Abstracts

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Fats and oils

MICROBIAL SYNTHESIS OF 1β- AND 15β-HYDROXYLATED BILE ACIDS. K. Carlström, D.N. Kirk, and J. Sjövall (Hormone Laboratory, Department of Obstetrics and Gynecology, Huddinge University Hospital, S-141 86 Huddinge, Sweden, Department of Pure and Applied Biochemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden, Chemistry Department, Westfield College, Hampstead, London NW3 7ST, England, and Department of Physiological Chemistry, Karolinska Institutet, S-104 01 Stockhom Sweden) *J. Lipid Res. 22*:1225–1234 (1981). Lithocholic, deoxycholic, and cholic acids were incubated with the molds *Absidia coerulea* Bain (CBS 105.08, I), three strains of *Rhizoctonia solani* Kühn (CBS 130.14 (II), CBS 341.36 (III), CBS 325.47 (IV)), and *Penicillium* species ATCC 12556 (V). The products were analyzed by gas-liquid chromatography and partially characterized by gas-liquid chromatography–mass spectrometry. Lithocholic acid was most extensively hydroxylated while cholic acid yielded only traces of hydroxylated metabolites. I, II, III, and V hydroxylated at position C-1; I at C-6; I and II at C-7; III at C-12; and I, II, IV, and V at C-15. Hydroxylations at unidentified positions were observed for all molds, and oxidoreductions at C-7 and C-12 were carried out by I, II, and III. The two major trihydroxycholanoates formed by V from deoxycholic acid were characterized by chemical, mass spectrometric, and nuclear magnetic resonance techniques as 1β,3α,12α- and 3α,12α,15β-trihydroxy-5β-cholanoic acids. The former bile acid was identical with the 1,3,12-trihydroxycholanoic acid present in human urine.

TITRATION OF THE PHASE TRANSITION OF PHOSPHATIDYLSERINE BILAYER MEMBRANES: EFFECTS OF pH, SURFACE ELECTROSTATICS, ION BINDING, AND HEAD-GROUP HYDRATION. Gregor Cevc, Anthony Watts, and Derek Marsh (From the Max-Planck-Institut für biophysikalische Chemie, Abteilung Spektroskopie, D-3400 Göttingen, Federal Republic of Germany) *Biochemistry 20*:4955–4965 (1981). The dependence of the gel-to-fluid phase transition temperature of dimyristoyl- and dipalmitoylphosphatidylserine bilayers on pH, NaCl concentration, and degree of hydration has been studied with differential scanning calorimetry and with spin-labels. On protonation of the carboxyl group (pK_2^{app} = 5.5), the transition temperature increases from 36 to 44°C in the fully hydrated state of dimyristoylphosphatidylserine (from 54 to 62°C for dipalmitoylphosphatidylserine), at ionic strength J = 0.1. In addition, at least two less hydrated states, differing progressively by 1 H_2O/PS, are observed at low pH with transition temperatures of 48 and 52°C for dimyristoyl- and 65 and 68.5°C for dipalmitoylphosphatidylserine. On deprotonation of the amino group (pK_3^{app} = 11.55) the transition temperature decreases to ~15°C for dimyristoyl- and 32°C for dipalmitoylphosphatidylserine, and a pretransition is observed at ~6°C (dimyristoylphosphatidylserine) and 21.5°C (dipalmitoylphosphatidylserine), at J = 0.1. No titration of the transition is observed for the fully hydrated phosphate group down to pH ≥ 0.5, but its affinity for water binding decreases steeply at pH ≤ 2.6. Increasing the NaCl concentration from 0.1 to 2.0 M increases the transition temperature of dimyristoylphosphatidylserine by ~8°C at pH 7, by ~5°C at pH 13, and by ~0°C at pH 1. These increases are attributed to the screening of the electrostatic titration-induced shifts in transition temperature.

ASSYMMETRY OF LIPID DYNAMICS IN HUMAN ERYTHROCYTE MEMBRANES STUDIED WITH IMPERMEANT FLUOROPHORES. Uri Cogan and David Schachter (From the Department of Physiology, Columbia University College of Physicians and Surgeons, New York, New York) *Biochemistry 20*:6396–6403 (1981). The synthesis, purification, and application of five membrane-impermeant derivatives of pyrene are described. Each probe consists of a membrane-impermeant moiety, either an oligosaccharide or glutathione, linked to pyrene via a connecting arm. Intact human erythrocytes and leaky ghost membranes prepared from them were treated with the probes to label, respectively, the outer membrane leaflet and both leaflets. Motional freedom of the pyrene fluorophores in the membrane was assessed by estimation of the steady-state polarization of fluorescence, the excited-state lifetime, and the excimer/monomer fluorescence intensity ratio. The fluorescence anisotropy of each impermeant derivative was lower in the outer as compared to the inner hemileaflet, whereas the corresponding excited-state lifetimes were similar. Excimer formation was consistently greater in the outer leaflet. The results demonstrate that the impermeant fluorophores experience greater motional freedom ("fluidity") in lipid domains of the outer as compard to the inner leaflet of the human erthrocyte membrane.

