

From Washington (Continued from page 724A)

Mixtures of two or more of the basic products could be labeled "vegetable protein product," or "plant protein product" with specific ingredients identified in the mandatory ingredient statement on the package.

Consumer-oriented products designed to resemble and to substitute for meat, seafood, poultry, eggs, or cheese must match one of six nutritional profiles developed by FDA for specific types of products. Products failing to match the FDA profiles would have to be labeled "imitation." Products meeting nutritional profiles would be labeled "vegetable (plant) protein product," with optional addition of "textured" or "texturized" as well as a physical description term.

Thus an artificially flavored product that is a substitute for ham could be identified as "artificially ham-flavored vegetable protein product," the FDA said.

Products made up of traditional foods and vegetable proteins also are covered. A fish chowder in which vegetable protein substitutes completely for the fish could be labeled "chowder made with artificially fish-flavored vegetable protein product," the FDA said. A macaroni-and-cheese-type product in which vegetable proteins are partially substituted for cheese could be termed, "macaroni casserole, made with cheese and vegetable protein product." Specific types of vegetable protein used would be listed in the ingredient declaration.

The nutritional profiles spell out vitamin and mineral requirements in each of six product categories. FDA said it was "immaterial" whether these ingredients are added by the vegetable protein manufacturer or the final food fabricator, as long as the consumer product meets the profiles. If final food fabricators add the ingredients, the protein producers will have fewer products to make, label, and keep in inventory. If final guidelines say producers have to add the vitamins and minerals, it will mean a sixfold increase in number of products handled.

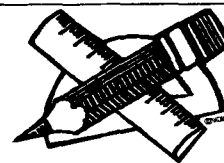
Details: *Federal Register*, Friday, July 14, 1978, pp. 30471-30491.

The Interagency Testing Committee of the Environmental Protection Agency published its final rules on health and safety study reporting regulations on July 18, 1978. The list of substances covered remains the same as proposed, but there have been some changes in definitions of terms and some changes in scope of the regulation. For example, the types of studies required to be reported now include all studies of any toxicities, not just the five categories originally specified. To cut down on duplicate submissions, the regulation now says manufacturers, processors, or distributors only need to submit reports done by or for them for substances actually manufactured, processed, or distributed by them. Studies already submitted to federal agencies or indexed in specified abstract services need not be submitted. The new regulation took effect Aug. 17, 1978. Details: *Federal Register*, Tuesday, July 18, 1978, p. 30984.

The EPA has published final rules for tolerances in food and on raw agricultural commodities for the insecticide O-ethyl O-(4-(methylthio)phenyl) S-propyl phosphorodithioate. The rule sets a tolerance of one part per million in cottonseed oil and cottonseed hulls, 0.5 part per million in raw cottonseed; 0.01 part per million on meat, fat, and meat by-products of cattle, goats, hogs, horses, poultry, and sheep; and 0.001 parts per million in eggs and milk. Details: *Federal Register*, Tuesday, July 25, 1978, pp. 32129, 32133.

The FDA has proposed removing gum guaiac from the list of direct human food ingredients that are considered safe. The FDA said it has not been able to find any evidence that the substance, at one time used in edible fats and oils as a preservative, is now being used in food products. Comments on the proposal will be accepted until Sept. 26, 1978. Details: *Federal Register*, Friday, July 28, 1978, p. 32819. The proposal would not affect the substance's use as an antioxidant in food packaging materials or in resinous and polymeric coatings.

Abstracts



EDITOR: S. KORITALA • ABSTRACTORS: J.C. Harris, M.G. Kokatnur, F.A. Kummerow, G. List, B. Matijasevic, K.D. Mukherjee, D.B.S. Min, R.A. Reiners, and P.Y. Vigneron

• Drying Oils and Paints

TUNG OIL SUBSTITUTE FOR PRINTING INK. O. Nitidandhaprabhas, *Am. Inkmaker* 55(9), 39 (2 pp) (1977). The properties of oil extracted from the fruit nuts of *Parinari anamense* Hance are briefly discussed. The oil contains a significant amount of elaeostearic acid (three conjugated double bonds), making it suitable for curing by UV irradiation or air drying, and may be converted into an alkali-sol. adduct by cooking 100 pts. oil with 10-20 pts. fumaric acid at 200° C. for 1 hr. under nitrogen. The temp. is then raised to 210° C. for 30 mins, then reduced to 180° C. for 30 mins. An intaglio ink formulation using this adduct is suggested. The ink may be wiped from printing plates by a mixture comprising 1% each of caustic soda and sulphonated castor oil. (World Surface Coatings Abs. No. 432)

CASTOR OIL MARKET. L.J. Jubanowsky, *Am. Paint J. Conv. Daily* 62(16), 20 (2 pp) (1977). Market trends over the last 5 years are briefly reviewed, mainly in terms of U.S. imports from Brazil (the principal supplier) and some other countries. (World Surface Coatings Abs. No. 432)

INKS BASED ON INDIAN SARDINE OIL. P.C. Chatterjee, *Am.*

Ink 55(11), 34 (2 pp) (1977). The properties of inks based on varnishes prepared by cooking rosin-modified phenolic resins with sardine oil are examined, it being concluded that the inks show commercial promise provided care is taken to control odours during preparation. (World Surface Coatings Abs. No. 432)

MISCIBILITY OF RESINS AND WAXES. J. Verseau, *Coating* 10(12), 343-4 (1977). The miscibility of various classes of natural and synthetic resins and waxes, e.g. for use in different types of coating compositions, printing inks, insulating compositions, paper coating compositions, heat-sealing compositions, etc, are indicated. (World Surface Coatings Abs. No. 432)

UTILISATION OF SOME NON-TRADITIONAL OILS IN SURFACE COATINGS. S.B. Dabhade and B.B. Gogte, *Paint India* 27(6), 17-21 (1977). A review is made of the use of unexploited non-edible oils in paints, including neem oil, nahor oil, walnut oil, gokharu oil, tobacco oil and kamala oil. (World Surface Coatings Abs. No. 430)

REVIEW OF 1977: OILS AND OILSEEDS. Anon. *Polym. Paint Col. J.* 167(3964/5), 1020-2 (1977). Raw material price trends in 1976 and 1977 for linseed oil, soyabean oil, etc, are

shown. At the end of 1977, the price of soyabean oil had fallen to that at the end of 1976, while the price of linseed oil had fallen well below its 1976 level. (World Surface Coatings Abs. No. 430)

• Edible Proteins

EFFECT OF SOY PROTEIN FLAKES AND ADDED WATER ON MICROBIAL GROWTH (TOTAL COUNTS, COLIFORMS, PROTEOLYTICS, STAPHYLOCOCCI) AND RANCIDITY IN FRESH GROUND BEEF. S.G. Thompson *et al.*, *J. Food Sci.* 43, 289-91 (1978). Ground beef and soy-beef mixtures were stored at 3°C, and microbiological enumerations and thiobarbituric acid (TBA) measurements conducted to investigate the effect of soy protein concentrate on ground beef shelf life. Soy-beef formulations have lower oxidation rates than the ground beef mixtures as indicated by lower thiobarbituric acid (TBA) values. At the end of 6 storage days, the soy-beef formulations had higher numbers of staphylococci, coliforms, proteolytics, and total organisms, but this was usually not statistically significant. The ground beef and tap water mixtures consistently gave the lowest counts of all enumerations.

• Fats and Oils

STEROL CONTENT OF FOODS OF PLANT ORIGIN. J.L. Weihrauch and J.M. Gardner, *J. Am. Diet. Assoc.* 73, 39 (1978). Available data on phytosterols from the world's literature have been compiled and summarized. Our compilation shows that plant oils are excellent sources of phytosterols. Nuts and seeds contain moderate levels, and fruits and vegetables generally contain the lowest concentrations of plant sterols. Analyses of the minor sterols, namely the Δ^5 - and Δ^7 -phytosterols, have become available only recently.

MASS FRAGMENTOGRAPHIC DETERMINATION DOCOSENOIC ACID IN RAPESEED OILS. R. Blomstrand *et al.*, *Lipids* 13, 283-8 (1978). A highly sensitive and accurate reference method for determination of docosenoic acid (mainly erucic acid, 22:1n-9) in different rapeseed oils is described. A fixed amount of [^{14}C] erucic acid methyl ester (about 1 μg) is added to a fixed amount of oil. After treatment with sodium methoxide/methanol reagent and extraction with hexane, the amount of unlabeled erucic acid is determined from the ratio between the recordings at m/e 320 and m/e 322 obtained after analysis with a combined gas chromatograph-mass spectrometer equipped with a MID (multiple ion detector). The two ions used correspond to the M-32 peak in the mass spectrum of unlabeled and [^{14}C] labeled erucic acid methyl ester. The relative standard deviation of the method is about 1.8%. The method was compared with a gas chromatographic method for determination of erucic acid.

FORMATION OF VOLATILE FLAVOR COMPOUNDS IN GREEN BEANS FROM LINOLEIC AND LINOLENIC ACIDS. B.O. de Lumen *et al.*, *J. Food Sci.* 43, 698-702 (1978). Both green beans and seeds formed n-hexanol, n-hexanal and 1-octen-3-ol as the principal volatile compounds from U- ^{14}C -labeled linoleic acid but at proportionately different ratios. With U- ^{14}C -labeled linolenic acid, green beans developed mainly *trans*-2-hexenal, 1-penten-3-ol, 3-penten-1-ol, *trans*-2-hexenol and *cis*-3-hexenol, while seeds produced largely 1-penten-3-ol and 3-penten-3-ol with a small amount of *trans*-2-hexenal. Green beans showed the highest lipoxygenase activity of the several fruits and vegetables compared. Though alcohol oxidoreductase was relatively low, rapid reduction of aldehydes/ketones to alcohols was found in green beans. Green bean lipoxygenase was inhibited by cyanide and a small fraction appeared to be quite heat stable, compared to alcohol oxidoreductase which was much more unstable. The optimal activity of green bean lipoxygenase was found to be at pH 5.8.

OCCURRENCE OF NOVEL BRANCHED-CHAIN FATTY ACIDS IN REFSUM'S DISEASE. J.T. Dulaney *et al.*, *Biochim. Biophys. Acta* 529, 1-12 (1978). Two novel branched-chain fatty acids, which appear to be unsaturated analogs of phytanic acid, have been observed in sera and urine of patients with Refsum's disease. They occur in both phospholipids and neutral lipids, and have been isolated and characterized.

ANALYSIS OF CAROTENOID AND PORPHYRIN PIGMENTS OF GEO-CHEMICAL INTEREST BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. S.K. Hajibrahim, *et al.*, *Anal. Chem.* 50, 549-53

(1978). High-performance liquid chromatography (HPLC) is shown to be a powerful tool in the analysis of carotenoid and porphyrin pigments. Columns packed with 5- μm irregular silica gel particles by a high density and high constant pressure method allow efficient separation of mixtures of total nonsaponifiable carotenoids from recent sedimentary situations. Good reproducibility of retention times (within 2%) is achieved in the gradient elution mode. However, attention must be paid to reequilibration of the column after each injection by washing with the less polar solvent for a minimum of 15 min (for carotenoids) or of 30 min (for porphyrins). HPLC appears to be useful in "fingerprinting" petroporphyrin distributions in crude oil.

INDIVIDUAL LIPIDS AND PROXIMATE ANALYSIS OF VARIOUS FOODS. 2. FRANKFURTERS AND OTHER MEAT AND POULTRY PRODUCTS. D.R. Newkirk *et al.*, *J. Agric. Food Chem.* 26, 348-50 (1978). Samples of all-beef-pork, and chicken frankfurters as well as various other meat and poultry products were purchased from several area supermarkets. The samples were analyzed for water, total fat, fatty acids, protein, ash, and sterols. Cholesterol values ranged from 7 to 100 mg/100 g of product. The fat content of the products varied from 2 to 30 g/100 g of product. All of the product were compared with respect to proximate analysis and sterol and fatty acid content.

MAGNETIC NONEQUIVALENCE WITHIN THE FATTY ACYL CHAINS OF PHOSPHOLIPIDS IN MEMBRANE MODELS: ^1H NUCLEAR MAGNETIC RESONANCE STUDIES OF THE α -METHYLENE GROUPS. M.F. Roberts *et al.*, *Biochemistry* 17, 935-42 (1978). The existence of a large chemical shift difference between the α -methylene groups of the two fatty acyl chains of phospholipids in Triton X-100/phospholipid mixed micelles has been demonstrated using ^1H NMR. This difference between the two α -methylene groups in the Triton mixed micelle system (0.09 ppm) is now compared with that observed for synthetic short-chain phospholipids which exist as monomers (0.03 ppm for dihexanoylphosphatidylcholine) and those which form micelles (0.09 ppm for dioctanoylphosphatidylcholine).

STEROID TOTAL SYNTHESIS. 11. (+)-ESTR-4-ENE-3,17-DIONE FROM A CHIRAL LACTONE. M. Rosenberger *et al.*, *J. Org. Chem.* 43, 1550-5 (1978). Optically pure (+)-estr-4-ene-3,17-dione and (-)-estra-4,9-diene-3,17-dione have been synthesized from the prochiral 5,9-diketoheptanoic acid via the lactone 11. The selective microbiological reduction of 10 produced optically pure 11, which was converted to the masked Mannich base 16 and subsequently condensed with 2-methylcyclopentane-1,3-dione to give predominantly the *trans* diene 17. This key intermediate was then transformed into (+)-estr-4-ene-3,17-dione via 24 and also to (-)-estra-4,9-diene-3,17-dione by the cyclization of the polyketone 20.

ALTERATIONS IN THE ULTRAVIOLET ABSORPTION SPECTRA OF STEROIDS UPON BINDING TO SERUM PROTEINS. S.D. Stroupe and U. Westphal, *Biochemistry* 17, 882-7 (1978). Difference spectra of progesterone-binding globulin (PBG) complexes with progesterone and testosterone were measured. The contributions of steroid and protein to the difference spectra were resolved by use of 5 α -pregnane-3,20-dione and dihydrotestosterone to compensate for the perturbation of PBG. The absorption spectra of seven bound steroids all showed increased extinction coefficient, sharpened absorption bands, a small blue shift, and an increased area implying an enhanced transition moment. This is in contrast to the steroid complexes with low affinity binders, human serum albumin, and α_2 -acid glycoprotein, which exhibit decreased extinction coefficients and reduced transition moments.

