level. The highest dose, 400 mg/day, proved to have the best lipid-lowering effects. On this therapy the elevated LDL-cholesterol fell by 28% in type IIA + B patients, and the elevated VLDL-TG by 65% in type IIB + IV patients. The HDL/VLDL + LDL-cholesterol ratio increased significantly in all groups, in particular in type IV patients (from 0.19 to 0.28, $P<0.001$). Fenofibrate and clofibrate were each given for 2 months in random order, and the effects on lipoproteins compared. Significant differences were: higher HDL-cholesterol in type IIA on clofibrate, lower LDL-cholesterol in type IIB on fenofibrate, lower TG and cholesterol in both VLDL and LDL in type IV on fenofibrate, combined with higher HDL-cholesterol on this drug. Thus, fenofibrate seems to be an efficient lipid lowering drug with 400 mg/day as an optimal dosage under our conditions.

BRADYKININ AND ANGIOTENSIN II ACTIVATION OF ARACHIDONIC ACID DEACYLATION AND PROSTAGLANDIN E_2 FORMATION IN RABBIT KIDNEY. HORMONE-SENSITIVE VERSUS HORMONE-INSENSITIVE LIPID POOLS OF ARACHIDONIC ACID. M. Schwartzman, E. Liberman and A. Raz (Dept. of Biochemistry, George S. Wise Center of Life Sciences, Tel Aviv University, Tel Aviv, Israel) *J. Biol. Chem. 256*(5):2329-33 (1981). The isolated perfused rabbit kidney was used to characterize the hormone-sensitive lipid pool from which esterified arachidonic acid is hydrolyzed by the vasoactive hormone bardykinin. The kidney lipids were labeled with radioactive arachidonic acid and the specific

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activities (counts per min/ng) of the prostaglandin E_2 released with or without hormone stimulation were compared. The results indicated the following. 1) The prostaglandin E_2 fractions obtained in the absence or presence of bradykinin originate from two functionally different pools of esterified arachidonate, designated the hormone-insensitive and the hormone-sensitive pools, respectively. 2) Exogenous arachidonate is incorporated mainly into the hormone-insensitive pool. 3) The rate of arachidonate incorporation into the hormone-sensitive pool is relatively slow and is controlled by the extent to which the pool has been depleted by prior hormone stmulation. Characterization of the hormone-sensitive pool by specific labeling with radioactive arachidonic acid would thus be best achieved under conditions which induce prior release of non-radioactive acid from this pool.

CHOLESTEROL ESTERIFICATION OF MECHANICALLY INDUCED AORTIC LESIONS IN NORMOLIPEMIC PIGS. I.H. Craig, G.G. Jamieson and M. Foldes (Departments of Medicine and Surgery, University of Adelaide, South Australia 5000, Australia) *Artery* 7(5):404-418 (1980). Intimal thickenings were produced in the abdominal aortae of normolipemic pigs by longitudinal mechanical injury. In two-week lesions there was increased I-^{14}C-oleic acid incorporation into esterified cholesterol (CE), accompanied by lipid droplet formation. In twelve-week lesions, lipid droplets had largely disappeared, and the incorporation of 1-^{14}C-oleic acid into CE was similar to that of normal aorta. Increased esterification of cholesterol with oleic acid may be a protective mechanism during the early phases of the arterial repair reaction. Regression of the lesions may be associated with the return of cholesterol esterification activity to normal.

PUBLICATIONS ABSTRACTED

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