POST-TRANSLATIONAL CLEAVAGE OF MUCOCYST PRECURSORS IN TETRAHYMENA. Tucker Collins and James M. Wilhelm (From the Department of Microbiology, University of Rochester Medical Center, Rochester, New York 14642) *The Journal of Biological Chemistry 256*(20):10475–10484 (1981). Pulse-chase experiments utilizing intact *Tetrahymena* revealed that at least six proteins (molecular weights, 61,000, 56,000, 51,000, 48,000, 42,000, and 38,000) were unstable and underwent proteolytic cleavage during the first 20 min of the chase period. At least 9 product polypeptides (molecular weights, 45,000, 41,000, 25,000, 21,000, 20,000, 18,000, 17,000, 16,000, and 15,000) appeared during the chase. The proposal that the product polypeptides were actually mucocyst constituents was supported by a variety of observations. First, treatment of whole cells in a complete media with dibucaine caused precursor cleavage and product accumulation with kinetics that were consistent with previous morphologic observations on mucocyst formation. Second, the product polypeptides were enriched in a cell fraction containing just cortical components and amorphous material consistent with aggregated mucus. Third, the labeled product polypeptides in the cortex comigrated with partially purified labeled mucus obtained by dibucaine treatment of whole cells. Fourth, one-dimensional peptide mapping of the 45-kilodalton product confirmed that the post-translationally derived product in whole cell pulse-chase experiments was similar to the purified products in the cortex fraction and in dibucaine-released mucus. Two-dimensional peptide mapping of the ^{125}I-labeled tryptic peptides of three pairs of products in the cortex and mucus further suggested a strong homology. The cleavage of mucocyst precursors was blocked by agents which deplete ATP levels and by *N*-tosyl-L-phenylalanyl chloromethyl ketone. Preliminary structural relationships were established between some of the precursors and products by one-dimensional peptide mapping. Models for the biogenesis of mucocysts are discussed, and it is proposed that the additional sequence information present in the precursors may be required for the intracellular transport of these proteins or their insertion and assembly within the mucocyst.

10-UNDECENOIC ACID: A VERSATILE SYNTHON FROM CASTOR OIL. V.S. Dalavoy & U.R. Nayak (National Chemical Laboratory, Poona 411008) *Journal of Scientific and Industrial Research 40*:520–528 (1981). The central theme of the present review is 10-undecenoic acid(1), an odd-carbon fatty acid, which is not found in natural oils and fats, but is produced by the pyrolysis of castor oil, an important indigenous agricultural oil of commerce. It is, therefore, necessary to consider some salient features of castor oil and its unique fatty acid, ricinoleic acid(2) (the former constitutes the industrial base for 10-undecenoic acid, while the latter is the immediate precursor for 1) before discussing the chemistry of the title compound.

MAGNETIC NONEQUIVALENCE OF THE TWO FATTY ACID CHAINS IN PHOSPHOLIPIDS OF SMALL UNILAMELLAR VESICLES AND MIXED MICELLES. Jacqueline De Bony and Edward A. Dennis (From the Department of Chemistry, University of California at San Diego, La Jolla, California 92093) *Biochemistry 20*: 5256–5260 (1981). Magnetic nonequivalence of the two acyl chains in phospholipids has been shown by ^1H NMR in several kinds of micelles [Roberts, M.F., Bothner-By, A.A., & Dennis, E.A. (1978) *Biochemistry, 17*, 935]. The chemical shifts of the two α-methylene groups have been assigned, and a difference of about 0.1 ppm between the two chains has been interpreted as a difference in the position of the two fatty acid chains relative to the interface. This conclusion has now been extended to phospholipid systems closer to biological membranes in the form of small unilamellar vesicles prepared by sonication of phospholipid dispersions. Several kinds of phospholipid vesicles are compared, including phosphatidylserine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidic acid, and phosphatidylcholine. By using suitable resolution enhancement techniques, it was possible to overcome the broad

lines in the spectra and observe the *sn*-1 and *sn*-2 α-methylene protons in most of the phospholipid vesicles. The two α-methylene protons on the *sn*-2 chain are nonequivalent, and the parameters of the AB pattern can be deduced ($\Delta\nu_{AB}$ = 0.002 ppm, J_{AB} = 17 Hz). In contrast, the α-methylene protons of the *sn*-1 chain are not magnetically distinguishable.

INTERACTION OF RHODOPSIN WITH TWO UNSATURATED PHOSPHATIDYLCHOLINES: A DEUTERIUM NUCLEAR MAGNETIC RESONANCE STUDY. Alan J. Deese, Edward A. Dratz, F.W. Dahlquist, and Michael R. Paddy (From the Division of Natural Sciences and Chemistry Board of Studies, University of California, Santa Cruz, California 95064 (A.J.D. and E.A.D.), and the Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon 94703 (M.R.P. and F.W.D.)) *Biochemistry 20*:6420–6427 (1981). Rhodopsin, prepared free of native lipid, was reconstituted with two unsaturated and specifically deuterated phosphatidylcholines: the minimally unsaturated 1-(16,16,16-trideuteriopalmitoyl)-2-palmitoleoyl-*sn*-glycero-3-phosphocholine [(CD_3-16:0)(16:1)PC] and a highly unsaturated phosphatidylcholine typical of that found in native rod outer segment (ROS) membranes, 1-(16,16,16-trideuteriopalmitoyl)-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine [(CD_3-16:0(22:6)PC]. Deuterium magnetic resonance (^{2}H NMR) spectra of these membranes and dispersions of the lipids alone were obtained at 23.0 MHz by using the quadrupolar echo technique. The apparent quadrupolar splittings are slightly less and the spectral features are somewhat broadened in the presence of the protein. Moment analyses of these spectra show that in the fluid phase the presence of rhodopsin at near physiological concentrations (1:1 w/w) does not change the average orientational order of either lipid. Rhodopsin does affect the structure of the bilayer, however, by causing an increase in the spread of the distribution of orientational order parameters about the average. Several interesting differences are observed in the phase behavior of the two lipids in the absence of rhodopsin. The orientational order of (CD_3-16:0)(16:1)PC and (CD_3-16:0)(22:6)PC is markedly different in the phase transition region, even though these two lipids have surprisingly similar phase transition temperatures. The 22:6-containing lipid exhibits a relatively large hysteresis (8–9°C) in its phase transition, while no hysteresis is observed for (CD_3-16:0)(16:1)PC. Further, the phase transition for (CD_3-16:0)(22:6)PC occurs over a much smaller temperature range than that for (CD_3-16:0)(16:1)PC. Overall, these results are consistent with our previous ^{1}H, ^{13}C, and ^{31}P NMR studies of native ROS membranes and ROS lipids: rhodopsin does not produce a long-lived, highly ordered population of lipids.