A CONVENIENT SYNTHESIS OF 3-KETO BILE ACIDS BY SELECTIVE OXIDATION OF BILE ACIDS WITH SILVER CARBONATE-CELITE. K. Tserng, *J. Lipid Res.* 19, 501-4 (1978). A number of 3-keto bile acids were synthesized by the selective oxidation of bile acid methyl esters with silver carbonate-Celite in refluxing toluene. The pure 3-keto bile acids were isolated simply by filtering the reaction mixture and concentrating the filtrate. The relation of the bile acid structure to the oxidation rate is also discussed.

PREPARATION OF 24(R)- AND 24(S)-5 β -CHOLESTANE-3 α ,7 α ,24-TRIOLS AND 25(R)- AND 25(S)-5 β -CHOLESTANE-3 α ,7 α ,26-TRIOLS BY A HYDROBORATION PROCEDURE. B. Dayal *et al.*, *J. Lipid Res.* 19, 191-5 (1978). This report describes a new and

convenient method for the preparation of 5β -cholestane- $3\alpha,7\alpha,24$ -triol (24R and 24S) and 5β -cholestane- $3\alpha,7\alpha,26$ -triol (25R and 25S) starting from 5β -cholestane- $3\alpha,7\alpha,25$ -triol. In each case the bile alcohols epimeric at C-24 and C-25 were resolved by analytical and preparative thin-layer chromatography and characterized by gas-liquid chromatography, infrared, proton magnetic resonance, and mass spectrometry. These epimeric bile alcohols will be useful for biological studies of chenodeoxycholic acid biosynthesis.

SYNTHESIS OF SOME UNSATURATED LACTONES AND THEIR RELATIONSHIP TO DEEP-FAT FRIED FLAVOR. W.A. May *et al.*, *J. Food Sci.* 43, 1248-52 (1978). Seven unsaturated lactones, viz., the lactones of 4-hydroxy-nonenic acid, with the double bond at the 2 and 3 position, respectively, 4-hydroxy-octenoic acid with the double bond at the 2 and 3 position, respectively, 5-hydroxy-4-nonenic acid, 5-hydroxy-2-octenoic acid, and 5-hydroxy-2-undecenoic acid, were synthesized. Their chemical structures were confirmed by their infrared and mass spectra. Organoleptic evaluation indicated that γ -lactones with unsaturation at the 2 or 3 positions imparted a characteristic deep-fat fried flavor to cottonseed oil when added at 2.5 ppm. Furthermore, γ -lactones with unsaturation at the 3 position could improve the flavor of margarine and snack foods.

DETERMINATION OF DOUBLE BOND POSITIONS OF UNSATURATED FATTY ACIDS BY A CHEMICAL IONIZATION MASS SPECTROMETRY COMPUTER SYSTEM. T. Murata *et al.*, *J. Lipid Res.* 19, 172-6 (1978). After stereospecific oxidation, trimethylsilylated methyl esters of mono- and diunsaturated fatty acids were analyzed by combined gas-liquid chromatography-chemical ionization mass spectrometry. The positions of original double bonds were deduced from the fragment ions produced by the cleavage of the carbon-carbon bond between two trimethylsilyl ethers. The diastereoisomers of diunsaturated fatty acids may also be distinguished from each other by comparing the intensities of the fragment ions formed by the loss of trimethylsilyl function from the characteristic ions.

LIPID DISTRIBUTIONS IN GREEN LEAF PROTEIN CONCENTRATES FROM FOUR TROPICAL LEAVES. S. Nagy *et al.*, *J. Agric. Food Chem.* 26, 701-6 (1978). Protein concentrates were prepared from the green leaves of four tropical plants: chaya, sorghum x sudan, cassava, and saurpous; and the lipid classes, sterols, and fatty acids of those concentrates were studied. About three-fourths of the green protein lipids were neutral lipids, one-fifth to one-fourth were glycolipids, and less than one-twentieth were phospholipids. After saponification of the total lipids about one-third were fatty acids, less than one-third were nonsaponifiables, and about one-third were "residuals" not extracted by hexane. Sterols were identified as cholesterol, stigmasterol, campesterol, β -sitosterol, and isofucosterol. The presence of acylated galactosyl lipids indicated the presence of glycolipid-hydrolyzing and acyl-transferring enzymes in the expressed leaf juices.

HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF AUTOXIDIZED LIPIDS: I. METHYL OLEATE AND LINOLEATE. W.E. Neff *et al.*, *Lipids* 13, 415-21 (1978). Autoxidized methyl oleate and linoleate were reduced with NaBH_4 and fractionated with a preparative high-pressure liquid chromatography (HPLC) reverse phase column. Products characterized from reduced-oxidized oleate included monohydroxy- and dihydroxyoctadecenoates, dihydroxy- and epoxyoctadecenoates. Products characterized from reduced-oxidized linoleate included hydroxy-*cis,trans*- and *trans,trans*-octadecadienoates, monohydroxy-, dihydroxy-, trihydroxy-, epoxyhydroxy-, and epoxyoctadecenoates. Quantitation of oxidation products by HPLC was in agreement with gas chromatography of trimethylsilyl ether derivative. Epoxyoctadecenoate in oleate and epoxy- and epoxyhydroxyoctadecenoates in linoleate were the most abundant secondary oxidation products. Some mechanisms are discussed to explain formation of these secondary products.

SYNTHESIS OF LECITHIN ANALOGUES BY MEANS OF CYCLIC ENEDIOL PHOSPHATES. DERIVATIVES OF 1-OCTADECANOL AND OF CHOLESTEROL. F. Ramirez *et al.*, *J. Org. Chem.* 43(12), 2331-4 (1978). Alkylphosphorylcholines have been synthesized as analogues of the natural phospholipid lecithin (phosphatidylcholine). The hydrolysis is performed in aqueous acetonitrile, in the presence of triethylamine, and gives the alkylphosphorylcholine zwitterion as a crystalline monohydrate after silica gel chromatography. A second method of synthesis reverses the sequence in which the choline chloride and

the lipophilic alcohols are phosphorylated and affords the same alkylphosphorylcholines but in lower yields than the first method.

TOTAL SYNTHESIS OF STEREOSPECIFIC SPHINGOSINE AND CERAMIDE. Y. Shoyama *et al.*, *J. Lipid Res.* 19, 250-9 (1978). A small-scale synthesis of the four sphingosine stereoisomers (D-erythro, L-erythro, D-threo, and L-threo) and lignoceroyl D- and L-erythro-sphingosines, which is suitable for synthesis of tritium-labeled compounds, is described. The conversion of the doubly labeled ceramide to 3-keto derivative is also described.

EVALUATION OF POLY S-179 AS A STATIONARY PHASE FOR THE GAS-LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY OF BILE ACID METHYL ESTER ACETATES. P.A. Szczepanik *et al.*, *J. Lipid Res.* 19, 280-3 (1978). The stationary phase Poly S-179 has been found to offer distinct advantages over the previously reported SP-525 for the gas-liquid chromatographic separation of bile acid methyl ester acetates. Relative retention times of these bile acid derivatives are compared on the two phases.

PROXIMATE ANALYSIS, FATTY ACID AND AMINO ACID COMPOSITION OF NIGELLA SATIVA L. SEEDS. V.K. Babayan *et al.*, *J. Food Sci.* 43, 1314-5 (1978). Proximate analysis of *Nigella sativa* L. seeds showed a composition of 21% protein, 35.5% fat, 5.5% moisture, 3.7% ash and the rest being total carbohydrate. Fatty acid analysis of the extracted oil was determined using gas-liquid chromatography; it showed 56% linoleic acid, 24.6% oleic acid, 12% palmitic acid, 3% stearic acid, 2.5% eicosadienoic acid, 0.7% linolenic acid, and 0.16% myristic acid. Traces of few unidentified fatty acids were also found. Amino acid analysis of the seed protein hydrolysate by gas chromatography of the n-propyl, N-acetyl derivatives showed the presence of 15 amino acids including 9 essential amino acids. Given the significant content of fat, protein and minerals in the seeds, it should be investigated as a source of these nutrients and its potential applications in flavoring various types of food.

• Biochemistry and Nutrition

STIMULATION OF ESTERIFIED CHOLESTEROL ACCUMULATION IN TISSUE CULTURE CELLS EXPOSED TO HIGH DENSITY LIPOPROTEINS ENRICHED IN FREE CHOLESTEROL. G.H. Rothblat, L.Y. Arbo-bast, and E.K. Ray (Dept. of Physiol./Biochem., The Med. College of Pennsylvania, 3300 Henry Ave., Philadelphia, PA) *J. Lipid Res.* 19, 350-8 (1978). Human high density lipoprotein enriched in free cholesterol was obtained by exposing the lipoprotein to lipid dispersions having a free cholesterol/lecithin molar ratio greater than two. The metabolism of cholesterol was studied in tissue culture cells exposed to normal and cholesterol-enriched lipoproteins. Incubation of Fu-5-AH rat hepatoma cells in medium containing cholesterol-enriched lipoprotein resulted in the accumulation of cellular cholesterol whereas normal high density lipoprotein produced no change in cellular content. The data indicate that the lipid composition of a lipoprotein can regulate free cholesterol uptake and esterification as well as cellular cholesterol content.

IDENTIFICATION OF THE SULFOLIPIDS IN THE NON-PHOTOSYNTHETIC DIATOM NITZSCHIA ALBA. R. Anderson *et al.*, *Biochim. Biophys. Acta* 528, 89-106 (1978). The four major sulfolipids in the non-photosynthetic marine diatom, *Nitzschia alba*, were isolated in pure form and their structures were established spectrometrically and by identification of their hydrolysis products as (a) 24-methylene cholesterol sulfate, (b) 1-deoxyceramide-1-sulfonate, (c) phosphatidyl sulfocholine (a sulfonium analogue of phosphatidylcholine) and (d) sulfoquinovosyl diglyceride. The major characteristic fatty acids of the sulfolipids were: for the deoxyceramide sulfonate, 16:0 (26%) and 16:1- Δ^3 -*trans* (64%); for the sulfonium analogue, 14:0 (30%), 18:1 (12%), 18:2 (two species, respectively), 14:0 (9%, 22%), 16:0 (16%, 28%), 18:1 (8%, 22%), 20:5 (42%, 23%) and 22:6 (14%, 2%). Traces of lyso-derivatives of sulfoquinovosyl diglyceride and phosphatidyl sulfocholine were also detected. The deoxyceramide sulfonate and the phosphatidyl sulfocholine represent novel membrane lipid components not previously detected in other organisms.

They may however have a widespread distribution in marine diatoms and perhaps in marine organisms generally.

5-(¹²⁵I) IODONAPHTHYL AZIDE, A REAGENT TO DETERMINE THE PENETRATION OF PROTEINS INTO THE LIPID BILAYER OF BIOLOGICAL MEMBRANES. T. Bercovici and C. Gitler, *Biochemistry* 17, 1484-9 (1978). 5-(¹²⁵I) Iodonaphthyl 1-azide is shown to be a useful reagent for the determination of the extent of penetration of proteins into the lipid bilayer of biological membranes. The label can readily be made highly radioactive and stored for reasonable times or repurified and then used. In the dark it has a high partition coefficient into membrane lipids. It has a high extinction coefficient for the light-mediated conversion of the azide into the reactive nitrene. It can be activated by short periods of light at wavelengths which membrane proteins and lipids do not absorb so that their radiation damage is minimal. The light-generated nitrene inserts covalently with very high efficiencies into the membrane components.

INTERACTION OF AN AMINE OXIDE DETERGENT WITH LECITHIN VESICLES AS STUDIED BY NUCLEAR MAGNETIC RESONANCE. K. Beyer and M. Klingenberg, *Biochemistry* 17, 1424-30 (1978). The interaction of an amine oxide detergent with single bilayer lecithin vesicles was investigated with proton and phosphorus magnetic resonance. The addition of the detergent micelles to vesicle suspensions leads to rapid detergent incorporation into the vesicle bilayer, resulting in a heterogeneous vesicle population. Initially, some vesicles take up the equivalent of one detergent micelle, whereas others contain no detergent. Subsequently, the detergent is distributed between the vesicles by vesicle-vesicle collisions. Measurements of proton spin-lattice relaxation times confirmed that the internal architecture of the vesicle bilayer is almost unaffected by the incorporated detergent.

DETERMINATION OF CONJUGATED BILE ACIDS IN HUMAN BILE AND DUODENAL FLUID BY REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. C.A. Bloeh and J.B. Watkins, *J. Lipid Res.* 19, 510-3 (1978). A simple method using reverse-phase liquid chromatography is presented for resolution and quantitation of the major conjugated bile acids of man, including the glycine and taurine conjugates of the dihydroxy bile acids, chenodeoxycholic and deoxycholic acid. This provides a sensitivity sufficient for analysis of dilute duodenal and gallbladder bile with minimal sample preparation.

CHARGE-PULSE RELAXATION STUDIES WITH LIPID BILAYER MEMBRANES MODIFIED BY ALAMETHICIN. G. Bohem and R. Benz, *Biochim. Biophys. Acta* 507, 262-70 (1978). Charge-pulse relaxation studies with the alamethicin-lipid membrane system reveal a triphasic decay of membrane voltage. At short times (resolution time 2 μ s), where a voltage decay due to the orientation of alamethicin dipoles from the interface into the membrane interior ("gating current") could possibly be expected, only a slow decrease with a time constant determined by the bare membrane conductance occurs. After approximately 1 ms (depending on the experimental conditions) the formation of alamethicin pores starts, leading to an increase in the voltage decay rate. When the characteristic voltage V_c^* is approached, pores close and after passing V_c^* is determined as a function of the initially applied voltage V_a , alamethicin and KCl concentration. Since the membrane voltage decreases continuously, the system does not reach the equilibrium states obtained at constant voltages. Taking the presented experimental results into account the estimate of the electrical potential at the functional membrane of photosynthesis induced by a saturating single turnover flash of $\Delta\Phi$, \approx 105-135 mV (Zickler, Witt and Bohem (1976) FEBS lett. 66, 142-148) is changed to $\Delta\Phi$, \approx 200 mV.

POLYMORPHIC PHASE BEHAVIOUR OF LIPID MIXTURES AS DETECTED BY ³¹P NMR. EVIDENCE THAT CHOLESTEROL MAY DESTABILIZE BILAYER STRUCTURE IN MEMBRANE SYSTEMS CONTAINING PHOSPHATIDYLETHANOLAMINE. P.R. Cullis and B. De Kruijff, *Biochim. Biophys. Acta* 507, 207-18 (1978). ³¹P NMR is a useful analytical technique for the study of the polymorphic phase behaviour of hydrated phospholipids in excess water. Such possibilities arise due to lateral diffusion of phospholipids which, in non-bilayer phases, produce additional motional averaging mechanisms. This results in distinctive ³¹P NMR spectra for lipids in the bilayer phase, hexagonal (H_{II}) phase, or phases such as the inverted micellar, cubic, or rhombic. These results are discussed with regard to the observation that biological membranes contain-

ing high concentrations of phosphatidylethanolamine contain little or no cholesterol. It is suggested that cholesterol may be excluded because its presence would disrupt bilayer structure. It is proposed that the bilayer structure of lipids in biomembranes may be in dynamic equilibrium with other available phases. In the light of such possibilities a mechanism of "flip-flop" phenomena in biological membranes is suggested.

STRUCTURAL EFFECTS OF MYELIN PROTEOLIPID APOPROTEIN ON PHOSPHOLIPIDS: A RAMAN SPECTROSCOPIC STUDY. W. Curatolo *et al.*, *Biochemistry* 17, 1802-7 (1978). Raman spectroscopy indicates that the myelin proteolipid apoprotein (PLA) perturbs the configuration and thermal behavior of the acyl chains of phospholipids. Spectral data in the 1000-1150-cm⁻¹ (C-C stretching) region for a dimyristoyllecithin (DML)/PLA recombinant indicate that, below the lipid order-disorder transition, the number of DML acyl chain trans conformers is reduced in the presence of PLA. Above the transition, the DML acyl chains are prevented by PLA from completely attaining the extensive gauche conformation.

¹³C NMR STUDIES ON [4-¹³C] CHOLESTEROL INCORPORATED IN SONICATED PHOSPHATIDYLCHOLINE VESICLES. B. De Kruijff, *Biochim. Biophys. Acta* 506, 173-82 (1978). 90.5 MHz ¹³C NMR linewidth measurements were performed on mixed sonicated [4-¹³C] cholesterol/phosphatidylcholine vesicles of different fatty acid composition. From the Dy³⁺-induced shift of the C₄ resonance of cholesterol it suggested that this part of the molecule is localized in the ester bond region of the bilayer. The local motion of the cholesterol ring system is restricted and independent of fatty acid composition. At cholesterol concentrations below 30 mol% the ring system becomes more immobilised when the fatty acids of the phosphatidylcholine molecules enter the gel state.

EFFECT OF ICED STORAGE ON FREE FATTY ACID PRODUCTION AND LIPID OXIDATION IN MULLET MUSCLE. J.C. Deng, *J. Food Sci.* 43, 337-40 (1978). The influence of two periods of iced storage followed by up to 12 months frozen storage on development of free fatty acid and oxidative rancidity was studied. Mullet held in the round for 1 day and 7 days iced storage were processed into various forms of mullet flesh: mullet in the round, boneless skin-on and skin-off fillets with and without antioxidant treatment and were then stored at -18°C. Generally, higher free fatty acid levels and less lipid oxidation (TBA and peroxide value) were observed in frozen mullet fillets (with and without skin) with a longer period of iced storage, except for the frozen skin-on fillets which showed no significant difference in oxidation between the two periods of iced storage. However, frozen, antioxidant-treated (immersed in 0.25% TBHQ + 2% ascorbic acid) fillets with the longer period of iced storage had less free fatty acid production than fillets with the shorter period of iced storage. Both free fatty acid and oxidative rancidity were higher in the frozen sample with 7 days iced storage than the samples with 1 day iced storage.

THE RELATIVE CONTRIBUTION OF PROPIONATE, AND LONG-CHAIN EVEN-NUMBERED FATTY ACIDS TO THE PRODUCTION ON LONG-CHAIN ODD-NUMBERED FATTY ACIDS IN RUMEN BACTERIA. B. Emmanuel, *Biochim. Biophys. Acta* 528, 239-46 (1978). The relative contribution of propionate, and long-chain even-numbered fatty acids to the production of long-chain odd-numbered fatty acids in six pure cultures of rumen bacteria were studied, using single and double isotope procedures. Both propionate and even-numbered fatty acids were converted to odd-numbered fatty acids, presumably by elongation of 2-C atoms and α -oxidation reactions, but even-numbered fatty acids were much more effective. The occurrence of α -oxidation was further confirmed by relatively large quantities of ¹⁴CO₂ produced when (1-¹⁴C) palmitate was used in the incubation medium. The incorporation of propionate into odd-numbered fatty acids was markedly reduced in the presence of palmitate, or stearate. The significance of the operation of α -oxidation is discussed in relation to the energy conservation in rumen bacteria.

OCCURRENCE OF SULFATED 5 α -CHOLANOATES IN RAT BILE. H. Eriksson *et al.*, *J. Lipid Res.* 19, 177-86 (1978). Bile acids in bile from male and female rats with cannulated bile ducts have been analyzed by repetitive scanning gas-liquid chromatography-mass spectrometry after initial fractionation of conjugate classes on diethylaminohydroxypropyl Sephadex LH-20. Sex differences were observed in the amounts and types

of bile acids in the sulfate fraction. The proportion of total bile acids excreted as sulfates was higher in female (0.9–1.3%) than in male (0.1–0.2%) rats. Most of the sulfated bile acids had a 5 α configuration, allochenodeoxycholic acid being the major compound in bile from female rats. This bile acid was also present in the nonsulfate fraction but could not be found in bile from male rats. The results indicate that gas-liquid chromatography-mass spectrometry has to be used to provide sufficient specificity in the bile acid analyses. Thus, compounds from the sulfate fraction having the retention times of cholic and chenodeoxycholic acid derivatives were found to be due to derivatives of the 3 β ,5 α -isomers of these bile acids.

THE EFFECT OF THE LIPID COMPOSITION ON THE PARTITION OF LIPOSOMES IN AQUEOUS TWO-PHASE SYSTEMS. E. Eriksson and P. Albertsson, *Biochim. Biophys. Acta* 507, 425–32 (1978). Liposomes have been partitioned in aqueous two-phase systems consisting of water, dextran, poly(ethylene glycol), salt, and buffer. Liposomes were used as a model system in order to determine the contribution of the lipids on the partition of membrane particles. The liposomes were composed of phospholipids with different polar head groups and different degrees of unsaturation. The role of cholesterol was also investigated. The polar head group of the phospholipid plays a dominant role in determining the partition of liposomes, while the degree of unsaturation is of less importance, thereby indicating that partition in two-phase systems is a surface dependent method. Incorporation of cholesterol in liposomes reduces differences in partition between liposomes of various composition.

THE PICOMOLE DETERMINATION OF FREE AND TOTAL CHOLESTEROL IN CELLS IN CULTURE. J.G. Heider and R.L. Boyett, *J. Lipid Res.* 19, 514–8 (1978). An enzymatic, fluorometric method for the determination of free and total cholesterol in cells in culture is presented. The method is simple, requiring one reagent that includes all of the enzymes and a second reagent that increases the pH, which enhances the fluorescence of the product. It is rapid, in that free cholesterol can be read in 5 minutes and total cholesterol after 20 minutes. The precision of the method is greater than that obtained from gas-liquid chromatography.

STEROL SYNTHESIS: CHEMICAL SYNTHESSES, SPECTRAL PROPERTIES, AND METABOLISM OF 5 α -CHOLEST-8(14-EN-3 β ,15 β -DIOL AND 5 α -CHOLEST-8(14)-EN-3 β ,15 α -DIOL. S. Huntton *et al.*, *J. Biol. Chem.* 253, 775–82 (1978). 5 α -Cholest-8(14)-en-3 β ,15 α -diol and 5 α -cholest-8(14)-en-3 β ,15 β -diol were prepared by lithium aluminum hydride reduction of 3 β -benzoyloxy-5 α -cholest-8(14)-en-15-one. 3 β -Benzoyloxy-5 α -cholest-8(14)-en-15 β -ol was prepared by sodium borohydride reduction of 3 β -benzoyloxy-5 α -cholest-8(14)-en-15-one. The absolute configurations of the 15-hydroxyl functions were previously established by x-ray crystallographic analysis of the 3 β -p-bromobenzoate derivative of 5 α -cholest-8(14)-en-3 β ,15 β -diol. In the present work the utility of chromatographic, mass spectral, optical rotation, and nuclear magnetic resonance spectral data to determine the absolute configuration of the 15-hydroxyl functions was explored. The results presented herein indicate that the use of empirical approaches involving chromatography or mass spectrometry were not valid in the case under consideration. The use of nuclear magnetic resonance and optical rotation studies, as described herein, appear to provide very simple approaches to the determination of configuration of $\Delta^{14,15}$ -hydroxysterols.

A ^1H NMR STUDY OF THE EFFECTS OF METAL IONS, CHOLESTEROL, AND N-ALKANES ON PHASE TRANSITIONS IN THE INNER AND OUTER MONOLAYERS OF PHOSPHOLIPID VESICULAR MEMBRANES. G.R. Hunt and L.R.H. Tipping, *Biochim. Biophys. Acta* 507, 242–61 (1978). A novel method is described which allows study of the effect of various asymmetries across a phospholipid bilayer. High resolution, ^1H NMR spectra of dipalmitoyl phosphatidylcholine single-bilayer vesicles are obtained at various temperatures in the presence of the lanthanide ions Pr $^{3+}$ and Gd $^{3+}$. The spectra are used to detect separately the phase transitions which occur in each monolayer of the bilayer. Dipalmitoyl phosphatidylcholine vesicles containing 0–50 mol% cholesterol are studied using the same techniques and the effect of increasing concentration of cholesterol on each monolayer is observed. The data obtained indicate that even for a symmetrical distribution of cholesterol its presence can reverse the effect

of a metal ion concentration gradient on the relative fluidity of the two monolayers. At 15 mol% cholesterol the bilayer is very susceptible to lysis at temperatures near the phase transition.

A NEW CHROMATOGRAPHIC APPROACH TO THE RESOLUTION OF INDIVIDUAL GANGLIOSIDES. GANGLIOSIDE MAPPING. M. Iwamori and Y. Nagai, *Biochim. Biophys. Acta* 528, 257–67 (1978). Anion-exchange column chromatographies on DEAE-Sephadex, DEAE-Sephacrose and QAE-Sephadex were tested for fractionation of ganglioside-molecular species. DEAE-Sephacrose gave the best resolution, with good separation of mono-, di-, tri-, and even tetrasialogangliosides. Even minor gangliosides could be resolved and detected by silica gel thin-layer chromatography of successive fractions of effluent from a DEAE-Sephacrose column. In this two-step chromatographic system, the first step of elution from the column depends on differences in anionic charge and the second step of development on a silica gel plate depends on differences in polarity.

ETHANE AND ETHYLENE FORMATION BY MITOCHONDRIA AS INDICATION OF AEROBIC LIPID DEGRADATION IN RESPONSE TO WOUNDING OF PLANT TISSUE. J.R. Konze and E.F. Elstner, *Biochim. Biophys. Acta* 528, 213–21 (1978). During aerobic incubation of potato slices or potato mitochondria at acidic pH, ethane and ethylene in a ratio of approx. 50:1 are generated from an endogenous substrate. Both ethane and ethylene production are stimulated by the addition of α -linolenic acid. Ethane formation from linolenic acid is a radical mechanism dependent on oxygen and is not significantly influenced by mitochondrial electron transport. Ethane production may represent a sensitive marker for membrane damage.

A RAPID AND QUANTITATIVE METHOD FOR THE ISOLATION OF GANGLIOSIDES AND NEUTRAL GLYCOSPHINGOLIPIDS BY DEAE-SILICA GEL CHROMATOGRAPHY. S.K. Kundu and S.K. Roy, *J. Lipid Res.* 19, 390–5 (1978). DEAE-silica gel has been shown to be an improvement over DEAE-Sephadex for the quantitative isolation of gangliosides and neutral glycosphingolipids from animal tissues or cells. Preliminary results indicated that it can also be used for protein separation. Direct comparative studies of DEAE-silica gel with DEAE-Sephadex showed preferences for the former for the following reasons: i) faster flow rate; ii) more rapid equilibration with the starting buffer; iii) easier regeneration; iv) more economical; and v) a lesser susceptibility to microbial attack.

VULNERABILITY OF KETO BILE ACIDS TO ALKALINE HYDROLYSIS. G. Lepage, *et al.*, *J. Lipid Res.* 19, 505–9 (1978). Rigorous alkaline hydrolysis of the two primary (cholic and chenodeoxycholic) and of the two preponderant secondary (deoxycholic and lithocholic) bile acids found in bile led to excellent recoveries. Such was not the case with 11 different keto bile acid standards. Recoveries for a number of standards were unacceptably low and a variety of artefactual products were tentatively identified by gas-liquid chromatography. Keto bile acids bearing a keto group on C-3 were particularly vulnerable. In view of these findings, quantitative and qualitative data reported on biological specimens submitted to saponification in ethanol, methanol, or even in water are of questionable significance.

SMALL-ANGLE X-RAY SCATTERING AND DIFFERENTIAL SCANNING CALORIMETRY STUDIES ON REVERSIBLY MODIFIED HUMAN-SERUM LOW DENSITY LIPOPROTEINS. L. Mateu *et al.*, *Biochemistry* 17, 1436–40 (1978). Small-angle x-ray scattering diagrams of human serum low density lipoprotein (LDL) were recorded at several temperatures in solutions of different freezing points. It was found that modifications of the x-ray patterns observed on cooling the lipoprotein samples below 0°C are due to reversible alterations of the LDL surface structure induced by the freezing process (independent of temperature). With both intact and partially dehydrated LDL, differential scanning calorimetry (DSC) carried out in the body temperature range revealed a heat absorption characteristic of the transition from a liquid crystal to an isotropic liquid phase of cholesteryl esters within the lipoproteins. However, small-angle x-ray scattering diagrams recorded with the same LDL sample before and after the partial removal of water were found to be very different: the scattering curve for intact LDL showed a strong band centered at (36Å) $^{-1}$ which disappeared upon drying and reappeared upon restoring the water. Our results suggest that the presence of this signal

strongly depends on the molecular structure of the lipoprotein surface.