SENSORY PROPERTIES AND LIPID OXIDATION IN PRERIGOR PROCESSED FRESH PORK SAUSAGE. D.L. Drerup, M.D. Judge and E.D. Aberle (Dept. of Animal Sciences, Purdue Univ., West Lafayette, IN 47907) *J. Food Sci. 46*(6):1659–1661 (1981). Fresh pork sausage prepared from prerigor ground and salted meat had higher pH, lower cooking losses, higher juiciness scores, and less easily fragmented cooked patties than that prepared from post-rigor ground and salted meat. Sausage from prerigor ground-post-rigor salted meat was intermediate in these properties to prerigor ground and salted and postrigor ground and salted products. Prerigor grinding and salting reduced the rate of autoxidation (TBA number) during storage at 0°C contrasted to oxidation in sausage that was salted postrigor after either prerigor or postrigor grinding.

SYNTHESIS OF A SATURATED LIPID HYDROPEROXY-CYCLIC PEROXIDE. E.N. Frankel, D. Weisleder and W.E. Neff (Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S.D.A., Peoria, IL 61604) *J.C.S. Chem. Comm. 15*:766–769 (1981). A simple one-step method has been developed for the synthesis of a saturated hydroperoxyepidioxide by allowing the methanesulphonate of methyl ricinoleate to react with 90% H_2O_2 in diethyl ether; the stereochemistry of the cyclic peroxide product has been established.

DEGRADATION OF LINOLEIC ACID HYDROPEROXIDES BY A CYSTEINE · $FeCl_3$ CATALYST AS A MODEL FOR SIMILAR BIOCHEMICAL REACTIONS: III. A NOVEL PRODUCT, *trans*-12,13-EPOXY-11-OXO-*trans*-9-OCTADECENOIC ACID, FROM 13-L(*S*)-HYDROPEROXY-*cis*-9,*trans*-11-OCTADECADIENOIC ACID. H.W. Gardner and C.G. Crawford (Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604) *Biochimica et Biophysica Acta 665*:126–133 (1981). Degradation of 13-L(*S*)-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid by a cysteine · $FeCl_3$ redox couple in the presence of O_2 resulted in the formation of a number of oxygenated fatty acids, among which was a novel, optically active product, *trans*-12,13-epoxy-11-oxo-*trans*-9-octadecenoic acid. A pathway for formation of the epoxyoxoene via an epoxyhydroperoxyene intermediate is proposed.

DEGRADATION OF LINOLEIC ACID HYDROPEROXIDES BY A CYSTEINE · $FeCl_3$ CATALYST AS A MODEL FOR SIMILAR BIOCHEMICAL REACTIONS: I. STUDY OF OXYGEN REQUIREMENT, CATALYST AND EFFECT OF pH. H.W. Gardner and P.A. Jursinic (Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604) *Biochimica et Biophysica Acta 665*:100–112 (1981). 1. A redox reaction between cysteine and linoleic acid hydroperoxide was catalyzed by $FeCl_3$ at pH values under 6. This reaction was assayed by the rate of O_2 uptake, and resulted in formation of mainly oxodiene, epoxyhydroxyene and epoxyoxoene fatty acids as well as cystine and oxides of cysteine. 2. The rate of O_2 uptake was dependent upon the relative concentrations of $FeCl_3$, linoleic acid hydroperoxide and cysteine. Rate dependence on $FeCl_3$ concentration yielded a sigmoid curve, but rate dependence on concentrations of either cysteine or linoleic acid hydroperoxide was best described by a Lineweaver-Burk double reciprocal plot. The possible participation of a linoleic acid hydroperoxide-cysteine-$FeCl_3$ complex is discussed. 3. About one-half mol of O_2 was absorbed per mol of linoleic acid hydroperoxide, whereas O_2 uptake per mol of cysteine amounted to one-fourth mol. A mechanism is proposed to account for the O_2 uptake stoichiometry.

DEGRADATION OF LINOLEIC ACID HYDROPEROXIDES BY A CYSTEINE · $FeCl_3$ CATALYST AS A MODEL FOR SIMILAR BIOCHEMICAL REACTIONS: II. SPECIFICITY IN FORMATION OF FATTY ACID EPOXIDES. H.W. Gardner and R. Kleiman (Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604) *Biochimica et Biophysica Acta 665*:113–125 (1981). 1. The degradation of linoleic acid hydroperoxide by cysteine and $FeCl_3$ resulted in formation of a number of oxygenated fatty acids, among which isomeric epoxyoxooctadecenoic and epoxyhydroxyoctadecenoic acids were major products. Pure isomeric hydroperoxides, either 13-L(*S*)-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid or 9-D(*S*)-hydroperoxy-*trans*-10,*cis*-12-octadienoic acid, were transformed into either 12,13-epoxides or 9,10-epoxides, respectively. 2. From 13-L(*S*)-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid, the epoxides were identified as *trans*-12,13-epoxy-9-oxo-*trans*-10-octadecenoic acid, *trans*-12,13-epoxy-9-hydroxy-*trans*-10-octadecenoic acid, *cis*-12,13-epoxy-9-oxo-*trans*-10-octadecenoic acid, *trans*-12,13-epoxy-*erythro*-11-hydroxy-*cis*(*trans*)-9-octadecenoic acid and *trans*-12,13-epoxy-*threo*-11-hydroxy-*cis*(*trans*)-9-octadecenoic acid. 3. The 12,13-epoxides were found to be optically active, indicating that the chiral center of the 13-L(*S*)-hydroperoxy carbon was retained. 4. Although many epoxy fatty acids previously have been identified as linoleic acid hydroperoxide products, this study reports a more complete structural analysis of the various epoxides and allows an assessment of the mechanisms of their formation from hydroperoxides.