PULMONARY SURFACTANT SYNTHESIS. A HIGHLY ACTIVE MICRO-SOMAL PHOSPHATIDATE PHOSPHOHYDROLASE IN THE LUNG. R.D. Mavis *et al.*, *J. Lipid Res.* 19, 467-77 (1978). Lung cell-free homogenate, which contains about twice the units of phosphatidate phosphohydrolase per mg of protein compared to liver, was fractionated by differential centrifugation and the fractions were assayed for phosphatidate phosphohydrolase and marker enzymes of endoplasmic reticulum, mitochondria, and lysosomes. Over 60% of the lung phosphatidate phosphohydrolase was associated with the endoplasmic reticulum, compared to 50% of the total liver enzyme. The presence of a high activity of phosphatidate phosphohydrolase in lung endoplasmic reticulum is consistent with the rapid synthesis of pulmonary surfactant phosphatidylcholine.

USE OF DEUTERATED PHOSPHOLIPIDS IN RAMAN SPECTROSCOPIC STUDIES OF MEMBRANE STRUCTURE. I. MULTILAYERS OF DIMYRISTOYL PHOSPHATIDYLCHOLINE (AND ITS $-D_{84}$ DERIVATIVE) WITH DISTEAROYL PHOSPHATIDYLCHOLINE. R. Mendelsohn and J. Maisano, *Biochim. Biophys. Acta* 506, 192-201 (1978). The temperature dependence of the Raman spectrum has been studied for binary phospholipid mixtures of dimyristoyl phosphatidylcholine (and its chain deuterated $-D_{84}$ derivative) with distearoyl phosphatidylcholine. Two distinct melting regions are observed for the 1:1 mole ratio mixture. The C-H stretching vibrations of the distearoyl component respond to the melting of the dimyristoyl component, an apparent consequence of alterations in the lateral interactions of the distearoyl chains.

STUDIES ON VITAMIN D (CALCIFEROL) AND ITS ANALOGUES. 14. ON THE 10,19-DIHYDROVITAMINS RELATED TO VITAMIN D_2 INCLUDING DIHYDROTACHYSTEROL. A. Mourine and W.H. Okamura, *J. Org. Chem.* 43, 1653-6 (1978). Vitamin D_2 (1a), its benzoate (1b), 5,6-*trans*-vitamin D_2 (2a), and its benzoate (2b) were each treated with 9-borabicyclo[3.3.1]nonane and then oxidized with basic hydrogen peroxide to afford the following pairs of stereoisomeric 10,19-dihydrovitamin D_2 's: 3a-4a, 3b-4b, 5a-6a, and 5b-6b, respectively. Catalytic reduction of 1a and 2a afforded the stereoisomeric pairs 3d-4d and 5d-6d, respectively. The four benzoylaxo alcohols 3b-6b were each individually converted to their *p*-toluene-sulfonates 3c-6c, respectively, and then each diester was subjected to lithium triethylborohydride reduction to afford 3d, 7, 5d, and 8, respectively. Finally, saponification of 4b produced 4a and 6b gave 6a. How these chemical transformations including spectral analyses definitively establish the absolute configurations of 3-6 is discussed. The relationship of 3d-6d to the substances referred to in the old literature as DHT₂, DHV₂-II, DHV₂-III, DHV₂-IV, and DT-66 is also discussed.

CHARACTERIZATION OF TRIMETHYLSILYL DERIVATIVES OF CEREBROSIDES BY DIRECT INLET-CHEMICAL IONIZATION MASS SPECTROMETRY. T. Murata *et al.*, *J. Lipid Res.* 19, 370-4 (1978). Submicrogram quantities of trimethylsilyl derivatives of cerebroside obtained from the spleen of a patient with Gaucher's disease and from bovine brain were analyzed by direct probe inlet-chemical ionization mass spectrometry, using isobutane as the reagent gas. Quasimolecular (QM^+ , $M + 73$) and other recognizable fragment ions produced by the successive elimination of trimethylsilanol and sugar residue gave useful information about fatty acid compositions. It was concluded that structural information and molecular species determination could be obtained from small amounts of purified cerebroside.

INTERACTION OF BILIRUBIN WITH LIPIDS STUDIED BY FLUORESCENCE QUENCHING METHOD. S. Nagaoka and M.L. Cowger, *J. Biol. Chem.* 253, 2005-11 (1978). The interaction of bilirubin and various lipids was studied by a static fluorescence quenching method. A quenching equation was developed to determine the binding constant. This method was tested first by a 5-dimethylaminonaphthalene-1-sulfonyl (dansyl) bovine albumin system and then applied to a fluorescent probe containing lipid. Both systems can yield binding parameters. Of the lipids tested, sphingomyelin showed the highest association constant; this may be related to the central nervous system toxicity of this bile pigment.

THE STERIC REQUIREMENTS FOR THE METABOLISM OF STEROLS BY TETRAHYMENA PYRIFORMIS. W.R. Nes *et al.*, *J. Biol. Chem.*

253, 2361-7 (1978). Cultures of *Tetrahymena pyriformis* were incubated with a series of sterols which differed from one another in the steric character of their side chains. (E)-17(20)-Dehydrocholesterol in which C-22 is fixed to the right (trans with respect to C-13, right-handed) in the usual view of the molecule was converted to its $\Delta^{5,7,17(20)}$ and $\Delta^{5,7,17(20),22}$ derivatives; however, the organism did not significantly metabolize the "left-handed" (Z)-17(20)-dehydrocholesterol in which C-22 is cis-oriented with respect to C-13. We interpret this to imply a stereochemical requirement for a right-handed side chain, i.e. a side chain with C-22 on the right side. The ability of the organism to metabolize the trigonal and therefore planar (E)- $\Delta^{17(20)}$ and (E)- $\Delta^{20(20)}$ -sterols proves that tetrahedral character at C-20 is not necessary and that no bulk in the perpendicular plane is required.

SYNTHESIS OF ACYL PHOSPHATIDYLGLYCEROL FROM PHOSPHATIDYLGLYCEROL IN ESCHERICHIA COLI K-12: EVIDENCE FOR THE PARTICIPATION OF DETERGENT-RESISTANT PHOSPHOLIPASE A AND HEAT-LABILE MEMBRANE-BOUND FACTOR(S). M. Nishijima *et al.*, *Biochim. Biophys. Acta* 528, 107-18 (1978). The conversion of phosphatidylglycerol to acyl phosphatidylglycerol by extracts of *Escherichia coli* K-12 strains was examined under various conditions. The maximum rate of conversion was observed at pH 7.2 in the presence of 50% (v/v) diethyl ether and 10 mM/CaCl₂. This conversion was found to involve two sequential reactions: (1) The formation of 2-acyl glycerophosphoglycerol and 2-acyl glycerophosphoethanolamine from phosphatidylglycerol and phosphatidylethanolamine, respectively, by detergent-resistant phospholipase A in the presence of Ca²⁺ and (2) transfer of the acyl group of 2-acyl lysophospholipid to phosphatidylglycerol by a heat-labile factor(s) in the presence of diethyl ether. Neither fatty acid, acyl-CoA nor 1-acyl lysophospholipid could act as an acyl donor for phosphatidylglycerol. The heat-labile factor(s) was found in both the inner membrane and supernatant fractions.

MECHANISM OF THERMAL ADAPTATION OF MEMBRANE LIPIDS IN TETRAHYMENA PYRIFORMIS NT-1: POSSIBLE EVIDENCE FOR TEMPERATURE-MEDIATED INDUCTION OF PALMITOYL-CoA DESATURASE. Y. Nozawa and R. Kasai, *Biochim. Biophys. Acta* 529, 54-66 (1978). The regulatory mechanism of a key enzyme, palmitoyl-CoA desaturase, involved in the adaptation to temperature shift was investigated by labeling *Tetrahymena pyriformis* cells with [¹⁴C]palmitic acid. The rate of conversion of [¹⁴C]palmitate to [¹⁴C]palmitoleate was shown to be dependent on incubation temperature and also to be maximal at 2 h after the shift 39.5 to 15°C. Addition of cycloheximide before the temperature shift produced no increase in desaturation of [¹⁴C]palmitate after the shift. These data would provide evidence for temperature-triggered increase of palmitoyl-CoA desaturase level and are also discussed in relation to membrane fluidity.

INTERFACIAL OXIDATION OF α -TOCOPHEROL AND THE SURFACE PROPERTIES OF ITS OXIDATION PRODUCTS. G.S. Patil and D.G. Cornwell, *J. Lipid Res.* 19, 416-22 (1978). DL- α -Tocopherol spread on an acidic subphase as a gaseous monolayer was oxidized slowly to a derivative that was identified by thin-layer chromatography as α -tocopherylquinone. The derivative generated the same II-A isotherm as α -tocopherylquinone. When the subphase contained gold chloride, α -tocopherol was oxidized rapidly and quantitatively to α -tocopherylquinone. DL- α -Tocopherol spread on a basic subphase as a gaseous monolayer was oxidized slowly to a mixture that contained α -tocopherol, a quinone, and a nonpolar derivative. The natural occurrence of both tocopherylquinone and the spirodienone ether suggests that several types of oxidant stress are found in biological systems. One type of oxidant stress may involve the peroxy radical generating tocopherylquinone; a second type may involve hydroxyl radical-hydroxide ion generating the spirodienone ether.

CONVERSION OF DIPHOSPHATIDYLGLYCEROL TO BIS(MONOACYLGLYCERYL)PHOSPHATE BY LYSSOMES. B.J.M. Poorthuis and K.Y. Hostetler, *J. Lipid Res.* 19, 309-15 (1978). Diphosphatidyl(1',2',3'-¹⁴C)glycerol (cardiolipin) is converted to bis(monoacylglyceryl) phosphate when incubated in vitro with rat liver lysosomes at pH 4.4. The stereochemical configuration of the product is unknown. This reaction probably takes place via lysophosphatidylglycerol, one of the major products of diphosphatidylglycerol hydrolysis by lysosomes. These results support our previous proposal that bis(monoacylglyceryl) phosphate formation may require the interaction of lysosomes

with other membranes that contain the substrates for the reaction. The stereochemistry of bis(monoacylglyceryl)phosphate biosynthesis is discussed.

CHARACTERIZATION OF MIXED MICELLES OF PHOSPHOLIPIDS OF VARIOUS CLASSES AND A SYNTHETIC, HOMOGENEOUS ANALOGUE OF THE NONIONIC DETERGENT TRITON X-100 CONTAINING NINE OXYETHYLENE GROUPS. R.J. Robson and E.A. Dennis, *Biochim. Biophys. Acta* 508, 513-24 (1978). The synthesis and high-pressure liquid chromatographic purification of the homogenous nonionic surfactant p-(1,1,3,3-tetramethylbutyl)phenoxy monoxyethylene glycol (OPE-9) in quantities suitable for membrane solubilization studies is reported. Micelles of OPE-9 and mixed micelles of OPE-9 with dimyristoyl and dipalmitoyl phosphatidylcholine as well as phosphatidylserine, phosphatidylethanolamine, lysophosphatidylcholine, sphingomyelin, and palmitic acid were characterized by column chromatography on 6% agarose. The mixed micelle size was found to be relatively independent of the absolute concentration of surfactant over a four-fold range if the mole fraction of phospholipid is kept constant. The usefulness of the OPE-9/phospholipid mixed micelle system for lipolytic enzyme substrates and membrane-related studies is considered.

BINDING OF CYTOCHROME b_5 TO CHOLESTEROL-CONTAINING PHOSPHATIDYLCHOLINE VESICLES. M.A. Roseman *et al.*, *Biochim. Biophys. Acta* 507, 552-6 (1978). Cytochrome b_5 was found to bind readily to sonicated vesicles containing as much as 0.8 mol cholesterol per mol egg phosphatidylcholine. This observation conflicts with the suggestion of Enomoto and Sato ((1977) *Biochim. Biophys. Acta* 466, 136-147) that cholesterol prevents binding of this protein to erythrocyte membranes.

CYCLIC PEROXIDES FROM A SOYA LIPOXYGENASE-CATALYSED OXYGENATION OF METHYL LINOLENATE. M. Roza and A. Francke, *Biochim. Biophys. Acta* 528, 119-26 (1978). Incubation of methyl linolenate with an aqueous extract of soybean flour at neutral pH gives the hydroperoxyendoperoxides 16-hydroxyperoxy-13,15-endoperoxylinolenate and 9-hydroxyperoxy-10, 12-endoperoxylinolenate as the principal oxygenation products in addition to monohydroperoxides. Lipoygenase I (EC 1.13.11.12) does not catalyze the oxygenation under this condition. The enzyme contributing most to the formation of the hydroperoxyendoperoxides is assumed to be a high molecular mass lipoygenase aggregate.

SURFACE AREAS OF LIPID MEMBRANES. M.A. Schwartz and H.M. McConnell, *Biochemistry* 17, 837-40 (1978). Upon photolysis, alkyl pentacyanocobaltate complexes generate alkyl radicals which react rapidly and specifically with nitroxide radicals, and which do not penetrate phospholipid bilayers. By measuring the loss of paramagnetic resonance signal intensity when multilamellar liposomes containing a small amount of spin-labeled lipid are exposed to these radicals, we have measured the proportion of lipid on the external surface of liposomes. We have shown that liposomes prepared under specified conditions from dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and binary mixtures of dipalmitoylphosphatidylcholine and cholesterol all have the same proportion of external lipid.