SPECIFIC TRITIUM LABELING OF GANGLIOSIDES AT THE 3-POSITION OF SPHINGOSINES. Riccardo Ghidoni, Sandro Sonnino, Massimo Masserini, Paolo Orlando, and Guido Tettamanti (Department of Biological Chemistry, The Medical School, University of Milano, 20133 Milano and Radiochemical Center, Catholic University, Roma, Italy) *J. Lipid Res. 22*:1286–1295 (1981). GM1 and GD1a gangliosides, treated with 2,3-dichloro-5,6-dicyano benzoquinone (DDQ) in the presence of Triton X-100 and in a toluene medium were specifically oxidized at the 3-position of sphingosine. Direct proof that the label was at C-3 of long chain bases was given by reoxidation with DDQ, which completely removed the label, and by ozonolysis, after which label was retained on the oligosaccharide-containing fragment. More than 99% of incorporated radioactivity was carried by the long chain bases. The radiochemical purity of labeled gangliosides was greater than 95% and the specific radioactivity was 1.25 and 1.28 Ci/m mol for [^{3}H]GM1 and [^{3}H]GDIa, respectively.

MEMBRANE PHOSPHOLIPID SYNTHESIS IN *ESCHERICHIA COLI*: PURIFICATION, RECONSTITUTION, AND CHARACTERIZATION OF *sn*-GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE. Phillip R. Green, Alfred H. Merrill, Jr., and Robert M. Bell (From the Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710) *The Journal of Biological Chemistry 256*(21)11151–11159 (1981). The membrane-bound *sn*-glycerol-3-phosphate acyltransferase of *Escherichia coli* was purified to near homogeneity from Triton X-100 extracts of membranes from strain VL3/pVL1 This strain contains a hybrid plasmid bearing the *pls*B gene, a structural gene of the enzyme, and overproduces *sn*-glycerol-3-phosphate acyltransferase activity more than 10-fold. At each stage of the purification performed in buffers containing Triton X-100, reconstitution of enzyme activity with phospholipids was necessary. Chromatography on Matrex Gel Red A, Octyl-Sepharose CL-4B, and hydroxylapatite yielded preparations containing a single band of 83,000 apparent molecular weight

upon polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Final reconstitutable specific activities in excess of 6 μmol/min/mg were achieved. Palmitoyl-CoA, oleoyl-CoA, palmitoyl-acyl carrier protein (ACP), and *cis*-vaccenoyl-ACP thioesters were substrates. The apparent K_m for *sn*-glycerol 3-phosphate was 150 μM with either palmitoyl-CoA or palmitoyl-ACP. Higher V_{max} values and lower apparent K_m values were observed for thioesters containing saturated fatty acids than were observed for thioesters containing unsaturated fatty acids. Lysophosphatidic acid was the only product produced. Assay conditions for the reconstituted enzyme were optimized. Bovine serum albumin and additional phospholipids appeared to lessen inhibition by palmitoyl-CoA. Reconstitution with *E. coli* phospholipids was complete in 10 min at 0°C. Activity depended on the amount and type of phospholipids employed. Some preference for phosphatidylglycerol and cardiolipin was observed. The *sn*-glycerol-3-phosphate acyltransferase, an integral cytoplasmic membrane protein, contained about 56% hydrophobic amino acid residues.

REDUCTION OF FATTY ACIDS TO ALCOHOLS IN ROE OF GOURAMI *(TRICHOGASTER COSBY).* Thomas W. Griffith, Donald M. Sand and Hermann Schlenk (The Hormel Institute, University of Minnesota, Austin, MN 55912) *Biochimica et Biophysica Acta 665*:34–39 (1981). Reduction of fatty acids to alcohols in gourami roe homogenates and fractions thereof was studied. The reducing activity is associated with the microsomal fraction. Activation of the acid and NADPH as reducing cofactor are required. The optimal pH for reduction is between 6.5 and 7.5. Reduction rates were highest for palmitic acid and were about half of that for oleic and linoleic acids. In contrast to the equal reduction rates of the latter acids in vitro, the percentages of oleyl and linoleyl alcohols in wax esters are greatly different in vivo. Very small amounts of aldehyde are found during the reduction and some substrate label is incorporated into the phospholipids. The traces of triacylglycerols in roe lipids are not markedly labelled. In homogenates, newly formed as well as added substrate alcohol is efficiently incorporated into wax esters. Roe homogenate is capable also of oxidizing fatty alcohol to acid. In contrast to reduction, oxidation proceeds with either $NADP^+$ or NAD^+ as cofactor. Only a small portion of the newly formed acid is esterified.

POLAR GROUP CONFORMATION OF 1,2-DI-*O*-ALKYLGLYCEROPHOSPHOCHOLINES IN THE ABSENCE AND PRESENCE OF IONS. H. Hauser, W. Guyer and F. Paltauf (Department of Biochemistry, ETH Zürich, Switzerland and Institut für Biochemie der Technischen Universität Graz, A-8010 Graz, Austria) *Chemistry and Physics of Lipids 29*:103–120 (1981). The conformation of the glycerophosphocholine (GPC) group of various 1,2-di-*O*-alkyl and 1,2-diacylglycerophosphocholines forming small micelles or single-bilayer vesicles in H_2O has been studied by NMR in the absence and presence of lanthanide ions. In the absence of lanthanides the motionally averaged polar group conformation of 1,2-di-*O*-alkylglycerophosphocholine (dialkyl-GPC) is similar to that of the diacyl compound. The replacement of the ester linkages in diacyl phosphatidylcholine by ether bonds has therefore no significant effect on the conformation and segmental motion of the glycerophosphocholine group. This conformation is found to be independent of the state of aggregation, i.e., the main features are the same below and above the critical micellar concentration (CMC). The determining factor must therefore be the intramolecular energetics. Within the experimental accuracy the conformation of dialkyl-GPC in the presence of lanthanide ions is also the same as that of the corresponding diacyl compound.

A NEW METHOD FOR THE DETRITYLATION OF 1,2-DIRADYL-3-*O*-TRITYLGLYCEROLS. A. Hermetter and F. Paltauf (Institut für Biochemie und Lebensmittelchemie, Technisch Universität Graz, Schlögelgasse 9, A-8100 Graz, Austria) *Chem. Phys. Lipids 29*(2): 191–195 (1981). The 3-*O*-trityl derivatives of 1,2-diacylglycerols, 1-*O*-alkyl-2-acylglycerols and 1,2-di-*O*-alkylglycerols are detritylated with boron trifluoride-methanol in methylene chloride. The reaction is complete within 30 min at 0°C. Acyl migration does not occur.

PERMEABILITY PROPERTIES OF UNILAMELLAR VESICLES CONTAINING CHOLINE PLASMALOGENS AND COMPARISON WITH OTHER CHOLINE GLYCEROPHOSPHOLIPID SPECIES. A. Hermetter and F. Paltauf (Institut für Biochemie und Lebensmittelchemie, Technische Universität Graz, A-8010 Graz, Austria) *Chem. Phys. Lipids 29*(3):225–233 (1981). The rates of non-electrolyte and ion diffusion across bilayer membranes consisting of choline plasmalogens or of their alkyl and acyl analogs were studied. The influx of [^{14}C] glucose, $^{86}Rb^+$ and $^{36}Cl^-$ into small unilamellar vesicles made from a semisynthetic choline plasmalogen and from synthetic diacyl, alkylacyl and dialkyl analogs with comparable side chain compositions were measured. Rates of glucose and Rb^+ diffusion are about equal in alkenylacyl- and diacyl-glycerophosphocholine (GPC) bilayers, but are reduced in dialkyl-GPC membranes; the permeability coefficients correlate with the packing densities of the respective choline glycerophospholipids in monolayers at the air water interface. Rates of chloride diffusion are consistently higher in membranes formed from phospholipids containing alkenyl or alkyl ether bonds as compared to the diacyl analogs. The phospholipid side chain composition markedly affects glucose and Rb^+ diffusion. Incorporation of cholesterol (30 mol%) into choline plasmalogen membranes reduces their solute permeability by approximately 70%. Thus, the choline phospholipid-cholesterol interaction, as far as it is reflected in reduced bilayer permeability, is not influenced by the presence of the alkenylether bond of plasmalogens.

IDENTIFICATION OF β-D-MANNOSYLCERAMIDE IN HEPATOPANCREAS OF THE FRESH-WATER BIVALVE, *HYRIOPSIS SCHLEGELII.* Taro Hori, Mutsumi Sugita and Hiromi Shimizu (Department of Chemistry, Faculty of Liberal Arts and Education, Shiga University, Otsu, Shiga 520, Japan) *Biochimica et Biophysica Acta 665*:170–173 (1981). A mannosylceramide was isolated by preparative thin-layer chromatography on a 3% borate-impregnated silica gel plate from a monohexosylceramide fraction of the hepatopancreas of the fresh-water bivalve, *Hyriopsis schlegelii*. It contained only mannose as the sugar component and the ceramide moiety contained mainly sphingosine and palmitic acid. Anomeric configuration of the sugar moiety was determined by enzymatic hydrolysis with β-D-mannosidase. The concentration of this glycolipid was 5% of the total monohexosylceramide fraction of the hepatopancreas.

A 2-PHASE LIQUID SCINTILLATION ASSAY FOR GLYCOLIPID SYNTHETASES. A.V. Hospattankar and N.S. Radin (Mental Health Research Institute, Dept. of Psychiatry and Dept. of Biol. Chem., Univ. of Michigan, Ann Arbor, MI 48109) *Lipids 16*(10):764–766 (1981). Glycolipid synthetases can be assayed conveniently by incubating the lipid substrate with the radiosugar-labeled nucleotide in a small plastic scintillation vial. At the end of the incubation period, water and perchloric acid are added, then *n*-butanol, then a toluene-based scintillation cocktail. The radioactive lipid partitions into the scintillation fluid, leaving excess sugar nucleotide in the aqueous phase. Only a small fraction of the total radioactivity in the aqueous layer is detectable. This method is illustrated for ceramide:UDP-glucose glucosyltransferase. The approach should be applicable to other lipid synthetases that can be assayed with a radioactive hydrophilic substrate.

COMPOSITION OF THE ESSENTIAL OIL OF SOILING DENT CORN. T. Kami (Faculty of Applied Biological Science, Hiroshima University, Fukuyama 720, Hiroshima, Japan) *J. Agric. Food Chem. 29*(9–10):909–911 (1981). The essential oil of soiling dent corn was isolated by steam distillation of the fresh grass with a yield of 0.0031%. The essential oil was analyzed by combined gas chromatography-mass spectrometry and gas chromatographic comparison with authentic specimens, and 90 compounds consisting of hydrocarbons, aldehydes, ketones, alcohols, esters, acids, phenols, and miscellaneous were identified. Quantitative analysis was further carried out on the essential oil, and soiling dent corn oil was relatively rich in hydrocarbons and aldehydes.

SYNTHESIS AND CHARACTERIZATION OF A NOVEL CHOLESTEROL NITROXIDE SPIN LABEL: APPLICATION TO THE MOLECULAR ORGANIZATION OF HUMAN HIGH DENSITY LIPOPROTEIN. J.F.W. Keana, T. Tamura, D.A. McMillen, and P.C. Jost (Dept. of Chem. and Instit. of Molecular Biology, Univ. of Oregon, Eugene, Oregon 97403) *J. Am. Chem. Soc. 103*(16):4904–4912 (1981). The purpose of this study was to synthesize a cholesterol nitroxide that closely mimics cholesterol in its physical and biological properties and to utilize this molecule to probe the nature of cholesterol-protein interactions in human high density lipoprotein particles. The rigidly labeled cholesterol nitroxide (Δ^5-3β-hydroxy steroid) was prepared by addition of isohexylmagnesium bromide to nitrone. Nitroxide was also converted into its oleate ester and tritiated analogue (sp act., 1:6 Ci/mMol). Nitroxide both served as a substrate for cholesterol oxidase and also entered into the lecithin-cholesterol acyl transferase reaction, albeit with an efficiency less than that of cholesterol itself. The extent of hydrolysis of nitroxide oleate by cholesterol esterase was about the same as that of cholesterol oleate, suggesting that toward this enzyme, nitroxide behaved like cholesterol. Cholesterol nitroxide was readily incorporated into human high density lipoproteins. Analysis of the ESR line shape showed the presence of cholesterol-lipid and cholesterol-protein contacts in HDL_3. These results are in contrast to the conclusion of other investigators, namely, that cholesterol is excluded from the immediate vicinity of a membrane protein that penetrates through cholesterol-containing phospholipid bilayers.