INDIVIDUAL LIPID AND PROXIMATE ANALYSIS OF VARIOUS FOODS. 3. POTATO CHIPS AND CORN SNACK FOODS. A.J. Sheppard *et al.*, *J. Agric. Food Chem.* 26, 346-8 (1978). Twenty different brands of potato chips, corn puffs, and corn chips were purchased from food stores in the Washington, D.C., and Davis CA areas. These samples were analyzed in duplicate for water, total fat, protein, ash, fatty acids, sterols, and *cis,cis*-methylene interrupted polyunsaturated triglycerides. The data show that considerable variation between brands exists in the fatty acid and sterol patterns in both the potato chip and corn snack food groups. The data indicate that some brands were processed using vegetable oils, whereas others were processed using animal fats. The portion of total polyunsaturated fatty acids that is *cis,cis*-methylene interrupted ranged from about 50% to nearly 100%, depending upon the brand being tested. The lipid content of both potato chips and corn snack foods is so variable between brands that the use of mean values in food composition tables to calculate dietary intake would not accurately reflect actual intake.

FACTORS AFFECTING THE AUTOXIDATION OF LIPIDS IN MECHANICALLY DEBONED FISH. D.A. Silberstein and D.A. Lillard,

J. Food Sci. 43, 764-6 (1978). Hemoglobin, myoglobin, total heme pigments, and nonheme iron concentrations were measured in phosphate buffer (pH 7.1) extracts of mechanical and hand deboned mullet, *Mugil cephalus*, to determine the factors which induce a greater prooxidant activity in mechanically deboned fish. Mechanical deboning increased the hemoglobin and nonheme iron contents but had very little influence on the amount of myoglobin in the deboned fish flesh. Oxygen uptake studies using oleic acid as a substrate revealed that the hemoprotein content had an influence on the prooxidant activity of the buffer extracts of the deboned fish. Studies using purified myoglobin and hemoglobin as prooxidants indicated that myoglobin had a greater catalytic effect than hemoglobin on the oxidation of oleic acid. It was concluded that, in addition to the concentration of total heme pigments, the hemoglobin to myoglobin ratio should be considered when determining the influence of hemoproteins on the oxidative stability of deboned fish.

LABELLING OF GLYCEROLIPIDS IN THE COTYLEDONS OF DEVELOPING OILSEEDS BY (^{14}C)-ACETATE AND (^3H)-GLYCEROL. C.R. Slack *et al.*, *Biochem. J.* 170, 421-33 (1978). 3-sn-Phosphatidylcholine was identified as the major lipid in cotyledons from the developing seeds of soya bean, linseed and safflower when tissue was steamed before lipid extraction. The proportion of oleate in this lipid decreased markedly and that of the polyunsaturated C_{18} fatty acids increased when detached developing cotyledons were incubated for up to 3 h. Similar but less pronounced changes occurred in diacylglycerol, which had a fatty acid composition resembling that of the 3-sn-phosphatidylcholine from cotyledons of the same species. The results suggest a rapid turnover of 3-sn-phosphatidylcholine during triacylglycerol accumulation in developing oilseeds, and are consistent with the operation of a biosynthetic route whereby oleate initially esterified to the phospholipid is first desaturated, then polyunsaturated fatty acids transferred to triacylglycerol, via diacylglycerol. The possible role of oleoyl phosphatidylcholine as a substrate for oleate desaturation is discussed.

STUDIES ON A PHOSPHOLIPASE B FROM *PENICILLIUM NOTATUM* SUBSTRATE SPECIFICITY. J. Sugatanti, N. Kawasaki and K. Saito, *Biochim. Biophys. Acta* 529, 29-37 (1978). The action of a highly purified phospholipase B from *Penicillium notatum* on 1-O-alk-1'-enyl-2-acyl-, 1-O-alkyl-2-acyl-, 1,2-diacyl-, 1-acyl and 2-acyl-sn-glycero-3-phosphocholine, monoacyl-, diacyl- and triacylglycerols, cholesteryl oleate and *p*-nitrophenyl acetate was studied. The hydrolysis products of the monoethermonoacylglycerophospholipids were identified as fatty acids, 1-O-alk-1'-enyl-sn-glycero-3-phosphocholine and 1-O-alkyl-sn-glycero-3-phosphocholine > 1-O-alk-1'-enyl-2-acyl-sn-glycero-3-phosphocholine > 1-O-alkyl-2-acyl-sn-glycero-3-phosphocholine. 1-Acyl-sn-glycero-3-phosphocholine was hydrolyzed about 15 times faster than 2-acyl-sn-glycero-3-phosphocholine. Monoacylglycerols were hydrolyzed at the optimal pH 4.0, but diacyl- and triacylglycerols were not hydrolyzed at various pH values between 4.0 and 9.0. Cholesteryl oleate and *p*-nitrophenyl acetate were not hydrolyzed.

STUDIES ON THE HYDROGEN BELTS OF MEMBRANES: III. GLYCEROL PERMEABILITY OF DIHYDROSPHINGOMYELIN-CHOLESTEROL MEMBRANES. L.J. Tirri *et al.*, *Lipids* 13, 267-9 (1978). The permeability of an N-oleoyldihydrospingomyelin bilayer against glycerol was similar to that of a bilayer of phosphatidylcholine with identical effective hydrophobic chain length. Cholesterol at 1:1 molar ratio reduced the permeability, and also reduced the energy of activation of glycerol penetration, an effect not found for diesterphosphatidylcholine with cholesterol. The higher level of the ground state of the entropy of activation for permeability can be interpreted in terms of a hydrogen belt model which postulates lipid-lipid hydrogen bonding in membranes and explains the effect found as a disturbance of the hydrogen belt structure. Dihydrospingomyelin can be considered to function as an "extender" in the hydrogen belt network.

PROTEOLYTIC DIGESTION IN THE ELUCIDATION OF THE STRUCTURE OF LOW DENSITY LIPOPROTEIN. R.B. Triplett and W.R. Fisher, *J. Lipid Res.* 19, 478-88 (1978). The apoprotein (apoB) of low density lipoprotein (LDL) is reported to be a large polypeptide, and it is proposed that there are two similar-sized subunit proteins in LDL. When apoB is isolated under conditions that minimize artifactual proteolysis, only a single, large molecular weight protein appears on polyacrylamide gel

electrophoresis in SDS. To investigate the organization of apoB as it exists within native LDL, limited proteolysis with trypsin has been used as a structural probe. Thus, the different quantities of lipid bound in these various LDL must interact with apoB so that the same regions of the apoprotein are exposed to the action of trypsin in these different molecular weight lipoproteins.

GLYCERIDE SYNTHESIS BY FOUR KINDS OF MICROBIAL LIPASE. Y. Tsujisaka *et al.*, *Biochim. Biophys. Acta* 489, 415-22 (1977). Apart from their usual mechanism of action, lipases from *Aspergillus niger* and *Rhizopus delemar* also catalyzed the synthesis of glycerides from oleic acid and glycerol. Lipases from *Geotrichum candidum* and *Penicillium cyclopium* were inactivated by oleic acid, but were stable in the presence of casein, albumin or buffer of appropriate pH. Lipases from *Aspergillus niger* and *Rhizopus delemar* synthesized glycerides from, not only fatty acid, but dibasic acids and aromatic acids, making ester bonds only at position 1 and 3 of glycerol. In contrast, lipases from *Geotrichum candidum* and *Penicillium cyclopium* synthesized glycerides only from long chain fatty acids, and made ester bonds at all three available positions of the glycerol molecule.

INTERACTION OF ACETYLCHOLINE RECEPTOR AND ACETYLCHOLINESTERASE WITH LIPID MONOLAYERS. T. Wiedmer *et al.*, *Biochem. Biophys. Acta* 506, 161-72 (1978). The interaction of acetylcholine receptor and acetylcholinesterase with lipid monolayers was followed by measuring changes in surface pressure. When injected into the subphase of a lipid monolayer, the proteins caused increases in surface pressure from 5 to 10 dynes/cm, indicating a penetration of protein into the monolayer. At pH values below the isoelectric point of the proteins the incorporation was improved. The same was observed when Ca^{2+} (2 mM) was added. The presence of the enzyme in the mixed film could be demonstrated by using diiso[^3H]propyl fluorophosphate-labelled acetylcholinesterase as well as by measuring enzyme activity. Acetylcholine receptor was shown to be present in the mixed film by using a complex made of the receptor and α -[^3H] neurotoxin.

LIPID SYNTHESIS IN ISOLATED INTESTINAL CELLS. K.M. Mohamed Shakir *et al.*, *J. Lipid Res.* 19, 433-42 (1978). Since the small intestine contributes significantly to serum cholesterol and very low density lipoprotein levels, acute regulation of lipid synthesis was investigated in isolated rat intestinal cells incubated in Krebs-Ringer bicarbonate buffer with 5 mM glucose and (^{14}C)acetate or $^3\text{H}_2\text{O}$. Incorporation of (^{14}C)acetate into cellular lipids was 6- to 8-fold greater in crypt than in villus cells. We conclude that ethanol stimulates intestinal lipid synthesis; however, in sharp contrast to their inhibition of lipid synthesis in hepatocytes and adipocytes, catecholamines, glucagon, and dibutyl cyclic AMP do not inhibit lipid synthesis in intestinal cells.

EFFECT OF GLUCOSE OR OIL SUPPLEMENTATION ON LIPOGENIC ENZYMES IN OVERFED CHICKS. N. Shapira *et al.*, *J. Nutr.* 108, 490-6 (1978). Chicks of a light breed, aged 26 days, were force-fed by intubation for 10 days. The feed administered consisted of a basal low-fat closed formula cereal based diet and of supplements of either glucose or soybean oil supplied in isoenergetic amounts. Ad libitum-fed chicks served as controls. At the end of the experiment, the weights and lipid contents of carcass, livers and abdominal adipose tissue were determined, as was the glucose and lipid content of plasma. Activities of citrate cleavage and malic enzymes increased in proportion to the amount of carbohydrates force-fed in excess of the control intake. These and previous results show that chicks adapt to excess carbohydrate intake by both liver enlargement and increased activity of lipogenic enzymes.

A MODEL FOR GANGLIOSIDE BEHAVIOUR IN CELL MEMBRANES. F.J. Sharom and C.W.M. Grant, *Biochim. Biophys. Acta* 507, 280-93 (1978). Gangliosides from beef brain have been spin-labeled using two different attaching groups and employed to investigate the physical nature of ganglioside behaviour in membranes. Results obtained using EPR spectroscopy indicate that, in phosphatidylcholine bilayers at physiological pH, ganglioside oligosaccharide chains are quite mobile and show a measurable tendency towards cooperative interaction amongst themselves. We suggest that the source of this interaction is the formation of H-bonds between sugar residues in adjacent ganglioside molecules. We predict that laterally mobile carbohydrate-bearing components of cell surfaces will show a tendency to cluster about

complex glycoprotein arrays, especially if the species involved bear accessible carboxylic acid functions.

DYNAMIC PROPERTIES OF HUMAN HIGH DENSITY LIPOPROTEIN APOPROTEINS. J. Shepherd *et al.*, *J. Lipid Res.* 19, 383-9 (1978). This study was designed to identify a method for the measurement of human high density lipoprotein subfraction (HDL_2 and HDL_3) metabolism. Apolipoproteins A-I, A-II, and C, the major HDL apoproteins, were radioiodinated and incorporated individually into HDL_2 and HDL_3 in vitro. Using a double label technique, the turnover of apoA-I in HDL_2 and HDL_3 was measured simultaneously in a normal male. The apoprotein exchanged rapidly between the two subfractions, evidence by equilibration of their apoA-I specific activity. Radiolabeled apoA-II, incorporated into the subfractions, showed a similar exchange in vitro. Overall, the study indicates that apoA-I, apoA-II, and the C proteins exist in equilibrium between HDL_2 and HDL_3 . This phenomenon precludes their use as probes for HDL subfraction metabolism in humans.

THE IN VITRO INTERACTION OF HUMAN APOLIPOPROTEIN A-1 AND HIGH DENSITY LIPOPROTEINS. J. Shepherd *et al.*, *Biochim. Biophys. Acta* 489, 486-501 (1977). Radioiodinated human apolipoprotein A-1, when incubated with plasma lipoproteins, associates exclusively with high density lipoproteins. It does not interact with very low density lipoproteins or low density lipoproteins. Binding is rapid, being complete within 10 min, and is not affected by variation of pH within the range 6.0-9.5 or of temperature over the range 0°-37°C. At equimolar concentrations of apolipoprotein A-1 and high density lipoproteins, 0.58 mol of the apoprotein bind per mol of high density lipoproteins. Binding increases progressively with apolipoprotein A-1 concentration up to an apolipoprotein A-1:high density lipoprotein molar ratio of 20:1, when each mol of lipoprotein binds 7.62 mol of apoprotein.

ISOLATION AND CHARACTERIZATION OF TWO THREONINE-POOR APOLIPOPROTEINS OF HUMAN PLASMA HIGH DENSITY LIPOPROTEINS. V.G. Shore *et al.*, *Biochemistry* 17, 2174-9 (1978). Two apolipoproteins that are minor components normally of human plasma lipoproteins were discovered. They comprise up to 25% or more of the apolipoproteins in high density lipoproteins of some individuals with lipoprotein abnormalities associated with certain metabolic diseases and in individuals treated with amphotericin B for coccidiomycosis infection. The lipid moiety of the abnormal HDL was richer in triglycerides and similar in phospholipid content to that of normal HDL.

THE EFFECT OF THE BASAL DIET ON THE TRUE METABOLIZABLE ENERGY VALUE OF FAT. I.R. Sibbald and J.K.G. Kramer, *Poult. Sci.* 57, 685-91 (1978). An experiment was made to measure the effect of the basal diet on the true metabolizable energy (TME) value of beef tallow. Sixteen diets were arranged as a 4 x 4 factorial with four basal diets (wheat and soybean; corn and soybean; wheat, soybean and meat; wheat, soybean and fish) and four levels of added tallow (0,5,10,15%). Each diet was assayed for TME 8 times and the TME value of the tallow was calculated by difference. Chloroform:methanol extracts of the basal diets were fractionated into neutral and phospholipids. The fatty acid compositions of total, neutral and phospholipid fractions were determined. The tallow supplemented diets were assayed for fatty acids. The TME value of the tallow decreased with the level of dietary inclusion ($P < 0.01$) and also differed according to the basal diet with which it was fed. The corn:soy basal gave higher TME values for the tallow than did the other three basals. There was no significant interaction between the basal diets and the level of fat inclusion. There were significant linear relationships between the TME value of the tallow and the amounts of phospholipid and linoleic acid per unit weight of dietary fat.