ALKALINE HYDROLYSIS OF PHOSPHOLIPIDS IN MODEL MEMBRANES AND THE DEPENDENCE ON THEIR STATE OF AGGREGATION. Charlotte Read Kensil and Edward A. Dennis (From the Department of Chemistry, University of California at San Diego, La Jolla, California 92093) *Biochemistry 20*:6079–6085 (1981). The rate of alkaline hydrolysis of phospholipids in different model membranes was studied as a probe of the phospholipid conformation and packing and for a better understanding of the effect of phospholipid aggregation on hydrolysis by phospholipase A_2. The products of hydroxide attack on phosphatidylcholine were free fatty acids and glycerophosphorylcholine, with lysophosphatidylcholine as an intermediate. The kinetics of phospholipid hydrolysis could be analyzed as a pseudo-first-order reaction by having the hydroxide concentration in large excess. Egg phosphatidylcholine dispersed in Triton X-100 mixed micelles at a mole ratio of 8:1 detergent:phospholipid was hydrolyzed with a second-order rate constant of 14.7×10^{-3} s^{-1} M^{-1}. Egg phosphatidylcholine dispersed in single bilayer vesicles and multibilayers was hydrolyzed at rates 7–11- and 3-fold lower, respectively. The calculation of these rates had to take into account the extent of inaccessibility of the inner layers to hydroxide which was measured by the fluorescence of a pH-sensitive probe (pyranine) trapped inside the vesicles and multibilayers. The monomeric phospholipid dihexanoylphosphatidylcholine was hydrolyzed at a rate 7-fold higher than when this lipid was incorporated into Triton X-100 micelles. An Arrhenius plot of the hydrolysis of dipalmitoylphosphatidylcholine vesicles indicated that the phospholipid phase transition exerted a small but detectable effect on the rate of hydrolysis. As with phospholipase A_2, hydroxide-catalyzed hydrolysis rates depend critically on the aggregation state of the phospholipid.

SYNTHESIS OF (22*R* AND 22*S*)-3α,7α,22-TRIHYDROXY-5β-CHOLAN-24-OIC ACIDS AND STRUCTURE OF HAEMULCHOLIC ACID, A UNIQUE BILE ACID ISOLATED FROM FISH BILE. Kenji Kihira, Yukari Morioka, and Takahiko Hoshita (Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima, Japan) *J. Lipid Res. 22*:1181–1187 (1981). (22*R* and 22*S*)-3α,7α,22-trihydroxy-5β-cholan-24-oic acids were synthesized, starting from chenodeoxycholic acid, in order to establish the chemical structure of haemulcholic acid, which has been found in certain fish as the major bile component. Oxidative decarboxylation of diformoxylated chenodeoxycholic acid with lead tetraacetate yielded 24-nor-5β-chol-22-ene-3α,7α-diol, which was hydroxylated to form a mixture of (22*R* and 22*S*)-24-nor-5β-cholane-3α,7α,22,23-tetrols. Lead tetraacetate oxidation of the mixture yielded 3α,7α-dihydroxy-23,24-dinor-5β-cholan-22-a1. A Reformatsky reaction of the dihydroxydinorcholanal with bromoacetate resulted in the formation of a mixture of (22*R* and 22*S*)-3α,7α,22-trihydroxy-5β-cholan-24-oic acids. The bile acids epimeric at C-22 were resolved by silica gel column chromatography, and their configurations at C-22 were assigned by a modification of Horeau's method and ^{13}C-nuclear magnetic resonance spectroscopy. By direct comparison with synthetic bile acids, the naturally occurring haemulcholic acid was shown to be (22*S*)-3α,7α,22-trihydroxy-5β-cholan-24-oic acid.

CHOLESTEROL CONTENT OF BEEF BONE MARROW AND MECHANICALLY DEBONED MEAT. J.E. Kunsman, M.A. Collins, R.A. Field and G.J. Miller (Animal Science Div., Univ. of Wyoming, Leramie, WY 82701) *J. Food Science 46*(6):1785–1788 (1981). Marrow from cervical, lumbar and femur bones of 5 steers and 5 cows fed only on native range (grass fed) and 5 steers and 5 cows fed a finishing ratio (grain fed) was analyzed for cholesterol content. The cholesterol content of the marrow was significantly different when diet or anatomical locations were compared. Bovine marrow from grass-fed animals averaged 119.6 mg/100g and marrow from grain-fed animals averaged 150.6 mg/100g marrow. The cholesterol content of marrow from the cervical, lumbar, and femur was 190.1, 124.1 and 91.0 mg/100g marrow, respectively, Mechanically deboned meat (MDM) and beef lean had a mean cholesterol content of 153.3 and 50.9 mg/100g tissue. Spinal cord material in MDM can account for the increased concentration of cholesterol in some MDM samples over the values for lean and marrow.