GLYCOSPHINGOLIPIDS IN HUMAN COLONIC ADENOCARCINOMA. B. Siddiqui *et al.*, *J. Biol. Chem.* 253, 2168-75 (1978). Glycosphingolipids were analyzed from colonic tumors and histologically normal adjacent tissues, obtained at surgery from eight patients. Detailed analysis was carried out on six neutral glycolipids from normal colonic mucosa and cancerous tissues. Their structures were established by permethylation, use of glycosidases, and immunological methods. Sulfogalactosylceramide, the major sulfoglycolipid in normal and colonic cancerous tissues, was increased in tumors.

EFFECT OF DIETARY OIL, CHOLESTEROL, AND SOYSTEROLS ON THE LIPID CONCENTRATION AND FATTY ACID COMPOSITION OF EGG YOLK, LIVER AND SERUM OF LAYING HENS. J.S. Sim and D.S. Bragg, *Poult. Sci.* 57, 466-72 (1978). Effect of dietary lipid factors (saturated and unsaturated oil, zoo and phytosterols) on the lipid concentration and fatty acid composition of egg yolk, liver and serum of the laying hen were studied. Single Comb White Leghorn laying hens, at 30 weeks of age, were fed two basal diets containing 8.0% hydrogenated coconut oil (HCO) or safflower oil (SFO), with or without supplemental cholesterol (1.0%), soysterols (2.0%) or combination of both. When HCO basal diet was fed, both liver weight and lipid content were significantly ($p < .01$) increased as compared to hens fed the SFO diet. Cholesterol feeding increased total lipid content in liver and serum, whereas soysterol feeding reduce or diminish lipid accumulation caused by the cholesterol treatment. Both dietary cholesterol and soysterols alter the fatty acid composition of liver, serum and egg yolk lipids by increasing oleic acid and decreasing palmitic and/or stearic acids. These changes were significantly greater upon feeding cholesterol than soysterols. However, the simultaneous feeding of cholesterol with soysterols exerted the least effect on the fatty acid composition.

EFFECT OF CLOFIBRATE ON IN VIVO TRIGLYCERIDE PRODUCTION AND CLEARANCE IN GENETICALLY HYPERLIPEMIC RATS. C. Simonelli and R.P. Eaton, *Atherosclerosis* 29, 269-75 (1978). Seventeen hyperlipemic and 17 normolipemic littermate Zucker rats were treated with clofibrate or normal saline to determine the effect of this drug upon hepatic triglyceride (TG) production and peripheral TG disposal. Peripheral Intralipid clearance was not different in hyperlipemic rats relative to control animals. Hyperlipemic animals demonstrated abnormally elevated TG production in the fasted state which was not corrected with clofibrate administration. Following treatment, peripheral Intralipid clearance was increased 100% in hyperlipemic rats, but unchanged in normolipemic animals. These observations provide further evidence that the predominant lipid reducing action of clofibrate is manifested only in the hyperlipemic state, and predominantly upon peripheral lipid disposal.

LIPID COMPOSITION OF THE GASTRIC MUCOUS BARRIER IN THE RAT. A. Slomiany *et al.*, *J. Biol. Chem.* 253, 3785-91 (1978). The lipid composition of the "mucous barrier" of rat stomach was investigated. Cellular mucus of the mucous cells from gastric epithelium and surface mucus from gastric mucosa were obtained by perfusion in vivo of Ghosh-Lai rat stomachs with 2 M NaCl. Lipids extracted from dialyzed and lyophilized 2M NaCl perfusates and 0.9% NaCl (saline) controls were quantitatively separated into single components by means of two-dimensional thin layer chromatography and compared. These data indicate that mucous barrier in addition to mucins contains considerable quantities of lipids of which glyceroglucolipids are the most prominent components.

PROPERTIES OF CHOLESTERYL OLEATE AND TRIOLEIN IN MIXED MONOLAYERS AT THE AIR-WATER INTERFACE. J.M. Smaby and H.L. Brockman, *J. Lipid Res.* 19, 325-31 (1978). The properties of cholesteryl oleate and triolein in mixed monolayers at the air-water interface have been measured between 24 and 37°C. Analysis of force-area curves obtained as a function of the mol fraction of cholesteryl oleate indicates that at relatively low surface pressures these compounds are miscible in two dimensions up to a limit of about 0.5 mol fraction. At higher pressures either cholesteryl oleate or both lipids are expelled from the monolayer to form a bulk phase which is in rapid equilibrium with the surface phase. Comparison of our results with the bulk properties of these lipids suggests that the expelled cholesteryl oleate exists as a smectic mesophase and thus the system may provide a model for studying the transfer of molecules between the interior and surface of lipid deposits of the type found in atherosclerotic lesions.

DEACYLATION OF ACETYL-COENZYME A AND ACETYLCARNITINE BY LIVER PREPARATIONS. A.M. Snoswell and P.K. Tubbs, *Biochem. J.* 171, 299-303 (1978). The breakdown of acetylcarnitine catalysed by extracts of rat and sheep liver was completely abolished by Sephadex G-25 gel filtration, whereas the hydrolysis of acetyl-CoA was unaffected. Acetyl-CoA and CoA acted catalytically in restoring the ability of Sephadex-treated extracts to break down acetylcarnitine, which was therefore not due to an acetylcarnitine hydrolase but to the sequential action of carnitine acetyltransferase and acetyl-CoA

hydrolase. Some 75% of the acetyl-CoA hydrolase activity of sheep liver was localized in the mitochondrial fraction. Two distinct acetyl-CoA hydrolases were partially purified from extracts of sheep liver mitochondria. Both enzymes hydrolysed other short-chain acyl-CoA compounds and succinyl-CoA (3-carboxypropionyl-CoA), but with one acetyl-CoA was the preferred substrate.

PROTEOLYSIS OF VERY LOW DENSITY LIPOPROTEIN IN PERFUSED LUNG. C.E. Sparks *et al.*, *Biochim. Biophys. Acta* 529, 123-30 (1978). Perfusion of homologous 125 I-labeled rat very low density lipoprotein through isolated rat lungs in the presence of heparin resulted in apoprotein proteolysis. At least the apoprotein C was degraded into two peptides smaller than 7500 daltons as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The lung uptake of radioactivity was small and due mainly to the presence of the larger of the two peptides. The lung protease was not active against an 125 I-labeled albumin substrate and was not released into the medium by heparin.

THE FORMATION OF LIPID-LINKED SUGARS AS INTERMEDIATES IN GLYCOPROTEIN SYNTHESIS IN RABBIT MAMMARY GLAND. B.K. Speake and D.A. White, *Biochem. J.* 170, 273-83 (1978). The incorporation of D-(1- 14 C)mannose, D-(2- 3 H)mannose and N-acetyl-D-(1- 14 C)-glucosamine into glycoproteins and lipid-linked intermediates of mammary explants obtained from lactating rabbits was studied. The amount of radioactivity incorporated into lipid-linked intermediates was very low compared with the incorporation into protein. Most of the radioactivity incorporated into the chloroform/methanol-soluble fraction was present as neutral lipid. Radioactivity from D-(2- 3 H)mannose was incorporated mainly into the fatty acid moiety, whereas radioactivity from D-(1- 14 C)mannose and N-acetyl-D-(1- 14 C)glucosamine was present in the glycerol moiety of triacylglycerol.

SYNERGISTIC EFFECTS OF DIETARY CARBOHYDRATE AND CHOLESTEROL ON SERUM LIPIDS AND LIPOPROTEINS IN SQUIRREL AND SPIDER MONKEYS. S.R. Srinivasan *et al.*, *Am. J. Clin. Nutr.* 31, 603-13 (1978). Serum lipid and lipoprotein responses to diets with a high level of simple carbohydrate (69% w/w sucrose) and a low level of saturated fat (5% w/w butter-coconut oil, polyunsaturated/saturated fatty acid ratio 0.03) containing 0, 0.1, and 1.0 mg/kcal added cholesterol was studied in five squirrel (*Saimiri sciurea*) monkeys. Variations in response produced by altering the nature of dietary carbohydrate (sucrose versus dextrin) and the fat (polyunsaturated/saturated fatty acid ratio, 0.03 versus 1.5) in the above diets were studied in three groups (five per group) of spider monkeys (*Ateles sp.*). These results emphasize the varied response of serum lipids and lipoproteins to dietary changes in carbohydrate, fat, and cholesterol that might have a bearing on experimental atherosclerosis.

INTERACTIONS OF NATIVE AND MODIFIED HUMAN LOW DENSITY LIPOPROTEINS WITH HUMAN SKIN FIBROBLASTS. D. Steinberg *et al.*, *Biochim. Biophys. Acta* 528, 199-212 (1978). 125 I-labeled low density lipoprotein (LDL) covalently bonded to Sepharose beads was not degraded by normal human fibroblasts nor did it trigger inhibition of sterol synthesis. The Sepharose beads loaded with LDL bound very tightly to the surface both of normal fibroblasts and fibroblasts from a subject with homozygous familial hypercholesterolemia; control Sepharose beads (activated sites covered with glycine) did not adhere to either cell type. LDL was extracted by a modification of the method of Gustafson so as to remove essentially all cholesterol, cholesterol ester and triglyceride. This modified LDL was bound, internalized and degraded as well as or better than native LDL. However, it failed to suppress sterol synthesis. These results provide additional evidence that the sterol moiety of the LDL is the key component affecting sterol synthesis. They also imply that the neutral lipids of LDL play a minor role in the binding of LDL to cell membranes and that the apoprotein rather than molecular size and shape is the critical factor.

EFFECT OF VARIOUS TRIGLYCERIDES ON BLOOD AND TISSUE CHOLESTEROL OF CALVES. J.W. Stewart *et al.*, *J. Nutr.* 108, 561-6 (1978). Blood and tissue cholesterol responses were measured in six groups of four calves each fed for 24 weeks reconstituted nonfat dry milk in which 30% of calories was derived from one of the following: soybean oil (SBO), beef tallow (T), medium-chain triglycerides (MCT), and 1:1 soybean-oil/tallow (SBO/T) combination. Two groups of four

calves each also were fed SBO-milk plus dry feed and T-milk plus dry feed, respectively. Blood cholesterol concentration was significantly greater in SBO-fed calves than in T-fed calves. Calves fed MCT-milk had significantly lower blood cholesterol than calves fed T- or SBO-milk. Significant differences in cholesterol concentration were noted for the liver, perianal fat, and for omental fat.

THE ESSENTIALITY OF VITAMIN D METABOLITES FOR EMBRYONIC CHICK DEVELOPMENT. M.L. Sunde *et al.*, *Science* 200, 1067-9 (1978). Laying hens maintained on 1,25-dihydroxyvitamin D₃ as their sole source of vitamin D produce eggs which appear normal but which produce embryos having a defective upper mandible and which die at 18 to 19 days of embryonic life. Hens maintained on 25-hydroxyvitamin D₃, on the other hand, produce normal embryos. Hens fed a vitamin D deficient diet produce eggs which develop the same embryonic defect. Injection of the affected eggs from the 1,25-dehydroxyvitamin D₃ fed hens with vitamin D₃, 25-hydroxyvitamin D₃, or 1,25-dihydroxyvitamin D₃ greatly increases the percentage of normal embryos. It therefore appears that 1,25-dihydroxyvitamin D₃ is not transferred from hen to egg in sufficient amounts to support embryonic development and that vitamin D or its metabolites, or both, are necessary for normal chick embryo development.

CHOLESTEROL ABSORPTION AND STEROID EXCRETION IN CHOLESTEROL-FED GUINEA PIGS. M.G. Traber and R. Ostwald, *J. Lipid Res.* 19, 448-56 (1978). Cholesterol absorption was studied in groups of guinea pigs fed diets containing 0, 0.1%, or 1% cholesterol. A similar proportion of tracer cholesterol was absorbed regardless of the cholesterol content of the diet. Furthermore, the proportion of tracer cholesterol absorbed by individual animals did not change when the cholesterol-free diet was changed to one containing 1% cholesterol. Excretion of total and of neutral steroids was measured in guinea pigs fed 0 or 1% cholesterol-containing diets. The 1% cholesterol-fed guinea pigs increased the excretion of steroids 3-fold over control levels. However, they absorbed more dietary cholesterol than they excreted in any form.

IODINE LABELED HUMAN AND RAT LOW-DENSITY AND HIGH-DENSITY LIPOPROTEIN DEGRADATION BY HUMAN LIVER AND PARENCHYMAL AND NON-PARENCHYMAL CELLS FROM RAT LIVER. T.J.C. Van Berkel *et al.*, *Biochim. Biophys. Acta* 529, 138-46 (1978). The abilities of homogenates of human liver, rat liver parenchymal cells, rat liver non-parenchymal cells and total rat liver to catabolize human and rat iodinated high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined by measuring the amount of trichloroacetic acid-soluble (non-iodine) radioactivity liberated upon incubation at the optimum pH of 4.2. The results indicate that a high proportion of the total rat liver capacity for lipoprotein degradation is localized in the non-parenchymal liver cells and this, together with the active endocytic activity, suggests an important role of these liver cells in hepatic lipoprotein catabolism.

GALACTOLIPID FORMATION IN CHLOROPLAST ENVELOPES. I. EVIDENCE FOR TWO MECHANISMS IN GALACTOSYLATION. A. Van Bewouw and J.F.G.M. Wintermans, *Biochim. Biophys. Acta* 529, 44-53 (1978). Two different enzymes for galactosylation occur in isolated chloroplast envelopes of spinach leaves, UDPgalactose-diglyceride galactosyltransferase and galactolipid-galactolipid galactosyltransferase. The first enzyme is responsible for the biosynthesis of monogalactosyl-diglyceride, UDPgalactose being donor of the galactosyl moiety. The second enzyme is responsible for the biosynthesis of digalactosyldiglyceride and higher homologues. It was concluded that the second enzyme does not require the presence of UDPgalactose, but that galactosyl transfer proceeds by direct exchange of galactosyl groups between molecules of galactolipids, or via unknown lipid intermediates, not detected in our system.