CALORIMETRIC INVESTIGATIONS OF SATURATED MIXED-CHAIN PHOSPHATIDYLCHOLINE BILAYER DISPERSIONS. Jeffrey T. Mason, Ching-hsien Huang, and Rodney L. Biltonen (From the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22908) *Biochemistry 20*:6086–6092 (1981). A series of saturated mixed-chain phosphatidylcholines were prepared whose *sn*-2 acyl chains are two, four, six, and eight carbon atoms shorter than the *sn*-1 acyl chain. The calorimetric behavior of multilamellar bilayers of these phosphatidylcholines in excess water is investigated. The phosphatidylcholines display cooperative phase transitions which are dependent upon both the difference in chain length and the position of the acyl chains on the glycerol backbone of the phospholipid. A model is proposed which suggests that the thermotropic behavior of the mixed-chain phosphatidylcholines results from progressively greater interdigitation of the acyl chains of the phospholipid across the bilayer center, in the gel state, as the chain-length difference is increased beyond a minimum value. The disruptive effect of the terminal methyl groups of the fatty acyl chains upon the bilayer packing stability is also stressed. Dispersions of some of the mixed-chain phosphatidylcholines display transition endotherms which appear to be composites of two or more individual transition peaks. The dependence of this behavior on the thermal history of the dispersions is investigated. It is proposed that these peaks arise from the ability of the phosphatidylcholines' acyl chains to pack in more than one interdigitated conformation in the gel state.

RAMAN AND FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDIES OF THE INTERACTION BETWEEN GLYCOPHORIN AND DIMYRISTOYLPHOSPHATIDYLCHOLINE. R. Mendelsohn, R. Dluhy, T. Taraschi, D.G. Cameron, and H.H. Mantsch (Department of Chemistry, Olson Laboratories, Neward, New Jersey 07102) *Biochemistry 20*(23):6699–6706 (1981). Glycophorin from the human erythrocyte membrane has been isolated in pure form and reconstituted into large unilamellar vesicles with 1,2-dimyristoyl-3-*sn*-phosphatidylcholine at lipid/protein mole ratios ranging from 50:1 to 200:1. The effect of protein on the phospholipid phase transition has been monitored by Raman and Fourier transform infrared spectroscopy and differential scanning calorimetry. No evidence for an immobilized higher melting lipid component is observed. The gel to liquid-crystalline phase transition is significantly broadened and shiften to lower temperatures as the proportion of protein is increased, while the pretransition is abolished. At all temperatures, the mobility of the acyl chains is increased by the addition of protein while interchain lateral interactions are disrupted. However, there is no evidence for a significant change in the conformational order at low temperatures (~5°C) or in the liquid-crystalline phase.

CHIROPTICAL PROPERTIES OF *S*-ALKYL DERIVATIVES OF 1-THIOGLYCEROL. P. Michelsen, B. Hersløf, and B. Akesson (Division of Organic Chemistry 1, Chemical Center, University of Lund, Research Laboratory, AB Karlshamns Oljefabriker, Karlshamn and Dept. of Clinical Chemistry, University Hospital, Lund, Sweden) *Chem. Phys. Lipids 29*(2):177–189 (1981). Several racemic and optically active *S*-alkyl derivatives of 1-thioglycerol have been synthesized. From 3-*S*-tetradecyl-3-thio-*sn*-glycerol was converted into the corresponding sulphoxide and sulphone. The chiroptical properties of these compounds were investigated by ORD and CD. Such measurements can be used in studies on the metabolism of enantiomeric acylglycerols.

ELECTRON NUCLEAR DOUBLE RESONANCE OF RADICALS PRODUCED BY THE PbO_2 OXIDATION OF α-TOCOPHEROL AND ITS MODEL COMPOUND IN SOLUTION. K. Mukai, N. Tsuzuki, K. Ishizu, S. Ouchi, and K. Fukuzawa (Dept. of Chemistry, Faculty of Science, Ehime University, Matsuyama 790 and Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi-1, Tokushima 770, Japan) *Chem. Phys. Lipids 29*(2):129–135 (1981). The electron nuclear double resonance (ENDOR) spectra of chromanoxyl radicals obtained by the PbO_2 oxidation of α-tocopherol and its model compound were observed in *t*-butylbenzene, and the proton hyperfine coupling constants were correctly determined. Each of the two β- and γ-methylene protons in the chromanoxyl ring shows an equivalent hyperfine splitting, suggesting that the heterocyclic ring attached to the aromatic ring are coplanar with the plane of the aromatic system. A comparison of the hyperfine couplings in α-tocopheroxyl radical and its model shows that the introduction of a long-isoprenoid-chain in the α-tocopheroxyl in place of a methyl group in the model compound has very little effect on the unpaired spin distribution or molecular structure of the chromanoxyl skeleton. The results of McLachlan molecular orbital (MO) calculations were found to be in satisfactory agreement with the 'experimental' spin densities evaluated from the hyperfine coupling constants.

A FACILE METHOD FOR THE PREPARATION OF 1-*O*-ALKYL-2-*O*-ACETOYL-*SN*-GLYCERO-3-PHOSPHOCHOLINES (PLATELET ACTIVATING FACTOR). T. Muramatsu, N. Totani, and H.K. Mangold (Federal Center for Lipid Research, Institute for Biochemistry and Technology—H.P. Kaufmann-Institute, Piusallee, D-4400 Münster (Federal Republic of Germany) *Chem. Phys. Lipids 29*(2):121–127 (1981). Biologically active 1-*O*-alkyl-2-*O*-acetoyl-*sn*-glycero-3-phosphocholines are prepared in good yields from ratfish *(Hydrolagus colliei)* liver oil, an inexpensive natural product, that contains over 70% neutral ether lipids.