PHASE TRANSITIONS IN PHOSPHOLIPID MODEL MEMBRANES OF DIFFERENT CURVATURE. P.W.M. Van Dijk *et al.*, *Biochim. Biophys. Acta* 506, 183-91 (1978). Nuclear magnetic resonance, light scattering and freeze fracturing electron microscopic techniques were used to characterize the size of unilamellar phospholipid vesicles of 1,2-dimyristoyl-sn-glycero-3-phosphocholine. Differential scanning calorimetric and light scattering analyses showed that very small unilamellar vesicles obtained by the sonication method exhibit a downward shifted,

largely broadened phase transition with a slightly decreased enthalpy change when compared with multilayered liposomes. Furthermore it was shown that ice-water transitions of the systems caused abrupt fusion of the lipid structures.

DESCRIPTION OF OBESITY IN THE PBB/LD MOUSE. S.U. Walkley *et al.*, *J. Lipid Res.* 19, 335-41 (1978). A new strain of obese mouse, the PBB/Ld, has been studied in terms of fat pad cellularity, serum insulin and blood glucose levels, and response to gold thioglucose injections. Age-matched C57B1/6J mice were used as controls. Adipocyte size and number in the major fat depots were determined at various ages from weaning to maturity in the PBB/Ld and C57B1/6J strains. Results indicated that obesity in the PBB/Ld was due to hypertrophy of adipocytes in retroperitoneal and subcutaneous fat depots and to hypertrophy and hyperplasia in the epididymal fat pad. PBB/Ld mice also developed hyperinsulinemia and hyperglycemia and these findings have been discussed in terms of the developmental changes in fat pad cellularity.

THE FORMATION OF LIPID-LINKED SUGARS BY CELL-FREE PREPARATIONS OF LACTATING RABBIT MAMMARY GLAND. D.A. White, *Biochem. J.* 170, 479-86 (1978). A lactating rabbit mammary gland microsomal system catalysed the incorporation of mannose from GDP-(U-¹⁴C)mannose into three endogenous acceptors, (i) polyprenyl phosphate mannose, (ii) lipid-linked oligosaccharide and (iii) protein. Synthesis of polyprenyl phosphate mannose was stimulated by addition of dolichol phosphate to the incubation medium and was reversed by addition of GDP. The product had properties identical with those of authentic dolichol phosphate mannose. The oligosaccharides derived from acid hydrolysis of the lipid-linked oligosaccharide fraction were of six, eight and nine to ten monosaccharide units, the octasaccharide being the major species formed. The oligosaccharide appeared to be attached to the lipid via a pyrophosphate bridge, since strong alkaline hydrolysis liberated an oligosaccharide phosphate. Polyprenyl phosphate mannose served as a mannose donor to lipid-linked oligosaccharides and protein. When added as exogenous substrate it gave rise to a lipid-linked oligosaccharide of about six units. Incorporation of radioactivity in protein was low, but polyacrylamide-gel electrophoresis of the protein fractions indicated that polypeptides of mol. wts. 115,000, 75,000 and 33,000 were labeled.

MECHANISM OF COUMARIN ACTION: SIGNIFICANCE OF VITAMIN K EPOXIDE REDUCTASE INHIBITION. D.S. Whitton *et al.*, *Biochemistry* 17, 1371-7 (1978). Vitamin K functions in a microsomal carboxylation reaction that converts glutamyl residues in precursor proteins to γ -carboxyglutamyl residues in the products of this reaction. The same liver microsomal preparations that carry out this carboxylation also convert the vitamin to its 2,3-epoxide (epoxidase activity) and reduce the epoxide to the vitamin (epoxide reductase activity). The effect of the coumarin anticoagulant Warfarin on these reactions has been studied. These data support the theory that the vitamin K-vitamin K epoxide interconversion is a physiologically important cycle of the vitamin and that the action of Warfarin as an anticoagulant might be to block this cycle. The physiologically important reducing agent which is replaced by dithiothreitol in these *in vitro* studies has not been identified.

ACTION OF PHOSPHOLIPASES A₂ ON PHOSPHATIDYLCHOLINE BILAYERS. EFFECTS OF THE PHASE TRANSITION, BILAYER CURVATURE AND STRUCTURAL DEFECTS. J.C. Wilschut *et al.*, *Biochim. Biophys. Acta* 508, 185-96 (1978). We examined the action of porcine pancreatic and bee-venom phospholipase A₂ towards bilayers of phosphatidylcholine as a function of several physical characteristics of the lipid-water interface. The results lead to the general conclusion that structural irregularities in the packing of the substrate molecules facilitate the action of phospholipases A₂ on phosphatidylcholine bilayers. Within the phase transition and with bilayers containing structural defects these irregularities represent boundaries between separate lipid domains. The stimulatory effect of strong bilayer curvature can be ascribed to an overall perturbation of the lipid packing as well as to a change in the phase-transition temperature.

IN VITRO BIOSYNTHESIS OF PHOSPHOLIPIDS BY CHONDROCYTES AND MATRIX VESICLES OF EPIPHYSEAL CARTILAGE. R.E. Wuthier *et al.*, *Biochemistry* 17, 1431-6 (1978). Matrix vesicles are

extracellular structures involved in endochondral calcification. They have a phospholipid composition distinct from that of chondrocytes from which they appear to be derived, but controversy exists concerning their origin. To elucidate the pathways involved in their formation, phospholipid biosynthesis by chondrocytes and matrix vesicles, either in tissue slices or as isolated fractions, was studied utilizing ¹⁴C-labelled lipid precursors: acetate, palmitate, eicosatrienoate and L-serine. Although matrix vesicles were enriched in SPH, PS, and the lyso forms, none of these showed enhanced biosynthesis by either chondrocytes or matrix vesicles. This indicates that selective degradation of phospholipids and shedding of the modified membrane are involved in matrix vesicle formation.

CHARACTERIZATION OF THE MICROSOMAL STEROID-8-ENE ISOMERASE OF CHOLESTEROL BIOSYNTHESIS. N. Yamaga and J.L. Gaylor, *J. Lipid Res.* 19, 375-82 (1978). Rat liver microsomes contain an enzyme that catalyzes the isomerization of the nuclear double bond of steroids from the 8(9) position to the 7(8) position. The enzyme is most active with zymosterol, 5 α -cholesta-8,24-dien-3 β -ol, which is a precursor of cholesterol. Properties of the microsomal isomerase have now been studied, and preliminary data are reported on both regulation of enzymic activity and first steps in the solubilization of the enzyme from membranes. Isomerase activity is destroyed by phospholipase A digestion, high concentration of bile salts, and solvent extraction, all of which are known either to remove phospholipid or to alter microsomal membrane integrity. On the other hand, isomerase remains active in the presence of a mild, nonionic detergent, Triton WR-1339; thus, solubilization with nonionic detergents is under study.

A MODEL FOR STUDYING LCAT REACTION: IN VITRO CHOLESTEROL ESTERIFICATION IN PIG OVARIAN FOLLICULAR FLUID. J.K. Yao *et al.*, *Lipids* 13, 225-31 (1978). Phosphatidylcholine acyltransferase (lecithin:cholesterol acyltransferase or LCAT; EC 2.3.1.43) activity was found to be present in pig ovarian follicular fluid (POFF), in addition to pig serum

(PS). The cholesterol esterification rate in both POFF and PS is linear with incubation time up to 2 hr. It is concluded that the LCAT of POFF, as well as that of plasma, is specific for individual fatty acids rather than for the fatty acid composition of phosphatidylcholine. The fatty acid concentration of lysophosphatidylcholine decreased during prolonged incubation times (6 to 21 hr) suggesting that the increased lysophosphatidylcholine formed as a product of the LCAT reaction may be reused as substrate for the LCAT reaction or for hydrolysis by lysophosphatidylcholine hydrolase.

ACTION OF SURFACE-ACTIVE SUBSTANCES ON BIOLOGICAL MEMBRANES. II. HEMOLYTIC ACTIVITY OF NONIONIC SURFACTANTS. B.Y. Zaslavsky *et al.*, *Biochim. Biophys. Acta* 507, 1-7 (1978). The hemolytic action of commercially available nonionic surfactants and synthesized polyoxyethylene fatty acids and mercaptans on human erythrocytes was measured. It is shown that the hemolytic power of the detergents depends on the mutual effect of the hydrophobic and hydrophilic fragments of the agent molecule and does not depend on the hydrophile-lipophile balance of the compounds. It is concluded that when the correlation between the hydrophile-lipophile balance values and a membrane effect the capacity of the surfactants this indicates that the effect is caused not by destruction of the membrane but by some rearrangement of the membrane structure accompanying the surfactant adsorption.

CHEMICAL CHARACTERIZATION OF EGG YOLK MYELIN FIGURES AND LOW-DENSITY LIPOPROTEINS ISOLATED FROM EGG YOLK GRANULES. T.D. Garland and W.D. Powrie, *J. Food Sci.* 43, 1210-4 (1978). Myelin figures (MF) and low-density lipoprotein (LDLg) in yolk granules were isolated from the sub-pellicle fraction formed during the supercentrifugation of a granule dispersion in 10% NaCl (Garland and Powrie, 1978). The MF and LDLg fraction were analyzed for total nitrogen, total phosphorous, total lipid, cholesterol and phospholipids. MF fraction possessed components which were similar to those for LDLg, but concentrations of the components were

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different. Phosphatidyl choline was the predominant phospholipid in both MF and LDLg, and small amounts of phosphatidyl ethanolamine, lysophosphatidyl ethanolamine and sphingomyelin were present.

ACID-LABILIZATION OF STEROLS FOR EXTRACTION FROM YEAST. R.A. Gonzales and L.W. Parks, *Biochim. Biophys. Acta* 489, 507-9 (1977). A wild type strain of yeast, *Saccharomyces cerevisiae*, pretreated with a mild acid hydrolysis, exhibited a 4-fold increase in sterol yield upon saponification and extraction. This increased yield is reflected in both major and minor sterols (ergosterol; zymosterol) and sterol esters.

LOCALISATION AND CHARACTERIZATION OF THE FATTY ACID SYNTHESIZING SYSTEM IN CELLS OF GLYCINE MAX (SOYBEAN) SUSPENSION CULTURES. H.G. Nothelfer *et al.*, *Biochim. Biophys. Acta* 489, 370-80 (1977). In course of a study of fatty acid synthetase in higher plants, non-green cell suspension cultures of *Glycine max* (soybean) served as model tissues. For the first time, a fatty acid synthesizing system was characterized in cell cultures of higher plants and was found to be solely located in proplastids of the cells. Under the conditions of the experiment, only small amounts of polyunsaturated fatty acids, the main fatty acid components of this organelle, were synthesized. In respect to fatty acid synthesis, the non-green cell suspension culture resembles photosynthetic leaf tissue.

LIPOSOMAL MEMBRANES I. CHEMICAL DAMAGE OF LIPOSOMAL MEMBRANES WITH FUNCTIONAL DETERGENT. J. Sunamoto *et al.*, *Biochim. Biophys. Acta* 510, 52-62 (1978). The interaction and reaction between liposomal membrane and a functional detergent, *N*-hexadecyl-*N*-(imidazol-4-yl)methyl-*N,N*-dimethylammonium chloride hydroperchlorate (Im-I), have been investigated in conjunction with the leakage of bromothymol blue encapsulated as a marker in the bilayers of liposomes. Im-I carries an imidazole moiety and was expected to behave as a simple lipase model.

REGULATION OF FATTY ACID COMPOSITION OF CYTIDINE DIPHOSPHATE DIACYLGLYCEROL BY ACYL TRANSFER REACTIONS. W. Thompson and G. MacDonald, *J. Biol. Chem.* 253, 2712-5 (1978). Cytidine diphosphate monoacylglycerol was prepared by the action of snake venom phospholipase on cytidine diphosphate diacylglycerol and shown to be readily acylated by rat liver microsomes. Acylation was monitored by spectrophotometric assay or by measuring the incorporation of radioactivity from ¹⁴C-labeled acyl-CoA esters. The product was identified as CDP-diacylglycerol. The acylation reaction was time-dependent, showed a linear response to microsomal protein concentration and had a pH optimum of 7.0. The high rates of acylation of CDP-monoacylglycerol obtained in these experiments suggest that this is an important mechanism for regulating the fatty acid composition of the liponucleotide in rat liver.

STRUCTURE OF NEUTRAL GLYCOLIPIDS IN BOVINE THYROID TISSUE. G. Van Dessel *et al.*, *Biochim. Biophys. Acta* 528, 399-408 (1978). Four asialo glycolipid fractions have been isolated from bovine thyroid glands. The structures were elucidated by partial hydrolysis, periodate oxidation, permethylation analysis and sequential enzymatic degradation studies. The following structures were identified: GL-1a glucosyl- β -(1 \rightarrow 1)ceramide; GL-1b galactosyl- β -(1 \rightarrow 1)ceramide; GL-2 galactosyl- β -(1 \rightarrow 4)glucosyl- β -(1 \rightarrow 1)ceramide; GL-3 galactosyl- α -(1 \rightarrow 4)galactosyl- β -(1 \rightarrow 4)glucosyl- β -(1 \rightarrow 1)ceramide; GL-4 *N*-acetylgalactosaminyl- β -(1 \rightarrow 3)galactosyl- α -(1 \rightarrow 4)galactosyl- β -(1 \rightarrow 4)glucosyl- β -(1 \rightarrow 1)ceramide.

PROTEIN-LIPID INTERACTIONS IN CYTOCHROME OXIDASE FROM SACCHAROMYCES CEREVISIAE. EFFECTS OF DETERGENTS AND RECONSTITUTION OF ENZYME ACTIVITY BY PHOSPHOLIPIDS BY USING CHOLATE-MEDIATED EXCHANGE. M. Virji and P.F. Knowles, *Biochem. J.* 169, 343-53 (1978). Cytochrome oxidase, purified from the yeast *Saccharomyces cerevisiae*, was shown to have associated phospholipid, cholate or detergent, which could be varied by dialysis or (NH₄)₂SO₄ precipitation of the protein. Cholate and the detergents Triton X-100 and V_{max}, but not the K_m, for ferrocytochrome *c* as the cholate concentration was varied indicate that cholate increases the number of exposed active sites of the enzyme. Cholate was used to introduce chosen phospholipids into the lipid environment of yeast cytochrome oxidase. Kinetic studies clearly showed that cholate can mediate exchange of exogenous for endogenous phospholipid.

PHOSPHOLIPID-DEACYLATING ENZYMES OF RAT STOMACH MUCOSA. M.K. Wassef *et al.*, *Biochim. Biophys. Acta* 528, 318-30 (1978). Rat stomach mucosa exhibited three distinguishable phospholipid-deacylating enzyme activities: lysophospholipase, phospholipase A₁ and phospholipase A₂. Phospholipases A₁ and A₂ retained about 50% of their activities by heating at 75° for 10 min. At 100°, phospholipase A₁ retained 22% of its activity, whereas phospholipase A₂ retained only 7%.

ABSORPTION OF SYNTHETIC, STEREOCHEMICALLY DEFINED ACYLGLYCEROLS IN THE RAT. B. Akesson *et al.*, *Lipids* 13, 338-43 (1978). The stereochemistry of fat digestion and absorption was investigated in rats with thoracic duct fistulas, after feeding synthetic triacylglycerol or alkyldiacylglycerol. After feeding 1,2-dilauroyl-3-oleoyl-*sn*-glycerol, dilauroyl-oleoylglycerol and lauroyldioleoylglycerol were the most abundant chyle triacylglycerols. Positional analysis of the fatty acid distribution and the absence of optical activity indicated that the following structures dominated: *rac*-1,2-dilauroyl-3-oleoylglycerol and *rac*-1,3-dioleoyl-2-lauroylglycerol. The appearance of oleic acid with different labels in chyle and intestinal lipids did not differ, indicating the absence of stereospecificity in fat digestion. Possible explanations for the low absorption are discussed.

CHEMICAL AND ENZYMIC STUDIES ON THE CHARACTERIZATION OF INTERMEDIATES DURING THE REMOVAL OF THE 14 α -METHYL GROUP IN CHOLESTEROL BIOSYNTHESIS. THE USE OF 32-FUNCTIONALIZED LANOSTANE DERIVATIVES. M. Akhtar *et al.*, *Biochem. J.* 169, 449-63 (1978). By using cell-free preparations of rat liver it was shown that the removal of the 14 α -methyl group (C-32) of steroids containing either a $\Delta^{7(6)}$ or a $\Delta^{8(9)}$ double bond is attended exclusively by the formation of the corresponding 7,14- and 8,14-dienes respectively (structures of types III and VIII). Cumulative evidence from a variety of experimental approaches has led to the deduction that $\Delta^{8(9)}$ -steroids are not involved as intermediates on the major pathway of cholesterol biosynthesis. The metabolism of (32-³H)anost-7-ene-3 β ,32-diol (structure of type I) results in the formation of radioactive formic acid, no labelled formaldehyde being formed. A detailed overall pathway for the 14 α -demethylation in cholesterol biosynthesis is considered and proposals about the mechanism of individual steps in the pathway are made.

METABOLISM OF EICOSA-11,14-DIENOIC ACID IN RAT TESTES. EVIDENCE FOR Δ^8 -DESATURASE ACTIVITY. D.H. Albert and J.G. Coniglio, *Biochim. Biophys. Acta* 489, 390-6 (1977). The metabolism of (¹⁴C)eicosa-11,14-dienoic acid was investigated in rat testes in vivo and in vitro. Intratesticular injection of (1-¹⁴C)eicosa-11,14-dienoic acid resulted in the appearance of radioactivity (4-30% of ¹⁴C in total fatty acids) in 20-carbon trienoic fatty acids and a small amount (2-3.5%) in arachidonic acid. These results are compatible with a limited desaturation of eicosa-11,14-dienoic acid to eicosa-8,11,14-trienoic acid and provide evidence for Δ^8 desaturase activity in rat testis.

1,2-PROPANEDIOL-INDUCED CHANGES IN PLASMA AND TISSUE LIPIDS OF RATS. M.K.P. Amma *et al.*, *Lipids* 13, 455-7 (1978). Oral administration of 1,2-propanediol to rats in a daily dose of 1 ml of 28.4% aqueous solution per 100 g body weight for 30 days caused a significant decrease in the total lipids, fatty acids, phospholipids, and triglycerides of plasma, liver, and heart. The cholesterol content in plasma decreased while that in the tissues increased significantly. The accumulation of cholesterol in tissues tends to discourage long term use of 1,2-propanediol even by the oral route.

HIGH DENSITY LIPOPROTEIN DISTRIBUTION. RESOLUTION AND DETERMINATION OF THREE MAJOR COMPONENTS IN A NORMAL POPULATION SAMPLE. D.W. Anderson *et al.*, *Atherosclerosis* 29, 161-79 (1978). In an earlier report we identified at least 3 major components within the serum total HDL distribution of normal subjects. In the present study, the serum concentrations of these components, which we designate HDL_{1s}, *d* 1.063-1.100 g/ml; HDL_{2s}, *d* 1.100-1.125 g/ml; HDL_{3s}, *d* 1.125-1.200 g/ml are determined for 160 clinically-screened subjects from a normal population sample. This determination involves graphically fitting reference schlieren patterns for each of the three components to the subjects' total HDL schlieren patterns with the aid of a computer.

A QUANTITATIVE ANALYSIS OF FINE STRUCTURE AND DRUG METABOLISM IN LIVERS OF CLOFIBRATE-TREATED YOUNG ADULT

AND RETIRED BREEDER RATS. L.E. Anthony *et al.*, *J. Lipid Res.* 19, 154-65 (1978). The effects of clofibrate on the fine structure and drug-metabolizing capacity of livers of normo-lipidemic young adult virgin (YA) and hypercholesterolemic retired breeder (RB) male rats were measured by morphometric and biochemical procedures. The oral administration of clofibrate for 7 days significantly increased liver weight and reduced the cholesterol concentrations in the serum and liver tissue in both groups of animals. The hepatic triglyceride (TG) concentration and the volume of cytoplasmic lipid droplets, presumably TG, as well as the serum TG concentration, increased only in the drug-treated RB rats. The results suggest that there is no positive correlation between the hypocholesterolemic response to clofibrate and the degree of subcellular changes in the hepatocytes and that this hypo-lipidemic drug elicits a minimal effect on the concentrations of the components of the hepatic microsomal drug-metabolizing system.

ANTIOXIDANTS IN NEOPLASTIC CELLS: I. CHANGES IN THE ANTIOXIDATIVE CAPACITY OF MOUSE NEUROBLASTOMA CELLS MEASURED BY A SINGLE-PHASE ASSAY. R.M. Arneson *et al.*, *Lipids* 13, 383-90 (1978). Cultured mouse neuroblastoma cells exhibit a striking increase in antioxidative capacity during the transition from logarithmically dividing cells to nondividing, neurite-bearing cells. Two physically separable phenomena are involved: (a) the membrane pellet of neurite-bearing cells is highly resistant to lipid peroxidation, and (b) the postmicrosomal supernatant of these cells inhibits peroxidation in rat liver mitochondria and other biological membranes. A precise, single-phase assay has been developed for assessing antioxidant levels in lipid extracts. By means of this assay, the increase in membrane resistance to lipid peroxidation has been correlated with a threefold increase in the antioxidant activity of the neuroblastoma neutral lipid fraction. This finding implies that generation of a neutral lipid antioxidant (or antioxidants) is involved in the profound increase in antioxidative capacity which occurs in differentiating neuroblastoma cells.

ANTIOXIDANTS IN NEOPLASTIC CELLS: II. ISOLATION AND PARTIAL CHARACTERIZATION OF A PHENOLIC ANTIOXIDANT FROM DIFFERENTIATED MOUSE NEUROBLASTOMA CELLS. R.M. Arneson and J.D. Wander, *Lipids* 13, 391-5 (1978). The generation of an antioxidant has been shown to be associated with the dramatic increase in resistance to lipid peroxidation which occurs during the differentiation of mouse neuroblastoma cells in culture. The antioxidant has been isolated from the neuroblastoma neutral lipid fraction and partially characterized by means of low-resolution and high-resolution mass spectrometry and other lines of evidence. All presently available information suggests that this antioxidant is a highly aromatic, monosubstituted phenol having the molecular formula $C_{15}H_{14}O_2$.

RUMINAL HYDROGENATION OF CHOLESTEROL. J.R. Ashes *et al.*, *J. Lipid Res.* 19, 244-9 (1978). Cholesterol was hydrogenated by anaerobic incubation with sheep rumen fluid for periods up to 20 hr. The principal product of cholesterol hydrogenation was identified as coprostanol. Cholesterol could be protected against in vitro ruminal hydrogenation by encapsulation in a matrix of formaldehyde-treated casein. Formaldehyde-treated casein-cholesterol preparations were also shown to be protected against hydrogenation in vivo and, when supplements containing 1 g per day of protected or unprotected cholesterol were fed to sheep over a period of 8-9 weeks, there were marked differences in the plasma cholesterol response. The plasma cholesterol of the sheep fed protected cholesterol increased by at least 60%. The plasma cholesterol of the sheep fed unprotected cholesterol also tended to increase during the first 5 weeks of supplementation but thereafter declined to almost control levels at 8 weeks.

A COMPARISON OF MOLECULAR PROPERTIES OF HEPATIC TRIGLYCERIDE LIPASE AND LIPOPROTEIN LIPASE FROM HUMAN POST-HEPARIN PLASMA. J. Augustin *et al.*, *J. Bio. Chem.* 253(9), 2912-20 (1978). Hepatic triglyceride lipase was isolated from human post-heparin plasma by the method of Ehnholm *et al.* using modification which increased the specific activity 12-fold to approximately 3,000 μmol of free fatty acid/h/mg of protein. Lipoprotein lipase with similar specific activity was prepared from the same plasma samples using heparin and concanavalin A affinity chromatography. The molecular weight

of hepatic triglyceride lipase (69,000) was slightly greater than that of lipoprotein lipase (67,000) as determined by polyacrylamide electrophoresis in sodium dodecyl sulfate-containing buffers. These proteins had identical amino acid compositions, terminal amino acid residues, and tryptic peptide maps. However, the differences previously described regarding optima of pH and ionic strength and the requirement for apolipoprotein CII (only for lipoprotein lipase) were maintained in the highly purified state. It was found that both proteins contain approximately 8% carbohydrate. Antisera prepared in goats selectively precipitated each activity. Other antisera prepared in chickens reacted with both enzymes, suggesting a common antigenic determinant.

ACUTE EFFECT OF DIETARY THERAPY OF TYPE IV HYPERLIPOPROTEINAEMIA ON THE SERUM AND LIPOPROTEIN CONCENTRATIONS AND RELATIVE COMPOSITION. D. Ballantyne *et al.*, *Atherosclerosis* 30, 79-87 (1978). In a detailed study the acute effect of diet and hospital admission on the plasma and lipoprotein lipid concentrations and composition was studied in 28 patients with type IV hyperlipoproteinaemia. Within 6 days there were significant falls in the mean serum cholesterol and triglycerides, very low density lipoprotein (VLDL) cholesterol and triglycerides and in high density lipoprotein triglycerides. These changes were accompanied by a significant rise in the mean low density lipoprotein (LDL) cholesterol concentrations. Using multiple regression models highly significant predictions of the change in VLDL triglyc-

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eride ($R^2 = 0.71$) and LDL cholesterol ($R^2 = 0.47$) were obtained utilising the pre-treatment lipoprotein levels as independent variables. Since elevated LDL cholesterol concentrations are associated with atherosclerotic disease such models may have important therapeutic applications.

FEEDING STUDIES IN WEANLING RATS WITH DORSOMEDIAL HYPOTHALAMIC LESIONS: EFFECT OF HIGH FAT AND HIGH CARBOHYDRATE DIET AND NUTRIENT COMPLETENESS ON FOOD CHOICE AND INTAKE. L.L. Bernardis *et al.*, *J. Nutr.* 108, 753-8 (1978). Weanling and mature rats with dorsomedial hypothalamic lesions (DMNL) show profound hypophagia. The present study was conducted to assess whether the hypophagia in the DMNL rat might be due to a disruption of systems subserving correct food (nutrient) choice, taste, or the sensing of dietary consistency. To this end, DMNL rats and sham-operated controls were exposed to three dietary regimens. In conjunction with previous findings of normal body composition, growth hormone and insulin levels and normal gluconeogenesis in DMNL rats, the data are consistent with the hypothesis that DMN lesions "reset" food intake and/or body weight to a lower level at which the animal can subsist on a smaller energy intake.

THE MEASUREMENT OF PHOSPHATIDATE PHOSPHOHYDROLASE IN HUMAN AMNIOTIC FLUID. J.E. Bleasdale *et al.*, *Biochim. Biophys. Acta* 528, 331-43 (1978). Phosphatidate phospho-

hydrolase (EC 3.1.3.4) activity can be found in late gestational human amniotic fluid and is thought to originate in type II alveolar cells of the fetal lungs where it plays an important role in lung surfactant synthesis. In the present study, phosphatidate phosphohydrolase activity was detected and characterized in a 105,000 × g pellet of amniotic fluid using either (^{32}P)phosphatidate or a water-soluble analog, 1-O-hexadecyl-*rac*-(2- 3H)glycerol 3-phosphate as substrate.

A NOVEL, SEMIAUTOMATED METHOD FOR THE ESTIMATION OF FREE FATTY ACID IN SERUM OR PLASMA. D.E. Bowyer *et al.*, *J. Lipid Res.* 19, 274-80 (1978). A modification of the semi-automated assay of Antonis for free fatty acid is presented. Free fatty acids are extracted from serum or plasma into di-*n*-butyl ether-2-methoxyethanol; the extract is almost free from phospholipids. The acids are analyzed in a portion of extract by a copper soap method using diphenylcarbazide. The extractant, being less dense than water, is easily separated from an aqueous phase both in the extraction of samples and in the assay of copper soaps. The assay is comparable in accuracy with well-tried titrimetric methods and is quicker and easier to operate. ●

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