ON THE THEORY OF DOMAIN FORMATION IN MIXED LIPID SYSTEMS. A.G. Petrov and H. Frischleder (AG Molekül-NMR, Sektion Physik der Karl-Marx-Universität DDR-7010 Leipzig, Linnéstr. 5 German Democratic Republic) *Chem. Phys. Lipids 29* (2):165–176 (1981). A theory of formation of one-component, circular domains in mixed lipid monolayers was developed using the language of the thermodynamics of micellar formation and the notion of the wedge-like molecular asymmetry, developed earlier. Some justifications concerning the bilayer case were also done. After suitable approximations essentially two geometrical parameters of the aggregated molecules—head group equilibrium area and hydrophobic part equilibrium area—were sufficient to discuss a number of properties of the aggregation process such as critical aggregation concentration, life times, curvature, size and size distribution of the domains. The influence of the boundary energy on the aggregation behaviour was demonstrated and the cases of partial and total demixing above the critical concentration were distinguished. The theoretical results were compared with experimental data.

KINETICS AND MECHANISM OF ASSOCIATION OF HUMAN PLASMA APOLIPOPROTEINS WITH DIMYRISTOYLPHOSPHATIDYCHOLINE: EFFECT OF PROTEIN STRUCTURE AND LIPID CLUSTERS ON REACTION RATES. H.J. Pownall, Q. Pao, D. Hickson, J.T. Sparrow, S.K. Kusserow, and J.B. Massey (Dept. of Medicine, Baylor College of Medicine, Houston, TX) *Biochemistry 20*(23):6630–6635 (1981). We have used a series of lipid-associating proteins with similar pI's and with molecular weights between 2280 and 28000 to study the mechanism of lipid-protein association. All of these polypeptides spontaneously associate with dimyristoylphosphatidylcholine (DMPC) to give quasi-discrete products. The reaction of the apoproteins with unsaturated lecithins is slow, if it occurs at all. Our data support the Kanehisa-Tsong cluster model of lipid permeability in many of its qualitative aspects. These are (a) that the rate of lipid-protein association increases with decreasing polypeptide molecular weight, (b) that there is a small temperature dependence for the rate of association of small peptides with DMPC but with large polypeptides the temperature at which association with lipid is rapid is confined to the solid → fluid transition temperature (T_C) of DMPC, and (c) that the rate is asymmetric about T_C, with the change in the rate with respect to temperature below T_C being greater than at $T > T_C$. In addition, we have shown that unfolded monomeric proteins with a large number of exposed hydrophobic residues associate with DMPC faster than self-associated and/or folded proteins. Our data suggest that the association of some of the apoproteins with phospholipids is subject to kinetic control.

CHROMATOGRAPHY OF ACIDIC PHOSPHOLIPIDS ON IMMOBILIZED NEOMYCIN. F.B. St.C. Palmer (Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7) *J. Lipid Res. 22*:1296–1300 (1981). Columns of immobilized neomycin (reductively coupled to porous glass beads) were used to separate weakly acidic lipids as well as the polyphosphoinositides. All anionic lipids present in chloroform–methanol extracts, which had been washed first with acid and then with neutral salt solutions, were adsorbed. Phosphatidylserine and phosphatidic acid were eluted with chloroform–methanol–formic acid mixtures. Phosphatidylinositol and cardiolipin were eluted sequentially by very low concentrations (10–100 mM) of ammonium formate in chloroform–methanol–water. All three phosphoinositides were

Index to Advertisers

isolated from washed chloroform–methanol–KCl extracts of brain. Sulfatides eluted with the phosphatidylinositol. Phosphatidylinositol phosphate and phosphatidylinositol bisphosphate were eluted in turn by higher salt concentrations (0.4–1 M). The immobilized neomycin was used repeatedly but the capacity eventually declined. This loss was reversed by sodium borohydride reduction.

RAMAN SCATTERING IN BILAYERS OF SATURATED PHOSPHATIDYLCHOLINES AND CHOLESTEROL: EXPERIMENT AND THEORY. D.A. Pink, T.J. Green, and D. Chapman (Theoretical Physics Institute, St. Francis Xavier University, Antigonish, Nova Scotia, Canada B2G 1CO) *Biochemistry 20*(23):6692–6698 (1981). Raman spectroscopy has been applied to a model biomembrane structure in order to obtain information about the effect of cholesterol upon phospholipid hydrocarbon chain ordering. The intensity of the 1130-cm^{-1} Raman line obtained from a dipalmitoylphosphatidylcholine (DPPC) coarse aqueous dispersion was measured as a function of temperature for two concentrations, c, of cholesterol: c=0.15 and c=0.35. The contribution of cholesterol to this line was deduced. Intensities of all lines were taken as peak areas. By use of a theory for assigning Raman intensities to chain conformations as well as a model of lipid bilayers containing cholesterol, the temperature and concentration dependence of the 1130-cm^{-1} line was calculated. Good agreement with DPPC experimental data was obtained, and predictions are made for dimyristoylphosphatidylcholine. The experimental results are interpreted in terms of a DPPC-cholesterol phase diagram and the average number of gauche bonds per DPPC molecule.

X-RAY DIFFRACTION AND CALORIMETRIC STUDY OF ANHYDROUS AND HYDRATED *N*-PALMITOYLGALACTOSYLSPHINGOSINE (CEREBROSIDE). M.J. Ruocco, D. Atkinson, D.M. Small, R.P. Skarjune, E. Oldfield, and G.G. Shipley (From the Biophysics Institute, Departments of Medicine and Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118 (M.J.R., D.A., D.M.S., and G.G.S.), and the School of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 (R.S. and E.O.)) *Biochemistry 20*:5957–5966 (1981). Differential scanning calorimetry and X-ray diffraction of anhydrous and hydrated *N*-palmitoylgalactosylsphingosine (NPGS) show evidence of complex polymorphic behavior and interconversions between stable and metastable structural forms. Anhydrous NPGS exhibits three lamellar crystal forms (A, B, and B′) at temperatures below 143°C and a liquid-crystal form between 143 and 180°C before melting to an isotropic liquid at 180°C. The crystal B → liquid-crystal transition is accompanied by an enthalpy change, ΔH, of 11.2 kcal/mol of NPGS, while a relatively small enthalpy change (ΔH = 0.8 kcal/mol) marks the liquid-crystal → liquid transition.

PUBLICATIONS ABSTRACTED

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